WORLD ASSOCIATION FOR BUIATRICS

Istanbul 33rd WORLD BUIATRICS CONGRESS

WBC 2026

6-10 September 2026

Global Solutions for Animals & Environment

IN COLLABORATION WITH

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Volume -2-

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PREFACE

The Farm Animal Veterinary Medicine Association (FAVMA) – Türkiye is honored and pleased to host the 33rd World Buiatrics Congress (WBC) for the first time in Türkiye between the dates of 6-10 September 2026. This important scientific event is organized in coordination with the World Association for Buiatrics (WAB) and with the contributions of the European College of Bovine Health Management (ECBHM).

In recent decades, significant changes have occurred in the field of bovine medicine with bovine health and herd health management gaining more importance than curative medicine. Therefore, in creating the scientific program of our congress, we have given ample space to presentations covering diseases and treatments, as well as nutrition, production efficiency, preventive medicine, and herd health. The scientific sessions of the congress also cover the topics of sheep, goats, buffalo, and camelids, in addition to cattle.

In line with the theme "Global Solutions for Animals & Environment," the congress specifically addresses global warming, epidemics and zoonotic diseases, antimicrobial resistance, and the one health approach to solving these problems, which have increasingly threatened animal and human health in recent years. The scientific program includes 59 keynote speeches, 220 oral and 589 poster presentations, along with 3 workshops, one of which will be held on a farm. Additionally, specific to WBC2026 Istanbul, a round table discussion focusing on various aspects of transition management in dairy cattle has been organized. Furthermore, sponsors are organizing 3 satellite symposia and 8 satellite conferences. At the 33rd WBC 2026 in Istanbul, prizes were organised for the top three winners of the poster presentations by young researchers.

We hope that all participants will enjoy the pleasure and happiness of being in Istanbul, a city that bridges Asia and Europe with its history, natural beauty, gastronomy, and culture. Enjoy the city of Istanbul and the Turkish hospitality in WBC2026 Istanbul.

We would like to express our sincere gratitude to the esteemed scientists who delivered keynote presentations at the congress, to the researchers who contributed to the scientific program with oral and poster presentations, to the members of the Scientific Advisory Board, and to the session chairs. We thank everyone who showed interest in attending the congress and everyone who contributed to its success. Of course, we owe a debt of gratitude to all our sponsors who provided invaluable support in making this congress a reality.

We hope that WBC2026 Istanbul will showcase innovations in cattle and other ruminant health and diseases worldwide, and that it will enrich the experiences of our colleagues, benefiting all participants and the industry.

Sincerely,

Prof.Dr. Hasan Batmaz

On behalf of the Organizing Committee and the Scientific Committee

Organizing Committee wishes to thank our sponsors for the support

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33rd WORLD
BUIATRICS CONGRESS

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Istanbul



BIOTECHNOLOGY & SUSTAINABILITY

Supporting the wider pathway to net zero: Benchmarking carbon calculation within UK veterinary practice

David Hugh Black

VetSalus, Carlisle, United Kingdom

BACKGROUND and OBJECTIVE: Climate change is already affecting animal health, and achieving Net Zero greenhouse gas emissions by 2050 is essential to limit global warming to 1.5°C. Veterinary professionals operate at the animal–human–environment interface and are well positioned to contribute to climate mitigation through sustainable practice management. Carbon calculation provides a measurable starting point for emissions reduction within veterinary businesses. This collaborative project between Vet Sustain and the University of Surrey aimed to benchmark carbon emissions across UK farm animal and mixed species veterinary practices, identify key emission sources, and generate actionable insights to support sector-wide decarbonisation.

MATERIALS and METHODS: Nineteen UK veterinary practices (farm animal only and mixed species), some operating across multiple sites, were recruited through the British Cattle Veterinarey Association (BCVA) and XLVets. Participants attended an introductory webinar covering climate science and data collection procedures, followed by a summary webinar addressing data collection challenges and action planning. Emissions data were collected at practice level and analysed collaboratively by the University of Surrey and Vet Sustain, with expert guidance from Investors in the Environment (IiE). Ethical approval was granted by the University of Surrey. Carbon dioxide equivalent (CO_2e) emissions were benchmarked per practice and normalised by full-time equivalent employees (FTE-E) and full-time equivalent veterinarians (FTE-V). Emission sources included travel, energy and water use, waste, and anaesthetic and refrigerant gases.

RESULTS: Total CO_2e emissions increased proportionally with FTE size. Mean emissions were 3.88 tonnes CO_2e per FTE-E (range 1.49–7.31) and 7.51 tonnes CO_2e per FTE-V (range 4.14–21.01). Farm-only practices demonstrated higher per-capita emissions, primarily driven by ambulatory travel. In mixed practices, both ambulatory travel and building energy efficiency were significant contributors. Travel constituted the dominant emissions source across most practices, with additional contributions from energy, waste, and anaesthetic/refrigerant use. Variation between practices suggests substantial opportunity for targeted mitigation.

CONCLUSIONS: Benchmarking carbon emissions within veterinary practice is feasible and provides meaningful comparative metrics. Travel—particularly in ambulatory farm services—is the principal emissions hotspot, while building efficiency is a key factor in mixed practices. Normalising data by FTE-E and FTE-V offers complementary perspectives for performance comparison. Limitations of this study include small sample size, data collection variability, and exclusion of certain Scope 3 emissions (e.g., purchased goods, commuting). Systematic tracking of resource use, staff engagement, and expert-supported sustainability planning are recommended to advance the sector's contribution to Net Zero.

Keywords: carbon footprint, net zero, sustainability,

Mapping scientific, educational, and translational research on bovines: A systematic review and text mining approach

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BACKGROUND and OBJECTIVE: Bovines are essential models in biomedical research, veterinary education, and translational medicine due to their significant physiological similarities to humans. Despite their widespread use, a cohesive mapping of bovine-related literature across these diverse fields is lacking. This study systematically characterizes global research trends and identifies key scientific,

educational, and translational trajectories over nearly a century.

MATERIALS and METHODS: Following PRISMA guidelines, a systematic search was conducted in the Scopus® database (January 1928- March 2026).The search combined bovine-related terms (e.g., "cattle," "dairy") with keywords like "scientific research" and "translational application." From 6,442 records, 253 eligible articles were selected after dual independent screening. Descriptive statistics examined trends by year, geographic origin, and journal. Text mining in R utilized Term Frequency-Inverse Document Frequency (TF-IDF) for term relevance and Latent Dirichlet Allocation (LDA) for thematic structuring, selecting a five-topic model for optimal coherence.

RESULTS: Bibliometric mapping indicates a global output led by the United States (11.46%), China (6.72%), and UK (6.32%). An increasing publication trend was observed in the last century. Animals (5.14%), Pakistan Journal of Nutrition (4.35%), and Journal of Dairy Science (3.16%) emerged as the most prolific journals. Highly cited works range from foundational endocrine reviews (Reíter, 1980) to contemporary studies on thermal stress and animal welfare. TF-IDF analysis identified “cell”, “calv”, “vaccin”, “group”, “milk”, “cattle”, “concentr”, “anim”, “product”, “treatment” as core high-significance terms. The LDA model identified five distinct themes: 1) Livestock & Calving Management (n=52): Focusing on field-based observational studies and welfare protocols; 2)Translational Biomedicine & In Vitro Modeling (n=61): Highlighting the link between bovine cells and human health applications; 3) Dairy Systems & Sustainability (n=49): Mapping the institutional integration of dairy science; 4) Lactation Performance & Dietary Interventions (n=44): Concentrating on dietary treatments and milk yield optimization; and 5) Infectious Diseases & Vaccine Development (n=47): Addressing vaccine development and infectious disease management. Topic 2 emerged as the most relevant cluster, representing the highest volume of scientific production and showing the strongest statistical correlation (beta values) with biological terms such as "cell" and "culture".

CONCLUSIONS: This systematic review provides a comprehensive roadmap of bovine research, highlighting a clear shift from traditional agricultural productivity toward sophisticated biomedical applications. The prominence of translational research and high TF-IDF values for "cell" and "treatment" suggest that future efforts should prioritize a "One Health" approach, linking bovine health directly to human medical breakthroughs.

Keywords: Bovine, Traslational Medicine, Management, Sustainability, One Health, Text mining

BUFFALO AND CAMELIDS

Milk-derived EVs play a differential role in boosting innate immune functions and developmental processes on the basis of the lactation curve

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BACKGROUND and OBJECTIVE: Water buffaloes, more resistant to disease and parasites, have a higher capacity to adapt and survive in different environments with distinct topography, climate and vegetation. For this reason, they are a key source of livestock in southern Italy, where they are mainly bred for milk production, as their milk has a significantly higher nutrient concentration than cow's milk. It contains bioactive compounds, including antioxidants and anti-inflammatories, and is rich in fat, lipids, vitamins and extracellular vesicles (EVs). Milk-derived EVs (mEVs) offer diverse advantages: high stability in harsh

environments, making them efficient vehicles for intercellular communication and promising candidates for therapeutic delivery, and carrying a variety of bioactive molecules that contribute to immunity and development. This study aims to characterize mEVs throughout the water buffalo lactation curve and to evaluate their effects on bovine macrophages and buffalo peripheral blood mononuclear cells (PBMC) when derived from colostrum (Colo), 15-day-old milk (15-dom), and 60-day-old milk (60-dom).

MATERIALS and METHODS: The samples were isolated and characterized using various techniques, including Transmission Electron Microscopy, Confocal Microscopy, Nanoparticle Tracking Analysis and Flow Cytometry, enabling a multi-parametric analysis of individual EVs to obtain their concentration, size, and phenotype. EVs characterization is based on staining with anti-CD9 and -CD63 monoclonal antibodies. Finally, the lipid cationic dye (LCD) was used to trace intact EVs. Our FC experiments highlight CD9 and CD63 expression on Colo and milk samples, highlighting a higher EV concentration in Colo. Uptake experiments were conducted using PKH67-labelled EVs and revealed a greater internalization of Colo EVs at both 4h and 24h in BoMac cells, demonstrating major EV persistence over time. Phagocytosis in bovine macrophages was investigated by FITC-labelled Escherichia Coli (E. Coli), revealing no increase in phagocytosis but a minor cell adhesion of E. Coli due to pre-treatment with milk EVS, highlighted by CM. Of note, phagocytosis in buffalo macrophages was increased, particularly in cells pretreated with EV 60-dom.

RESULTS: Furthermore, the cargo composition was assessed and out of the total number of microRNAs (miRNAs) found in Colo (145) and milk-EVs (179), a significant number were found to be expressed in one specific sample type (10 Colo-EV-specific and 44 milk-EV-specific) and were completely absent (or present in negligible quantities) in the other one. These miRNAs contribute to immune tolerance, intestinal cell and neonatal intestinal barrier maturation.

CONCLUSIONS: These findings suggest that mEVs play a role in boosting innate immune functions and are beneficial for developmental processes. (RC IZSME 03/2023).

Keywords: Milk, extracellular vesicles, Bubalus bubalis

Are DST-F and P22 useful for the diagnosis of tuberculosis in water buffalo?

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BACKGROUND and OBJECTIVE: Tuberculosis (TB) is a reported zoonotic disease caused by bacteria of the *Mycobacterium tuberculosis complex*, affecting both domestic and wildlife species. The ante-mortem diagnosis in water buffalo relies primarily on the skin test, while the interferon-gamma release assay (IGRA) represents an official diagnostic test for granting and maintaining Officially Tuberculosis-Free (OTF) herd status, as provided by EU Delegated Regulations 2020/689 and 2020/688. Both tests assess cell-mediated immunity. This study aimed to benchmark the performance of novel antigens, P22 and DST-F, compared with purified protein derivatives (PPDB and PPDA), in both skin test and IGRA (using two commercial kits) in water buffalo.

MATERIALS and METHODS: Skin tests were performed on 508 buffaloes from 13 farms: 3 TB-infected (42 animals) and 10 OTF (466 animals). Animals received intradermal inoculation of PPDB, PPDA, P22 and DST-F. Skinfold thickness was measured before injection and at 72 hours later. For IGRA, 1453 blood samples were stimulated with PPDB, PPDA, P22, DST-F, PBS and PWM. Two kits

were used: KIT1 (optical density differences) and KIT2 (S/P% interpretation, cutoff ≥35%). ROC curves were generated to determine optimal cutoffs and calculate AUC to assess diagnostic accuracy.

RESULTS: At a 4 mm cutoff, skin test performance was compared with qualitative IGRA results using manufacturers' predefined cutoffs. Relative Specificity(Spr) was similar across antigens (≈97% with both kits). Relative Sensitivity(Ser) varied: with KIT1, PPDB showed 49% and P22 46%; with KIT2, both reached 59%. DST-F showed lower Ser (17–22%) compared with SITT and CITT. For IGRA, ROC analysis comparing KIT2 against KIT1 showed excellent diagnostic accuracy for PPDB (AUC=0.98) and DST-F (AUC=0.92), while P22 showed acceptable accuracy (AUC=0.76). Cohen's kappa indicated strong agreement between PPDB-KIT1 and PPDB-KIT2 (κ=0.87), but weak agreement between experimental antigens and standard tests. Optimal cutoffs were 15% for PPDB and DST-F, and 7% for P22.

CONCLUSIONS: Both kits showed comparable performance in identifying skin test-positive buffaloes. Although P22 and DST-F demonstrated high Spr, their Ser was lower than PPDB, especially for DST-F in skin tests. P22 showed promising IGRA performance, reaching sensitivity like PPDB at optimized cutoffs. Lower cutoffs (15% for PPDB and DST-F; 7% for P22) may improve diagnostic accuracy compared to 35%. DST-F showed perfect Spr in OTF herds, suggesting potential value in confirmatory testing. However, the low Ser of experimental antigens in skin tests limits their current standalone use. Further studies including post-mortem confirmed cases are needed to validate and refine diagnostic strategies for buffalo TB eradication programs.

Keywords: tuberculosis, water buffalo, DST-F, P22, IGRA, skin tests

Inside the limbs of the alpaca ({Vicugna pacos}): Comparative insights into proximal musculature

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BACKGROUND and OBJECTIVE: A detailed anatomical understanding of any species underpins meaningful comparative studies and informs our interpretation of evolutionary adaptation. For clinicians, anatomical knowledge directly supports diagnostic imaging, regional anaesthesia, pathophysiological reasoning, surgical planning, and overall patient management. However, comprehensive descriptions of proximal limb musculature in the alpaca (Vicugna pacos) remain limited. The Camelid Anatomy Project was established to expand the functional, comparative, and applied anatomical knowledge base for South American camelids.

MATERIALS and METHODS: The left fore- and hindlimb of a 4 year old female Huacaya alpaca were dissected, and findings were compared with corresponding musculature in other domestic species. The donated specimen was stored at −18 °C until use and thawed at room temperature prior to dissection.

RESULTS: The alpaca's forelimb musculature shared broad similarities with that of cattle, sheep, and horses, but exhibited several distinctive features. Notably, a well developed *subclavius* muscle was present, inserting along the cranial scapular border adjacent to the supraspinatus. Additional findings included extensive muscle adhesions and supplementary muscular extensions that likely enhance stability and facilitate flexion–extension movements of the shoulder. Key hindlimb characteristics included a broad, extensively attached fascia lata with an additional head; a clearly identifiable accessory head and distal bifurcation of the main belly of the middle gluteal muscle; the presence of an *articularis coxae* (typically absent in ruminants); and a comparatively less

developed *gluteobiceps* muscle than in sheep and cattle.

CONCLUSIONS: While the general limb musculature of the alpaca aligns with that of other domestic ungulates, numerous species specific variations were identified. Reductions and fusions within the hindlimb musculature may reflect adaptations for efficient movement and energy conservation, warranting further investigation. Forelimb features suggest a functional mechanism analogous to the equine stay apparatus. Overall, alpacas display unique muscular adaptations that support stability, flexibility, and energy efficient locomotion in challenging terrain.

Keywords: alpaca, anatomy, limb musculature

Clinical and parasitological control of sarcoptic mange in alpacas following oral fluralaner administration

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BACKGROUND and OBJECTIVE: Sarcoptic mange is a major dermatological disease in alpacas (*Vicugna pacos*) that could be fatal in severe cases. Current treatments are challenging due to lack of licensed drugs, need for repeated administrations, and variable efficacy. Fluralaner is an isoxazoline ectoparasiticide approved for mite infestations in dogs, cats, and poultry. In alpacas, its use remains empirical and supported only by a single case report describing successful treatment of sarcoptic mange, while herd-level efficacy and systemic drug concentrations have not been investigated. This study aimed to assess the clinical efficacy and to determine the serum concentrations following a single oral administration of fluralaner in an alpaca herd naturally affected by sarcoptic mange.

MATERIALS and METHODS: Seven adult female alpacas naturally affected by sarcoptic mange received a single oral dose of fluralaner in tablets (5.2 mg/kg) (approval n° 83_2023). At the time of treatment, all animals presented with intense pruritus and typical crusting skin lesions located in the perineal region, muzzle, and caudal thighs. Animals were monitored for three months. Every 15 days, clinical examinations and deep skin scrapings were performed. Animals were considered cured when clinical signs and skin lesions resolved and two consecutive parasitological examinations were negative. Additionally, blood samples were collected before drug administration and at predefined times from 1 hour up to 82 days to determine serum fluralaner concentrations using liquid chromatography mass spectrometry.

RESULTS: Pruritus resolved in all alpacas (7/7, 100%) by the first follow-up evaluation. Skin lesions progressively improved and completely resolved in five animals (71.4%), whereas two subjects (28.6%) showed marked improvement but persistent lesions. Parasitological negativity increased over time, from 28.6% after 15 days to 100% of animals at 60 days after treatment; however, recurrence of positivity was observed in one alpaca during the last follow-up (82 days). Overall, sustained parasitological cure was achieved in five alpacas, while two subjects remained intermittently positive. Peak serum fluralaner values were variable among animals (174.67-635.73 ng/mL) and occurred approximately two days after administration. The last measurable concentrations (1.30-12.30 ng/mL) were detected between 19 and 50 days after treatment. No adverse effects were observed in any animal throughout the entire follow-up period.

CONCLUSIONS: A single oral administration of fluralaner resulted in rapid clinical improvement, with resolution of pruritus in all animals, marked regression of skin lesions, and good treatment tolerance. However, parasitological relapse in some alpacas indicates that a single dose may not ensure consistent long-term control.

Keywords: Camelids, sarcoptic mange, fluralaner, dermatological diseases, pruritus, antiparasitics

CALF HEALTH

Can pH measures be used to estimate bacterial contamination of bovine colostrum?

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BACKGROUND and OBJECTIVE: Bacterial contamination of liquid colostrum for feeding to newborn calves is difficult to determine on farm. Highly contaminated colostrum may have health implications for calves, since bacterial species may be be pathogenic or interfere with absorption of immunoglobulins through neonatal enterocytes. Bacteria produce acid by products from their metabolism, therefore more highly contaminated colostrum is likely to be more acidic, based on lower pH measurement.

The objective of this study was to determine whether pH could be used as a proxy measure for bacterial contamination (using reference standard sheep blood agar plates to measure total bacteria counts (TBC) and MacConkey plates to measure total coliform counts (TCC)) of bovine colostrum.

MATERIALS and METHODS: One hundred and one samples were purposively selected (low, medium and high contamination) from previously frozen (at -20°C) bovine colostrum samples. Sample pH was measured using a pH meter in duplicate and bacteria counts were measured using the reference tests of 5% sheep blood agar and MacConkey agar plats for TBC and TCC, respectively. Univariable mixed models were constructed using farm as a random effect, pH as the outcome of interest and log TBC and TCC as the predictors of interest. A receiver operator characteristic (ROC) curve was created to determine the optimal threshold (using TBC and TCC as reference standards) to accurately predict bacterial contamination (≥100,000 CFU/mL TBC and ≥10,000 CFU/mL TCC) of BC using pH measures. The Youden statistic (Youden, 1950) was calculated based on the sum of the sensitivity and specificity, with equal weight given to both false positive and false negative results.

RESULTS: Small decreases in pH measurement were associated with increases in bacterial contamination of colostrum, particularly coliform species, which are those most implicated in disruption of IgG absorption from colostrum. There was a statistically significant relationship between pH measurements and log transformed bacterial measurements. pH as an estimate of bacterial contamination of colostrum lacked diagnostic accuracy for predicting contamination of colostrum due to low test sensitivity and specificity.

CONCLUSIONS: The pH meter lacked diagnostic accuracy at specified cutpoints, but there was a statistically significant relationship between pH measurements and log transformed bacterial measurements.. pH meters should measure pH to at least two decimal places to be of use to estimate bacterial contamination of colostrum, which needs to be considered when selecting devices for on-farm use. It would be useful to repeat this work using a pH meter in fresh (unfrozen) colostrum samples.

Keywords: Calves, Colostrum, Contamination, pH

A preliminary study of intraperitoneal fluid administration to neonatal calves

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BACKGROUND and OBJECTIVE: The application of intraperitoneal fluid therapy (IPFT) to dehydrated neonatal calves has been limited by the perceived risks, and unknown efficacy despite being cited as an effective rehydration route in practice. This study aims to demonstrate the efficacy and applicability of IPFT to bovine veterinary practice (buiatric neonatology). It is hypothesised IPFT will be an efficient and safe rehydration method in healthy neonatal calves.

MATERIALS and METHODS: Healthy calves aged between 4-14 days (n=4) were administered four litres of isotonic saline via the left paralumbar fossa into the peritoneum (IP). Control animals received no treatment (n=2). Blood and clinical parameters, and ultrasonographic measurements were collected

before and after fluid administration in all animals. Charles Sturt University Animal Ethics Committee approval A24038.

RESULTS: Fluid absorption from the abdominal cavity was indicated by girth diameter reduction and transabdominal ultrasonographic fluid depth reduction. Reduction in systemic total plasma protein, and increases in sodium and chloride concentrations indicated systemic fluid and electrolyte absorption. There were no alterations in clinically measured parameters, nor an elevation in measured serum amyloid A to suggest any adverse effects or inflammatory response occurred in the treated animals. One animal was euthanised 72 hours following IPFT due to prolonged recumbency and diarrhoea. Despite initially meeting the trial selection criteria, it was notably smaller and underconditioned compared to its cohorts. Post-mortem findings were indicative of a localised enteritis in the small intestine without indicators of peritonitis or evidence of internal injury subsequent to IPFT injection. Blood analytes suggested severe acidaemia was the most likely cause of the clinical signs that prompted euthanasia.

CONCLUSIONS: Findings indicate IPFT delivered fluid systemically to calves within a clinically useful timeframe. Moderately dehydrated calves are likely to benefit from IPFT, making it a viable treatment option in these animals[1].

1. Ferguson KP, Quinn CT, Moffat AG, Gunn AJ. Intraperitoneal fluid administration to neonatal calves. Am J Vet Res. 2025 Jul 24;86(10): ajvr.25.03.0103. doi: 10.2460/ajvr.25.03.0103. PMID: 40712651.

Keywords: intraperitoneal, fluid therapy, dehydrated calves, IPFT, bovine

Factors influencing treatment decisions and non-steroidal anti-inflammatory use in pre-weaned calves: Insights from a mixed-methods study of farmers and veterinarians

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BACKGROUND and OBJECTIVE: Pre-weaning calf mortality has been reported to have significant welfare, economic and sustainability impacts on farms, with the most common causes reported to be diarrhoea and respiratory disease (BRD). In some regions, legislation allows farmers to treat individual calves using veterinarian-prescribed antibiotics and non-steroidal anti-inflammatories (NSAIDs). Effective treatment depends upon farm staff's ability to recognise illness and treat promptly, with timely responses being critical for successful recovery.

OBJECTIVE: To identify factors influencing treatment choices in pre-weaned calves using a mixed method research approach.

MATERIALS and METHODS: Study 1: An online survey was used, featuring eight videos of pre-weaned calves displaying various clinical signs to determine the likelihood of respondents treating in sick calves. Data were analysed using descriptive statistics and multivariable logistic regression models.
Study 2: Veterinarian and farmer focus groups were used to identify underlying influences for NSAID use in sick calves through thematic analysis.

RESULTS: Study 1: After exclusions, 657 respondents (314 farmers and 343 veterinarians) from the UK and North America were analysed. Respondents were more likely to use NSAIDs for BRD than for diarrhoea. In individual calf scenarios, farmers with dedicated youngstock rearers, and female farmers were more likely to administer NSAIDs and electrolytes, while male farmers tended to favour antibiotic use. Farmers' treatment decisions were more influenced by specific clinical signs than veterinarians', suggesting greater reliance on intuition and variable interpretation of symptoms. Study 2: NSAID use was shaped b intrinsic factors, including animal welfare values, perceived treatment efficacy, and education,

and extrinsic factors including economic pressures, legislation, and accessibility. Although participants discussed the desire to "do the right thing" for the calf, barriers included limited NSAID knowledge, differing farm priorities, habitual practices, staff skill levels, and variable veterinary support. A recurring theme was the normalisation of poor calf health and the influence of previous disease experiences. The study revealed uncertainty around societal expectations for drug use and ambiguity regarding the role and purpose of injectable medicines. Veterinarians and farmers suggested a need for evidence-based, industry-led initiatives to support NSAID use.

CONCLUSIONS: There was a wide variation in treatment choices for calves, highlighting potential inconsistencies in recognising and responding to clinical signs. NSAIDs were underutilised in generic treatment protocols and for individual calves. The study highlighted strong intrinsic motivation to improve calf welfare. There were multiple barriers reducing the use on farm including limited knowledge; this led to a desire for increased education at an industry level.

Keywords: Animal welfare, Herd Health, Clinical practice, Calf, Treatment decisions, Disease management

Monitoring colostrum harvesting and distribution equipment cleanliness with adenosine triphosphate luminometry before and after recommendations to improve hygiene practices in french dairy farms

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BACKGROUND and OBJECTIVE: In addition to being a potential source of infection, bacterial contamination of colostrum can hinder the absorption of immunoglobulins in calves, leading to inadequate TPI. Feeding equipment has been identified as a major source of colostrum contamination. This observational study evaluated the effects of practical hygiene recommendations on cleanliness and bacterial contamination of equipment used to harvest and feed colostrum on 8 French dairy farms.

MATERIALS and METHODS: The study was conducted in 2 phases: before (phase 1) and after (phase 2) the implementation of farm-specific recommendations. During each phase, equipment cleanliness (e.g., robot-compatible buckets, milking buckets, transfer buckets, bottles, nipples, nipple buckets, drenchers, and esophageal tube feeders [ET]) was assessed using ATP luminometry (through direct surface [ATP-S] and rinsing liquid [ATP-L] swabbing, expressed in relative light units [RLU]), visual scoring, and culture-based bacteriological analysis. Fresh colostrum samples were also collected and analyzed during each phase. A self-reported questionnaire administered during phase 1 identified critical control points for colostrum contamination, which informed the formulation of practical hygiene recommendations. Contamination thresholds were defined as ≥100,000 cfu/mL for total bacterial count (TBC) and ≥10,000 cfu/mL for total coliform count (TCC).

RESULTS: Hygiene practices related to the operator, cow teats, and equipment varied considerably among farms, as did adherence to the recommended practices. Overall, lower RLU values were recorded on equipment surfaces during phase 2 compared with phase 1 (ATP-L: −1.51 log [±0.24], $P < 0.001$; ATPS: −1.15 log [±0.24], $P < 0.001$). These reductions were associated with good operator hygiene practices and the use of brand-new equipment. Lower levels of contamination of colostrum samples were found in phase 2 (TBC: −0.92 log [±0.19], $P < 0.001$; TCC: −1.14 log [±0.30], $P < 0.001$), associated with good operator hygiene practices. Significant correlations (Spearman's rho, rs) were observed between visual cleanliness scores and RLU values from both swabbing techniques, as well as between ATP-L RLU values and bacterial counts (TBC: rs = 0.744, 95% CI: 0.556–0.859, $P < 0.0001$; TCC: rs = 0.475, 95% CI: 0.183–0.690, $P < 0.002$). Based on ATP-L swab results, optimal luminometry cutpoints maximizing both sensitivity and specificity were identified at ≥6,950 RLU (sensitivity: 79.17%; specificity: 100%) for TBC,

and ≥11,912 RLU (sensitivity: 88.89%; specificity: 70.97%) for TCC.

CONCLUSIONS: This study demonstrates that targeted hygiene improvements can significantly reduce both equipment surface and colostrum bacterial contamination and support the use of ATP luminometry as a practical tool for hygiene monitoring on dairy farms.

Keywords: Adenosine Triphosphate, Cattle, Colostrum, Hygiene, Luminometry

Impact of increasing colostrum IgG concentration using a colostrum replacer on the apparent efficiency of IgG absorption in newborn Holstein-Friesian calves

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BACKGROUND and OBJECTIVE: Transfer of passive immunity via intestinal absorption of colostral immunoglobulins shortly after birth confers important health benefits. Prior work on enriching maternal colostrum (MC) with colostrum replacers has been mixed, with some protocols increasing serum IgG and others showing no improvement or decreases. This study evaluated whether enriching low-quality MC with bovine dried colostrum replacer powder affects the apparent efficiency of absorption (AEA) of IgG in newborn Holstein calves.

MATERIALS and METHODS: A longitudinal cohort study was conducted on a Dutch Holstein-Friesian dairy farm (July 2024–January 2025). Calves were assigned based on maternal colostrum quality measured by Brix (%): Non-supplemented MC (N-MC; Brix ≥ 28%) and Supplemented MC (S-MC; Brix < 28%), with supplementation using bovine dried colostrum replacer powder [Calf's Choice Total® 100, SCCL, Canada] mixed directly into fresh MC to target a final Brix of 28%. Approximately 360 g IgG was delivered within three hours after birth to all calves. Serum samples were collected 2–7 days post-birth to measure serum IgG (g/l). AEA% was calculated as [(50 kg)x(0.07)x(Serum IgG, g/l) / (Total IgG Delivered, g)] x 100. Linear regression assessed the relationship between the amount of supplemented IgG and AEA%. Analyses were performed in R.

RESULTS: Sixty-seven calves were enrolled (37 N-MC; 30 S-MC). Non-supplemented MC had higher IgG concentration than supplemented colostrum (102.0 ± 9.8 vs. 92.6 ± 12.0 g/l; p < 0.001). Calves in S-MC received more colostrum volume (4.2 ± 1.1 vs. 3.4 ± 1.2 l; p = 0.006). Total IgG delivered was numerically higher in S-MC (390.8 ± 118.8 g) than N-MC (349.1 ± 138.6 g), but not statistically different (p = 0.2). Serum IgG did not differ significantly between groups (22.4 ± 11.4 g/l S-MC vs. 24.6 ± 11.4 g/l N-MC; p = 0.4). AEA% was significantly lower in S-MC (21.1 ± 10.6%) compared to N-MC (28.4 ± 17.0%; p = 0.044). Furthermore, AEA% was negatively associated with the amount of IgG supplemented in the colostrum (Beta = -0.39, 95% CI -0.74; -0.07, p = 0.029).

CONCLUSIONS: If colostrum has less than 50 g IgG/l (Brix measurement below 22%), consider enriching it with extra antibodies before feeding it to newborn calves. Dried bovine colostrum can be used for this purpose. However, mixing colostrum powder with cow colostrum results in a decrease in AEA%, which implies that sufficient extra antibodies must be provided through the colostrum powder and/or colostrum volume to compensate for this decrease in absorption efficiency.

Keywords: Apparent efficiency of absorption, Passive transfer of immunity, Colostrum replacers, colostrum management, immunoglobulin

Low birthweight in calves: Risk factors and association with health traits, growth performance and survival in Argentinian dairy farms

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BACKGROUND and OBJECTIVE: In central Argentina, it is frequent to observe calves born with low bodyweight. The aim of the present study is to describe this phenomenon, understand the risk

factors and quantify the association of low birthweight with health traits, growth performance and survival.

MATERIALS and METHODS: Four large, confined farms were included in the present study. Holsteins singleton females born between December 2021 till July 2025 from a normal gestion length (260-300dd) and a known birthweight (BW) were enrolled (n=13314). Data included: birth date and weight, culling date, pneumonia and diarrhea occurrence, current daily gain at 3 (CDG3) and 6 months of age (CDG6), dam parity, gestation length and calf outcome. BW was divided into categories: low birthweight calves (LBW) with BW <33.5Kg (1 Standard Deviation from the overall BW mean) and normal BW (NBW). Differences among farm, season and dam parity were assessed using chi-square test. Risk factors for LBW and its association with neonatal diarrhea and pneumonia risk were calculated using logistic regression models. CDG3 and CDG6 least square means were calculated for LBW and NBW. A Cox's proportional hazard model was calculated to address LBW death risk in the first 120 days of life.

RESULTS: Calf mean BW was 37.88±4.26Kg. Overall LBW were 11%, with significant differences among farms (10.1%, 7.0%, 35.0%, 4.5%; p=0.001). LBW proportion was highest in Autumn (14.9%) and in calves born form LACT≥3 dams (12.7%). When risk factors analysis for LBW was performed, farm, gestation length and birth season variables were significant (p<0.001). Each day of gestation length decreased the risk for LBW by 7% (OR 0.93 95% CI 0.92-0.94), while autumn had higher odds for LBW compared to winter (OR 1.56; 95%CI 1.28-1.99), spring (1.6; 95% CI 1.18-2.15) and summer (2.2; 95% CI 1.7-3.0). Dam parity was not significant (p=0.28). LBW had higher odds for neonatal diarrhea (OR 1.32; 95% CI 1.14-1.52) but was not associated with pneumonia (p=0.78). LBW had slightly lower growth performance with 0.008 Kg (p=0.04), lower CDG3 and 0.020Kg (p<0.001) CDG6 compared to NBW. Risk of death was significantly higher for LBW compared to NBW (HR 1.42; 95% CI 1.15-1.74).

CONCLUSIONS: Calf LBW represents a significant issue, especially in certain farms and during the autumn season, possibly due to lack of heat-stress abatement strategies in dry cows. LBW calves had higher risk of diarrhea and lower survival risk, in conjunction with poorer growth performance. These insights may support the adoption of mitigation strategies.

Keywords: Calf, low birthweight, calf health, calf survival, calf growth

Lung ultrasonography used as a diagnostic test for bronchopneumonia diagnosis in calves: Systematic review and meta-analysis using a Bayesian latent-class modelling approach

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BACKGROUND and OBJECTIVE: Bronchopneumonia is one of the most common infectious disease in calves. There is currently no affordable perfectly accurate reference standard (RS) test. Lung ultrasonography (LUS) using various consolidation extension thresholds has emerged as a practical calf-side test that can be done routinely. However, information on this diagnostic test's accuracy is limited especially because no test reaches the accuracy of a gold-standard comparator test. The objective of this systematic review and meta-analysis was to determine the accuracy of LUS while adjusting for imperfect accuracy of available RS tests using a Bayesian latent class meta-analysis approach.

MATERIALS and METHODS: Three different databases were consulted using Ovid Medline, Web of Science Core Collection (Clarivate) and CAB Abstract. The full papers were then read and retained if allowing extraction of calves' cross-classification using at least one definition of LUS positive and LUS negative cases and one of the RS tests. Pooled diagnostic accuracy of LUS was calculated using a Bayesian latent class meta-analysis when at least 5 studies using the same LUS threshold and RS test were available.

RESULTS: Of the 875 studies screened, 26 reported at least one 2 x 2 table cross classifying calves according to LUS results (positive vs negative) and RS test (positive vs negative).The RS test included were Wisconsin Clinical Respiratory Score (WCRS, n=16), California Clinical Respiratory Score (n=4),

other clinical signs combinations (n=5), clinical score combined with bronchial lavage results (n=1), thoracic radiograph (n=1), expert panel diagnosis (n=1) and necropsy (n=1). Various LUS thresholds for test positivity were reported; however, 2 thresholds based on maximal consolidation depth (≥1cm and ≥3cm), were the most commonly reported in 12 and 8 studies, respectively. For LUS≥1cm positivity threshold, the median pooled sensitivity (95% BCI) and specificity were 68.4% (51.7-87.4%) and 91.2% (78.5-99.4%) respectively. For LUS≥3cm positivity threshold, the median pooled sensitivity and specificity were 58.2% (38.4-80%) and 95.8% (86.7-99.4%) respectively. The LUS appeared as a moderately sensitive test with a relatively high specificity. The models revealed heterogeneity that could not be further investigated due to the low number of available studies.

CONCLUSIONS: This meta-analysis allows to determine LUS accuracy accounting for the inaccuracy of RS test. Sensitivity was lower with higher uncertainty when compared with specificity. This study highlights gaps of knowledge and reporting issues such as standardization of LUS protocol, and limited information on respiratory pathogens in most studies.

Keywords: Bovine respiratory disease, lung consolidation, infectious disease, diagnosis

Burning questions: A comparative study of hot-iron vs. hot-air calf disbudding

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BACKGROUND and OBJECTIVE: Calf disbudding is a routine dairy procedure commonly performed with hot iron, though hot air method predominates in Norway. Both approaches rely on high temperatures to burn and destroy the horn-producing cells, thereby preventing horn growth. This study compares both techniques regarding wound healing, secondary infections, and overall calf health.

MATERIALS and METHODS: Holstein Friesian bull calves (n=22, aged=22–40 d) were randomly assigned to two thermal disbudding groups. Hot air disbudding was performed using a hot air gun in two steps. First, the skin over the horn bud was cauterized with hot air heated to 550°C for 10 s. Then, the heated skin patch was removed, and the horn bud itself was cauterized for 20 s at the same temperature. The hot-iron group received a single 15 s cauterization (600°C) without scooping. All calves were provided with sedation, local anesthesia, and analgesia. Wound healing (tissue-type scoring) and calf health (Wisconsin score) were monitored at 2 d, 7 d, and then weekly until 63 d post-procedure. To evaluate subclinical inflammation, blood samples were collected before the procedure (day 0), and at 3 h, 2 d, 7 d, 14 d, 21 d, and 35 d post-disbudding. Wound temperature was recorded using a thermal camera immediately after and again at 5 and 30 minutes post-procedure.

RESULTS: Time-to-event (survival) analysis of wound healing showed no significant differences between groups, although one calf in the hot-iron group was not fully healed (based on the presence of epithelial tissue) by day 63. Purulent discharge was

observed in 3 calves (5 horn buds) from the hot-iron group only. One calf in the hot-iron group exhibited profuse bleeding for 30 minutes post-procedure, while one calf in the hot-air group showed heavier bleeding on day 14 after the calf dislodged the necrotic tissue. Generalized estimating equation models were used to analyze skin temperature and blood parameters. Wound temperature decreased significantly at 5 and 30 minutes post-dehorning, with no differences observed between the groups. The disbudding method did not significantly affect Hp, SAA, Fb, or substance P concentrations, although significant temporal changes were noted for all parameters, except for Hp. The hot-iron group had significantly higher clinical scores on days 2 and 42 compared to the hot-air group (Mann-Whitney U test).

CONCLUSIONS: Although both methods had comparable healing times, wound infections were observed exclusively in the hot-iron group. This may be attributed to differences in wound type created by each respective technique.

Keywords: calf disbudding, thermal disbudding, hot iron, hot air, wound healing

Assessing the effect of systemic inflammation on recovery in dairy calves diagnosed with bovine respiratory disease

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BACKGROUND and OBJECTIVE: Failure to recover from bovine respiratory disease (BRD) after initial antimicrobial treatment occurs in approximately 20% to 40% of dairy calves, resulting in increased antimicrobial use, additional labor, and potential long-term performance consequences. Systemic inflammation at the time of diagnosis may influence disease resolution, yet its role in short-term recovery remains unclear. The objective of this prospective cohort study was to assess the effect of systemic inflammation at BRD diagnosis on disease recovery in pre-weaned dairy calves.

MATERIALS and METHODS: From August to December 2025, 120 grouped-housed dairy calves from Virginia Tech Dairy Farm had thoracic ultrasonography (TUS) weekly for diagnosis of BRD (TUS score ≥ 3). Upon BRD diagnosis, a blood sample was collected to measure serum concentrations of haptoglobin (Hp) and ceruloplasmin (Cp) as indicators of systemic inflammation. Calves were reassessed 8 ± 1 days after diagnosis and were classified as recovered if they had a TUS score of ≤ 2. At that time a second blood sample was collected to determine Hp and Cp concentrations. All calves diagnosed with BRD received the same treatment as per farm protocol. Only the first diagnosis of BRD per calf was considered for the study. Calves diagnosed and treated for BRD by farm personnel were not enrolled in the study. Milk intake information was collected from the calf barn automatic feeder.

RESULTS: A total of 28 dairy calves were diagnosed with BRD. Recovery occurred in 53.5% (13/28) of the calves. The average age at diagnosis was 46.5 d (SE = 4.8), and it was not different between recovered and not-recovered animals (P = 0.51). Serum Cp (P = 0.29) and Hp (P = 0.58) at diagnosis were not associated with recovery. The percentage of milk intake per day increased over time following BRD diagnosis (P < 0.01), and it was not different between recovered and non-recovered animals (P = 0.36). Serum Cp (P = 0.56) and Hp (P = 0.86) concentrations at diagnosis were not associated with the percentage of milk intake over time. A moderate positive correlation was observed between Hp concentrations at diagnosis and at the recovery assessment (r = 0.36, P = 0.07), suggesting that inflammatory responses may persist in some calves, although this association was not statistically significant.

CONCLUSIONS: These findings suggest that acute-phase protein concentrations at diagnosis may not be reliable predictors of short-term recovery. Larger studies are warranted to further investigate the role of persistent systemic inflammation in BRD outcomes.

Keywords: Youngstock, Pneumonia, Cattle, Treatment

Linking calves from fixed dairy farms to fixed fattening units and its impact on the need for antibiotic treatment of the calves

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BACKGROUND and OBJECTIVE: In Finland dairy surplus calves are typically transported from dairy farms to calf rearing units or fattening units at an early age. Since Finnish dairy farms generally are quite small, calves need to be collected from multiple dairy farms before arriving at the rearer. Commingling calves from different dairy farms could be a potential risk factor for Bovine Respiratory Disease (BRD) and use of antibiotics. The aim of this study was to find out if linking calves from fixed dairy farms to fixed fattening units would affect the need for antibiotic treatments (ABX).

MATERIALS and METHODS: Calves from fixed dairy farms (1 to 7 dairy farms per fattening unit) were transported to fixed fattening units. The fattening unit received 8-16 calves every other week in general, both bull and heifer calves. The data was collected from 10 different fattening units in Finland and includes the calves each unit received for a period of two years between 2018-2025. Calves transported to the fattening units during a period of one year before the intervention were used as historical control. ABX given in the fattening units until slaughter were recorded. Statistical analysis was performed using STATA/MP 18.0. A p-value < 0.05 was considered statistically significant. A logistic regression analysis was used to study the association between the need for ABX and linking calves from dairy farms to fattening units. The fattening farm and batch were used as random factors. Breed, sex and weight at transport from dairy farm were also considered in the analysis. The breeds were grouped into Holstein, Ayrshire, large and small mixed beef breeds and others.

RESULTS: Altogether 6558 calves were included in the analysis. 35.3% of controls received ABX compared to 24.2% of study calves. The statistical analysis indicates that study calves had lower odds of ABX compared to controls (coef -0.643, p=0.001). A larger weight at transport correlated with a lower risk for ABX (coef -0.030, p=0.001). Heifers had lower odds of receiving ABX compared to bull calves (coef -0.522, p=0.001). Large (coef -0.187, p=0.035) and small mixed beef breeds (-0.881, p=0.001) had also lower odds of receiving ABX compared to Holsteins.

CONCLUSIONS: Creating a system in which calves from fixed dairy farms are linked to fixed fattening units appears to lower the risk of ABX. This is likely due to the reduced commingling of animals, which, according to the literature, decreases both illness and stress in calves.

Keywords: BRD, Calf rearing, Commingling

The clubs study: Association of (c)linical signs, ultrasound-detected (lu)ng lesions, (b)ody weight, and (s)erum total protein in veal calves on arrival at a collection center with mortality during the rearing period

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BACKGROUND and OBJECTIVE: Mortality in veal calves is an important welfare and economic concern, particularly during the early post-arrival period, when calves are exposed to stressors that may increase susceptibility to disease and death. Early identification of high-risk calves at arrival could improve monitoring and management. The objective of this prospective cohort study was to evaluate the population-level associations between CLuBS parameters: (C)linical signs, (Lu)ng lesions, (B)ody weight, and (S)erum total protein, assessed at arrival at a collection center (CC) and subsequent mortality during both the first 30 days (early mortality) and the entire fattening period (total mortality).

MATERIALS and METHODS: The study was conducted at a CC located in Ghedi, Brescia, Italy, and included 934 veal calves, over a six-month enrollment period (January–July 2025). Immediately after arrival at the CC, they were weighted. After that, all calves underwent a clinical assessment including rectal temperature, fecal score, evaluation of umbilical structures, and joint examination and thoracic ultrasonography (TUS). Also, a blood sample was collected for each calf to determine serum total protein (STP). Associations between CLuBS variables at arrival and subsequent mortality were assessed using multivariable generalized estimating equation (GEE) logistic regression models accounting for clustering of calves within farms of origin. Results are presented as odds ratios (OR) with 95% confidence intervals (CI), and $P < 0.05$ was considered significant. The study protocol was approved by the Ethical Committee of the University of Milan (approval number 91_2025).

RESULTS: Overall mortality was 9.4% (32/934 early; 88/934 total). In multivariable GEE models, severe lung consolidation (≥3 cm) was the strongest predictor of mortality. Compared with calves with severe consolidation, those without lung lesions had significantly lower odds of early mortality (OR = 0.23; 95% CI: 0.09–0.55) and total mortality (OR = 0.30; 95% CI: 0.17–0.53). Arrival body weight was inversely associated with mortality, with a 9% reduction in early mortality odds (OR = 0.91; 95% CI: 0.84–0.97) and a 4% reduction in total mortality odds (OR = 0.96; 95% CI: 0.94–0.99) per additional kilogram. Abnormal fecal consistency at arrival was independently associated with total mortality; calves with normal feces had lower odds of death compared with those presenting watery feces (OR = 0.47; 95% CI: 0.25–0.86).

CONCLUSIONS: In conclusion, severe lung consolidation and lower arrival body weight were associated with increased mortality risk in veal calves, supporting the use of structured arrival screening protocols.

Keywords: veal calves, mortality, thoracic ultrasonography, body weight

Lung lesion dynamics, recovery and growth in dairy calves with bovine coronavirus and mixed bacterial respiratory infection

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BACKGROUND and OBJECTIVE: Bovine coronavirus (BCoV) is increasingly recognized in calf respiratory samples, however its impact on lung health and growth is less understood. This study characterized longitudinal lung ultrasound consolidation dynamics, recovery, and growth in dairy calves exposed to endemic BCoV and *Pasteurellaceae* infections.

MATERIALS and METHODS: A prospective cohort study was conducted in two dairy herds including 126 calves that were monitored from birth to one month of age twice weekly using thoracic ultrasonography and clinical respiratory scoring. Lung ultrasound scores (LUS) were defined as 0 (<1 cm), 1 (≥1 to <2 cm), 2 (≥2 to ≤3 cm), and 3 (>3 cm); calves with LUS 2 or 3 were treated with florfenicol and meloxicam. Recovery was defined as regression to LUS 0 within three subsequent examinations. Circulation of BCoV and *Pasteurellaceae* was confirmed at herd level. Average daily gain (ADG) was evaluated during the first 4 and 8 weeks of life. Study was funded by the Estonian Research Council (grant PRG2233).

RESULTS: Overall, 74.6% of calves developed lung consolidations (LUS > 0) at a median age of 16 days. However, all calves developed lung consolidations > 0 cm in depth at some point during the first month. Among calves with LUS > 0, 97% had subclinical and 3% clinical pneumonia. During the 4-week period, the highest recorded LUS was 2 for 40.5% of calves, followed by LUS 3 in 17.5% and LUS 1 in 16.7%. Among 94 calves with consolidations ≥1 cm, two lung quadrants were most commonly affected (38.3%),

followed by one (33.0%), three (23.4%), and four (5.3%) over 4 weeks. Among calves that developed LUS 1 (n = 46), 60.9% showed progression to higher LUS without treatment. Antimicrobial treatment increased recovery odds in calves with LUS 2 (odds ratio (OR) = 3.6; p = 0.016) and LUS 3 (OR = 28.9; p = 0.004) compared with untreated LUS 1 calves. Calves with three or four affected lung quadrants grew 87 g/day less during the first month (p = 0.07). Calves with lung consolidations detected at two visits grew 61 g/day less from birth to two months (p = 0.048).

CONCLUSIONS: BCoV and *Pasteurellaceae*-associated subclinical pneumonia was ubiquitous in the first month, with all calves showing lung consolidations. Untreated LUS 1 lung lesions often progressed to more severe pneumonia. Early detection and antimicrobial treatment of consolidations ≥2 cm were associated with improved recovery. Repeated lesion detection and multilobar involvement tended to impair growth.

Keywords: bovine respiratory disease, pneumonia, thoracic ultrasonography, calf health, recovery

Effects on clinical status, fecal microbiota, hydroelectrolytic, and acid-base balance in calves affected by neonatal diarrhea treated with two oral rehydration solutions with different tonicity: a randomized prospective clinical trial

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BACKGROUND and OBJECTIVE: Oral rehydration solutions (ORS) are the main treatment for moderate neonatal calf diarrhea (NCD). Data on the impact of ORS tonicity on clinical outcomes and fecal microbiota in naturally occurring NCD are limited. This study compared a commercial high-sodium ORS (CORS) and a home-made low-sodium ORS (HORS) in moderately ill calves.

MATERIALS and METHODS: Fifty calves with NCD, from a commercial dairy farm, were randomly assigned to HORS (25) or CORS (25) and monitored for 6 days (ethical approval no. 92_2025, University of Milan). Calves received 2 L of the respective ORS twice daily for 3 consecutive days. Clinical variables (vigour score, heart rate, respiratory rate, dehydration status, fecal score), blood gas parameters, and osmolarity were assessed at admission (T0) and follow-up (T1–T5). Fecal samples were collected for microbiota analysis. Data were analyzed using generalized estimating equation (GEE) models with group and time as covariates ($p < 0.05$).

RESULTS: Fecal scores were significantly influenced by both treatment (p = 0.046) and time (p < 0.001). The probability of exhibiting more severe fecal scores was higher in HORS group compared to CORS (OR = 1.817; p = 0.046). Compared with T5, earlier time points showed higher odds of elevated fecal scores (OR = 8.606; p < 0.001).Packed cell volume was significantly elevated in HORS group relative to the CORS group (OR = 1.149; p = 0.011), with higher values observed at admission and a significant decline over the duration of the study (p < 0.001). Potassium concentrations measured at admission were significantly greater in the HORS (OR = 1.089; p = 0.004), exhibiting a significant reduction over time (p < 0.001). No statistically significant differences were detected between treatment groups for the remaining clinical and laboratory parameters. Nonetheless, significant temporal improvements were documented for body temperature (p = 0.019), vigour score (p < 0.001), dehydration status (p < 0.001), bicarbonate (p < 0.001), sodium (p = 0.027), and glucose (p = 0.034). Fecal microbiota composition did not differ significantly between the treatment cohorts; however, significant temporal alterations were observed, indicating dynamic shifts in microbial diversity throughout the recovery process.

CONCLUSIONS: The findings of this study suggested that, although both ORS facilitated clinical improvement in calves with NCD, there were differences in the rates of recovery. In contrast to previous reports, low-sodium ORS did not provide greater benefits in maintaining fluid, electrolyte, and acid–base balance compared to high-sodium ORS in this study cohort.

Keywords: Neonatal calf diarrhea, Oral rehydration solutions, Therapy

Impact of different fat content in colostrum and transition milk on metabolic parameters in newborn calves

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BACKGROUND and OBJECTIVE: Colostrum and transition milk contain more fat than mature milk. While colostrum fat content varies considerably, its role in postnatal metabolic adaptation under cold conditions is not well understood. Calves born in cold seasons may benefit from higher fat intake as an energy source for thermoregulation. This study investigated the effects of different fat contents in colostrum and transition milk on energy and lipid metabolism in newborn calves.

MATERIALS and METHODS: Twelve calves (9 Holstein, 3 Holstein × Limousin; birth weight 41.3 ± 1.8 kg) were assigned to either a full-fat colostrum group (FF; n=6; 4.81% fat) or a low-fat group (LF; n=6; 0.58% fat). At 4 and 12 hours postpartum, calves received 2.5 L of the respective colostrum. On days 2 and 3, colostrum was diluted with bulk milk to simulate transition milk (5.17% fat in FF; 3.8% in LF). From day 4 onward, all calves were fed identical bulk milk (4.5% fat). Blood samples were analyzed for glucose, gamma-glutamyl transferase (GGT), aspartate aminotransferase (ASAT), cholesterol (CHOL), high-density lipoprotein (HDL), triglycerides (TG), non-esterified fatty acids (NEFA), and total protein (TP). Data (mean ± SEM) were analyzed using a mixed model (SAS 9.4) with group, time, and their interaction as fixed effects ($P < 0.05$). The average temperature during the study was 4.3°C (THI 42).

RESULTS: Plasma glucose increased in both groups during the first days but was significantly higher in FF calves, particularly during the first four days ($P < 0.05$). GGT activity was higher in LF calves ($P < 0.05$), peaking at 24 hours in both groups before declining. ASAT showed a similar temporal pattern but did not differ between groups ($P = 0.31$). Plasma CHOL, HDL, and TG concentrations were consistently higher in FF calves ($P < 0.05$). No significant group differences were found for NEFA ($P = 0.25$) or TP ($P = 0.27$).

CONCLUSIONS: Higher fat content in colostrum and transition milk improved glucose and lipid metabolism in newborn calves, indicating greater energy availability compared to calves receiving low-fat colostrum under cold conditions.

Keywords: Colostrum, Transition Milk, Fat Content, Newborn Calves, Energy Metabolism, Thermoregulation

Comparative analysis of serum proteomes among different passive transfer immunities in neonatal calves

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BACKGROUND and OBJECTIVE: Neonatal calf health critically depends on successful passive transfer of immunity (PTI) via colostrum. Although PTI is routinely assessed by serum IgG concentration, IgG values do not capture the broader systemic biological responses associated with different PTI outcomes, and the molecular mechanisms underlying these differences remain poorly defined. This study aimed to determine whether serum proteomic profiles at 48 hours postpartum discriminate calves with failed, adequate, and excellent PTI.

MATERIALS and METHODS: The study included 40 Holstein calves managed under identical farm conditions. Blood samples were collected before first colostrum intake (0 h) and at 48 h postpartum. Serum IgG concentrations were quantified using a bovine-specific ELISA (BIO K420, Biox, Belgium). Calves were classified according to 48-h serum IgG concentrations as Failed Passive Transfer Immunity (FPTI; <1000 mg/dL), Adequate Passive Transfer Immunity (APTI; 1000–2000 mg/dL), and Excellent Passive Transfer Immunity (EPTI; >2000 mg/dL). To maximize phenotypic contrast, five representative calves per group were purposively selected for

proteomic analysis. Serum samples obtained at 0 and 48 h were pooled within each PTI group and analyzed using LC–MS/MS in DIA mode (Waters M-Class UPLC coupled to Xevo G2-XS QTOF MS). Differentially abundant proteins were defined as those with $p < 0.05$ and fold change ≥1.2.

RESULTS: At 48 h postpartum, 80 serum proteins were identified. Pairwise comparisons showed that 18 proteins differed between APTI and FPTI, 22 between EPTI and APTI, and 13 between EPTI and FPTI, indicating broader proteomic divergence with increasing PTI. In the direct comparison between EPTI and FPTI calves, the most pronounced differences were observed for haptoglobin (27.75-fold increase in EPTI), followed by lipopolysaccharide-binding protein (7.27-fold decrease in EPTI) and pigment epithelium-derived factor (PEDF) (4.82-fold increase in EPTI). Importantly, haptoglobin and PEDF also showed marked post-colostral induction within the EPTI group (51.10-fold and 27.24-fold increases from 0 to 48 h), highlighting a strong PTI-dependent proteomic activation. Functional annotation of the 13 EPTI–FPTI discriminatory proteins revealed that most were associated with binding, catalytic, and immune-related functions.

CONCLUSIONS: Distinct serum proteomic profiles distinguish calves with failed, adequate, and excellent PTI, with EPTI calves exhibiting a markedly amplified post-colostral response. Several discriminatory proteins identified at 48 h, particularly haptoglobin and PEDF, may represent candidate biomarkers of successful passive immunity. As the biological roles of many detected proteins remain incompletely defined, further functional characterization may improve understanding of neonatal immune adaptation and support targeted monitoring strategies.

Keywords: proteomics, passive transfer immunity, calves

Diagnostic test study on procalcitonin as biomarker for sepsis in calves

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BACKGROUND and OBJECTIVE: Differentiating sepsis from other causes of critical illness in calves remains challenging when relying solely on clinical signs and routine blood examination. Procalcitonin (PCT) is repeatedly reported as a promising biomarker for sepsis in other species. Therefore, the objective of this study was to evaluate the diagnostic accuracy and prognostic value of PCT for the detection of sepsis in calves.

MATERIALS and METHODS: A total of 156 calves were included: 30 healthy calves, 45 non-critically ill calves (NCIC) with diarrhea, and 81 critically ill calves (CIC). Critical illness was defined as the presence of severe respiratory problems, cardiovascular abnormalities and/or neurological abnormalities. Of the CIC, 44 calves had diarrhea. Calves were clinically examined and blood cultures were taken. Serum PCT was measured by commercial ELISA assay. Mixed models and logistic regression were used for statistical analysis.

RESULTS: Of the CIC, 16 had clinically relevant bacteremia and were classified as confirmed sepsis. None of the NCIC (healthy and diarrheic calves combined) had clinically relevant bacteremia. With mixed models, no significant differences in PCT levels were observed neither between healthy calves and NCIC with diarrhea ($p = 0.41$) or CIC ($p = 0.23$), nor between NCIC with diarrhea and CIC ($p = 0.24$). In CIC, PCT concentration was significantly higher compared to NCIC ($p = 0.021$). The odds of critical illness in calves were increased in calves with PCT concentrations > 10.92 pg/mL (OR 3.1; 95% CI: 1.5–6.6; $p = 0.003$). Logistic regression demonstrated no significant difference between non-septic calves and calves with confirmed sepsis ($p = 0.35$), or between calves with or without diarrhea ($p = 0.91$),

pneumonia (p = 0.94), umbilical infection (p = 0.82), mortality (p = 0.56). Within the CIC group, there was no significant difference between the calves with and without sepsis (p = 0.58).

CONCLUSIONS: Although PCT concentrations differed significantly between CIC and NCIC, this distinction has limited clinical relevance, as critical illness can already be identified through clinical examination. Furthermore, the ROC-derived PCT cut-off for critical illness was notably low and approached the assay's limit of detection as specified by the manufacturer. Based on the results observed in our study population, combined with the current lack of commercially available rapid calf-side PCT tests, PCT does not appear to be a clinically useful biomarker for the diagnosis of sepsis in calves.

Keywords: antimicrobial stewardship, critically ill calves, blood culture

Objective spectral and amplitude characterization of normal and pathological lung sounds in pre-weaned dairy calves

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BACKGROUND and OBJECTIVE: Bovine respiratory disease is a major health challenge in pre-weaned dairy calves, negatively affecting animal welfare and farm sustainability. Thoracic auscultation remains a cornerstone diagnostic tool under field conditions; however, its diagnostic accuracy is limited by subjective interpretation of lung sounds. In human medicine, objective analysis of spectral and amplitude-related lung sound features has improved diagnostic performance and the development of artificial-intelligence tools. This study aimed to characterize and compare spectral and amplitude-related features of lung sounds in pre-weaned calves. We hypothesized that objective acoustic parameters differ among lung sound types.

MATERIALS and METHODS: Lung sounds were recorded using a digital stethoscope and saved as WAV files. Recordings were classified as normal (N), increased breath sounds (IBrS), increased bronchial sounds (IBS), crackles (C), or wheezes (W). Each recording was evaluated for quality based on the presence. Only high-quality recordings (clear lung sounds or with removable parasitic noise) were included. Frequency-related parameters: dominant frequency (DF), minimum frequency (MinF), and maximum frequency (MaxF) in Hz and amplitude-related parameters: total energy (TotE), mean energy (ME), and maximum energy (MaxE) in dB were extracted using Fast Fourier Transformation. Differences among categories were assessed using the Kruskal–Wallis test followed by Dunn's post hoc test ($p < 0.05$).

RESULTS: A total of 249 recordings (25% of all recordings) were included: 65 (26.1%) N, 62 (24.9%) IBrS, 48 (19.3%) IBS, 42 (16.9%) C, and 32 (12.9%) W. Dominant frequency (~1000 Hz) and MinF (~50 Hz) were similar across categories, whereas MaxF ranged from 1094±33 Hz (N) to 1132±41 Hz (W). TotE ranged from −6.7±4.9 dB in N to 4.8±6.3 dB in C; ME ranged from −53.7±5.0 dB in N to −43.7±5.0 dB in W; and MaxE ranged from −36.3±5.0 dB in N to −24.4±7.4 dB in C. Kruskal–Wallis analysis revealed significant differences for MinF, MaxF, and all amplitude-related parameters. Post hoc analysis showed significant differences in MinF between IBS and N, and in MaxF between IBrS and N and between W and N. Amplitude-related parameters consistently differentiated normal from pathological sounds, with significant differences observed between N and all pathological categories across TotE, ME, and MaxE.

CONCLUSIONS: These findings demonstrate that pathological lung sounds in pre-weaned calves differ objectively from normal sounds in both spectral and amplitude characteristics. Amplitude-related features showed the strongest discriminatory capacity, supporting their potential use in AI-based

tools to enhance the accuracy and standardization of thoracic auscultation in bovine practice.

Keywords: bovine respiratory disease, calves, thoracic auscultation, lung sound analysis, respiratory acoustics

An exploratory randomised controlled trial of a probiotic-postbiotic supplement on Cryptosporidium parvum shedding dynamics in pre-weaned Holstein heifer calves

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BACKGROUND and OBJECTIVE: *Cryptosporidium parvum* is a common and challenging-to-control cause of diarrhoea in pre-weaned calves, driving growing interest in probiotic approaches to support calf health; therefore, we evaluated a commercial *Enterococcus lactis*-based probiotic-postbiotic supplement (Precision Microbes). This study aimed to provide a preliminary comparison of *C. parvum* shedding parameters between supplemented calves and non-supplemented controls.

MATERIALS and METHODS: This exploratory, double-blinded, randomised controlled field trial was conducted on a commercial dairy farm in the United Kingdom. Holstein heifer calves (n=22 per pen/treatment group) were randomly assigned at birth to one of two adjacent pens. Calves in one pen received the oral supplement (at the dose recommended by the manufacturer) daily, while calves in the other pen received a placebo. Calves were examined 2-3 times weekly from birth to 6 weeks of age (16 examinations per calf), resulting in 635 examinations/faecal samples over a total of 19 visits. Faecal consistency was scored, and faecal samples were tested for *C. parvum* by antigen ELISA, and oocyst per gram (OPG) quantification with haemocytometers using phase-contrast microscopy. With a single pen per treatment group, analyses were descriptive; however, the intensive longitudinal sampling enabled detailed characterisation of *C. parvum* shedding dynamics.

RESULTS: Shedding dynamics and clinical observations appeared similar in both groups. All calves became infected with *C. parvum* (44/44), the earliest positive was detected at 7 days of age, and all calves were positive by 15 days. Median age at first positive was 11 (IQR 10-12) days. Peak shedding occurred at 13 (IQR 12-15) days, with peak intensity 6.5 (6.0-6.9) $\log_{10}$ OPG. Shedding duration (defined as first to last positive) was 6 (IQR 4-7) days. Earlier onset of shedding was associated with a longer shedding duration (ρ = -0.45, 95% CI [-0.67, -0.16], $P < 0.005$, n = 44), consistent with earlier infection with *C. parvum* being associated with a longer observed shedding period. Scour (faecal score ≥2) occurred in 121/635 (19.1%) examinations, and 41/44 (93.2%) calves had ≥1 scour episode.

CONCLUSIONS: In this exploratory on-farm study, probiotic-postbiotic supplementation was not associated with apparent differences in *C. parvum* shedding dynamics or clinical parameters. Intensive longitudinal sampling characterised infection timing, peak shedding, and shedding duration, supporting future study design, sampling schedules, and variance estimates for sample size calculations.

Keywords: Cryptosporidium parvum, shedding dynamics, probiotics, postbiotics, pre-weaned calf diarrhoea

Evaluating the effect of isolation on recovery from bovine respiratory disease in group-housed dairy calves

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BACKGROUND and OBJECTIVE: This study aimed to evaluate the effect of isolation on clinical recovery from bovine respiratory disease (BRD) in group-housing dairy calves.

MATERIALS and METHODS: From October 2025 to January 2026, 55 calves at the Virginia Tech Dairy Farm were moved from individual hutches to group housing with an automatic feeder at 14 ± 3d of age. At this time, calves received a clinical examination and thoracic ultrasonography (TUS). Calves meeting the BRD case definition (TUS ≥3) were excluded regardless of their clinical signs. In group housing, calves were clinically scored and assessed by TUS three times weekly. A total of 35 calves diagnosed with BRD were enrolled (day 0), weighed, treated according to farm protocol, and randomly assigned to isolation (ISO; n = 17) or group housing (CON; n = 18), using a blocked randomization by birth week and serum total protein measured at 2 days of age. Isolated calves were moved to individual hutches and fed twice daily, while CON calves remained at the calf barn. Weight measurement and respiratory examinations were performed on d 3, 5, and 7. Calves with TUS ≤ 2 on any exam day were considered recovered, and those in ISO were returned to group housing, whereas calves failing to improve by d 7 were classified as treatment failures. Recovery was analyzed with a general linear mixed model, time to recovery using a Cox proportional hazards model, and milk replacer consumption and average daily weight gain using a mixed repeated measures model

RESULTS: The mean age at diagnosis was similar between groups (ISO: 29.58 ± 7.11 days; CON: 29.66 ± 7.3 days). Twenty-three calves recovered during the treatment follow-up period (ISO, n = 12; CON, n = 11), with no difference in the odds (P = 0.35) or time (P = 0.14) to recovery between treatment groups. There was treatment-by-day interaction in milk consumption, with ISO calves consuming a greater proportion of their offered milk on days 1, 2, and 6 (P < 0.02) compared to the CON group. However, no treatment-by-day interaction was detected for average daily weight gain during the study period (P = 0.72).

CONCLUSIONS: These findings suggest that recovery outcomes were similar across treatment groups. Still, isolated calves exhibited greater milk intake on several treatment days, with no corresponding differences in average daily weight gain compared with group-housed calves.

Keywords: Bovine respiratory disease, Isolation, Clinical Recovery.

Association between mycoplasmopsis (M.) bovis status, herd-level risk factors, and clinical observations in German dairy operations

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BACKGROUND and OBJECTIVE: Mycoplasmopsis (M.) bovis is widely present in German dairy farms. Steppin et al. (2024) showed that in nearly 45% of German dairy farms, especially in large operations, M. bovis-antibodies (AB) are present. This study investigates the association between M. bovis-AB in bulk tank milk (BTM) and management practices, as well as clinical symptoms such as respiratory diseases and otitis in calves. If a correlation exists,

clinical symptoms could potentially be used as a presumptive diagnostic screening tool.

MATERIALS and METHODS: A total of 750 BTM samples were collected once from dairy farms across Germany by veterinary practitioners and herd managers between June 2023 and January 2026. The samples were tested for M. bovis-AB using an indirect ELISA test (ID Screen® Mycoplasma bovis, ID vet, France). Analyses were conducted at the Bavarian Animal Health Services laboratory (Poing, Germany) or by the private laboratory bovicare GmbH (Schönow bei Bernau, Germany). Veterinarians and farmers completed a short questionnaire (mostly yes/no options) regarding farm management (purchase of animals, bovine respiratory disease (BRD) vaccination) and the presence of clinical symptoms and observations in calves (such as BRD; otitis / "drooping ears"). Associations were analyzed via Odds Ratio (OR) /CI with $p<0.05$ (IBM SPSS, 29.0, USA).

RESULTS: Purchasing is a significant risk factor for M. bovis: OR 2.48 (CI 1.78-3.47 / p <.001). 27.0% of the herds (n =201) were non-closed herds. 36.1% of these herds were M. bovis-positive versus 18.6% among closed operations. BRD vaccination did not reduce M. bovis-AB occurrence (OR 1.95 / CI 1.45-2.63 / p<.001). The M. bovis-AB correlated positively with the presence of BRD symptoms in calves (OR 1.79 / CI 1.10-2.91 / p<.018). The distribution of calves without BRD symptoms was 64.6% for the group M. bovis-negative and 35.4% for the group M. bovis-positive. M. bovis-AB also correlated positively with the presence of otitis symptoms / "drooping ears" in calves (OR 2.62 / CI 1.91-3.58 / p<.001). The prevalence of otitis was 36.0% in the group M. bovis-negative and 64.0% in the group M. bovis-positive.

CONCLUSIONS: : M. bovis infection significantly increases the risk of otitis (head tilt, "drooping ears") and respiratory symptoms in calves. These observations can be used by practicing veterinarians and farmers to make a presumptive diagnosis of M. bovis; however, further diagnostic procedures such as PCR in bronchoalveolar lavage (BAL) or serology remain necessary.

Keywords: M.bovis, otits, bulk tank milk, BRD

Use of a calf ethogram to assess pain and discomfort in preweaned group housed dairy calves with bronchopneumonia

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BACKGROUND and OBJECTIVE: Bovine respiratory disease (BRD) seriously affects calf welfare. Currently, no standard methods assess welfare in calves with pneumonia. While ethograms are used in other species to link behavior to pain and health, they are largely unexplored in calves. This study aimed to determine the association between an ethogram and the individual behaviors with ultrasound confirmed pneumonia.

MATERIALS and METHODS: A cross-sectional study included 102 preweaned group-housed dairy calves aged 4 to 10 weeks from four Flemish dairy farms in February 2026. Calves were assessed for 33 binary negative behavioral parameters across seven categories and 9 positive behaviors. Total negative (max score = 33) and positive scores (max= 9) were calculated, and an overall ethographic score was obtained by subtracting negative from positive scores. Calves were evaluated with quick thoracic ultrasound (qTUS). Three different pneumonia definition based on lung consolidation depth were evaluated: any consolidation depth, ≥1 cm and ≥3 cm. Associations between individual behavioral parameters, total/negative/positive ethogram score, and pneumonia were analyzed by logistic regression.

RESULTS: The prevalence of a lung consolidation of any depth, ≥1 cm and ≥3 cm, was 60%. Fever (≥39.3 °C) was observed in 27% of calves, and 15.7% had clinical BRD. Of the 33 negative behavioral parameters, 18 were observed in less than three calves. The positive behaviours were more frequent, with only one (stretching when getting up) observed in two calves. The mean negative ethogram score was 1.3 ± standard deviation= 1.6 (min.-max.= 0-6) in healthy calves, and 1.98 ± 2.4 (0-10) in calves with lung consolidation ≥1 cm (p= 0.17), whereas the positive ethogram score was 2.8 ± 1.5 (0-6) and 2.8 ± 1.8 (0-8)

(p= 0.92). The total ethogram (pos-neg) was not different between healthy and pneumonic animals (1.5± 2.4 (-4 -6) vs. 0.85± (-10 – 8) (p= 0.15). Also, for severe pneumonia or lung consolidation of any depth no significant differences were found. Low head carriage while standing was the only individual behavior associated with consolidation of any depth (OR= 4.7 (95% confidence interval= 1.3-17.1) (p= 0.02) and consolidation >= 1 cm (OR= 2.7 (1.0-7.5; p= 0.049).

CONCLUSIONS: In the present population with mainly mild or subclinical pneumonia, behavioral parameters and ethogram scores did not differ between calves with and without ultrasound-confirmed pneumonia. Only low head carriage while standing was associated with lung consolidation, suggesting that mild lung lesions may not produce clear behavioral signs of impaired welfare.

Keywords: pneumonia, ethogram, dairy calves, welfare

Distribution and diagnostic accuracy of the Wisconsin clinical score across ultrasonographic pneumonia severity in veal calves

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BACKGROUND and OBJECTIVE: Bovine respiratory disease remains a major health challenge in veal production. Clinical scoring systems, particularly the Wisconsin score (WSC), are widely used in research but have low sensitivity for early pneumonia detection compared to thoracic ultrasound. Because the original WSC did not include lung lesion scoring, re-evaluation of its threshold for identifying calves with pneumonia is warranted. This study aimed to describe the distribution of WSC across mild, moderate, and severe pneumonia determined by quick thoracic ultrasonography (qTUS) in veal calves during both acute outbreaks and chronic endemic situations. A secondary objective was to optimize WSC thresholds for these settings.

MATERIALS and METHODS: A cross sectional study was done in veal calves. Clinical scoring (WSC), and qTUS were performed just before the start of the first antimicrobial group treatment for BRD one week after arrival (acute) and repeated in week 10-12 post-arrival (chronic). The qTUS score was used, differentiating pneumonia based on maximum consolidation depth into: "healthy"= no consolidation, "mild" <1cm, "moderate" ≤1-3cm, "severe" ≥3cm). Descriptive statistics and receiver operating characteristics (ROC) analysis were used for threshold determination.

RESULTS: A total of 1090 veal calves from 4 production rounds were included. In the acute stage, mean ± standard deviation (min–max) WSC for healthy, mild, moderate, and severe category were 1.8±1.5(0-8), 2.0±1.5(0-8), 2.4±1.7(0-9) and 3.5±3.5(0-11), respectively. In the chronic stage, these were 1.6±1.0(0-7), 1.6±1.0(0-5), 1.8±1.3(0-8) and 2.4±1.6(0-8), respectively. In the acute stage, the moderate and severe pneumonia groups differed significantly from other categories and from each other, while in the chronic stage only severe differed from the others. ROC-analysis identified WSC ≥ 3 as the optimal cutoff for all qTUS categories at both time points. In the acute stage, sensitivity/specificity/accuracy for qTUS thresholds ≥0.5cm, ≥1cm, and ≥3cm were 47.3/76.8/61.1%, 53.1/75.8/66.5%, and 65.9/71.1/70.1%, respectively. In the chronic stage, these were 24.8/88.7/61.0%, 28.8/88.4/69.1%, and 40.6/86.8/80.1%. The common cutoff ≥5 had lower sensitivity, higher specificity, and slightly lower accuracy. Among calves with WSC ≥3, qTUS classified 29.9%, 9.7%, 24.8%, 35.5% as healthy, mild, moderate, and severe in the acute stage, and 37.1%, 8.8%, 20.1%, 34.0% in the chronic stage.

CONCLUSIONS: WSC distributions overlapped substantially across qTUS classes in both acute and chronic stages, with generally lower scores in the chronic stage. Despite this, some categories differed significantly in WSC. The cutoff WSC ≥3 performed better than ≥5, but sensitivity remained unacceptably low, especially in the chronic stage.

Keywords: Calf health, qTUS, pneumonia, Wisconsin score

Pilot evaluation of bovine respiratory syndrome in suckling holstein calves using thoracic ultrasonography in Greek farms

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BACKGROUND and OBJECTIVE: Bovine Respiratory Disease (BRD) is a major cause of morbidity and mortality in young calves, and early and accurate diagnosis remains an important research issue. In addition to clinical examination, the use of thoracic ultrasonography is currently recommended for detecting lung lesions and assessing disease severity. The aim of this study was to conduct a pilot investigation of the effectiveness of combining these diagnostic methods in Greek dairy farms.

MATERIALS and METHODS: A total of 201 Holstein suckling calves from 23 farms were included in the study, divided into two age groups: 81 calves aged 22±6 days (Group A) and 120 calves aged 59±19 days (Group B). Calves were examined once. First, a clinical respiratory assessment was performed using the Wisconsin scoring system (WISC), including temperature, nasal and ocular discharge, cough, and ear position (disease defined as a score ≥4). Subsequently, thoracic ultrasonography (US.) was performed with a linear probe (7.5–10.0 MHz) on both right and left thoracic sides (1st–10th and 2nd–10th intercostal spaces, respectively), and lung lesions were scored on a 0-5 scale (lesions considered present at a US ≥2). The effects of colostrum management (quantity and time from birth to feeding) and calf vaccination against respiratory diseases were also evaluated. Statistical analyses were conducted using analysis of variance (ANOVA), linear models, and Pearson correlation coefficients, both overall and separately within the two age groups.

RESULTS: Analysis of variance showed that time of colostrum administration and vaccination significantly affected US ($P<0.05$). Early colostrum intake and vaccination were associated with lower US scores. No similar effect was observed for WISC scores. Linear models showed significant associations ($P<0.05$) between US scores and farm, age (positive), vaccination (negative), and WISC scores (positive). The correlation between clinical signs and presence of lung lesions was significant ($P<0.05$) but low ($r=0.30$ and 0.37 in group A and B, respectively). Additionally, notable discrepancies were observed: in Group A, 67% of calves with lung lesions showed no clinical signs, while 76% of calves with clinical signs had no lesions. In Group B, 28% of calves with lesions showed no clinical signs, while 56% of clinically affected calves had no lesions.

CONCLUSIONS: The combination of clinical evaluation and thoracic ultrasonography represents the most reliable approach for detecting and assessing BRD, as it allows identification of disease presence in the upper or lower respiratory tract, even in the absence of clinical signs.

Keywords: Bovine respiratory desease, calves, thoracic ultrasound

Effects of transition milk feeding on calves' fecal shedding of multi–drug resistant extended–spectrum β–lactamases–producing enterobacterales: A pilot study

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BACKGROUND and OBJECTIVE: Several studies have reported that providing transition milk (TM) may be strategic in the early feeding management in calves due to several beneficial effects, including improved weight gain, reduced odds of suboptimal clinical scores and decreased risk of infection. TM is classified as waste milk (WM), whose administration to calves is discouraged due to potential antimicrobial residues and effects on antimicrobial resistance (AMR) spreading and the increase of gastrointestinal diseases risk. Specific data on TM-related AMR remain limited, therefore this preliminary study aims to evaluate the effects of transition milk feeding on AMR patterns and prevalence of extended-spectrum beta-lactamases

(ESBL)-producing Enterobacterales and the effect on health status of dairy calves in Northern Italy.

MATERIALS and METHODS: From 4 farms located in Lombardy, 49 Holstein-Friesian calves, aged less than 21 days and with no previous antimicrobial treatment, were enrolled and grouped by TM-feeding practices after the first colostrum administration. In control farms, calves received milk replacer (MR) instead of TM. Faecal samples, collected from the rectal ampulla of each calf after a clinical examination, were enriched for 24h in Mueller–Hinton broth at 37°C and then plated on ESBL-selective media. Bacterial colonies were identified by MALDI-TOF-MS, and double disc synergy and antimicrobial susceptibility tests were performed on all presumptive ESBL-producing Enterobacterales. Calf-level statistical analyses were conducted in SPSS 30.0 using chi-square tests and an ordinal logistic regression to evaluate associations between TM-feeding, ESBL shedding, resistance class profiles, and the occurrence of diarrhoea, pneumonia, and antimicrobial treatment.

RESULTS: TM feeding was not associated with differences in the faecal shedding of ESBL-producing Enterobacterales ($P > 0.05$), which were highly prevalent in all enrolled farms. ESBL-producing Escherichia coli was the most detected bacterial species, in 82,4% of MR-fed calves and in 93,3% TM fed calves. However, TM-feeding was significantly associated with the number of AMR classes. Calves fed TM had lower odds of being classified in higher resistance categories than calves not fed TM (ordinal logistic regression: OR = 0.05, 95% CI: 0.01–0.23; $P < 0.001$). No significant differences were observed between the two groups in the incidence of diarrhoea, pneumonia, or antimicrobial treatment.

CONCLUSIONS: TM-feeding may influence the diversity and development of AMR of ESBL-producing Enterobacteralesin calves, but it has no significant effect on the rate of their fecal shedding. This suggests that early nutritional management could reduce AMR development in dairy calves, while the high ratio of ESBL-producing Enterobacterales likely reflects early environmental colonization.

Keywords: Transition milk, calf health, extended-spectrum beta-lactamases (ESBL)-producing-Enterobacterales, antimicrobial resistence

Bacteria in the umbilicus of neonatal dairy calves: Identification and antimicrobial susceptibility

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BACKGROUND and OBJECTIVE: Umbilical infections (UIs) are among the most common diseases in neonatal dairy calves. This study aimed at identifying the bacteria present in the umbilicus of newborn calves and calves with UIs. Furthermore, the susceptibility of selected bacteria to antibiotics commonly used in calves was determined.

MATERIALS and METHODS: The study was conducted from September 2022 to April 2024 on five dairy farms in Brandenburg, Germany. Farms were visited twice weekly over a twelve-week period. All calves up to 30 days of age were clinically examined weekly including a clinical assessment of the umbilicus for enlargement, heat, tough consistency, pain, and discharge. Calves born during the farm visits (n = 47) and calves diagnosed with UIs accessible for sampling (e.g., discharge, fistula) (n = 106) were sampled using swabs. All bacterial species were cultivated and subsequently identified by MALDI-TOF MS. Antimicrobial susceptibility testing was conducted for *Escherichia coli*, *Trueperella pyogenes*, *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, and *Klebsiella* spp. using broth microdilution and VITEK® 2 compact in accordance with CLSI standards. Minimal inhibitory concentrations (MICs) were determined.

RESULTS: Of 1,236 calves clinically examined, 144 (11.7%) were diagnosed with UIs. In both newborn calves and calves with UIs, more than 100 different bacterial species were identified, with significant differences between both groups. *Trueperella pyogenes*, *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus dysgalactiae* and certain obligate anaerobic bacteria (e.g., *Bacteroides* spp.) were strongly associated with UIs. The most frequently isolated bacterial species in both sampling groups was *E. coli*. Among all *S. aureus* isolates, 69.6% (n = 32) were methicillin-resistant. Streptococci and *Trueperella pyogenes* showed high susceptibility to antibiotics commonly administered to treat UIs (e.g., penicillin/benzylpenicillin). In contrast, all bacterial species tested exhibited low susceptibility to tetracycline, which is frequently used for navel care in Germany. In addition, *E. coli* isolates from UIs on farms using chlortetracycline spray for navel care showed significantly higher MICs for most antibiotics tested compared to isolates from farms using iodine. In contrast to isolates from newborn calves, *E. coli* isolates from UIs displayed significantly higher MICs to nearly all antibiotics tested.

CONCLUSIONS: The umbilicus of neonatal dairy calves harbors a broad diversity of bacteria. UIs were associated with shifts of the bacterial spectrum and reduced antimicrobial susceptibility compared to newborn calves. Moreover, the prophylactic use of chlortetracycline spray appears questionable given the low antimicrobial susceptibility rates observed and the potential risk of promoting antimicrobial resistance.

Keywords: dairy calves, umbilical infections, umbilicus, bacteria, antimicrobial susceptibility testing, antimicrobial resistance

The relationship between neonatal nutrition and the gut microbiome from birth to post-weaning

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BACKGROUND and OBJECTIVE: Neonatal calf nutrition is vital for health and development of not only the animal itself but also the gut microbiota. With different feeding strategies for colostrum and milk replacer utilised on farm, this study aims to identify whether duration of colostrum feeding, and functional protein content of the milk replacer have an effect on the neonatal calf faecal microbiome.

MATERIALS and METHODS: One-hundred Jersey heifers were enrolled (October 2021-July 2023) and allocated into 4 treatment groups, determining the duration of colostrum (1 or 4-days) and milk replacer type fed (isocalorific; 23.8% protein - two different levels of whey protein phospholipid concentrate (WPPC)). We hypothesised that 4-day colostrum feeding, and high WPPC milk replacer would positively influence gut microbiome development. All calves received 2.5 litres colostrum within 6 hours of birth (Brix value ≥22%), followed by 2.5 litres within 12 hours. Calves in the 1-day colostrum group received milk replacer from their 3rd feed, while calves receiving 4-days continued colostrum/transition milk (2.5 litres twice daily) for 3 more days, before transitioning to milk replacer. Milk replacer was fed at 165g/l; 2.5 litres twice daily. Standard husbandry procedures were carried out. Rectal swabs were taken at birth (pre-colostrum), 1 month and 10 weeks (post-weaning). Swabs were frozen to -20°C on farm and transferred to -80°C at the University facility within 6 weeks of sampling. DNA extraction was undertaken and microbial composition was assessed using 16S rRNA gene sequencing. Data analysis utilised ANOVA to analyse Alpha diversity (AD), PERMANOVA to assess Beta diversity (BD), ordination to visually analyse BD,

and Negative Binomial Regression to analyse differential abundance.

RESULTS: No difference was identified in the gut microbiome between treatment groups at birth. However, the herd moved farms halfway through the study (51 calves born at farm 1; 49 at farm 2), and farm of birth significantly influenced AD (Shannon) (P=0.003) and BD (Bray Curtis) (P=0.001) of the microbiome at birth, as did season of birth; BD (Bray Curtis) (P=0.002). The gut microbiome present at 1 month was significantly influenced by milk replacer type at the BD level; Bray Curtis (P=0.001) and weighted UniFrac (P=0.015).

CONCLUSIONS: Colostrum feeding duration had no high-level impact on gut microbiome, however sampling frequency might explain this. Feeding high WPPC milk replacer influenced microbiota at 1 month, with the effect seen being gone by post-weaning. The farm and season of birth significantly influenced the gut microbiome at birth.

Keywords: calf, colostrum, milk replacer, microbiome

Investigating neonatal calf diarrhoea vaccination and 'lean' management in commercial dairy farms using machine learning: growth rates, health and antimicrobial use

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BACKGROUND and OBJECTIVE: Control of neonatal calf diarrhoea (NCD) is important in prevention of later health problems and achieving more sustainable dairy production. Rotavirus/Coronavirus/E.coli vaccine (Fencovis®, Boehringer Ingelheim, Germany) is indicated for active immunization of pregnant heifers and cows to stimulate the development of antibodies against bovine rotavirus, bovine coronavirus and E. coli expressing F5 (K99) adhesin and to increase passive immunity of calves against NCD. In calves fed with colostrum and milk from vaccinated cows for the first week of life, laboratory studies conducted with challenge strains demonstrated that these antibodies prevent NCD alongside reduction of severity and shedding. Vaccination of dams to prevent NCD is inextricably linked with good colostrum management for neonatal calves. Multicentric commercial herd studies present challenges for study design but opportunities for wider systems understanding. The study objective was to evaluate the differences in growth rate, health and antimicrobial usage from 0-8 weeks of age between dairy and beef bred calves from non-NCD-vaccinated animals ('Control' calves) and calves from vaccinated dams combined with 'Lean' calf management improvements ('Test' calves) on commercial dairy farms.

MATERIALS and METHODS: Eight herds participated in this observational study, four in UK and four in Italy. A review of calf management was undertaken collaboratively on each farm. Animal observations including regular two-weekly weighing were performed by veterinarians and technicians alongside the farm team, prior to commencement of vaccination and management changes. 'Lean' management improvements prioritised practical methods for better colostrum management. Univariate and multivariate machine learning approaches were taken to the data analysis

RESULTS: There were significant differences between farms; univariate analyses were presented as eight case studies. 'Test' (vaccination with 'Lean' improvements in management) was significant at individual farm level for daily liveweight gain

(DLWG) and antimicrobial usage (AMU). Random forest multivariate modelling was used to investigate the interaction of features in this study. 'Farm' was the most significant feature affecting outcome DLWG, total health events and AMU, with 'country' most important feature affecting number of NCD events. However, vaccination together with 'Lean' improvements in management ('test') was the second most significant feature affecting growth rate changes of calves between control (n=387 calves) and test (n=349 calves) groups across all herds.

CONCLUSIONS: Calf health and growth are affected by complex multifactorial management features, which are challenging to model. However, machine learning approaches may offer insight in understanding feature importance of vaccination and 'Lean' management changes on commercial farms.

Keywords: Dairy, calves, diarrhoea, weight, antimicrobials, vaccination

Effect of waste milk on clinical health and immune cell profile in preweaned Holstein calves

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BACKGROUND and OBJECTIVE: Feeding waste milk (WM) to calves reduces losses but raises early-life health, microbial and antimicrobial concerns. This exploratory study evaluated WM or pasteurized waste milk (PWM) versus saleable milk (SM) on disease dynamics, growth, hematology, and blood gas parameters in preweaned Holstein calves

MATERIALS and METHODS: Thirty Holstein calves were assigned to WM, PWM (63°C, 30 min), or SM (n=10/group) from 7–63 days of age. Diarrhea (fecal score ≥2) and bovine respiratory disease (BRD; ≥2+ parameters) were assessed daily. Fecal dry matter (FDM <15%), complete blood count, and blood gases were measured on days 7, 21, 35, 49, and 63; weight was recorded weekly. During diarrhea, feces were analyzed by aerobic culture (MALDI-TOF MS), PCR, and Ziehl–Neelsen staining. BRD-positive calves underwent nasal culture Pasteurella multocida and Mannheimia haemolytica. Data were analyzed using linear mixed models ($P \leq 0.05$; trend: $0.05 < P \leq 0.10$)

RESULTS: Diarrhea was neonatal and similar among treatments. However, WM-fed calves had lower FDM (22.93 ± 5.37, 20.71 ± 7.30, and 13.26 ± 6.21 for SM, PWM, and WM; P = 0.02), and more dehydration tendency in PWM and WM (12.0%, 50.0%, 30.0%; P = 0.10). Escherichia coli (100%) and Cryptosporidium spp. (81.5%) were detected, viruses were absent. BRD tended to be more frequent in WM and PWM (24.19% SM; 34.26% PWM; 43.55% WM; P = 0.10), despite no respiratory pathogen isolation. Weekly body weight showed a treatment × age interaction (74.9 ± 2.1, 73.7 ± 2.1, and 75.3 ± 1.7 kg for SM, PWM, and WM; P = 0.04), being lower in WM at 21 (P = 0.03) and 28 days (P = 0.01). Lymphocyte were higher in WM-fed calves (SM: 4.0 ± 0.1; PWM: 4.2 ± 0.2; WM: 4.6 ± 0.1 ×10³/µL; P = 0.02), and monocytes in PWM (SM: 0.4 ± 0.0; PWM: 0.6 ± 0.1; WM: 0.5 ± 0.0 ×10³/µL; P = 0.05). Lactate showed a treatment × age interaction (SM: 1.6 ± 0.2; PWM: 1.9 ± 0.2; WM: 1.4 ± 0.1 mmol/L; P = 0.01), and potassium was higher in PWM and WM (SM: 4.3 ± 0.3; PWM: 4.5 ± 0.3; WM: 4.5 ± 0.3 mmol/L; P = 0.01)

CONCLUSIONS: WM feeding reduced FDM, impaired transient growth, and triggered immune activation. Elevated lymphocytes and monocytes suggest sustained early-life inflammation. Although pasteurization reduces microbial load, it fails to prevent these alterations, suggesting early WM exposure modulates immune and metabolic responses during critical developmental windows

Keywords: Diarrhea, bovine respiratory disease, fecal dry matter, leukocyte profile, metabolic parameters, neonatal calves

Development of the acid-base equilibrium during and after weaning in calves on a high plane of nutrition with high starch diet - an observational study

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BACKGROUND and OBJECTIVE: Feeding calves solid feed with high starch content during and after weaning is thought to increase the risk of overacidification. We aimed at following the development of the acid base balance in dairy calves fed on a high nutritional plane during and after weaning.

MATERIALS and METHODS: Fourteen calves kept in group pens were studied from 6 to 25 weeks of age. Calves were fed with up to 12 L of milk replacer and were weaned from the 7th to the 12th week of age. A calf-TMR formulated to meet or exceed nutritional requirements, with a starch content of 28-32% was offered ad lib. Blood and urine were obtained every two weeks. Blood gas analyses were conducted and net-acid-base excretion (NABE) was determined in urine.

RESULTS: Calves remained healthy with an average daily weight gain of 1.15 kg/d. At 14 weeks, calves on average weighed 138 kg and consumed between 5.7 and 7.9 kg DM TMR/d. No time effect was observed on blood base excess (BE) or pCO2, a numerical increase of the BE from 4.6 to 6.2 mEq/L was however observed. Urine pH increased from 6.3 to 8.1, with the steepest increase occurring between 10 and 12 weeks of age NABE showed a concomitant increase.

CONCLUSIONS: In this study solid feed with a starch content of 28-32% did neither cause compensated nor decompensated metabolic acidosis in calves on a high nutritional plane. Observed increases in urine pH and NABE are consistent with the metabolic transition from monogastric (calf) to ruminant. The effects of high-starch diets in the digestive tract remain to be described.

Keywords: calf, acid-base equilibrium, high starch diet

The effect of parenteral iron dextran on pre-weaning daily liveweight gains, antimicrobial treatment rates and mortality in whole milk fed dairy calves in the United Kingdom

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BACKGROUND and OBJECTIVE: Colostrum and cow's milk have very low iron concentrations in relation to recommended intake levels for calves. Several studies found subclinical iron deficiency anaemia pre-weaning in whole milk fed calves and increased daily liveweight gains in iron supplemented animals. The effect of iron supplementation on calf health is controversially discussed, with some authors stating health improvements while others claim that iron supplementation also feeds pathogens, leading to higher rates of diseases like neonatal calf diarrhoea. The objective of this study was to compare pre-weaning daily liveweight gains, mortality and antimicrobial treatment rates between calves supplemented with parenteral iron dextran to unsupplemented animals.

MATERIALS and METHODS: On two large dairy farms in the Southwest of England between November 2024 and January 2026 neonatal calves were enrolled between 2 and 10 days of age and randomly selected to either receive 1000 mg of iron as intramuscular iron dextran or no treatment. Farm staff were blinded about the treatment groups. Calves showing signs of any disease at enrolment were excluded from the study. Calves were weighed using weigh tapes at enrolment and around four and eight weeks later. All calves were fed natural whole milk from the same farm, on one farm for four weeks before changing to milk replacer, on the other farm for the entire pre-weaning period. Pre-weaning daily liveweight gains, mortality rates and antimicrobial treatment rates were compared between the treatment groups.

RESULTS: 765 calves were enrolled in total. Daily liveweight gains were significantly higher in iron supplemented calves. Over both farms total daily liveweight gains were 28 g/day higher in iron supplemented animals over the first four weeks as well as the first eight weeks. Farm H (feeding whole milk for four weeks only) showed a difference of 24 g/day for the first four weeks and 25 g/day for the

first eight weeks. For farm M (feeding whole milk only) the difference was 42 g/day for the first four weeks and 26 g/day for the first eight weeks. Mortality during enrolment was low at 1 % between the two farms, with no significant difference between the treatment groups. Antimicrobial treatment rates during enrolment did not differ significantly between the treatment groups, neither analysed as total nor for the individual farms.

CONCLUSIONS: The study confirms that iron supplementation increases daily liveweight gains in whole milk fed calves pre-weaning without negative effects on antimicrobial treatment rates or mortality.

Keywords: calves, whole milk, iron supplementation, growth rates, mortality, antimicrobial treatment rates

Effects of different levels of probiotic supplementation on growth performance and health status of pre-weaned holstein calves

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BACKGROUND and OBJECTIVE: The pre-weaning period is a critical stage in the life of dairy calves, during which gastrointestinal disorders, particularly diarrhea, are common and can negatively affect growth, health, and long-term productivity. In recent years, the use of probiotics has received increasing attention as a nutritional strategy to support gut microbial balance, enhance immune responses, and reduce the reliance on antibiotics in calf rearing systems. The present study aimed to evaluate the effects of different levels of the probiotic Biolence on performance and health status of pre-weaned Holstein calves.

MATERIALS and METHODS: A 75-day experiment was conducted using pre-weaned Holstein calves randomly assigned to four treatments receiving 0, 1.5, 3, or 4.5 g/day of Biolence probiotic mixed in milk. Calves were housed individually under similar management conditions and fed whole milk with the assigned probiotic level, while calf starter and water were provided ad libitum. Feed intake, body weight, and growth measurements were recorded during the trial. Health status and diarrhea incidence were monitored daily. Blood samples were collected to evaluate selected metabolites, and fecal samples were analyzed for bacterial populations including Clostridium, Escherichia coli, and Lactobacillus. Data were analyzed using a completely randomized design to compare treatment effects.

RESULTS: Probiotic supplementation influenced several performance and health parameters in pre-weaned Holstein calves. Calves receiving 3 g/day of Biolence showed significantly higher dry matter and organic matter intake compared with the control group. This level of supplementation was also associated with improved growth performance, particularly greater body length development during the experimental period. Blood metabolite analysis indicated that calves supplemented with 3 g/day had higher concentrations of glucose and cholesterol, suggesting improved metabolic status and nutrient utilization. In contrast, the highest probiotic level (4.5 g/day) increased the feed conversion ratio, indicating lower feed efficiency compared with the moderate supplementation level. Health observations showed that probiotic supplementation had a beneficial effect on gastrointestinal health. The incidence of diarrhea was significantly reduced in calves receiving 3 and 4.5 g/day of the probiotic compared with the control treatment. Analysis of fecal microbial populations also revealed a reduction in the abundance of Clostridium in these groups. However, probiotic supplementation did not significantly affect the populations of Escherichia coli or Lactobacillus in fecal samples.

CONCLUSIONS: Overall, the results indicate that moderate probiotic supplementation, particularly 3 g/day, improved feed intake, growth indicators, metabolic status, and intestinal health in pre-weaned Holstein calves, while excessive supplementation did not provide additional benefits and could reduce feed efficiency.

Keywords: Probiotic, Holstein calves, Pre-weaning period, Growth performance, Gut health, Diarrhea incidence

CATTLE WELFARE

Quality of life methodology, a good option for animal welfare assessment - a pilot study

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BACKGROUND and OBJECTIVE: Test theory, human quality of life methods and literature covering causal and effect indicators for latent variables were applied in order to identify the most appropriate way to measure animal welfare (AW) as a subjective experience of an animal.

MATERIALS and METHODS: Welfare assessments performed during annual veterinary visits for 5 554 animal groups, animal register data and slaughter records for 104 996 bovine carcasses slaughtered in Finland in 2021 by Atria Plc were used as a pilot data to test the reviewed methods. Generalized structural equation models were built to study various welfare dimensions (WD) as latent variables and associations between them. A theoretical model was built to describe how various WD were expected to be related. A set of indicators of different WD was included in the model. Possible confounding factors, like production type and breed class were controlled in the model as well. Separate models were made for slaughtered dairy cows, suckler cows, heifers and bulls. Models were run using generalized structural equation models in Stata 16.0 intercooled software.

RESULTS: Altogether, nine different welfare related latent variables (LV) were found. They were named based on the included indicators: 1) disease prevention, 2) housing, 3) nutrition, 4) food health, 5) health, 6) behavior, 7) welfare risk, 8) meat production ability and 9) overall welfare. LVs and connections between them as well as confounding factors varied between animal groups. LV1, LV3 and LV5 were found for heifers and bulls, LV2 and LV7 for all groups, LV4 for heifers and dairy cows, LV6 for bulls, LV8 for bulls and dairy cows and LV9 for heifers only. Due to the methodology, it was possible to control for, the effect of assessing veterinarian, which increased the accuracy of obtained farm specific latent variables.
Internal consistency, content and structural validity of the used welfare scheme were estimated to be tolerable. Anyhow, a restricted number of used behavioral indicators and their cumulation to the best values limited the range of LV6 and LV9.

CONCLUSIONS: The study revealed many ways to develop the used welfare assessment scheme. It should include more effect indicators for AW, defined as a subjective experience. Each item of the scheme should be specified precisely to be either effect or causal indicator. The applied methodology also questioned the need for item level repeatability in an AW scheme. The methodology was found to be a promising way to develop on-farm welfare schemes.

Keywords: animal welfare, quality of life, generalized structural equation model

Influence of a stressful manipulation on substance P and cortisol concentrations, and on behavior in calves

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BACKGROUND and OBJECTIVE: Cattle, as prey animals, only show subtle signs for pain. Therefore, pain assessment is difficult, and pain management is a major welfare problem. An objective parameter to assess nociception in cattle is substance P (SP), which is a neurotransmitter involved in processing noxious information to the brain. However, research in humans and laboratory animals found that not only nociception but also stress increases SP concentrations. The objectives of this pilot study were to assess plasma substance P (PSPC), plasma cortisol (PCC), and salivary substance P (SSPC) concentrations, as well as vital signs, behavioral parameters and activity in calves submitted to either a stressful or no stimulation.

MATERIALS and METHODS: This study included 24 healthy male German Simmental calves (aged 43 ± 1 days). Calves were randomly assigned to either control (CON) or trial (STRESS) group. Calves in STRESS were submitted to a stressful stimulation consisting of restrainement in a crush and an ultrasonographic examination of the heart. CON

calves remained in their igloo for the duration of the trial. Samples for assessment of PSPC, PCC, and SSPC were taken before (baseline and at 0 min), during (5 min to 30 min), and after (35 min to 2 hours) the stimulation. Vital signs and behavior were recorded during the stimulation for a duration of 35 minutes, and before each blood sampling. Activity was assessed over 24 hours.

RESULTS: PSPC and PCC did not differ significantly between groups. In STRESS, PSPC decreased during the stressful stimulation, and were significantly lower at 5 min and at 20 min compared with at baseline. SSPC were below the sensitivity of the ELISA in 34% of samples and were significantly lower in STRESS compared with CON at 0, 5, 10, 20, and 30 min, and at 2 hours. Also, SSPC were significantly lower at 30 min compared with at baseline in STRESS. PSPC and SSPC did not correlate. In CON, PCC were significantly lower at 10 and 25 min, and in STRESS at 20 min compared with at baseline. Occurrences of "ear movements", "limb movements", and "urination" during stimulation were significantly higher in STRESS compared with CON. Activity did not differ between groups.

CONCLUSIONS: Acute stress without nociception or tissue damage decreased PSPC in healthy calves, contrary to previous findings. Saliva samples do not seem to be a suitable alternative to determine SP concentrations in cattle.

Keywords: Fleckvieh, nociception, pain, ultrasonographic examination, welfare

Study on the prevalence of bovine tail lesions and associated local hypersensitivity

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BACKGROUND and OBJECTIVE: The bovine tail is a sensitive anatomical structure that is frequently manipulated when handling dairy cattle. When performed improperly—such as through forced twisting—tail manipulation can result in painful injuries. This study aimed to determine the prevalence and characterize the main types of tail lesions in dairy cows, as well as to assess the presence of secondary hypersensitivity associated with these injuries.

MATERIALS and METHODS: Data were collected from dairy farms in mainland Portugal and in the Azores. In the first part of the study 2,667 cows from 87 farms were assessed for the presence of tail lesions and their association with the milking system used in the Azores: mobile milking machine, milking parlours with a pit, and milking parlours without a pit. In the second stage, the tails of 381 cows were palpated at the base, middle, and distal regions to evaluate pain-related responses. Finally, an original tail-compressing algometer was used in 76 cows to compare sensitivity between normal, injured, and amputated tails at the middle and distal regions.

RESULTS: Farms using mobile milking machines showed the highest prevalence of tail fractures (10.2%), followed by parlours with a pit (6.1%) and parlours without a pit (3.6%). Significant variability in lesion prevalence was observed among farms, with some presenting more than 40% of cows affected by at least one type of tail lesion. Tail palpation revealed a positive association between the presence of lesions and the intensity of behavioural reactions. Lesions were unevenly distributed along the tail, occurring most frequently in the middle region. However, cows exhibited stronger reactions to palpation of the distal tail. Algometer assessment of the mid-tail region indicated a trend toward increased sensitivity in cows with tail lesions, regardless of lesion location. Algometric compression also revealed hypersensitivity in the stumps of amputated tails.

CONCLUSIONS: Dairy farms using mobile milking machines exhibited a notably high prevalence of tail lesions, likely due to the absence of adequate restraint structures, which may encourage the use of improper immobilisation techniques such as tail twisting. Behavioural responses to palpation and algometric testing indicate increased sensitivity in both injured and amputated tails. These findings provide evidence of hyperalgesia associated with iatrogenic tail injuries and highlight handling practices that may lead to chronic pain, thereby substantially compromising dairy cow welfare. It also shows important differences between farms, suggesting that forceful handling can derive from human carelessness.

Keywords: dairy cow, tail lesion, pain, hyperalgesia

A retrospective pilot assessment of claw lesions occurrence in Italian dairy farms under a national surveillance initiative (2023–2025)

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BACKGROUND and OBJECTIVE: Hoof lesions represent a major welfare and productivity constraint in dairy cattle, yet epidemiological data from Italy remain limited. This pilot study aimed to quantify lesion prevalence, distribution, and incidence within a structured national claw health monitoring framework over the period 2023–2025.

MATERIALS and METHODS: This retrospective study evaluated hoof lesion prevalence and anatomical distribution in Italian dairy cows (2023–2025). Records from two professional hoof trimmers included 72,692 trimming events from 153 farms and 58,446 cows (29,663 unique cows). For each record, farm ID, cow ID, trimming date, lesion diagnosis, and anatomical location were available. Lesions, based on ICAR score, were coded as binary (1 = lesion present; 0 = no lesion). Data were analysed at cow, farm, visit, foot (front/hind), and claw (medial/lateral) levels, and farm-level prevalence and per-cow lesion frequency were derived. Lesion risk was assessed using a multilevel mixed-effects logistic regression model (binomial, logit link) with claw as a fixed effect and random intercepts for farm and cow. Visit size was stratified into quartiles to evaluate its association with lesion prevalence. Trimming sessions were divided into quartiles (Q1 to Q4) based on the number of cows trimmed per session.

RESULTS: Overall cow-level prevalence was 38.8%, it decreased from 56.4% in Q1 (median 26 cows/visit) to 31.9% in Q4 (median 131 cows/visit). Farm-level prevalence similarly decreased from 64.9% (Q1) to 40.9% (Q4); mean 50.4%. The most frequently observed conditions were digital dermatitis (31.4% of all lesions), sole haemorrhage (16.3%), sole ulcers (14.4%) and corckscrew lesion (9.4%). The mean incidence rate of new lesions across farms was approximately 4.99% per screening period. Anatomical position significantly influenced lesion risk ($p < 0.001$). Compared with the Front Lateral claw, lesion odds were highest in the Hind Lateral claw (OR = 14.16; 95% CI: 13.24–15.15), followed by the Front Medial claw (OR = 9.05; 95% CI: 8.46–9.68) and the Hind Medial claw (OR = 1.75; 95% CI: 1.62–1.89)

CONCLUSIONS: In conclusion, this multi-farm retrospective study demonstrates a heterogeneous distribution of hoof lesions in Italian dairy herds. These findings emphasise the need for targeted prevention strategies and support the value of hoof-trimming data for future cow- and herd-level risk-factor studies, as well as national-scale genetic analyses aimed at improving claw health and animal welfare in dairy cattle.

Keywords: Hoof lesions, Italian dairy, Prevalence, Mixed-effects logistic regression

Pre-transport risk factors for severity of respiratory associated symptoms in unweaned calves following long-distance transport

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BACKGROUND and OBJECTIVE: Protecting calf welfare during long-distance transport requires early identification of animals most likely to develop transport-associated respiratory disease. Although calves may meet legal fitness requirements, substantial variation exists in their susceptibility to post-transport illness. This study aimed to

determine how pre-transport health indicators and predisposing genetic or environmental factors relate to post transport respiratory disease signs in unweaned calves transported from Ireland to The Netherlands

MATERIALS and METHODS: Two cohorts of legally transport fit calves were enrolled during commercial journeys in April and May 2022. Pre-transport assessments included thoracic ultrasound (TUSpre), clinical respiratory scoring (CRSpre), age, body weight, breed, sex, and source (farm or livestock mart). Post transport outcomes included the highest recorded thoracic ultrasound score (TUS), clinical respiratory score (CRS), neutrophil, monocyte, and neutrophil: lymphocyte (N: L) ratio, and the lowest recorded lymphocyte count and total immunoglobulin concentration measured between arrival and three weeks post-arrival. These peak values were selected to reflect the point at which disease expression was greatest.

c: Calves with a favourable TUSpre of 0 developed significantly lower post-transport TUS, neutrophil counts, and N: L ratios than calves with a TUSpre of 2 (all $p < 0.05$). CRSpre did not predict any post-transport respiratory disease indicators (all $p > 0.2$). Among predisposing factors, Holstein-Friesian calves showed higher post-transport TUS and CRS, neutrophilia, monocytosis, and elevated N: L ratios compared with Holstein-Friesian × Beef calves (all $p < 0.05$). Lower body weight was associated with post-transport neutrophilia and higher N: L ratios (both $p < 0.05$). Slight increases in respiratory disease signs were observed in Cohort 1 calves, females, and calves sourced from livestock marts, although these patterns were inconsistent across variables.

CONCLUSIONS: A high pre transport thoracic ultrasound score, dairy breed, and low body weight emerged as key risk factors for the development of respiratory disease signs following long distance transport in calves that were otherwise deemed legally fit for transport. Incorporating TUSpre into pre transport fitness assessments may improve the identification of calves at elevated risk and support more targeted welfare focused transport decisions

Keywords: calf, transport, respiratory, ultrasound

Voluntary liquid intake of unweaned calves after an 11 hour fast: Implications for feeding during long distance transport

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BACKGROUND and OBJECTIVE: In the European Union, anticipated legislation is expected to mandate the feeding of unweaned calves during long-distance transport (>8h). While typical feed consists of milk (replacer), the suitability of electrolyte solutions as a temporary substitute is under discussion due to their easier on board handling and provision on livestock vehicles. Feed should be provided during a one hour stationary stop, yet the voluntary intake of different liquid types during such breaks is unknown. This study aimed to assess voluntary liquid intake by calves after 11 hours of fasting during simulated transport and to describe associated short-term physiological responses.

MATERIALS and METHODS: This pilot study was conducted over six weeks in 2025 at Aarhus University (Tjele, Denmark) with 32 unweaned calves. Four-to-eight calves per week were catheterised at T-5h and fed 3L of milk replacer at T-2h. At T0h, they were moved to individual pens (at 20°C). At T9h, calves were health-scored, blood sampled and subsequently offered one of seven ad libitum liquids for 1h: water; electrolytes ([VE-] 17, 26, or 35 g/L, or 26 g/L + concentrate [VE26+C]); or milk replacer (room temperature [COLD] or 40°C [WARM]). After 30 min access, they were assisted to the teat. Blood sampling was repeated at T11h, T13h, and T15h and analysed for glucose, BHB, NEFA, and sodium. Data were analysed in SAS OnDemand.

RESULTS: Milk resulted in the highest numerical liquid intakes, followed by electrolyte solutions, while water was least consumed. Average intake was 4.1L for WARM, 4.3L for COLD, 3.1L for VE35, 3.8L for VE26, 2.4L for VE26+C, 1.7L for VE17, and 0.4L for

WATER. At least one calf in every treatment group consumed < 0.5L, indicating substantial individual variability and possible refusal behaviour. Overall intake tended to differ between treatments (p = 0.07), with a tendency for VE26 calves to drink more than WATER calves (p = 0.08; estimated mean 4.5L vs. 0.2L). High individual variation and small sample sizes limited statistical power, and no differences in plasma glucose, BHB, NEFA, or sodium were detected.

CONCLUSIONS: Calves showed a preference for milk and electrolyte solutions over water during a 1-hour feeding opportunity after 11 hours of fasting. Water alone appears inadequate to ensure meaningful voluntary intake under these conditions. Physiological effects showed large individual differences and did not stand out between treatments. Larger studies under commercial transport conditions are needed to determine whether different liquid types can maintain hydration, physiological homeostasis, and be feasibly delivered on livestock vehicles.

Keywords: Calf transport, feed deprivation, hunger, welfare

Dopamine receptor D4 gene polymorphism and personality traits in Holstein-Friesian cattle

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BACKGROUND and OBJECTIVE: Understanding the genetic background of individual behavior in dairy cattle is paramount for advancing sustainable breeding programs and optimizing management practices. Phenotypic variations in individual coping styles, which significantly impact health, welfare, reproductive efficiency, and adaptability to modern farming systems, are increasingly recognized as genetically influenced traits. This study aimed to investigate the association between polymorphisms in the dopamine D4 receptor (DRD4) gene and various personality traits reflecting coping styles in Holstein-Friesian heifers.

MATERIALS and METHODS: Eighty-two non-lactating Holstein-Friesian heifers underwent a comprehensive behavioral assessment, including tests of approachability, fear of people, reaction to novel objects, impulsivity, and aggressivity, along with DRD4 gene polymorphism analysis from blood samples (G/T substitution at position 140). Principal component analysis (PCA) was conducted to assess the differentiation of DRD4 genotypes based on the measured behavioral traits.

RESULTS: Of the 82 animals, 80 produced successful PCR reactions, yielding three distinct DRD4 genotypes. The numbers of animals at the three DRD4 genotypes were N(GG)=29, N(GT)=36, and N(TT)=15. Groups were similar in age, body condition score, body weight, and days of pregnancy. Two principal components (PCs) were identified, with PC1 accounting for the majority of the variance and explaining the separation between groups. In the first PC, the component loadings, in decreasing order of absolute value, were: novelty seeking, aggressivity, and impulsivity. Within the second component, the most determining variables were the fearfulness-measured values, followed by the immobility reaction. The first component effectively separated the TT genotype from the GG and GT genotypes. This distribution suggests that animals with the TT genotype displayed higher levels of novelty seeking (approaching and touching novel objects) and aggressivity.

CONCLUSIONS: There are differences in DRD4 polymorphisms among behavioral traits in dairy cattle. DRD4 is also linked to coping styles in dairy cattle, as reflected in differences in novelty-seeking, aggressiveness, and reactivity to humans. Our results underscore the potential of genomic approaches to unravel the complex genetic architecture of temperament and stress coping in dairy cattle, offering valuable tools for genetic improvement programs aimed at optimizing animal welfare and productivity.

Keywords: dairy cattle, dopamine receptor D4, gene polymorphism, personality

The impact of early life social housing on the play behaviour of dairy calves

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BACKGROUND and OBJECTIVE: Early life housing conditions play a critical role in shaping the development, health and overall welfare of dairy calves. The growth, health and behavioural impacts of social housing, in either pair or group settings, are widely reported. However, the short and long term impacts of early socialisation on calf welfare and particularly on play behaviour, which is a known positive welfare indicator, are not well understood. This study therefore aimed to use accelerometer-derived measures of calf play behaviour as an indication of the immediate and long-term welfare impact of different early life housing conditions

MATERIALS and METHODS: A total of 96 female dairy calves were recruited from four commercial dairy herds in Scotland and assigned to individual, paired or group housing at birth. Play behaviour was measured using IceTag accelerometers in the same cohort of calves across two experimental periods: neonatal play recording in calves aged 24 to 72 hours old and weaned play recording in calves aged between three to five months old. Statistical analysis was performed using mixed-effects negative binomial regression models.

RESULTS: Compared to calves housed individually from birth, pair-housed calves performed significantly more neonatal play behaviour (IRR = 1.29; p = 0.002). Similarly, when compared to calves housed individually from birth, group-housed calves performed significantly more neonatal play behaviour (IRR =1.43; p < 0.001). There was no significant difference in levels of neonatal play behaviour between group versus pair-housed calves (IRR = 1.11; p = 0.334). There was no correlation between levels of neonatal and weaned calf play. Additionally, there was no impact of different early life housing experience on weaned calf play behaviour with no significant difference in weaned play between paired vs. individually reared calves (IRR = 1.21; P = 0.381); group vs. individually reared calves (IRR = 1.03; P = 0.789) or group vs. pair reared calves IRR = 0.91; P = 0.599).

CONCLUSIONS: This work adds to the growing body of evidence supporting social housing of dairy calves and indicates that housing calves in either pairs or groups from birth provides a more positive early life welfare experience than individual housing. Though further investigation of the factors influencing weaned calf play is warranted, the lack of lasting impact of early play experience and early life housing on future play behaviour in this study may indicate that play in dairy calves is a dynamic behaviour influenced by current welfare status rather than a developed behaviour.

Keywords: calf welfare, play behaviour, social housing

Developing an animal welfare assessment protocol for extensive beef cow-calf systems in New Zealand and Namibia: Categorisation and scoring of welfare assessment measures

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BACKGROUND and OBJECTIVE: In New Zealand and Namibia, beef cattle are reared extensively, and beef is exported to high-value markets. These markets require high production and welfare standards. The study aimed to develop a practical protocol to assess the welfare of extensive beef cattle on farms across New Zealand and Namibia and to describe the categorisation of animal welfare measures into scores

MATERIALS and METHODS: A protocol based on the Welfare Quality and the University of California (UC) Davis Cow-Calf protocols, with some additional indicators specific to New Zealand, was trialled on one farm, and 50 measures were identified as potential welfare indicators. The protocol was

subsequently tested on 25 farms (3,366 cows), resulting in a suitable 32-measure protocol. Assessments were conducted on cows in yards during pregnancy testing, and a questionnaire-guided interview gathered information on cattle health and management practices. For each measure, categorical thresholds were established to distinguish acceptable from unacceptable welfare to guide when intervention was needed at the individual measure level, rather than create an aggregated 'score' for each farm. These imposed thresholds were compared to thresholds derived from farm data. The New Zealand protocol was subsequently tested on a semi-commercial Namibian village farm with 5 separate herds. This test excluded one non-relevant measure (tail fecoliths) and identified nine additional measures (40 measures in total). The protocol was tested on 55 beef farms across three production systems (17 commercial, 20 semi-commercial, and 18 communal village) and was found to be both feasible and applicable.

RESULTS: In New Zealand, most of the imposed thresholds were retained. Thresholds for dirtiness, faecal staining, and fearful/agitated and running behaviours were excluded because they were deemed to have limited welfare impact or were expected due to the unfamiliarity with yarding of pasture-based cattle. In Namibia, commercial farms generally met the thresholds identified in the New Zealand study, but many semi-commercial and communal herds did not. This was partly due to the severe drought that occurred in Namibia. It was proposed to modify measures related to good feeding and mortality thresholds during droughts, but keep the original thresholds for "normal conditions".

CONCLUSIONS: The protocol needs further validation with more assessors and farms in both countries. Still, it can serve as a basis for evaluating and promoting beef cow welfare across the beef industry. The follow-up study aims to analyse and refine threshold categorisation by gathering stakeholders' opinions to include their perspectives in the final assessment protocols.

Keywords: Beef Cattle, Welfare Assessment, Measures Categorisation, New Zealand, Namibia, Extensive system

Evaluation of the relationship between hoof diseases and productivity in dairy cattle

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BACKGROUND and OBJECTIVE: Hoof diseases are among the most important health problems in dairy cattle and are a major cause of lameness, which negatively affects feed intake, animal welfare and milk production. In severe cases, advanced disease necessitates the culling of previously healthy and high-yielding animals. The objective of this study was to evaluate the impact of various hoof diseases on milk productivity and composition, while identifying the primary risk factors contributing to their occurrence.

MATERIALS and METHODS: From a single large cattle farm 50 cows that were showing lameness were selected, sorted by a lameness scale from 1 to 5 and then treated for their specific pathologies such as white line disease, digital dermatitis (DD), sole ulcers, laminitis, interdigital hyperplasia, and phlegmon. Data on the cows were obtained from herd management software, which recorded milk production before the onset of lameness, during the lameness episode, and four weeks after treatment. The analysed parameters included milk fat, protein, fat-to-protein ratio (FPR), average daily milk yield, lactose content, and somatic cell count (SCC).

RESULTS: The research demonstrated that with the exception of milk fat ($p = 0,84$), all milk properties, as well as milk yield itself, were significantly negatively affected by lameness. Analysis revealed that for every increase in lameness score by 1, daily milk yield decreased by an average of 3,35 kg/d. ($p = 0,00145$). Average milk yield dropped from 35,03 kg/d. in healthy cows to 22,72 kg/d. during the lameness period ($p = 0,0014$) and recovered by 11,46 kg/d. post treatment ($p < 0,001$). Regarding milk composition, lactose and protein levels decreased by 0,92% ($p < 0,001$) and 0,68% ($p < 0,001$) respectively. As a result of this, the FPR increased by 0,31 during illness ($p < 0,001$).

CONCLUSIONS: Bovine hoof pathologies significantly impair both milk volume and quality, resulting in a 12,31 kg/d yield reduction alongside

significant declines in lactose (0,92%) and protein (0,68%) concentrations. The relationship between lameness scores and yield loss highlights that even moderate cases (score 2, affecting 52% of the study group) cause severe economic losses. Data identifies high-yielding cows managed on concrete flooring with poor hygiene as the most likely for contracting these hoof diseases.

Keywords: Dairy cows, Milk yield, Lameness, Locomotion scoring, Animal welfare

Veterinary surgeons' perceptions of cattle welfare in the Norwegian on-farm emergency slaughter system

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BACKGROUND and OBJECTIVE: On-farm emergency slaughter (OFES) is used more in Norway than any other European country, accounting for >4% cattle slaughter. It permits non-transportable cattle to undergo OFES for human consumption after an accident under specific regulatory conditions (Regulation (EC) 853/2004). The requirement for an accident to have occurred is currently under review by the EU. Norwegian veterinarians' perceptions of OFES on cattle welfare is unstudied. This study aimed to describe veterinarians' attitudes towards the welfare consequences of OFES.

MATERIALS and METHODS: A web-based questionnaire with multiple-choice and open-ended questions was distributed to veterinarians practising in Norway in 2023, addressing demographics, welfare attitudes, acceptable waiting times, and competence with on-farm killing. Data were analysed using descriptive statistics; open-ended welfare arguments were thematically coded.

RESULTS: Three hundred and one veterinarians responded (264 private practitioners [PP], 37 Norwegian Food Safety Authority veterinarians [OV]. Ninety-two percent considered OFES always or often beneficial for welfare, with PP more positive (94%) OV (78%). Of 164 respondents providing welfare-related open-text arguments, the principal welfare benefits cited were that OFES promoted faster decision making and ensured safe euthanasia. Prolonged waiting for the slaughterman and/or treatment, a restrictive regulatory framework (particularly for chronically lame cattle) were the perceived negative animal welfare aspects of OFES. Seventy-five percent of respondents considered <12 hours an acceptable maximum wait from AMI to killing, with over a third recommending >3 hours. Published data show a median waiting time from AMI to death of 2.5 hours, with 5% of cattle waiting >20 hours. For fractures, dystocia, and prolapse, the majority considered <3 hours the maximum acceptable wait, yet over 25% of animals waited more than 4.3, 2.5, and 3.1 hours for the respective condition. For recumbency, the majority accepted a waiting time of <6 hours (75% are dead within 5.75 hours of AMI), whilst for lameness, 96% accepted waits >6 hours (75% dead within 5.6 hours).

CONCLUSIONS: Norwegian veterinarians are overwhelmingly positive about OFES as a welfare measure, principally because it incentivises timely decision-making. However, a gap exists between what veterinarians consider acceptable waiting times and the times some animals actually experience. Many Veterinarians felt that strict regulations regarding the acceptability of animals for OFES is detrimental for animal welfare. This is pertinent given the discussion within the EU on permitting OFES if an animal cannot be transported to a slaughterhouse irrespective of the reason, thereby removing the current requirement for an accident.

Keywords: Animal welfare, emergency slaughter, veterinarian, beef

DIAGNOSTIC IMAGING AND LABORATORY

Main hepatic vein spectral doppler waveform and doppler parameters in healthy and brisket disease diagnosed Holstein cows for determining tricuspid valve regurgitation and the differences with other hepatic pathologies

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BACKGROUND and OBJECTIVE: Brisket disease (BD) and tricuspid valve regurgitation (TVR) in cattle at high altitudes is often linked to barometric hypoxia. Accurate diagnosis can be challenging, necessitating reliable non-invasive cow-sided methods. Evaluation of hepatic vein blood flow with spectral doppler ultrasonography is an accepted method used in human and small animal medicine to determine TVR in animals with right congestive heart failure (RCHF)

MATERIALS and METHODS: This study hypothesized that pulsed-wave Doppler ultrasonography (PWD) could effectively assess hepatic vein blood flow patterns to diagnose tricuspid regurgitation associated with RCHF in cattle at high altitudes and differentiate it from other hepatic pathologies. The study included 82 Holstein cows from ten dairy farms (2,500-3,000 m.a.s.l.) in Colombia, comprising 60 healthy cows, 14 cows diagnosed with BD and tricuspid regurgitation, and 8 cows with other hepatic conditions. Blood flow velocities in the main hepatic vein were measured, and Doppler parameters, including the Pulsatility Index (PI) and flow patterns, were analyzed and compared between groups

RESULTS: Significant differences ($p<0.05$) were found in Doppler parameters between healthy cows and those with tricuspid regurgitation. Healthy cows exhibited predominantly monophasic, hepatofugal flow, whereas cows with tricuspid regurgitation showed biphasic flow with retrograde S waves. The PI was significantly higher in cows with tricuspid regurgitation (mean ± SD: 3.91 ± 4.5) compared to healthy cows (0.61 ± 0.39; $p<0.05$). No alterations in hepatic vein flow were observed in cows with other liver diseases.

CONCLUSIONS: PWD ultrasonography of the main hepatic vein is a valuable non-invasive cow-sided tool for diagnosing tricuspid regurgitation associated with RCHF in cattle at high altitudes diagnosed with BD. Identifying specific changes in hepatic vein flow patterns facilitates earlier and more accurate diagnosis, allowing for timely intervention and improved management of RCHF in high-altitude livestock

Keywords: doppler, pulsed wave doppler, brisket disease, cow, tricuspid valve regurgitation

Quantitative ultrasonographic analysis of luteal tissue for early pregnancy prediction in Holstein cows

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BACKGROUND and OBJECTIVE: Early pregnancy detection in dairy cows is critical for improving reproductive efficiency and farm management. Ultrasonographic evaluation of the corpus luteum (CL) is commonly used but largely depends on subjective interpretation. Quantitative image analysis may provide objective indicators of luteal function. This study aimed to determine whether quantitative measurements of CL size, pixel intensity, and echotexture from ultrasound images can distinguish pregnant from non-pregnant cows after artificial insemination (AI), and to identify predictive parameters according to days after insemination.

MATERIALS and METHODS: From July 27, 2021, to June 3, 2022, transrectal ultrasonographic videos were collected from 1,568 Holstein cows at 32 commercial dairy farms. Cow identification number, parturition date, and insemination date were recorded. Among them, 422 cows with a visible CL and between 7 and 30 days after AI were selected.

Ultrasound image frames were extracted using VLC media player and annotated with the Supervisely labeling tool. Quantitative image analysis was performed using custom Python software to measure CL width, height, area, pixel mean (pmean), and pixel heterogeneity (phetero; SD) from grayscale pixel intensity values (0–255). Echotexture features were calculated using gray-level co-occurrence matrix (GLCM) analysis, including contrast, dissimilarity, homogeneity, angular second moment, energy, and correlation. CLs were classified as compact CL or cavitary CL (CCL); in CCL cases, luteal tissue excluding the cavity was analyzed. Statistical analyses were conducted using R (version 4.1.2) with the Mann–Whitney U test and optimal cut-off determination using the maxstat package.

RESULTS: Significant differences between pregnant and non-pregnant cows were observed in luteal tissue parameters. Luteal tissue area was greater in pregnant cows than in non-pregnant cows (465.35 vs. 398.50 mm²) at 13–21 days after AI ($P < 0.001$). Pixel heterogeneity of luteal tissue was lower in pregnant cows (14.14 vs. 18.18) at 22–27 days after AI ($P = 0.004$). Luteal tissue contrast from GLCM analysis also differed between groups (43.64 vs. 48.41) at 21–30 days after AI ($P < 0.016$).

CONCLUSIONS: Quantitative ultrasonographic analysis showed that luteal tissue provides stronger predictive indicators of pregnancy than overall CL measurements. Moreover, optimal predictive parameters vary with days after insemination. These findings support the development of an explainable computer-aided diagnosis system integrating quantitative luteal analysis with deep learning–based ovarian ultrasound segmentation for early pregnancy prediction in dairy cows.

Keywords: cow, ultrasound, reproduction, pregnancy diagnosis, quantitative analysis, echotexture

Space allowance influenced ultrasound fat deposition pattern and muscle growth of the longissimus dorsi in beef heifers

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BACKGROUND and OBJECTIVE: Space allowance could induce stress conditions in beef cattle farming negatively affecting animals' growth. The *longissimus dorsi* ultrasound is a method for assessing their performances which evaluate both fat and muscle components by backfat thickness (BFT), muscle thickness (DEPTH), ribeye area (RIBEYE), and intramuscular fat (IMF). European recommendations suggest a space from 2.4 to 5.5 m²/animal. However, a minimum space of 13 m²/animal is suggested for heavy animals to reduce group stress. The aim of the study was to evaluate beef heifers housed at two stocking densities (10.3 vs. 5.54 m²/animal) using *longissimus dorsi* ultrasound.

MATERIALS and METHODS: 54 Charolais heifers of 13 months were enrolled (protocol number of ethical approvals 17498/2024). Animals were randomly divided into indoor boxes at arrival composing: high stocking density group (HIGH_D; two boxes with 13 animals/box (5.54 m2/animal), N=26) routinely adopted in the farm, and low stocking density group (LOW_D; four boxes with 7 animals/box (10.3 m2/animal), N=28) as a compromise between animals' and farmer's needs. Blood sampling and *longissimus dorsi* ultrasound evaluations were performed at arrival (D0), and after 30, 60, 90 and 120 days (D30, D60, D90, and D120) until slaughtering. The animal body weight (BW; Kg) was measured at D0, D60, and D120 and average daily gain (ADG; Kg/day) was then calculated among time-points. Finally, the carcass yield (%) was obtained by the slaughter house. Statistical analysis was conducted with

linear mixed models, a p≤0.05 was used as significant, and 0.05<p≤0.10 as trend.

RESULTS: The LOW_D had lower neutrophils (p=0.012) and greater lymphocytes (p=0.008) between D60 and D120, and lower monocytes (p=0.031) between D90 and D120. Even if these results were within ranges, their trend suggested a slight greater stress in HIGH_D. The LOW_D had also greater BWD120 (p=0.007), ADGD120_D0 (p=0.073), ADGD120_D60 (p<0.001), carcass yield (p=0.023), DEPTH (6.45-7.30 vs. 5.84-6.58 cm; SEM=0.14; p=0.021) and RIBEYE (77-102 vs. 69-86 cm2; SEM=2.01; p<0.001) from D30, BFT from D60 (1.10-1.38 vs. 0.91-1.05 cm; SEM=0.04; p<0.001), and IMF from D90 (6.69-7.74 vs. 5.92-6.93 %; SEM=0.16; p=0.015) until D120 compared to HIGH_D. These results could be related to lower competitive feeding behavior, and greater animals' movement with an improvement in the musculoskeletal system development in LOW_D.

CONCLUSIONS: In conclusion, this study confirmed that space allowance affects beef heifer performance and health with animals housed at 10.3 m²/animal had better growth outcomes, ultrasound fat deposition pattern and muscle growth, and slight lower stress condition.

Keywords: Space allowance, Beef cattle, Muscle ultrasound, Growth, Stress

Associations of clinical and thoracic ultrasonographic findings with necropsy-confirmed pneumonia in cattle under two years of age

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BACKGROUND and OBJECTIVE: Bovine respiratory disease (BRD) is a major cause of morbidity in cattle under two years of age, including young calves and feedlot cattle. Diagnosis based solely on clinical examination remains challenging under field conditions, which has led to an increasing use of thoracic ultrasonography (TUS) as a complementary diagnostic tool. While the diagnostic performance of TUS has been widely assessed in young calves, data remain scarce in older cattle, in which the cranial part of the lungs is not accessible to TUS examination. This study aimed to investigate the relationships between clinical signs, ultrasonographic findings, and post-mortem lung lesions in cattle under two years of age. In addition, the diagnostic performance of TUS for detecting BRD was evaluated, both alone and in combination with clinical findings.

MATERIALS and METHODS: A prospective study was conducted between September 2023 and January 2025 in two French veterinary schools. Forty-two cattle under two years of age were included. All animals underwent a complete clinical examination, followed by bilateral TUS from the fourth to the eleventh intercostal spaces within 24 hours before necropsy. Consolidations ≥ 1 cm in depth were recorded. A post-mortem examination was performed to assess the location and extent of pneumonic lesions. Associations between clinical, ultrasonographic, and necropsy findings were analysed using Fisher's exact test and the chi-square test. Sensitivity, specificity and Youden indices were calculated using either ultrasonography or necropsy as the reference standard.

RESULTS: Twenty-eight of the 42 cattle had pneumonic lesions at necropsy. TUS alone yielded 20 true positives, 3 false positives, 8 false negatives and 11 true negatives (sensitivity: 0.71; specificity: 0.79). Dyspnoea, spontaneous coughing and abnormal auscultation were significantly associated with TUS consolidation and lung lesions at necropsy ($p < 0.05$) but showed limited sensitivity (0.43 - 0.57) and good specificity to detect lung lesions (0.89 – 0.95). Among strategies combining TUS and clinical findings, the best diagnostic performance was obtained when TUS was combined with abnormal auscultation (sensitivity: 0.61; specificity: 1.00). Overall, compared with clinical examination alone, combining TUS with one clinical sign yet improved specificity but lowered sensitivity than TUS alone. Adding additional clinical signs did not improve performance further.

CONCLUSIONS: In our population of cattle under two years of age, TUS showed good diagnostic performance to detect lung lesions and outperformed individual clinical signs. Combining TUS with selected clinical findings increased specificity but reduced sensitivity.

Keywords: BRD, TUS, Necrospy, Diagnostic accuracy

Effect of lipomobilisation on hyperbilirubinemia in dairy cows

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BACKGROUND and OBJECTIVE: Hyperbilirubinemia is frequently observed in dairy cows with lipid mobilization and hepatic lipidosis and is often interpreted as an indicator of impaired liver function. Since serum bilirubin and non-esterified fatty acids (NEFA) share hepatic uptake pathways, competition between these lipophilic compounds may influence circulating bilirubin levels. The aim of this study was to evaluate the relative effects of serum NEFA concentrations and hepatic triacylglycerol content (LTAG) on serum bilirubin in dairy cows.

MATERIALS and METHODS: Thirty-six Holstein–Friesian cows were studied: 10 clinically healthy cows (5.8 ± 1.2 years) and 26 cows with left displaced abomasum prior to surgery (4.8 ± 1.3 years, 1–6 weeks postpartum). Hepatic triacylglycerol content was measured enzymatically in liver biopsy samples. Serum NEFA was determined using an automated analyzer. Bilirubin fractions (total bilirubin (TB), unconjugated bilirubin (UCB), and conjugated bilirubin (CB)), were quantified by high-performance liquid chromatography (HPLC). All variables except LTAG were log-transformed to achieve normal distribution. Correlations between parameters were calculated, and multiple linear regression analysis was performed using SAS. The proportional contributions of NEFA and LTAG to TB variance were estimated by partitioning the sums of squares of the regression model.

RESULTS: LTAG ranged from 10–150 mg/g fresh weight, serum NEFA from 150–2500 µmol/L, and TB from 2–28 µmol/L. Significant correlations were observed between LTAG and NEFA (r = 0.64), TB (r = 0.68), UCB (r = 0.68), and CB (r = 0.44), and between NEFA and TB (r = 0.83), UCB (r = 0.80), and CB (r = 0.51) (all $p < 0.01$). The regression model (log TB = −1.65 + 0.67 log NEFA + 0.004 LTAG) explained 74% of serum TB variance ($r^2 = 0.74$, $p < 0.001$). Partitioning the sums of squares indicated serum NEFA accounted for 36%, LTAG for 5%, and their interaction for 58% of the explained variance. After proportional allocation, serum NEFA explained about 87% and LTAG 13% of serum TB variance.

CONCLUSIONS: In cows with lipid mobilization and hepatic lipidosis, serum bilirubin concentrations provide limited information on liver function. Hyperbilirubinemia is primarily explained by competition between NEFA and bilirubin for hepatocellular uptake rather than impaired excretory capacity caused by liver dysfunction. Conjugated bilirubin concentrations did not indicate cholestasis in these cows.

Keywords: cow, bilirubin, liver function, hepatic lipidosis, lipomobilization

Accuracy of determining the activity of the corpus luteum using color-doppler ultrasonography in lactating dairy cows

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BACKGROUND and OBJECTIVE: Accuracy of determining the activity of the corpus luteum (CL) based on presence or absence of luteal blood flow (LBF) using color-Doppler ultrasonography was investigated in lactating Holstein dairy cows.

MATERIALS and METHODS: Between days 35–48 postpartum (day 0), cows were examined with transrectal B-mode ultrasonography to determine the mean of maximal and perpendicular diameters of CL on frozen images. Cows (n=204 [primiparous: n=128; multiparous: n=76]) with a CL ≥ 15 mm were enrolled. Luteal size (LS) was recorded, and presence or absence of LBF was assessed using transrectal color Doppler ultrasonography (EasyScan®, ESG,

IMV, Belshill, Scotland, UK). Based on LS measurements on day 0, cows were classified into groups depending on a predefined cutoff (≥ 20 mm) and a cutoff derived from ROC analysis (>22.5 mm). Three days later (day 3), all cows were re-examined to determine LS and presence or absence of LBF. At least approximately 10% of luteal area with distinct color flow signals especially condensed LBF at the base of CL was accepted as presence of LBF. In case of undetected CL on day 3, LS was accepted as 0 mm, and LBF was accepted as absent. Based on LBF status on days 0 and 3, frequencies of true positives (present on both days), false positives (present on day 0, absent on day 3), false negatives (absent on day 0, present on day 3), and true negatives (absent on both days) were determined.

RESULTS: Chi-square analyses revealed that true positive (21.1%, 8/38; 51.8%, 86/166; 35.3%, 36/102; 56.9%, 58/102), false positive (21.1%, 8/38; 20.5%, 34/166; 17.6%, 18/102; 23.5%, 24/102), false negative (21.1%; 8/38; 10.2%, 17/166; 14.7%, 15/102; 9.8%, 10/102) and true negative (36.8%, 14/38; 17.5%, 29/166; 32.4%, 33/102; 9.8%, 10/102) values differed (P=0.41; P<0.01; P<0.01; P<0.08) within each group according to LS below predefined cutoff (15 mm ≤ LS < 20 mm; n=38), equal to or above predefined cutoff (LS ≥ 20 mm; n=166), equal to or below cutoff derived from ROC (15 mm ≤ LS ≤ 22.5 mm; n=102) and above cutoff derived from ROC (LS > 22.5 mm; n=102) based on comparison of LBF between two examinations, respectively.

CONCLUSIONS: In conclusion, assessing LBF in cows with LS ≥ 20 mm may improve detection of LBF. As higher LS and cutoff values increase true positives but reduce true negatives, highlighting potential for early LBF prediction and better reproductive management in dairy cows.

Keywords: Cow, corpus luteum, blood flow

GENETICS AND BREEDING

Influence of PITX2 and LHX3 genetic markers on growth characteristics and litter size in saanen goats

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BACKGROUND and OBJECTIVE: Growth and reproductive traits in goats are key determinants of herd productivity and economic sustainability. Genetic variation in these traits enables the selection of superior individuals and drives genetic improvement in breeding programs. LIM homeodomain (LHX) proteins are regulators of neurogenesis, and the LHX3 gene is particularly important for pituitary and nervous system development. The LHX3 protein regulates the transcriptional activity of GH, PRL, POU1F1, FSHβ, and TSHβ genes. The PITX2 gene plays a significant role in cell proliferation, differentiation, hematopoiesis, and organogenesis and is involved in pituitary gland cell differentiation by activating the promoters of FSHβ and PRL genes. This study aimed to investigate the relationship between the LHX3 (g.8035G>C) and PITX2 (g.18353T>C) polymorphisms, which have been reported to be associated with differences in yield and growth-reproductive characteristics in goats.

MATERIALS and METHODS: In this study, blood samples were collected from 211 Saanen goats, and DNA isolation was performed using the "Thermo Scientific GeneJET Genomic DNA Purification Kit." Genotyping was performed using PCR-RFLP. The relationship between growth and reproductive characteristics, including birth weight, litter size, daily live weight gain, total live weight gain, weight for initial breeding, body length, chest circumference, rump width, rump height, and wither height, was examined. Associations between genotypes and traits were evaluated using a general linear model (GLM).

RESULTS: In this context, this study demonstrated for the first time that the LHX3 (g.8035G>C) polymorphism is significantly associated with litter size ($P<0.05$). This association may be explained by the biological role of LHX3, a LIM-homeobox transcription factor that plays a critical role in pituitary gland development and endocrine regulation. LHX3 is involved in the differentiation and function of hormone-producing cells in the anterior pituitary, including those responsible for the secretion of gonadotropins such as follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Since these hormones are key regulators of follicular development, ovulation, and overall reproductive performance, genetic variation within the LHX3 gene may influence litter size through its effects on the hypothalamic–pituitary–gonadal axis. No statistically significant association was found between the PITX2 (g.18353T>C) polymorphism and any of the 10 phenotypic traits examined.

CONCLUSIONS: These results may enhance understanding of the genetic basis of growth and reproductive traits in goats and highlight the examined genes as potential markers for selection in breeding programs. Compared with cattle breeding, genetic analyses in small ruminants are relatively limited; therefore, identifying genotype–phenotype associations is particularly important.

Keywords: Goat, Growth, Reproduction, LHX3, PITX2.

Comparative transcriptomic responses to Treponema exposure in fibroblasts from heifers with different genetic merit for digital dermatitis resistance

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BACKGROUND and OBJECTIVE: Digital dermatitis (DD) is one of the major causes of lameness, negatively affecting animal health and welfare, leading to significant economic losses due to decreased milk yield, increased treatment costs, reduced reproductive performance, and early replacement. *Treponema* spp. plays a key role in the pathogenesis of this polybacterial disease. Host genetic resistance to DD has been reported. However, the molecular mechanisms underlying DD susceptibility and host-pathogen interactions have not been fully elucidated. The objective of this research was to compare the gene expression of primary fibroblasts from heifers with different genomic estimated breeding values (GEBVs) for DD resistance following exposure to sonicated *Treponema* spp.

MATERIALS and METHODS: For this purpose, 323 maiden heifers were followed for a four-month observation period, with their hind feet assessed for DD using the M-stage scoring system. GEBVs for DD resistance were obtained from the official UK national evaluation database (AHDB, 2020), and 15 heifers were categorized into three groups according to genetic merit and clinical history. These groups were defined as follows: healthy heifers with high genetic merit (HGH), low genetic merit heifers that

have recovered (LGIH), and chronically affected heifers with low genetic merit (LGCI). Primary fibroblast cultures were derived from foot skin biopsies from all heifers in the three groups. Fibroblast monolayers were incubated for six hours with either control medium, *Salmonella typhimurium* lipopolysaccharide (LPS), sonicated *Treponema phagedenis* extract (T320A), or a sonicated Treponema mix (*T. pedis, T. medium, T. phagedenis*). Following incubation, RNA was extracted from the cells and submitted for bulk RNA sequencing.

RESULTS: Our results indicate that fibroblasts from heifer groups with low-genetic-merit (LGIH and LGCI) exhibited significantly more differentially expressed genes (DEGs) and distinct gene expression patterns compared to the high-genetic-merit healthy (HGH) group. The findings suggest that transcriptional regulation in susceptible animals is more extensive or less controlled. Additionally, pathway analyses revealed enrichment of immune response, Toll-like receptor activation, cytokine-mediated responses, extracellular matrix organization, and wound-healing processes.

CONCLUSIONS: Our findings suggest that the host's genetic merit may significantly influence cellular responses to DD-related *Treponema* spp. Additionally, the in vitro model developed in this study provides a valuable tool for investigating host-pathogen interactions involved in the pathogenesis of digital dermatitis. Overall, these results offer novel insights into genetic resistance to DD by elucidating host-pathogen interactions and support breeding strategies to improve cattle health, welfare and productivity.

Keywords: Digital dermatitis, GEBV, Treponema, RNA-seq

GLOBAL WARMING AND ENVIRONMENT

Blood-based indicators of enteric methane emissions in dairy cows during early lactation

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BACKGROUND and OBJECTIVE: Enteric methane emissions from dairy cattle represent a significant source of agricultural greenhouse gases and are a primary focus of climate mitigation strategies. This study aimed to examine the correlation between enteric methane emissions and blood metabolic and inflammatory parameters in early-lactation dairy cows, and to determine if deviations from physiological reference ranges are associated with variations in methane production.

MATERIALS and METHODS: The research carried out on a dairy farm in Lithuania, involving 42 clinically healthy multiparous Holstein cows from calving to 100 days postpartum. Enteric methane emissions were systematically assessed utilising a portable methane detector. Blood samples were collected weekly and analysed for β-hydroxybutyrate (BHB), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), and urea levels. An observational design utilising repeated measures was employed, with correlation, regression, and linear mixed-effects models applied to evaluate the relationships between blood parameters and methane emissions.

RESULTS: Cows were categorised for each biomarker as either within the physiological reference interval (Group 1) or outside the reference interval (Group 2). Methane emissions demonstrated significant variability among BHB groups: cows with elevated BHB (≥ 1.2 mmol/L) emitted 87.8% more methane than those within the reference range (537.89 ± 65.21 vs. 286.41 ± 10.49 ppm × m; $p < 0.05$). Methane levels were comparable between the AST- and LDH-based groups; however, cows with elevated GGT (≥ 50 U/L) demonstrated a 25.2% increase in methane compared to those with normal GGT activity ($p <$

0.05). Correlation analysis revealed a moderate positive correlation between methane and BHB (r = 0.54, p < 0.01) and a weak positive correlation with urea (r = 0.21, p < 0.05). In multiple regression analysis, BHB was identified as a significant positive predictor of methane (β = 0.55, p = 0.001), whereas LDH served as a significant negative predictor (β = −0.21, p = 0.033). ROC analysis revealed that BHB displayed exceptional discriminatory capacity for identifying high methane emitters (AUC = 0.889).

CONCLUSIONS: This study elucidates a distinct correlation between metabolic status and enteric methane emissions in clinically healthy dairy cows during early lactation. Increased BHB consistently correlated with heightened methane production and remained a significant positive predictor of methane in multivariable modelling, while LDH exhibited an inverse correlation. Elevated GGT levels correlated with increased methane emissions, underscoring the significance of liver-related metabolic stress in methane fluctuations. The findings suggest that metabolically compromised cows may significantly contribute to enteric methane emissions.

Keywords: methane emission, cattle, metabolic biomarkers, early lactation

Subclinical ketosis in early-lactation dairy cows increases greenhouse gas emissions

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BACKGROUND and OBJECTIVE: Methane (CH_4) from ruminant livestock is a major contributor to global greenhouse gas (GHG) emissions. Early-lactation cows experience negative energy balance, leading to mobilization of body reserves. Subclinical ketosis (SCK) is a common metabolic disorder immediately after calving that can compromise feed efficiency, productivity, and rumen fermentation. The aim of the study was to investigate the impact of SCK on GHG output and production efficiency.

MATERIALS and METHODS: A total of 60 multiparous Holstein-Friesian cows were monitored from 3 to 28 days in milk. Blood β-hydroxybutyrate (BHB) concentrations were measured at 7, 14, 21, and 28 DIM to divide cows as healthy controls (CTR; BHB<1.0 mmol/L at all time points; n=43) or affected by SCK (KET; BHB≥1.0 mmol/L at least once; n=17). Individual CH_4, CO_2, and H_2 emissions were recorded daily using an automated system during voluntary feeding, that measures gas concentrations in the breath and eructation of animals. Emission data were expressed both as absolute daily production and normalized per kilogram of milk or dry matter intake (DMI) to assess efficiency-related effects. Statistical analysis was performed using linear mixed-effects models, with health status, time, and their interaction as fixed effects and cow as a random effect. Differences were considered significant at p≤0.05.

RESULTS: Cows affected by SCK produced 65.2% more CH_4 than healthy ones (402.8±26.6 vs. 243.8±17.7 g/day), corresponding to 10.96±0.72 kg CO_2-equivalents/day compared with 6.60±0.48 kg/day in CTR cows. When normalized per kilogram of milk, CH_4 output was nearly doubled in KET cows (12.8±1.2 vs. 7.7±0.8 g/kg), indicating reduced feed-to-milk conversion efficiency. Total CO_2 emissions were lower in KET cows (11035±593 vs. 12225±416 g/day), but including CH_4-derived CO_2 equivalents showed an overall higher GHG footprint for diseased animals. The H_2 emissions were reduced in KET cows, reflecting greater utilization by methanogenic archaea in the rumen. Temporal analysis revealed that the increase in CH_4 was particularly evident within the first five days after diagnosis, indicating a metabolic shift in rumen fermentation. The DMI was consistently lower in KET cows, confirming reduced feed efficiency.

CONCLUSIONS: The findings of this study have important implications for the environmental sustainability of dairy production. The SCK significantly affects enteric CH_4 emissions and the overall carbon footprint of early-lactation dairy cows. Preventing or mitigating SCK could provide dual benefits, improving both metabolic health and milk production. These results highlight the importance of integrated metabolic and nutritional management strategies to enhance both animal welfare and the sustainability of dairy systems.

Keywords: Ketosis, GHG, Methane

Microclimate dynamics in a dairy cow shed: Indoor–outdoor comparisons of temperature, humidity and temperature humidity index

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BACKGROUND and OBJECTIVE: Cattle welfare and production can be affected by microclimatic conditions, which can vary between locations inside a shed, influenced by factors such as distance from fresh air inlets and areas of animal crowding (e.g. collecting yard and milking parlour). Most studies that have investigated environmental impacts on dairy cow production have used weather station data, which may not be an accurate representation of the microclimate inside a shed. The aims of this study were to compare the microclimate in sheds to the outdoor environment in a 1-year period and to assess how the presence of animals affects the microclimatic conditions within sheds.

MATERIALS and METHODS: The study was conducted on a 900-cow dairy farm in Scotland. The sheds were steel-framed and covered by spaced corrugated composite sheets. The sides were open or consisted of widely spaced vented corrugated metal panels. The sheds were positioned in an east-west orientation. The cows were milked 3 times a day. Milking and fresh cows were kept in a free stall cubicle system and a straw yard, respectively. An environmental sensor was placed just above cow level in six pens in three sheds. Data on temperature, relative humidity and light intensity were collected every 30 min for a year. Outdoor environmental data was accessed from satellite information models for temperature and relative humidity at the hight of 2 meters.

RESULTS: All parameters showed seasonal and between pen variation. The mean temperature inside the sheds was 1.9 to 4°C higher than outdoor. On average the inside air was 10 to 16% less humid than outdoor. The inside temperature humidity index (THI) was 3.7 to 7.1 units higher than outdoor. When animals were being milked, the temperature and THI in the pens dropped by 0.7-0.8 °C and 1.2-1.5 units, respectively, while the temperature and THI in the collecting yard increased by 3.3°C and 5.2 units, respectively.

CONCLUSIONS: The environment in the sheds varied depending on animal presence, as suggested by the increase in temperature and THI in the collecting yard. The lower moisture inside the sheds shows that relative humidity is more closely associated with temperature than with the presence of animals. The results suggest that weather station data might underestimate the microclimatic conditions that cows are exposed to when assessing environmental impacts on dairy cow production.

Keywords: dairy, cows, microclimate, environment, shed,

HERD HEALTH MANAGEMENT AND ECONOMICS

BRSV surveillance in Europe with the DECIDE barometer: Establishing epidemic thresholds

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BACKGROUND and OBJECTIVE: Timely identification of bovine respiratory syncytial virus (BRSV) epidemics could enhance prevention

measures and enable objective comparison across regions and years. While threshold-based surveillance methods like the Moving Epidemic Method (MEM) are well established in human medicine, their applicability in veterinary surveillance remains unexplored. Therefore, the objective of this study was to evaluate whether epidemic thresholds for BRSV in four European countries could be determined using MEM.

MATERIALS and METHODS: Anonymized diagnostic test results from individual and pooled respiratory samples submitted for BRSV testing in cattle with clinical signs were collected from Belgian (2016-2025), Dutch (2018-2023), French (2017-2023) and Irish (2021-2023) laboratories. Analyses were performed overall and per country using an adapted MEM implementation in R-studio. Weekly positive sample rates were calculated, and epidemic weeks were defined as the shortest consecutive period containing 90% of seasonal cases. Epidemic thresholds were calculated as the 95th percentile of pre-epidemic weekly positivity rates. Threshold performance was evaluated using leave one season out cross validation, estimating sensitivity (Se), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV). Weeks with less than 5 analysed samples were excluded from the analysis.

RESULTS: Overall BRSV positivity rate within submitted samples was 18.3% (2841/15530), ranging from 15.9% in Ireland and 16.3% in the Netherlands to 16.7% in Belgium and 31.0% in France. The overall epidemic threshold was calculated at 34%. Country specific thresholds varied between countries i.e. 33% in Belgium, 47% in France, 9% in Ireland and 27% in the Netherlands. Across datasets, Sp remained consistently high (89 to 97%), whereas Se was low (26 to 42%). For the overall model, Se was 26% and Sp was 95%, resulting in a PPV of 82% and an NPV of 61%. In Belgium, Se and Sp were 33% and 97%. In France, Se and Sp were 27% and 89%. In the Netherlands, Se was highest at 42% and Sp was 91%. Reliable cross validation metrics could not be estimated for Ireland due to the limited number of seasons.

CONCLUSIONS: Moving Epidemic Method (MEM) can be applied to multi-country BRSV laboratory surveillance data and provides reproducible epidemic thresholds with consistently high Sp and acceptable PPV. The conservative nature of the threshold limits Se, indicating that the method is better suited for confirming epidemic activity than for early detection. Overall, MEM offers a transparent and standardised method for veterinary epidemic monitoring, although performance depends on the availability of sufficient historical seasons and consistent sampling intensity.

Keywords: BRSV, Epidemic Thresholds, Veterinary surveillance, Respiratory disease in cattle

Veterinary education interventions supporting rural and bovine practice: A scoping review

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BACKGROUND and OBJECTIVE: A persistent shortage of rural and food-animal veterinarians represents a global challenge with implications for herd health, food production systems, and rural economies. While multiple structural factors influence workforce distribution, veterinary education plays a critical role in shaping graduates' readiness and willingness to enter bovine practice. The objective of this scoping review was to synthesise published evidence on veterinary school–based interventions associated with recruitment and retention of veterinarians in rural and food-animal practice, and to identify key gaps in the evidence base.

MATERIALS and METHODS: A scoping review was conducted following established methodological guidance. Searches were performed in CAB Abstracts, MEDLINE, and Scopus in July 2025. Peer-reviewed English-language studies published from 1970 onwards were included if they addressed rural or food-animal veterinary recruitment or retention in relation to veterinary students or veterinary training programmes. Data were extracted and analysed descriptively, with studies categorised by design and thematic focus. **RESULTS:** Thirty-nine studies met the inclusion criteria, the

majority of which were cross-sectional survey studies. Four primary categories of educational intervention were identified: outreach activities, admissions processes, mentorship and experiential learning, and academic programming. Prior exposure to livestock production, both before and during veterinary training, was consistently associated with interest in rural and food-animal practice. Mentorship and extramural clinical experiences were frequently identified by students as critical for developing technical competence, confidence, and professional identity relevant to bovine practice. Species-focused academic programmes, particularly bovine-oriented electives, were described; however, direct evidence linking these initiatives to long-term workforce retention in rural practice was limited.

CONCLUSIONS: Veterinary medical colleges have multiple opportunities to influence the rural and food-animal veterinary workforce through targeted educational interventions. However, the current evidence base is largely descriptive and lacks longitudinal evaluation of workforce outcomes. Strengthening the evidence linking educational strategies to sustainable bovine veterinary service provision is essential for addressing long-term herd health, animal welfare and food-animal veterinary capacity.

Keywords: Veterinary education, recruitment, retention, scoping review, bovine practice

Development of a novel approach to interpreting Mycobacterium avium subspecies paratuberculosis (MAP) Bovine Milk ELISA results and its use to monitor Johne's disease at the herd level and predict individual cow outcomes

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BACKGROUND and OBJECTIVE: Johne's disease is difficult to manage/control, due to low test sensitivity, imperfect test specificity and the long delay between infection and testing positive. Many farms have reduced Johne's prevalence to low levels (2-3%), largely through not breeding or culling positive cows, but progress beyond this point is challenging. The current UK strategy for Johne's control in dairy herds is focused on the use of milk ELISA results with individual tests defined as positive or negative. Results are combined to classify an animal as being at low, medium or high risk of having Johne's disease. The system does not consider cow or farm level factors, despite strong evidence that these can influence test results. This research aimed to investigate approaches to refining the interpretation of Johne's test data to optimise disease management on farm.

MATERIALS and METHODS: Data from 47 UK dairy herds with 220,179 individual MAP ELISA results from 37,782 cows with concurrent dairy herd improvement tests were collated and cleansed. Animal 'states' were determined using the TotalVet© herd health analysis software, prior to anonymisation and export in a csv format suitable for further analysis using "Python" and "R". Approaches to adjusting ELISA titre results taking into account individual cow and herd level characteristics were explored. Unsupervised clustering (a Hidden Markov model) was used to refine the interpretation of test results and develop an approach to predicted individual cow outcomes. Finally, a Pattern Tool was developed to aid practitioners in the monitoring and management of Johne's Disease on Dairy Farms.

RESULTS: Unsupervised clustering identified four clusters; two (C1 and C2) mapped closely to cows defined as low risk and one (C4) to cows defined as high risk by the current UK classification system. However, in the remaining cluster (C3) approximately 25% of cows were currently defined as medium/high risk and the remaining 75% as low risk – we termed these "hidden high-risk" (HHR) cows. Further research demonstrated that HHR cows were more likely to produce offspring that went on be MAP positive than C1 cows. Furthermore, heifers born to C1 cows were more likely to be MAP positive when born in the 'presence' of HHR cows.

CONCLUSIONS: This research offers new insights into Johne's Disease management on dairy farms, particularly in low prevalence herds. It offers an early warning system for predicting emergence of

disease in individual animals and thereby the ability to make more informed management decisions.

Keywords: Johne's Disease, MAP, Machine Learning

Implementation of preventive medicine practices: Developing a practical collaborative framework to support implementation on ruminant farms

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BACKGROUND and OBJECTIVE: Despite increasing evidence for the benefits of multiple preventive medicine practices (PMP) in ruminant farming, uptake often remains limited. In other fields, implementation frameworks are successfully used to facilitate the adoption of evidence-based practices - yet they are currently underutilised in veterinary medicine. This pilot study explored the application of a novel implementation framework (NIF) to guide and enhance the uptake and integration of PMP on ruminant farms, while simultaneously informing further development of the NIF itself.

MATERIALS and METHODS: Guided by the NIF, and facilitated by the researcher, farmer–veterinarian pairs engaged in one or two planning discussions, followed by implementation of selected farm specific intervention(s) over 6–9 months. Pairs also participated in mid point monitoring and end point review discussions. In all discussions, specific topic areas were explored and questions asked, linked to each stage of the NIF – including for example, farm context, individual motivations, consideration of potential interventions, and skill level of personnel. Final interviews were conducted separately with farmers and veterinarians to reflect on the NIF and the process followed. Discussions were audio recorded, transcribed, and analysed using qualitative content analysis to explore the implementation process. Proctor's Implementation Outcomes was used to guide evaluation of the implementation of the NIF.

RESULTS: Initial analysis indicates successful implementation occurred on farms following the NIF, including effective uptake of new vaccines and achievement of farm specific health and productivity targets. Moreover, analysis of the process followed demonstrated both alignment and divergence from the initial NIF and broader implementation theory. This indicated that while some aspects of implementation were readily met through on-farm discussions, others—such as goal setting and targeted follow up —were challenging. A variety of factors impacted on the ability of pairs to engage with aspects of the NIF, including the personalities of individuals involved, type of intervention and time available. These findings enabled further development of the NIF, to reflect what is practical to achieve on farms, alongside what is key to support effective implementation.

CONCLUSIONS: The NIF facilitated collaborative decision making between farmers and veterinarians and supported uptake of a range of PMPs. In cases where implementation was unsuccessful, the NIF identified potential explanations for this. The NIF demonstrates promise in enhancing adoption of PMP on ruminant farms. Future work should focus on efficacy testing in a broader range of settings.

Keywords: Implementation science, Preventive medicine, Veterinary, Framework, Human behaviour change

Reproductive consequences of switching synchronization programs in commercial Holstein Friesian dairy herds

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BACKGROUND and OBJECTIVE: Background and Objective reproductive efficiency is a key determinant of biological longevity and economic sustainability in high-producing Holstein-Friesian dairy herds. Hormonal synchronization programs are widely used to improve control over ovulation and insemination timing and to increase the proportion of cows inseminated. In commercial practice, these programs are often modif ied when fertility declines or management strategies change. However, long-term field data describing the reproductive consequences of switching synchronization programs remain limited. The objective of this study was to evaluate reproductive outcomes associated with switching synchronization programs in Holstein-Friesian dairy herds over a five-year period.

MATERIALS and METHODS: Materials and Methods A multicenter retrospective study was conducted in 5 commercial Holstein-Friesian dairy herds between January 2021 and December 2025. Herds were included if they maintained continuous electronic reproductive records, performed pregnancy diagnosis at 32–42 days after insemination, and documented at least one sustained synchronization program switch. Synchronization strategies were classified as Ovsynch-based protocols, Double-Ovsynch, progesterone-based programs, or targeted reproductive management systems using automated estrus monitoring. Program switches were categorized as intensification, targeted adoption, or simplification. After data quality control, 15000 lactations and 26000 insemination events were analyzed. Main outcomes were pregnancy per artificial insemination, 21-day pregnancy rate, submission rate, and days open. Statistical analyses included interrupted time-series models, mixed-effects logistic regression, and Cox proportional hazards models adjusted for parity, body condition score, postpartum disease, season, temperature-humidity index, year, herd, and protocol compliance.

RESULTS: Results Four synchronization program-switch events were identified across the participating herds. Mean protocol compliance increased from 82.4% before switching to 93.1% after switching. The herd-level 21-day pregnancy rate increased by 2.3 percentage points following program change and showed a modest positive trend thereafter. Pregnancy per artificial insemination increased from 37.6% to 40.8%. Intensification and targeted adoption were associated with higher odds of pregnancy, whereas simplification did not significantly affect fertility outcomes. Submission rate increased from 57.8% to 61.9%, with the greatest improvement observed after adoption of targeted reproductive management. Median days open decreased from 123 to 114 days. Improvements were most evident at first service and tended to plateau after 12–18 months. Heat stress remained a significant factor reducing fertility.

CONCLUSIONS: Conclusions Switching synchronization programs in commercial Holstein-Friesian dairy herds was associated with moderate improvements in reproductive performance. Benefits were linked to improved protocol compliance, higher submission rate, better pregnancy outcomes, and shorter time to conception, although long-term fertility gains remained limited by environmental and metabolic factors.

Keywords: dairy cows, estrus synchronization, reproductive performance, pregnancy rate, herd reproductive management

Pregnancy associated glycoproteins as markers of late embryonic mortality in dairy cows

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BACKGROUND and OBJECTIVE: Late embryonic mortality, occurring between days 18 and 42 of gestation, is an important reproductive and economic problem in dairy cattle. Embryonic loss reduces reproductive efficiency, prolongs calving intervals, and generates economic losses. Early detection is difficult as clinical signs are usually absent. Pregnancy associated glycoproteins (PAGs) are placental proteins secreted by trophoblast cells and detectable in maternal circulation during early pregnancy. Their concentrations reflect placental activity and embryo viability, making them potential indicators of pregnancy status and embryonic loss. This study aimed to characterize changes in PAG concentrations in blood plasma, milk, and urine of cows with late embryonic mortality and to evaluate the diagnostic usefulness of radioimmunoassay.

MATERIALS and METHODS: Seventy-five Black and White dairy cows with Holstein Friesian genetics (body condition score 3.0–3.5) from a herd of 550 animals were included. Cows were housed in a free-stall system, fed a total mixed ration, and milked three times daily. Estrus was synchronized using cloprostenol, and artificial insemination was performed 12 hours after estrus detection. Pregnancy was confirmed by ultrasonography between days 35 and 40 after insemination. Animals were assigned to three groups: cows maintaining pregnancy (C, n = 35), non-pregnant cows (NC, n = 20), and cows with late embryonic mortality diagnosed between weeks 5 and 7 (LEM, n = 20). Blood plasma samples were collected weekly until week 15. PAG concentrations were measured using a heterologous radioimmunoassay.

RESULTS: The incidence of late embryonic mortality was 18%. PAG concentrations in cows maintaining pregnancy increased progressively with gestation, reflecting normal placental development. In cows with late embryonic mortality, PAG concentrations increased temporarily in early gestation, then declined after embryo loss. At approximately the fifth week, clear differences were observed: 17 of 20 LEM cows had PAG <2.5 ng/ml, while all cows maintaining pregnancy had concentrations >2.5 ng/ml, and 28 of 35 exceeded 4.5 ng/ml. Statistically significant differences between groups became evident later, with markedly lower PAG concentrations in LEM cows detected in weeks 9 and 11 after insemination.

CONCLUSIONS: Measurement of PAG in blood plasma using radioimmunoassay enables confirmation of late embryonic mortality approximately 4–5 weeks after embryo loss. Monitoring PAG concentrations provides a useful tool for retrospective detection of embryonic loss and evaluation of reproductive performance in dairy herds.

Keywords: pregnancy-associated glycoproteins, late embryonic mortality, dairy cows, reproductive biomarkers

Should she stay or should she go now? Ranking of cull cows based on net present value implemented in dairy management software

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BACKGROUND and OBJECTIVE: Decisions regarding culling dairy cows are complex and many factors influence the decision. Farmers may aim to keep the most profitable cows, but profitability can be difficult to assess and are often made in a nonprogrammed fashion, relying partly on intuition. To support the day-to-day decision of which cows are candidates

for culling, ranking of the cows based on the Net Present Value (NPV) has shown to be useful. The NPV estimates the discounted, future cash flows of a cow, including both revenues and costs. The objective of this project was to implement an NPV ranking of cows in the Danish Dairy Management Software, DMS, supplementing the existing data on performance with an estimation of each cow's future economic outcome.

MATERIALS and METHODS: A dynamic, stochastic and mechanistic simulation model (the SimHerd Model) was used in a large simulation study of cash flows of different cow types. The SimHerd Model simulates a dairy herd in weekly steps; representing production, events and state of each individual cow in the herd. The simulated cow types were based on parity, lactation stage, milk yield, plus reproductive and health status. All cow types were simulated in different herd types characterised by milk yield, culling level and reproductive performance. Based on the simulated data, response surface models (RSMs) were developed to predict the NPV of individual cow types in the different herd types. In the Danish DMS, the cow level data on milk recordings, reproduction events and health registrations are used as input for the RSM models. The individual NPV is calculated after each monthly milk recording for all lactating cows with milk recordings >70 DIM. The herd and cow specific NPV are converted into values on a ranking index from -100 to +100.

RESULTS: In January 2026, all dairy farmers with access to DMS were presented with the ranking index in a dashboard together with supplemental information regarding the cow's performance in the current lactation. Cows not eligible for culling, due to medicine use or near calving, are marked. The user can filter which group of cows are of interest. The dashboard has been well received with predominantly positive response to the new tool from the farmers.

CONCLUSIONS: Ranking of culling candidates based on the NPV was successfully implemented in a dairy management software with national reach. Farmers find the add-on with the future economic value useful in combination with the historic data on the cows.

Keywords: Culling, Net Present Value, Economic

Economic comparison of alternative mastitis therapies in the commercial setting

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BACKGROUND and OBJECTIVE: Mastitis is the most prevalent and costly disease in dairy production. We compared cure outcomes of 3 treatments for first-case clinical mastitis in a commercial dairy: BOL (plant-derived bolus, AHV®), ATB (ceftiofur intramammary antibiotic), and spontaneous cure (CON; no treatment) from an economic perspective. We hypothesized that cure outcomes would not differ between BOL and ATB relative to CON for the first case of mastitis within a lactation, but economic impacts would vary with treatment.

MATERIALS and METHODS: Animals diagnosed with clinical mastitis were enrolled across four seasons and included both primiparous and multiparous animals. Cows were randomly assigned to 1 of the 3 treatments: BOL (n=230), ATB (n=201), or CON (n=215). Outcomes evaluated included clinical cure (no clinical signs at 28 d post-treatment), mastitis recurrence, occurrence of other diseases, reproductive performance, milk production to 190 days in milk, and somatic cell score (SCS) at 60 and 90 d post-treatment.

RESULTS: Preliminary results indicate that clinical cure rate was affected by treatment (ATB 84.9 ± 3.2%; BOL 72.4 ± 3.5%; CON 77.4 ± 3.6%, P=0.04). Milk production did not differ across treatments (ATB 7242 ± 120 kg; BOL 7132 ± 118 kg; CON 7313 ± 134 kg, P=0.60), but at 60 and 90 d post-treatment, BOL and CON had lower SCS than ATB (60 d: 4.5 ± 0.22 vs. 4.6 ± 0.22 vs. 5.6 ± 0.23; P<0.05; 90 d: 3.9 ± 0.22 vs. 4.4 ± 0.25 vs. 4.7 ± 0.23; P=0.06). Treatment did not affect mastitis recurrence (ATB 23.5 ± 3.7%; BOL 33.0 ± 4.0%; CON 32.6 ± 4.4%, P=0.17), occurrence of other diseases, i.e. ketosis, metritis, retained placenta, pneumonia, digestive, and hoof injuries (P=0.54), or days open (P=0.61). Times bred tended to be different across treatments (ATB 1.9 ± 0.1; BOL 2.0 ± 0.1; CON 2.2 ± 0.1, P=0.09). Saleable milk was higher in BOL and CON than ATB (7,233 ± 104 kg vs. 7,290 ± 110 kg vs. 6906 ± 109 kg, P=0.02), with greater milk income for BOL and CON compared with ATB (3506 ± $50.9 vs. 3572 ± $54.0 vs. 3366 ± $53.6, P=0.01).

CONCLUSIONS: These findings suggest that although antibiotic treatment improved clinical cure, overall production, health, and reproductive outcomes were similar across treatments. Reductions in SCS and increased saleable milk in BOL and CON cows meant higher milk income versus ATB. The high spontaneous cure rate suggests that treatment may not always be necessary for first-case clinical mastitis.

Keywords: Mastitis, losses, therapy

Prevalence, economics, and management drivers of dairy cubicle bedding systems in the UK: A survey of 228 farms cows

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BACKGROUND and OBJECTIVE: This study aimed to quantify the prevalence, primary selection drivers, and comparative weekly costs of dairy bedding materials in UK herds to inform veterinary advisory strategies regarding farm infrastructure.

MATERIALS and METHODS: A comprehensive online questionnaire was distributed to UK dairy farmers in 2024 via the Müller Milk & Ingredients supplier network. The survey was hosted on the JISC online platform. In total, 228 responses were recorded. The survey captured data on herd demographics, housing facilities, bedding material types, procurement methods, and primary decision-making drivers. To determine practical farm-level economics, an extrapolated "cost per cow per week" was calculated by multiplying the average cost per application (derived from the reported weight of material applied) by the most common application frequencies for each material

RESULTS: The average respondent farm milked 247 lactating cows and housed 35 dry cows. The vast majority of lactating cows (97%) and a majority of dry cows (63%) were housed in cubicles. For lactating cows, 67% of farms utilized a combination of rubber padding layered with temporary bedding. The most prevalent temporary bedding material was wood shavings (56%), followed by sand (20%), straw/hay (15%), and recycled manure solids (2%). For dry cows, straw was the most prevalent choice (40%). When identifying the primary reasons for selecting a specific bedding material, respondents overwhelmingly cited cow welfare and cleanliness. Economic and environmental factors were secondary, with nearly 70% of farmers expressing an unwillingness to pay a premium for environmentally sustainable bedding. Economically, costs varied significantly when accounting for both raw material prices and application frequencies. Sawdust (applied mostly daily) represented the highest extrapolated cost at approximately £5.04 (~$6.75 / €5.80) per cow per week. Sand (applied mostly several times a week) averaged approximately £0.39 (~$0.52 / €0.45) per week. Straw (applied mostly daily) was highly economical at £0.07 (~$0.09 / €0.08) per week. Conversely, recycled manure solids (applied mostly daily) provided a net economic benefit of -£1.89 (-$2.53 / -€2.17) per week due to the offset market value of the manure.

CONCLUSIONS: Cow welfare and cleanliness remain the overriding priorities for UK dairy farmers when selecting bedding, outweighing environmental concerns. Despite wood shavings carrying the highest weekly operational costs, they remain the most widely used material for lactating cows. Given the substantial cost disparities between materials, bovine practitioners can leverage these baseline prevalence and weekly economic metrics to guide farmers toward cost-effective bedding strategies that optimize both cow comfort and farm profitability.

Keywords: dairy cows, cow welfare, bedding materials, farm economics, housing management

Can failure to voluntarily attend to the automatic milking system be used as a selection criterion for dairy cows?

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BACKGROUND and OBJECTIVE: Automatic milking systems (AMS) are based on the principle of voluntary milking behavior of dairy cows. However, in practice, some cows do not attend the milking robot on time despite being eligible for milking and

therefore need to be directed to the robot by farm personnel. Removing cows that do not voluntarily attend to the robot is one of the possible management strategies. The aim of this study was to compare cows that voluntarily attend to the automatic milking system with cows that do not attend to the robot on time and are brought to milking by farm personnel, in terms of milk yield and composition, milking characteristics, reproductive traits and some additional performance parameters.

MATERIALS and METHODS: The material of the study consisted of 230 lactating dairy cows located in the ANKAS Farm equipped with an automatic milking system (LELY). Cows were classified into two groups as -Cows voluntarily attending to the robot -Cows not attending to the robot on time and brought to milking by farm personnel Parameters evaluated in the study included milk yield, average milking duration, milk flow rate, milk temperature, rumination activity, milk fat percentage, milk protein percentage, fat-to-protein ratio, somatic cell count (SCC), calving interval, body weight and age at first calving. Normality of the data was evaluated using the Wilcoxon test and comparisons between groups were performed using the Independent Samples T-Test.

RESULTS: No statistically significant difference was found between the groups in terms of milk yield in both primiparous and multiparous cows. Average milking duration was significantly higher in cows voluntarily attending to the robot (3.03 min vs. 2.78 min, P=0.004). Milk protein percentage was found to be higher in cows not voluntarily attending to the robot (%3.25 vs. %3.18, P= 0.027). Calving interval tended to be longer in cows voluntarily attending to the robot (414.93 days vs. 386.08 days, P=0.068). No significant differences were observed between groups in terms of milk fat percentage, milk flow rate, somatic cell count, body weight, age at first calving or milk temperature.

CONCLUSIONS: The findings indicate that production performance parameters of cows that do not voluntarily attend to the automatic milking system are not sufficiently low to justify their removal from the herd. Therefore, failure to voluntarily attend to the milking robot does not appear to be a suitable selection criterion.

Keywords: Robotic milking system, Voluntary milking behavior, Milk yield, Selection criterion.

Analyzing large scale veterinary intervention data to support on farm veterinary decision making on fertility management

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BACKGROUND and OBJECTIVE: Worldwide the herd size of dairy farms is increasing, and veterinarians have a need for tools to convert the large amount of data available in these herds into useful information, that help in making decisions regarding herd health management. Vetwerk is a digital herd health management platform that integrates dairy production and health data from multiple sources into a single interface for veterinarians. It supports veterinarians in monitoring animal and herd performance, preparing and performing farm visits, and recording clinical observations through a structured digital workflow. By enabling centralized data access and standardized reporting, Vetwerk facilitates efficient, data-driven veterinary advise with respect to herd health management. This paper explores the potential of Vetwerk data collected over 5 years in >25 veterinary practices to answer clinically relevant questions to support veterinary herd health advisory work. As an example, we analyze the prognosis of cyclicity in cows that received a prostaglandin (PG) injection by quantifying the proportions of cows that are inseminated within one week after the injection, one cycle later, or not at all, as well as the pregnancy rates of these inseminations.

MATERIALS and METHODS: Over 2.3 million records on veterinary interventions on 171,878 cows in 745 farms served by 212 veterinarians during the years 2021 through 2025 were extracted from the database. Follow up data on insemination status and pregnancy status after PG injection are currently being extracted and will be linked to treatments with PG. Proportions and 95%CI will be calculated for the outcomes 'inseminated within one week'; 'inseminated in next cycle'; or 'not inseminated' and for each of these categories the proportion of cows pregnant after 56d.

RESULTS: Out of the full dataset, 7,068 relevant PG treatments of 5,182 cows on 377 farms were present, entered by 157 veterinarians. At this stage data on subsequent inseminations and pregnancy has been extracted and will be further analyzed.

CONCLUSIONS: Our work shows that systematic analysis of available data on veterinary interventions has great potential to help clarify questions regarding herd health management and to quantify the added value of interventions at the inter-farm and intra-farm level. This, combined with farm management data, creates powerful opportunities to develop the information needed to support herd health advisory work in dairy practice.

Keywords: Herd health, reproduction, veterinary advice

IMMUNOLOGY AND VACCINOLOGY

Statistical analysis of the clinical mastitis rate, somatic cell count, and antibiotic use before and after vaccination against Streptococcus uberis in twenty Dutch dairy farms

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BACKGROUND and OBJECTIVE: Streptococcus uberis is an important mastitis pathogen. Udder health problems caused by Streptococcus uberis often lead to increased antibiotic use, negatively impact animal welfare and milk quality, and result in significant economic losses. The objective of this poster is to analyse the effect of a mastitis vaccine against Streptococcus uberis on several udder health Key Performance Indicators (KPI's) across twenty commercial dairy farms in the Netherlands.

MATERIALS and METHODS: Twenty commercial dairy farms in the Netherlands implemented a mastitis vaccine against Streptococcus uberis. Vaccination protocol: three times primovaccination, four weeks apart, followed by a booster every six months. Vaccination was administered by a veterinarian. The farms included in the study have an average of 207 lactating cows (24-593). Two periods were compared: 12 months pre-vaccination and 12 months post-vaccination. The following KPI's have been analyzed; clinical mastitis rate (including new cases as well as recurrent cases) (18 farms), mastitis tubes for lactating cows (LC tubes) per cow in lactation (17 farms), Defined Daily Dose per Animal (ADDD) at farm level (20 farms), bulk tank somatic cell count (bSCC) (18 farms), percentage of animals with a new high somatic cell count (SCC) (19 farms). In the statistical analysis, a paired t-test has been performed to analyse the bSCC, and a Wilcoxon paired test has been performed on the other KPI's. For the analysis of the bSCC, a correction was applied. Since January 1, 2022, laboratories have adopted a unified standard for calibrating equipment used to determine cell counts. As a result, somatic cell counts in the Netherlands have been 12% higher since that date. A correction for this 12% increase was applied for eight farms.

RESULTS: Statistical analysis revealed that vaccination against Streptococcus uberis had significant improvements in all measured KPI's. The clinical mastitis rate decreased by 46.7% (p-value: <0.001), LC tubes/cow in lactation decreased by 35.6% (p-value: 0.008), ADDD at farm level decreased by 15% (p-value: 0.008), bSCC decreased by 11.4% (p-value: 0.009), the percentage of the animals with new high SCC decreased by 16.3% (p-value: 0.039).

CONCLUSIONS: This study confirms that mastitis vaccination against Streptococcus uberis has a positive impact, contributing to a decrease in the clinical mastitis rate, antibiotic use, and bSCC, as well as the proportion of new high-SCC cows. The results indicate that vaccination against Streptococcus uberis, implemented as part of a comprehensive control program, contributes meaningfully to mastitis prevention.

Keywords: milk quality, mastitis, vaccination, strep. uberis, udder health

Relationship between udder imbalance and survival probability in a Saanen dairy goat farm in the Netherlands

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BACKGROUND and OBJECTIVE: In dairy goat production systems, herd profitability is closely linked to animal longevity. Extending the productive life of animals, the initial investment for rearing replacements is spread over a longer period of milk output. This cost dilution, combined with sustained production, enhances profitability and contributes to the long-term viability of dairy farming systems. Udder health and morphology are factors influencing longevity. Udder conformation defects are one of the most common reasons for early culling, and mastitis—particularly chronic forms—can cause permanent mammary tissue damage. Consequently, mastitis may be considered a direct cause of culling in dairy goats. The objective of this study was to evaluate the association between udder imbalance and culling rate in dairy goats.

MATERIALS and METHODS: The study was conducted in December 2023 on a Saanen goat farm in the Netherlands, involving 3,164 lactating does. Udder imbalance was classified using a two-category system: goats with symmetrical udders ("No") and those showing visible asymmetry ("Yes"). Culling rates were assessed using individual farm records from April and August 2024 and January 2025. A goat was considered culled if it appeared at one time point but not in the subsequent record, suggesting permanent removal from the population. A logistic regression model was fitted to estimate survival probability, incorporating age, udder imbalance, and their interaction as explanatory variables. Statistical analyses were performed in R (v4.4.39) using the glm function, with significance set at $\alpha = 0.05$.

RESULTS: The culling rate was 34.75% in goats with udder imbalance, compared to 21.25% in goats without imbalance. This reflects a 64% higher culling risk for goats exhibiting udder asymmetry. Survival probability was significantly lower in goats with udder imbalance across all ages ($p < 0.001$), and the decline in survival was steeper over time, indicating faster removal from the herd. The negative effect became more pronounced in older animals, likely due to accumulated tissue damage and chronic mastitis. A previous study on the same farm also found that udder asymmetry was associated with decreased milk yield, particularly during the first year of lactation.

CONCLUSIONS: In conclusion, udder asymmetry is a key factor influencing longevity in dairy goats. Reducing the severity of mammary infections can decrease the occurrence of udder imbalance, leading to higher survival, improved milk production, and greater farm profitability.

Keywords: dairy goat, staphylococcus aureus, vaccinations, udder imbalance

Impact of mastitis vaccination on intramammary antibiotic consumption during lactation period in 10 Belgian, commercial dairy farms

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BACKGROUND and OBJECTIVE: Mastitis remains the most prevalent disease in dairy cattle and a major driver of antibiotic use in dairy production. In Belgium, udder health management accounts for a substantial antibiotic consumption on dairy farms, and reducing antibiotic use is a major concern for all stakeholders. Preventive strategies, such as mastitis vaccination, are increasingly implemented in the field. Given the limited availability of multi-farm data evaluating the impact of mastitis vaccination on antibiotic use under commercial Belgian conditions, the objective of this study was to assess the effect of mastitis vaccination on intramammary antibiotic tube consumption during lactation in Belgian dairy farms.

MATERIALS and METHODS: A retrospective, multi-farm observational before–and–after study was conducted across 10 Belgian dairy farms, using data from official registers on intramammary antibiotic tube use during lactation. Each farm served as its own control by comparing a 12-month pre-vaccination period (Y0) with the first 12-month

period under vaccination (Y1). Vaccination was implemented under veterinary supervision using a commercially available multivalent vaccine against Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli) and a monovalent vaccine against Streptococcus uberis (S. uberis), or a combination of both.
The vaccine against S. aureus and E. coli (6 farms) was administered as two injections at one-month intervals, followed by booster injections every 2.5 to 3 months in lactating cows, dry cows, and heifers. The vaccine against S. uberis (3 farms) was administered as three injections at one-month intervals, followed by booster injections every six months. On the farm, using both vaccines, booster injections followed the respective schedules of each vaccine. Intramammary antibiotic consumption during lactation was analysed as the absolute annual number of antibiotic tubes used per farm. Data were analysed using a linear regression model.

RESULTS: The average intramammary antibiotic tube consumption during lactation decreased significantly from 319.1 (± 214.7) tubes per farm per year in Y0 to 214.5 (± 160.2) tubes in Y1 ($p = 0.001$), corresponding to an average relative reduction of 32.8%. All farms included in the analysis showed a reduction in absolute antibiotic tube consumption, with an average decrease of 104.6 tubes per farm per year.

CONCLUSIONS: Mastitis vaccination was associated with significantly reduced intramammary antibiotic tube use during lactation in Belgian dairy farms, despite heterogeneity in systems, herd sizes, farm management practices, pathogen profiles, and vaccines. Given the mastitis' multifactorial nature, vaccination should be included within broader mastitis control programs to further improve udder health and support antibiotic reduction under field conditions.

Keywords: mastitis, milk quality, vaccination, e.coli, s. aureus

Longitudinal multicenter field trial evaluating the serological response to maternal vaccination against Cryptosporidium parvum in newborn holstein-friesian calves

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BACKGROUND and OBJECTIVE: Calf diarrhea caused by *Cryptosporidium parvum* is widespread and contributes substantially to neonatal calf mortality worldwide. A recently identified glycopeptide epitope on the parasite (Gp40), recognized by a monoclonal antibody, enabled development of a commercially available maternal vaccine intended to confer passive immunity to calves via colostrum. Pre-clinical studies indicated safety and efficacy. This field trial aimed to confirm vaccine uptake by measuring anti-Gp40 antibodies in neonatal calf serum and to evaluate vaccine safety under routine farm conditions.

MATERIALS and METHODS: A longitudinal multicenter cohort study was conducted from July 2024 to January 2025 on four commercial Holstein-Friesian dairy farms in the Netherlands and Luxembourg with verified *C. parvum* infection pressure and a history of neonatal cryptosporidiosis. Healthy cows and heifers 12 to 6 weeks pre-calving were randomly allocated to non-vaccinated controls or to receive two doses of the *C. parvum* vaccine 4–5 weeks apart. Calves born to study dams were enrolled as control calves (CC) or vaccinated calves (VC) according to dam status; calves were excluded if they received colostrum from a dam other than their own. Serum was collected 2–7 days post-birth and analyzed for total IgG (commercial ELISA, BIO K420) and anti-Gp40 antibodies (in-house ELISA). Veterinary clinicians reported any adverse events

following vaccination. Statistical analyses used R at a 5% significance level.

RESULTS: Among 153 calves (66 CC, 87 VC), mean total serum IgG did not differ between groups (CC: 13.69 ± 7.01 g/L; VC: 14.86 ± 7.03 g/L; p = 0.09). In contrast, anti-Gp40 antibody levels were significantly higher in calves from vaccinated dams (mean log2 titer VC: 16.43 ± 2.65) compared with controls (CC: 10.93 ± 1.47; p < 0.001). This difference remained when including the 42 calves with failure of passive transfer (serum IgG < 10 g/L), indicating enhanced Gp40-specific immunity regardless of overall IgG status. No vaccine-related adverse events were reported.

CONCLUSIONS: This multicenter field trial shows that maternal vaccination with a vaccine against *C. parvum* effectively increases Gp40-specific antibodies in neonatal calves, confirming vaccine uptake via colostrum and supporting passive protection against *C. parvum*. The absence of differences in total IgG underscores the specificity of the response, while elevated anti-Gp40 titers highlight a targeted enhancement of immune protection. Safety was confirmed under standard farm conditions, supporting maternal vaccination as a practical, safe strategy to help mitigate cryptosporidiosis in dairy herds.

Keywords: Cryptosporidiosis, GP40, neonatal diarrhea, dam vaccination

Separate milking for colostrum collection is associated with higher IgG concentration at first calf feeding than other milking methods

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BACKGROUND and OBJECTIVE: The aim of the study was to evaluate changes in colostrum IgG concentration between first milking and calf feeding and to determine how farm management practices influence this change on dairy farms. Changes in colostrum IgG concentration between first milking and calf feeding have not previously been studied.

MATERIALS and METHODS: The study was conducted on 72 Finnish dairy farms between March 2022 and December 2023. In total, 327 calves that received colostrum from their own dam were enrolled, with 1–13 calves per farm.
Farms recorded colostrum-related data and collected colostrum samples, which were frozen and transported frozen to the laboratory. IgG concentrations were determined using ELISA (Bovine IgG ELISA kit, Bethyl Laboratories, Inc., Texas, USA) in colostrum sampled immediately before the cow's first milking after calving and in the colostrum fed to the calf at first feeding. The difference in IgG concentration was calculated and used as the outcome variable to assess the effects of colostrum management practices.
Data were compiled in Microsoft Excel (Microsoft Office 365), and statistical analyses were performed using RStudio (Posit Software, PBC) and R (R Foundation for Statistical Computing). Linear mixed-effects models with herd as a random intercept were used for univariate analyses, and a combined forward and backward stepwise selection procedure was applied to build the final model.

RESULTS: The mean IgG concentration in colostrum samples collected from cows immediately before first milking was 79.9 g/L, and 22.5% of cows produced low-quality colostrum (IgG < 50 g/L). At the calf's first feeding, the mean IgG concentration was 63.8 g/L, and 37.6% of calves received low-quality colostrum. The mean change in colostrum IgG concentration was −18.9 g/L.
In the final mixed model, the milking method at first colostrum collection was significantly associated with colostrum IgG concentration: automatic milking systems were associated with lower IgG concentrations compared with separate milking (P<0.01). A greater volume of colostrum at first milking was associated with higher IgG concentration (P<0.01), whereas a longer interval between colostrum milking and calf feeding was associated with lower IgG concentration (P<0.01). Mean herd size was not associated with colostrum IgG concentration (P>0.05).

CONCLUSIONS: Automatic milking systems were associated with a greater decline in colostrum IgG concentration between first milking and calf feeding. A longer interval between colostrum collection and first feeding further increased IgG loss. Separate milking appears to be the most

reliable method for obtaining high-quality colostrum for calf feeding.

Keywords: dairy cow, colostrum, automatic milking system, separate milking, immunoglobulin, ELISA

Impact of Fasciola hepatica infection and triclabendazole treatment on the quality of the immune response to foot and mouth disease vaccination in cattle

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BACKGROUND and OBJECTIVE: *Fasciola hepatica* exerts potent immunomodulatory effects on the host, potentially compromising responses to concurrent infections or vaccination. While vaccination is crucial for controlling Foot-and-Mouth Disease (FMD), the impact of concurrent fasciolosis on the quality of vaccine-induced immunity remains poorly characterized. This study evaluated whether *F. hepatica* infection alters the long-term humoral memory stimulated by commercial inactivated FMD vaccines and assessed the efficacy of anthelmintic treatment (Triclabendazole, TCZ) in restoring immune competence.

MATERIALS and METHODS: Fifty-two calves (6-8 months old) were allocated to five groups: infected prior to primary vaccination (INF1); infected prior to vaccination and treated with TCZ (INF1-TCZ); infected post-vaccination (INF2); infected post-vaccination and treated with TCZ (INF2-TCZ); and uninfected/vaccinated controls (CTL). Infections were induced using a dose of 2 metacercariae per kg of body weight, and all animals received a vaccine booster 5 months after the primary dose (Day 150). Monitoring included biweekly haematology and faecal egg counts. Humoral responses were characterized by liquid-phase blocking ELISA (total titres) and an IgG1 Avidity Index (AI) to assess antibody quality.

RESULTS: Results indicated that total FMDV-specific antibody titres did not differ significantly between groups throughout the study. However, antibody quality was severely compromised. Specifically, the INF2 group exhibited a marked reduction in IgG1 Avidity Index (approx. 50% lower than controls) at Day 150 (pre-booster) in comparison with the CTL group. Furthermore, 14 days post-booster, the INF2 group failed to reach the AI levels observed in the CTL group ($p < 0.05$). Notably, calves treated with TCZ (INF2-TCZ) displayed AI values comparable to the CTL group, indicating a restoration of the affinity maturation process. While the INF1 group showed increased variability in AI, mean values did not differ significantly from the CTL group.

CONCLUSIONS: These findings demonstrate that active *F. hepatica* infection, particularly during the inter-vaccination period, impairs the maturation of antibody avidity without affecting total circulating titres. This dissociation suggests that routine serology might overestimate protection in infected herds. Crucially, timely TCZ treatment prevents this immunomodulation, highlighting that strategic parasite control is a prerequisite for sustaining effective vaccine-induced herd immunity in endemic regions.

Keywords: Foot and Mouth disease, vaccination, avidity

Vaccination prevents renal and genital leptospira colonisation in vaccinated ewes

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BACKGROUND and OBJECTIVE: Leptospirosis is a global zoonosis that impairs reproductive efficiency in ruminants, leading to economic losses through infertility and abortions, often linked to colonization of the genital tract. While systemic vaccination mitigates clinical signs, its efficacy against genito-urinary colonization remains disputed. This study aimed to assess the efficacy of three vaccine strategies against *Leptospira borgpetersenii* serovar Hardjo in preventing renal and genital leptospirosis in experimentally infected ewes.

MATERIALS and METHODS: This study was approved by the Ethical Committee for Animal Use of Federal Fluminense University. Twenty-four adult Santa Inês ewes were divided into four groups of six animals. Groups 1 and 2 received experimental vaccines (Vac1 & 2) containing the same antigen (*L. borgpetersenii* serogroup Sejroe, serovar Hardjo, strain Hardjobovis) but different adjuvants (aluminum hydroxide and mineral emulsion, respectively), while Group 3 received a Leptospira commercial injectable multivalent vaccine (Vac3) subcutaneously at the dose recommended for cattle by the manufacturer. Group 4 remained unvaccinated. After two vaccine doses (43 and 15 days prior to the experimental challenge), the animals were challenged on D0 via the genital route with a fresh culture containing 1×108 leptospires, using a speculum and a multidose insemination gun. Monitoring included serological tests (MAT) and quantitative qPCR analysis of urine and cervicovaginal mucus (CVM) on priming (D-43) and booster (D-15) vaccination, on D0 (challenge), and later on days D7, D14, D21, D30, and D60 post-infection. At the end of the study, all infected animals were treated with three doses of Streptomycin IM 25 mg/kg.

RESULTS: All animals were negative at D-43. In Groups 1, 2, and 3, it was possible to observe a sharp increase of antibody titers against serogroup Sejroe on D-15 (at the time of vaccine booster) in 100 % of the vaccinated animals (vaccinated vs unvaccinated $p < 0.001$). Although vaccination did not completely prevent renal colonization, the vaccines containing mineral adjuvant (Vac2) and the multivalent vaccine (Vac3) reduced the bacterial load and the number of infected animals. Vac2 alone elicited an immune response that cleared the genital infection of all ewes from D30 onwards ($p < 0.001$). Therefore, Vac2 was the only one able to interrupt bacterial shedding both via urine (100 %; 6/6) and via CVM (100 %; 6/6) from D30 onwards.

CONCLUSIONS: The study demonstrates that vaccine formulation, particularly the use of mineral adjuvants, plays a crucial role in protecting the genital tract against *Leptospira*, offering interesting potential for limiting reproductive failures despite incomplete renal protection.

Keywords: {Leptospira}, bovine genital leptospirosis, vaccine, adjuvant

Host immune responses to bovine digital dermatitis associated treponemes using Korean native black goats as ruminant model

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BACKGROUND and OBJECTIVE: Bovine digital dermatitis (BDD) is an infectious hoof disease in cattle but also reported in goats. This disease is strongly associated with several Treponema species. In BDD, host immune responses remain incompletely understood due to lack of laboratory model of infection. This study utilized the Korean Native Black Goats (KNBGs) as ruminant model to

investigate the host immune responses following challenge with BDD-associated treponemes.

MATERIALS and METHODS: Before challenge, hair on both hind legs were clipped above the hock and a superficial incision was made in the interdigital skin. A moist anaerobic wrapping technique was applied. Challenged animals were inoculated twice with a 2 × 10^9 cells/mL live Treponema mixture prepared by combining equal concentrations of T. phagedenis HNW1, T. pedis GNW45, and a putative novel Treponema species isolated from BDD lesions while controls received sterile PBS only. After challenge, animals were monitored daily for lameness and recorded as either lame or not lame. At D49, wraps were removed for gross lesion assessment and tissue biopsies were collected for histopathology, Treponema spp. quantification, and transcriptomic analysis. Weekly serum samples were analyzed for Treponema-specific IgG titers, TNFα, IFNγ, IL17, and serum amyloid A using ELISA.

RESULTS: Lameness was first observed in 66.7% (2/3) of challenged animals at D28 and reached 100% by D35–D49, whereas controls remained unaffected. At D49, 50% (3/6) of challenged hooves showed mild lesions characterized by reddening and bleeding while no lesions were observed in controls. Histologically, challenged tissues showed epidermal mononuclear cell infiltration, necrosis, and hemorrhage while controls exhibited weak infiltration of mononuclear cells. Lesion-positive hooves had significantly higher Treponema spp. loads (1,308.31 copies/mg) compared with healed hooves (93.71 copies/mg; $p < 0.05$). Treponema spp. DNA was undetectable in controls. Transcriptomic analysis revealed that challenged animals showed upregulation of epithelial stress markers (WFDC18, SERPINB3, SPRR2E, S100A7), neutrophil-associated genes (S100A9, S100A12, LTF), immunoglobulin related transcripts and downregulation of oxidative stress–related genes (alpha-tocopherol transfer protein). Notable enriched KEGG pathways in challenge animals included NOD-like receptor signaling, complement and coagulation cascades, and NET formation pathway linked to innate immune and inflammatory responses. Challenged animals also showed significantly higher and sustained Treponema-specific IgG titers and significant transient increases in TNFα, IFNγ, IL17 and SAA levels compared to controls ($p < 0.05$) indicating measurable systemic immune activation.

CONCLUSIONS: This study demonstrates that Treponema spp. challenge in KNBG elicits measurable clinical and immunological responses however it does not fully replicate the complexity of naturally occurring BDD.

Keywords: bovine digital dermatitis, Treponema, immune response, ruminant model, transcriptomic analysis

Clinical efficiency of a new vaccine for respiratory disease of cattle

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BACKGROUND and OBJECTIVE: Bovine respiratory disease (BRD) is leading cause of death in young cattle with high economic consequences. While the etiology of BRD is multifactorial, viruses play an initiating role, but bacteria are also involved in the clinical picture. The major viruses causing BRD are Infectious bovine rhinotracheitis virus (BoHV-1), Parainfluenzavirus-3 (PI-3), Bovine respiratory syncytial virus (BRSV), and Bovine viral diarrhea virus (BVDV). The most effective way to combat BRD is through effective vaccination programs. Due to updated GMP requirements, there are restrictions on the international trade of some vaccines. In this research, safety and efficiency studies were conducted on a candidate vaccine (Bovisave®, Vetal-Türkiye) that can be used for prevention against BRD.

MATERIALS and METHODS: Viral strains BoHV-1 /Cooper, PI-3 /SF-4, BVDV /NADL and BRSV /A51908 were subjected to basic R&D studies including inactivation curve, and adjuvant (Al[OH]3) ratio. *P. multocida* was also involved in the formula. The viruses were BEI inactivated while formaldehyde was used for inactivation of the bacteria. Candidate vaccine formulations (KSA-1, -2, -3) were tested on Guinea pigs. Pilot production batches were evaluated for safety and immunogenicity on cattle after laboratory animal trials. Antibody responses were identified by sero-neutralisation test. Potency testing for viral agents was also applied on immunized young cattle according to European Pharmacopea requirements. Mice potency testing was conducted for *P. multocida*.

RESULTS: Viral titers that were between Log10TCID: 5,37 and 8,00 were totally destructed by 8 hours after a gradual loss of infectivity. The ideal adjuvant ratio was found 10%. Pilot production series were successfully passed safety testing in mice and Guinea pigs, then applied in 1 month old calves, young animals and pregnant cows with no side effects. Blood parameters followed for 15 days pi were in normal ranges. Only slight rises were recorded in body temperature of some animals (7/15, 46,7.0%) after the prime vaccination, comparing to control group (3/15, 20.0%). It was also measured (3/5 60.0%) after booster dose vaccination. Regarding the immunogenicity, the earliest antibody rise was detected against BVDV (d7) while BoHV-1 antibodies first detected in d21 and long-lasted till >d180 for all the viruses. Prime-booster vaccination reduced the clinical findings and virus shedding after pathogen challenge. Clinical scores for BoHV-1, PI-3 and BRSV were 1, 0, and 1 in vaccinated animals, comparing the control groups 10, 6 and 7, respectively. Potency for *P. multocida* was also echieved.

CONCLUSIONS: This study has successfully demonstrated the safety and clinical efficiency of the tested vaccine (TÜBİTAK-TEYDEB project No: 3231735).

Keywords: Bovine respiratory disease, BRD, Vaccine, Safety, Immunogenicity, Prevention

Concurrent reduction in BRD mortality, lung lesion severity, and anti-infective treatments with use of a commercially available modified-live Mycoplasmopsis bovis vaccine in Limousin feedlot calves

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BACKGROUND and OBJECTIVE: Bovine respiratory disease (BRD) due to *Mycoplasmopsis bovis* is chronic, poorly responsive to antibiotics, and economically burdensome in feedlots. A commercially available live attenuated *M. bovis* vaccine was administered to Limousin cattle twice (Day 0 and Day 21). This study evaluated whether vaccination was associated with reductions in BRD related mortality, lung lesion severity, and anti-infective (AIF) treatment burden in Limousin calves. The primary objective was to determine whether vaccination with a modified live *M. bovis* vaccine reduces BRD related death loss and increases the proportion of calves reaching harvest. The secondary objectives were to assess whether vaccination reduces lung lesion severity at Day 35 (thoracic ultrasonography), associated AIF use, and BRD treatment costs.

MATERIALS and METHODS: The study was conducted in a French feedlot using weekly half batch allotment without commingling where pen was the experimental unit. Limousin intact male calves were assigned to vaccination group (Protivity®, Zoetis, n=439) or to an unvaccinated control group (n=426) against *M. bovis*. Cattle were fed for approximately 180 days. A thoracic ultrasonography subsample was assessed at Day 0 and Day 35. Health events, BRD diagnoses, mortality, anti-infective treatments, and costs were recorded. Categorical outcomes were analyzed with generalized linear mixed models (SAS GLIMMIX) and continuous outcomes with linear mixed models (SAS MIXED), including fixed effects for treatment, season, cohort week, and barn, with pen as a random effect.

RESULTS: BRD related death loss tended to be lower with vaccination (3.6% in controls vs 1.7% in vaccinated; P = 0.0720). AIF exposure and costs for BRD were significantly reduced: IM/SC volume decreased from 60.2 mL (control) to 48.8 mL (vaccinated) (P = 0.0031), and total IM/SC treatment cost from $55.51 to $39.95 (P = 0.0028). First episode BRD treatment cost declined from $44.57 to $33.12 (P = 0.0026), with drugs $35.56 to $24.78 (P = 0.0032) and labor $10.09 to $8.45 (P = 0.0217). Vaccinated Limousin calves showed a significantly more favorable distribution of right-side lung ultrasound scores at Day 35 (x^2 = 0.0144), with a favorable trend on the left side, in the Day35 subsample.

CONCLUSIONS: Vaccination with a modified-live *M. bovis* vaccine was associated with improved lung ultrasound scores at Day 35, significant reductions in AIF exposure and BRD treatment costs, and a favorable trend toward lower BRD related mortality. These converging outcomes indicate reduced clinical severity and support a clinically and

economically meaningful benefit of using *M. bovis* vaccination in Limousin feedlot systems.

Keywords: Mycoplasmopsis bovis, vaccine, bovine respiratory disease, feedlot

Efficacy and kinetics of an inactivated phase I Coxiella burnetii vaccine in sheep against various C. burnetii strains

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BACKGROUND and OBJECTIVE: Coxiellosis, caused by Coxiella burnetii, poses a significant threat to livestock. An effective vaccine must provide broad protection against diverse field strains. This study aimed to evaluate the protective efficacy and kinetic profile of an inactivated Phase I C. burnetii vaccine (Coxevac®, Ceva Santé Animale, Libourne, France) in sheep against a panel of heterologous C. burnetii strains.

MATERIALS and METHODS: Ten ELISA-seronegative C. burnetti ewes were split into two groups of 5 animal each and vaccinated with Coxevac® on D0 and D21 according to the manufacturer's instructions. In contrary to group1, animals from group 2 received a new full protocol 4 months later (D141 and D162). In addition, four C. burnetti-seronegative sheep as well as three naturally infected sheep were used as control groups. Blood samples were taken on vaccinated sheep 63 days after the second injection (D84) and again on D337 which corresponds to 316 days after the end of vaccination (group 1) or 175 days after the end of the second protocol (group 2). At each time point, harvested sera from all enrolled animals were subjected to ELISA screening followed by sero-neutralization assay using a panel of 7 strains (Nine-Mile as reference and six field strains previously isolated from zoonotic human cases (n=4) or ruminants (n=2). Protection was defined as a significant reduction in infection rate (IR) compared to an unvaccinated negative control group via one-sided Mann-Whitney U tests. Kinetics were assessed using paired Wilcoxon tests.

RESULTS: The range of infection rate was [32.4% – 36.3%] and [5.7% - 13.3%] for negative and positive controls respectively. Animals from the group 2 showed the most robust and sustained protection, with significant IR reductions of 80-88% against all 6 heterologous strains at both D84 and D337 ($p \leq 0.004$). The group 1 also showed strong initial protection (80-93% reduction, $p \leq 0.004$), which was maintained at D337 for all heterologous strains (70-74% reduction, $p=0.004$) but waned for the Nine-Mile reference strain ($p=0.421$). ELISA titers correlated with protection at early (D84) but not late timepoint.

CONCLUSIONS: Coxevac® vaccine induces potent and durable cross-neutralizing immunity against diverse heterologous field strains. Importantly, sero-neutralization can occur independently of ELISA seroconversion, highlighting the lack of sensitivity of this technique. Qualitative results of ELISA tests should therefore not be used to measure vaccine efficacy. The observed neutralization effect against various Coxiella burnetii strains suggests effective protection against these contemporary circulating isolates.

Keywords: Coxiella burnetii, vaccination, sero-neutralization, efficacy

Comparative field study of a new autogenous vaccine adjuvanted with Montanide™ against bovine papillomatosis

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BACKGROUND and OBJECTIVE: Bovine papillomatosis is a worldwide constantly growing pathology; in Italy it's possible to combat the spread of the pathology through the use of an autogenous vaccine authorized by the Ministry of Health and produced in the Pharmaceutical Laboratories of the

IZSUM "Togo Rosati" in Perugia. The main genotypes found in the Bovidae family in Italy are BPV-1, 2, 7 and 13. From our experience, BPV-1 appears to be the most frequently identified from lesions and surgically removed pathological material.

MATERIALS and METHODS: A novel inactivated autogenous viral vaccine adjuvanted with Montanide™ Seppic IMS1313 has been formulated and compared whit the traditional autogenous vaccine. The viral matrix was inactivated with formaldehyde 37% (0.04% v/v), triturated, homogenized and subjected to several centrifugation (5000 G) and triple purification steps by 1,2, 0,65 and 0,45-0,22 μm filter. The vaccine matrix was subsequently divided into two parts.One part was added with IMS 1313 adjuvant, while the other 50% of the vaccine was produced using the traditional method without the use of adjuvant. The administration protocol was a single "one-shot" administration for the IMS1313-adjuvanted product, while the traditional vaccine consisted of three doses administered two weeks apart. Three hundred and twenty adult cattle were involved in the trial to compare the field efficacy of the new vaccine and evaluate its safety. The group of 320 animals was divided into two using a treatment randomization system using software into which the animals' ear tags were entered. The vaccine was subjected to "in vitro" sterility control and "in vivo" toxicity tests in laboratory animals.The two types of vaccine were used on a farm extensively affected by a widespread form of bovine papillomavirus type 1 (BPV-1).

RESULTS: The results were extremely satisfactory in terms of safety (100%) and efficacy (82%). The "one shot" protocol showed slightly lower results than the traditional three inoculations (82% versus 96% remission) but allowed a significant reduction in costs and working times, aspects that affect the possibility of carrying out vaccination in some specific types of farms.

CONCLUSIONS: Studies are currently underway to further increase the efficacy of the single-dose vaccine, bringing it to the excellent rates of the traditional autogenous vaccine currently widely produced by the Perugia Vaccine Production Laboratory. Both formulations undoubtedly represent an excellent, well-tolerated and cost-effective metaphylactic strategy against Bovine Papillomavirus (BPV).

Keywords: papillomatosis, autogenous vaccine, cattle, BPV

Development and characterization of boron nitride nanoparticles as novel vaccine adjuvant candidates

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BACKGROUND and OBJECTIVE: Traditional vaccines often require effective adjuvants to enhance the immune response and ensure long-lasting protection. Boron nitride (BN) has emerged as a promising material for biomedical applications due to its unique chemical and physical properties. This study aimed to develop, characterize, and evaluate the potential of boron nitride nanoparticles (BN-NPs) as a novel adjuvant system for veterinary vaccine formulations, focusing on their structural stability, safety profile, and immunomodulatory effects.

MATERIALS and METHODS: BN-NPs were prepared using the nanoprecipitation method. Various formulations were developed by dissolving boron nitride in organic phases and dispersing them into aqueous phases containing stabilizing surfactants. The physicochemical properties were characterized using Zetasizer for particle size and zeta potential, Transmission Electron Microscopy (TEM) for morphology, and FT-IR for chemical stability. Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) was utilized for quantitative determination, encapsulation efficiency, and release studies. Sterilization was achieved via UV light exposure. Cytotoxicity was evaluated on primary lung and tracheal fibroblast cells using the MTT assay to determine the IC50 (half maximal inhibitory concentration) values. Finally, the in vivo immunogenic potential was assessed in a murine

model through intramuscular injections, followed by ELISA and flow cytometry to analyze IgG levels and immune cell populations (CD4, CD8, CD19, and various cytokines).

RESULTS: Physicochemical analysis revealed stable BN-NPs with a narrow size distribution and distinct zeta potentials. TEM imaging confirmed spherical and well-dispersed morphologies. ICP-MS analysis demonstrated a high production yield, and stability tests showed that the nanoparticles maintained their structural integrity and boron content for up to six months at 25°C. FT-IR spectra confirmed no undesired chemical interactions between the BN and the surfactants. In vitro assays indicated a safe profile at lower concentrations, with cell viability remaining above 50% at the selected IC50 dilution. In vivo studies showed that BN-NP groups exhibited significantly higher IgG levels compared to control groups at both 28 and 42 days ($P<0.05$). Flow cytometry confirmed the activation of the immune system, evidenced by high CD45 levels (>85%) and a notable increase in CD19 and IgM levels over time.

CONCLUSIONS: The results demonstrate that boron nitride nanoparticles can be successfully formulated as stable, non-toxic, and effective vaccine adjuvants. The significant enhancement of both humoral and cellular immune markers suggests that boron nitride nanoparticles are strong candidates for the next generation of veterinary vaccines. Their high stability at room temperature and potent immunogenic properties provide a rational basis for further evaluation in target animal species.

Keywords: vaccine, adjuvant, boron nitride, immune system

The application of recombinant antigens from Histophilus somni to prevent respiratory tract infections in dairy calves

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BACKGROUND and OBJECTIVE: Gram-negative, bacterial facultative pathogens play an important role in causing respiratory tract pathology in cattle. Our previous studies demonstrated that sera from animals vaccinated with two recombinant proteins derived from Histophilus somni (cytoplasmatic rHsp60 and outer membrane rOMP40) showed broad cross-reactivity with related antigens of several species of Pasteurellaceae and Enterobacteriaceae families. The aim of the study was to evaluate the immune response and protective effect of the vaccination of dairy calves with these antigens under field conditions.

MATERIALS and METHODS: Forty four dairy heifer calves (HF) originating from three farms were divided to two groups (22 calves each): C - control, A – vaccinated twice, sc with 10 µg rHsp60+20 µg rOMP40 antigens mixed with Emulsigen (20% v/v). Both groups were divided into two subgroups: calves born in summer (12) and in winter (10). Health status and monthly weight gain was observed to the age 6 months. In cases of BRD culture of nasal swab was performed and reactivity of serum antibody against BRSV, BHV-1, PI-3, BVD viruses and Mycoplasma bovis were estimated. Calves were vaccinated twice: first at the age of 5-6 weeks (P2), two weeks later moved to common calfbarn, second vaccination was two weeks after arrival (P3). Clinical score, body weight and blood samples were taken at P0 (24-72 hours of life), P1 (3-4 weeks), P2 (first immunization), P3 (second immunization), P4 (3 months), P5 (4 months), P6 (5 months), P7 (6 months old). Serum IgG1, IgG2 and IgM, haptoglobin, Serum Amyloid A, antibody against H.somni rHsp60, rOMP40, in IgG1, IgG2, IgM and IgA classes and plasma fibrinogen were measured in over mentioned time points. The immunoglobulin concentration was estimated in first colostrum whey.

RESULTS: Vaccinated calves showed BRD signs and seroconversion against respiratory tract viruses less frequently than control calves. Mean concentration of Ig in colostrum whey did not differ between groups. Serum Ig concentration after colostrum intake (P0) was slightly lower in experimental than in control calves (no statistically significant differences). Serum IgG1 and IgG2 antibodies reactivity with both antigens was significantly higher in experimental than in control animals in

samples P4 to P7. Serum IgG1 and IgG2 concentration increased markedly in control group since P3 to P7 as compared to A group.

CONCLUSIONS: Vaccination of calves with Histophilus somni rHsp60 and rOMP40 antigens induced significant rise of antibody to 6th month of life. The protective effect of vaccination was confirmed.

Keywords: Histophilus somni, rHsp60, rOMP40, dairy calves, BRD

Assessment of long term efficacy of RB51 vaccine by challenge of pregnant water buffaloes vaccinated at 6-9 months age

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BACKGROUND and OBJECTIVE: This pilot study evaluated the long-term efficacy of the RB51 vaccination protocol authorized in Italy, tested in pregnant water buffaloes (*Bubalus bubalis*) challenged 4 or 5 years post vaccination (ypv).

MATERIALS and METHODS: Sixteen female buffaloes (3-5 years) were enrolled: ten animals received the live attenuated *B. abortus* RB51 vaccine at a triple bovine dose (primary vaccination at 6-8 months of age followed by a booster after 30 days), while six animals served as unvaccinated controls. In study 1, challenge was performed at 4 ypv: six vaccinated and two control animals were bred and, at 6-7 months of gestation, transferred to an ABSL facility. Following a 10-day acclimation period, all animals were challenged *via* conjunctival inoculation with 107 CFU of *B. abortus* strain 2308. In study 2, four vaccinated and four control buffaloes were managed under the same conditions, but challenged at 5 ypv. Clinical monitoring and laboratory analyses were undertaken to assess humoral immune response (RBT, CFT, RB51-CFT, i-ELISA and c-ELISA), and cell mediated immunity (IFN-gamma release assay). Bacteriological investigations were conducted to assess infection outcomes and pathogen shedding during late gestation, parturition, and early lactation.

RESULTS: All animals, regardless of vaccination status, seroconverted to RBT, CFT, i-ELISA and c-ELISA with absent or low-titer reactivity in the RB51-CFT. Eight of ten vaccinated animals delivered vital calves. However, two animals challenged at 5 ypv exhibited premature delivery with neonatal mortality. Placental expulsion was normal in all vaccinated buffaloes and *Brucella* was not detected at delivery or from the calves that died after birth. Low number of *B. abortus* 2308 were isolated from the milk of one animal challenged 4 ypv, while positive vaginal swabs were recorded from day 2 to 17 *post-partum* in a buffalo challenged 5 ypv. In contrast, four out of six control animals aborted approximately two months post-challenge, while the remaining two delivered normally. *B. abortus* 2308 was consistently isolated from placenta, fetus, and milk in all aborting controls, whereas no bacterial isolation was obtained at delivery in the two full-term control animals.

CONCLUSIONS: These findings suggest that the RB51 vaccination protocol applied in Italy to young water buffaloes confers substantial long-term protection against abortion but not from infection and markedly prevents or reduces pathogen shedding. Thus, vaccinated animals may become infected but are unlikely to substantially contribute to onward transmission. These findings support the use of RB51 as a complementary toll to the test-and-slaughter strategy for brucellosis eradication in water buffaloes. "Research was co-funded by the European Union's Horizon Europe-101136346 EUPAHW".

Keywords: Brucellosis, Water Buffalo, RB51 Vaccine, Challenge study, Long-term immunity, Pathogen shedding.

INFECTIOUS DISEASES

Clinical, Pathological and Molecular Study of Lumpy Skin Disease in Al Muthanna Province/ Iraq

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BACKGROUND and OBJECTIVE: The first emergence of Lumpy skin disease (LSD) in Iraq was in Nineveh and Baghdad in August 2013.There are shortage publications on various aspect of LMD since first emergence report in 2015. Consequently, this study intends to describe various aspect of LSD outbreaks in Al-Muthanna governorate including the clinical presentation, pathological changes and molecular detection of LSD virus from skin lesions, the bitting ticks, and blood samples from infected cattle and buffaloes by extraction of DNA using conventional PCR assay.

MATERIALS and METHODS: This study was done in Al-Muthanna governorates including 257 cattle and 77 buffaloes, during the period extended from (10/2016 - 12/2017). Skin biopsy from nodular sitfast lesions were collected and divided into 2 pieces the first for histopathological investigation, while the second part for molecular studies. Ten ml Whole blood, ticks were collected for PCR extraction. DNA was extracted from ticks, skin nodules, and whole blood using the AcciPrep ® Genomic DNA extraction, kit according the instruction of the company. Conventional PCR was applied with forward P32 primer 5'-CGCGAAATTTCAGATGTAGTTCCA-3' and Reverse primer 5'- TGAGCCATCCAT TTTCCAACTC-3'. The PCR products examined by electrophoresis in 1% agarose gel, stained with ethidium bromide, and visualized under UV.

RESULTS: The suspected animals exhibited numerous lumpy/ urticaria-like lesions over entire body. All suspected animals showed fever, severe depression, anorexia, enlargement of pre-scapular and pre-femoral lymph nodes. Mastitis, loss of milk production, testicular swelling, orchitis, deep ulcerative skin lesions, lameness and swelling of the joints were also observed. The histopathological examination of the sitfast skin lesions showed severe acanthosis, orthokeratototic hyperkeratosis. Micro-vesicles formation and severe ballooning degeneration of the stratum spinosum were seen in the epidermal cells. Intracytoplasmic eosinophilic inclusion bodies were observed in degenerated epidermal cells, and some macrophages. The molecular investigation revealed positive samples with amplicon size 752 bp molecular weight. The percentages of positive cases in PCR were 75.87% (195) and 70.12% (54) for cattle and buffaloes respectively. This high percentages of positive cases suggests that the disease occurred as outbreak. The negative PCR results might be due to collection of samples during the convalescent stage.

CONCLUSIONS: This study approved the spreading LSD outbreaks in Al-Muthanna governorate / Iraq. In addition, it confirmed the diagnosis according to clinical, histopathological features and molecular assay. The author request more epidemiological studies in different Iraqi governorate aiming to control the distribution of the disease and legislate strict regulations to decrease spreading of the disease in Iraq.

Keywords: Al Muthanna, clinical, Molecular LSD,PCR

Acidification of raw whole milk for Inactivation of H5N1

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BACKGROUND and OBJECTIVE: Avian Influenza virus H5N1 2.3.4.4.b has recently been detected in cattle, with milk from infected animals reported to contain a high viral load, serving as a potential source for shedding and dissemination of this virus. Currently, pasteurization is the only widely recognized method for on-farm inactivation of H5N1 in milk. A current concern is that, according to USDA data, less than 50% of large dairy farms pasteurize non-saleable milk, with a much lower rate on medium and small dairy farms. The objective of this pilot study was to evaluate the effect of milk acidification to a pH of ~4.0 to 4.4 on the inactivation of low pathogenic avian influenza (LPAI) and highly pathogenic avian influenza (HPAI) in raw whole

milk. Initial trials in our study used the LPAI H6N2 virus as a surrogate for HPAI H5N1.

MATERIALS and METHODS: For the acidification trials, citric acid was used to acidify milk. To evaluate milk acidification, milk samples were inoculated with LPAI H6N2, and samples were collected before and after treatment at various time points. Evaluation of virus viability was conducted using specific-pathogen-free embryonated chicken eggs, and viral quantification was performed using real-time quantitative PCR. Three acidification experiments were conducted using milk spiked with LPAI H6N2. Given the positive outcome of inactivating LPAI with citric acid, a fourth trial was conducted with milk containing a high load of H5N1 from actively infected cows.

RESULTS: Our results indicated that milk acidification to a pH of 4.1-4.2 inactivated LPAI H6N2 and HPAI H5N1 viruses after 6 hours of treatment.

CONCLUSIONS: This is the first study to report the effectiveness of milk acidification in inactivating HPAI H5N1 in milk from animals infected with H5N1. We have an ongoing project using milk from farms naturally infected with H5N1, evaluating at a larger scale the milk acidification method for deactivating H5N1. We will present the findings from these ongoing trials.

Keywords: High Pathogenic avian influenza, dairy cows, milk acidification, HPAI inactivation

Fatal Mannheimia haemolytica polyserositis in dairy-replacement calves in the Netherlands; differences and similarities with veal calves

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BACKGROUND and OBJECTIVE: *Mannheimia haemolytica* (*M. haemolytica*) serotype A2 has emerged as an important cause of acute polyserositis (PS) in veal calves. Although most veal calves originate from dairy farms, *M. haemolytica* PS is rarely observed in dairy-replacement calves. The aim of this study was to compare the prevalence of *M. haemolytica* PS in veal and dairy calves and to gain more insight into the characteristics of *M. haemolytica* PS in dairy calves through a case study.

MATERIALS and METHODS: *A cross-sectional prevalence study*Data: 7344 necropsies from calves within the national cattle surveillance program.
Case study
On-farm samples were collected after post-mortem diagnosis of *M. haemloytica* PS in a third case from the same farm.
Samples
Samples comprised deep nasal swabs from 5 cows, 5 calves (0–2 weeks), 10 calves (2–16 weeks; affected group), broncho-alveolar lavage samples from 3 other calves from the affected group and the necropsy results of a PS case.
Tests • PCR for nine major respiratory pathogens: BRSV, PI3, BCoV, Influenza D, *M. haemolytica* A1/A6 and A2, *Mycoplasma bovis*, *Pasteurella multocida*, *Histophilus somni*
• Bacteriological culture
• Antimicrobial resistance (AMR) testing
• Whole genome sequencing (WGS) of *M. haemolytica*

RESULTS: From 2018-2024, the prevalence of *M. haemolytica* PS in necropsied veal calves (n=3697) was 10.0 % and in dairy youngstock (n =3647) 0.60%. Calves <2 weeks were PCR-negative for all tested pathogens. In group housed calves >2 weeks, positive PCR findings included: 85% *M. haemolytica* (69% A2, 31% A1/A6), 85% *P. multocida*, 31% BCoV, 15% PI3, and 8% *H. somni*. Adult cattle tested positive for *M. haemolytica* A1/A6 (40%) and *P. multocida* (20%). Six *M. haemolytica* isolates were tested for AMR and sequenced: The A2 isolate found in dairy calves was genetically similar to veal-calf A2 isolates, though showed no AMR, in contrast to >95% of A2 isolates from veal calves that carry multiple AMR genes.

CONCLUSIONS: For reasons not yet understood, *M. haemolytica* PS occurs far more often in veal calves than in dairy-replacement calves. Disease in both can be caused by genetically similar A2 strains, and AMR does not appear essential for disease development. The presence of a clonal A2 strain within group housed calves suggests calf to calf transmission. The prevalence of *M. haemolytica* was

high in the group-housed calves whilst in adult cattle and individually housed young calves on the same farm, fewer and no respiratory pathogens were detected, respectively.

Keywords: Mannheimia haemolytica polyserositis, serotypes, calves, prevalence, sequencing, antimicrobial resistance

Severe thrombocytopenia and spontaneous bleeding in young calves infected with bluetongue virus

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BACKGROUND and OBJECTIVE: Bleeding disorders are uncommon in cattle and frequently associated with thrombocytopenia. In calves, hemorrhagic syndromes are classically linked to bovine viral diarrhea virus (BVDV) infection or bovine neonatal pancytopenia. Bluetongue virus (BTV) infection is known to induce vascular damage and hemorrhagic manifestations, mainly in sheep, whereas severe hematological disorders are rarely reported in cattle. During the 2025 BTV epizootic in western France, unusual spontaneous bleeding episodes were observed in young calves. The objective of this report is to describe clinical, hematological, and pathological findings in those calves and to explore potential etiologies.

MATERIALS and METHODS: Four Holstein-Friesian calves aged 10–15 days, from different farms, were referred to the Clinic for Ruminants at Oniris VetAgroBio Nantes Veterinary College between September and October 2025 for spontaneous bleeding. Clinical examination, complete blood counts, blood smear examination, and coagulation testing were performed at admission. Whole blood RT-PCR assays were conducted for BTV serotypes 3 and 8, epizootic hemorrhagic disease virus (EHDV), and BVDV. Selected cases underwent bacteriological testing for *Salmonella spp*. All calves were necropsied, and bone marrow cytology was performed to assess platelet production.

RESULTS: All calves presented with skin bleeding, petechiae, and mucosal hemorrhages; three exhibited gastrointestinal bleeding (hematochezia or melena). Mild to severe anemia was present in all cases. Severe thrombocytopenia was documented in 3/4 calves at admission (<20 × 10^3/µL). The fourth calf showed marked thrombocytosis at admission, consistent with a rebound following previously documented thrombocytopenia. Coagulation times and fibrinogen concentrations were within reference ranges in all calves, consistent with preserved secondary hemostasis. All animals tested positive for BTV (BTV-8 (3/4) and BTV-3 (1/4)) and negative for BVDV and EHDV. *Salmonella* cultures were negative. No treatments were administered. Three calves died or were euthanized due to severe hemorrhage. One recovered clinically with subsequent reactive thrombocytosis and was euthanized due to biosecurity constraints and to allow post-mortem investigations. Necropsy revealed widespread hemorrhages in the three still ill calves. Bone marrow cytology showed marked megakaryocytic hyperplasia in all four calves, supporting a peripheral mechanism of thrombocytopenia.

CONCLUSIONS: This report describes severe thrombocytopenia with spontaneous bleeding in young calves infected with BTV-3 or BTV-8. Although causality cannot be definitively established, alternative major causes of hemorrhagic syndromes were excluded, and bone marrow findings support peripheral platelet destruction. These observations suggest a possible association between BTV infection and immune-mediated or virus-associated thrombocytopenia in calves.

Keywords: Bleeding disorders, Platelet disorders, Primary haemostasis, Bluetongue virus, BTV-3, BTV-8

Epidemiology and risk factors for Mycoplasma wenyonii and Candidatus Mycoplasma haemobos co-infection in dairy cattle in Canada

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BACKGROUND and OBJECTIVE: *Mycoplasma wenyonii* (MW) and *Candidatus Mycoplasma haemobos* (MH) are hemotrophic, epicellular bacteria increasingly detected in cattle. Subclinical infection might reduce productivity and influence susceptibility to other diseases. The objective of this study was to investigate their presence in dairy cattle in Ontario, Canada, and explore herd-level management practices associated with co-infection.

MATERIALS and METHODS: A pilot cross-sectional study was conducted on 10 commercial dairy farms in southwestern Ontario between December 2023 and May 2024. Thirty cows per herd were sampled between 21 and 28 days in milk. Whole blood was collected in EDTA tubes and submitted for gel-based PCR testing for MW and MH. A structured questionnaire captured herd-level management data including housing, animal purchases, vector control, needle use, and bedding materials. Wright-Giemsa-stained blood smears were evaluated in a subset of PCR-positive animals by a board-certified clinical pathologist to assess the diagnostic utility of microscopic examination. The primary outcome was the herd-level proportion of co-infected animals. Univariable linear regression identified candidate risk factors ($P < 0.20$), which were evaluated in a multivariable model using backward elimination, retaining significant variables ($P \leq 0.05$) and confounders. Interaction terms between biologically relevant variables were assessed.

RESULTS: A total of 297 cows were enrolled. The PCR-positive proportions were 63.3% for MW and 54.6% for MH, with 23.6% co-infected. Only 2.7% tested negative for both organisms. Blood smear evaluation in 28 PCR-positive animals did not detect organisms. Housing breeding-age heifers on sand was positively associated with co-infection (+19.20%; 95% CI: 7.54 to 30.86; $P < 0.01$). Housing weaning-age heifers on shavings tended to be associated with lower co-infection (-6.13%; 95% CI: -13.90 to 1.64; $P = 0.10$). An interaction was observed (-16.87%; 95% CI: -33.80 to 0.07; $P = 0.05$), with the combination of sand bedding for breeding-age heifers and shavings for weaning-age heifers associated with a lower percentage of co-infected animals, suggesting youngstock housing may influence herd infection dynamics.

CONCLUSIONS: These findings suggest hemotrophic mycoplasma infections are widespread in Ontario dairy cattle and predominantly subclinical. Management practices related to youngstock housing may influence herd-level co-infection risk. Increased awareness and targeted diagnostic testing may support earlier detection and inform prevention strategies including improved needle hygiene, vector control, and youngstock housing management. Blood smear evaluation demonstrated low diagnostic sensitivity for detecting infection in this study, supporting PCR as the preferred diagnostic method. Larger epidemiological studies are warranted to confirm risk factors and clarify the clinical and production impacts of subclinical infection.

Keywords: hemoplasma, subclinical infection, dairy herd health, epidemiology, vector-borne pathogens, diagnostic testing

The Scottish BVD eradication scheme: What have we achieved so far? Celebrating the successes and learning from the challenges

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BACKGROUND and OBJECTIVE: Since 2010, the Scottish Government has been working with industry, veterinary practitioners and scientific partners to eradicate bovine viral diarrhoea (BVD) virus in Scotland. The motives for eradicating BVD include improving the economic sustainability and welfare of cattle farming, in addition to reducing greenhouse gas emissions by increasing efficiency.

MATERIALS and METHODS: In phase 1, which ran from September 2010 to April 2011, the Scottish Government subsidised testing to encourage

farmers to determine their BVD status. Legislation was put in place for phase 2. This required all breeding herds to screen for BVD by February 2013 and annually thereafter. In phase 3, from January 2014, the herd status had to be declared at the point of sale and movement restrictions were put on untested herds and animals persistently infected with BVD. Phase 4 commenced in June 2015 with movement restrictions put on herds with a "not-negative" status. Animals entering herds from untested holdings were also required to be tested from this date. In phase 5, from December 2019, those holdings that chose to retain BVD virus positive animals for over 40 days were publicly listed and herds with a "not-negative" status for over 15 months were required to carry out a Compulsory BVD Investigation (CBI). The most significant elements of Phase 6 took effect from February 2026. There is now a requirement to BVD virus test calves born to dams that moved into a breeding herd during pregnancy. In addition, holdings that have not completed their CBI within 13 months are now considered "non-compliant" and publicly listed.

RESULTS: A survey carried out prior to the eradication scheme indicated that 40% of Scottish breeding herds had evidence of exposure to BVD. Currently 7.4% of breeding herds in Scotland are classed as "not-negative". To date 7,328 animals have been identified as persistently infected with BVD and over 99.9% of these have been culled. Only two herds are currently retaining a known BVD positive animal for more than 40 days. 33 herds are currently considered non-compliant.

CONCLUSIONS: The Scottish BVD eradication scheme has made considerable progress in the past fifteen years. The phased approach has ensured that all areas of the cattle industry have been aware of their increasing responsibilities. This presentation reviews the successes of the scheme to date and the challenges we have faced. With other nations moving forward with BVD eradication and introducing legislation, it is hoped that others can learn from the Scottish story.

Keywords: BVD, Eradication, Cattle, Scotland, United Kingdom

Epizootic hemorrhagic disease virus exposure in dromedary camels in Oman: A neglected threat at the livestock–wildlife–bovine interface

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BACKGROUND and OBJECTIVE: Epizootic hemorrhagic disease virus (EHDV) is an *orbivirus* transmitted by *Culicoides* midges that infects multiple ruminant species, but its occurrence and epidemiology in dromedary camels are poorly understood. Northern Oman has intensive multi-species livestock production, making EHDV relevant for bovine infectious disease control. This study aimed to estimate seroprevalence, identify risk factors and explore the spatial distribution of EHDV infection in dromedary camels in two northern Omani governorates.

MATERIALS and METHODS: A cross-sectional study was conducted between September 2020 and July 2021 in Al Batinah North and Al Batinah South governorates. Blood samples were collected from 415 apparently healthy camels from 50 holdings. Sera were tested for EHDV antibodies using a commercial VP7-based competitive ELISA (reported sensitivity 85.9%, specificity 99.7%), and apparent and true prevalence were estimated. Animal and management level risk factors were evaluated using univariable and multivariable logistic regression. Spatial clustering of seropositive holdings was assessed by average nearest neighbour statistics, with kernel density estimation and Getis-Ord Gi* used to visualise and test for local high-risk areas.

RESULTS: Overall, 40.2% of camels were seropositive, corresponding to a true prevalence of 46.7%, with similar levels in both governorates. Seropositivity was higher in camels >4 years, in animals in contact with other ruminants, and in leisure/breeding camels. In the multivariable model, age >4 years and contact with other ruminants remained independent predictors of infection. Positive holdings showed strong global spatial clustering, with higher densities along coastal agricultural corridors, although no significant local hot spots were detected.

CONCLUSIONS: There is substantial circulation of EHDV among dromedary camels in northern Oman, with older animals in mixed-species herds acting as important maintenance hosts in multi-ruminant systems. Incorporating camels into integrated EHDV surveillance, alongside vector monitoring and molecular characterisation of circulating strains, may strengthen early warning and control strategies for orbiviral diseases at the wildlife–camel–bovine interface.

Keywords: Epizootic hemorrhagic disease virus (EHDV), Dromedary camels, Seroprevalence, Risk Factors

Rapid risk assessment of the transboundary spread of foot-and-mouth disease virus serotype SAT1 across North Africa, Western and Central Asia, and South-Eastern Europe

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BACKGROUND and OBJECTIVE: Foot-and-mouth disease (FMD) remains one of the most economically significant transboundary animal diseases affecting cloven-hoofed livestock. In March 2025, outbreaks of FMD virus serotype SAT1 were first reported in Iraq, marking an unusual emergence of this serotype outside its traditional range in sub-Saharan Africa. Subsequent detections in other countries in the Near East region raised concerns about wider regional spread. A rapid risk assessment was conducted to evaluate the likelihood and potential impact of SAT1 introduction into currently unaffected countries across North Africa, Western and Central Asia, and South-Eastern Europe over from December 2025 to February 2026.

MATERIALS and METHODS: Twenty countries and territories adjacent or in close proximity to affected areas were included. The assessment followed FAO rapid risk-analysis guidelines and combined epidemiological data, livestock demographics, trade and movement patterns, vaccination status, veterinary service capacity, and expert consultation. Likelihood of introduction and potential impact were qualitatively scored using standardized scales, with uncertainty evaluated based on data availability and reliability.

RESULTS: The risk assessment concluded that the introduction of infection to livestock was very likely in three countries and likely in two countries within the following three months. Informal cross-border livestock movements, particularly of small ruminants that may show limited clinical signs while shedding virus, were identified as the principal pathway for spread. Limited use and availability of vaccines containing SAT1 strains and variable veterinary service capacity further increased vulnerability. Potential impacts were assessed as moderate to severe, reflecting substantial variation across livestock production systems. Countries where livestock contribute significantly to agricultural GDP and household livelihoods—such as Afghanistan, Pakistan, Libya, and the Syrian Arab Republic—would likely experience pronounced effects on food security, rural incomes and livestock value chains. In contrast, countries with smaller livestock sectors and stronger veterinary infrastructures may experience more localized economic impacts.

CONCLUSIONS: Despite moderate-to-high uncertainty due to under-reporting and limited data on informal movements, the findings highlighted the urgent need for strengthened surveillance, risk-based vaccination strategies, and regional coordination to mitigate the potential spread of SAT1. Following the publishing of the assessment, serotype SAT1 was confirmed in three countries considered at likely or very likely to be exposed during the timeframe.

Keywords: food-and-mouth disease, rapid risk assessment, transboundary animal diseases, surveillance, vaccination

Association between Staphylococcus aureus in waste milk and nasal carriage in dairy calves in Chile

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BACKGROUND and OBJECTIVE: *Staphylococcus aureus* is a major contagious pathogen causing bovine mastitis worldwide and is frequently isolated from dairy herds in southern Chile. The emergence of methicillin-resistant *S. aureus* (MRSA) represents a growing concern within a One Health framework. Waste milk (WM), commonly used to feed dairy calves, may contain mastitis-associated pathogens and antimicrobial residues, potentially acting as a vehicle for bacterial transmission. However, the prevalence of *S. aureus* and MRSA in calves and WM, as well as the role of WM feeding in calf colonization, remain poorly characterized. This study aimed to determine the prevalence of *S. aureus* and MRSA in dairy calves and WM, and to evaluate the genetic relatedness between isolates from these two sources on dairy farms.

MATERIALS and METHODS: A cross-sectional study was conducted in 40 dairy farms. During each visit, one WM sample (n = 40) used for calf feeding was collected. Additionally, nasal swabs were obtained from at least 10% of the calves present during the visit. Samples were subjected to an enrichment step in Mueller–Hinton broth with 6.5% NaCl, followed by incubation for 18 h at 37 °C. Then, cultures were plated onto Baird–Parker and Chromogenic agar for MRSA isolation. Identification of *S. aureus* was performed using an agglutination test and PCR (gene *nuc*). To evaluate the genetic relatedness between isolates from WM and calves, six farms with *S. aureus*-positive WM were selected. In these farms, isolates from WM and from positive calves were analyzed using RAPD-PCR (primer CRA22). Three isolates per positive sample were analyzed to compare genetic profiles.

RESULTS: A total of 589 nasal swabs from calves across 40 dairy farms were analyzed. MRSA was not detected in WM samples or in nasal swabs. *S. aureus* was isolated from 65 calves, corresponding to a prevalence of 11.0% (95% CI: 8.5–13.6%). At least one positive calf was detected in 30 of the 40 farms (75%). Among WM samples, 16 out of 40 (40%) were positive for *S. aureus*. Genetic comparison using RAPD-PCR was performed on isolates from six farms with *S. aureus*-positive WM. In five of the six farms, at least one calf isolate showed a genetic profile identical to that of isolates obtained from WM, suggesting a potential epidemiological link between the two sources.

CONCLUSIONS: These findings indicate that *S. aureus* is commonly present in both dairy calves and WM, and the detection of identical genetic profiles suggests that WM feeding may contribute to calf colonization.

Keywords: Staphylococcus aureus, Waste milk, Calves, Dairy farms, Molecular typing.

Calf serum MAP antibody titre as indicator of neonatal exposure to Johne's disease

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BACKGROUND and OBJECTIVE: Johne's disease (JD), caused by Mycobacterium avium subsp. paratuberculosis (MAP), is a chronic enteric infection of ruminants typically acquired early in life. Identifying calves exposed to MAP remains challenging. Circulating immunoglobulins detected shortly after birth are derived from colostrum and reflect maternal antibody transfer. If MAP-specific antibodies are present in colostrum, their detection in neonatal calf serum may provide an indirect indicator of exposure to MAP-positive dams and contaminated periparturient environments. This study evaluated whether MAP-specific antibodies are passively transferred from dam to calf and

whether neonatal calf serum titres reflect maternal JD status and efficiency of passive transfer.

MATERIALS and METHODS: Neonatal calves (n = 38) from a commercial Holstein–Friesian dairy herd were blood sampled within the first 10 days of life as part of routine monitoring of passive transfer. Serum total protein (STP) was measured using an optical refractometer as an indicator of colostrum immunoglobulin uptake. Residual serum samples were analysed for MAP-specific antibodies using an indirect ELISA, with results expressed as sample-to-positive (S/P) ratios. Calves with failure of passive transfer (STP < 55 g/L) were excluded, leaving 33 calves. Dam JD status was defined using historical quarterly milk ELISA results. Associations between calf MAP antibody titres, STP, and dam serological status were evaluated using multivariable linear regression.

RESULTS: Median calf age at sampling was 3 days (IQR 2–5), median STP was 70 g/L (IQR 61–79), and median MAP S/P ratio was 24.7 (IQR 16.0–40.2). Calf MAP antibody titres increased significantly with increasing STP ($p < 0.001$), indicating dependence on the efficiency of passive transfer. Calves born to MAP-seropositive dams had higher MAP antibody titres than calves from seronegative dams ($p = 0.025$). A significant interaction between STP and dam serostatus ($p = 0.011$) indicated that the relationship between passive transfer efficiency and MAP antibody titres differed according to maternal infection status, consistent with enhanced transfer of MAP-specific antibodies when the dam was seropositive.

CONCLUSIONS: MAP-specific antibodies are detectable in neonatal calf serum and are jointly determined by passive transfer efficiency and dam JD serostatus. These findings demonstrate the biological patency of MAP-specific antibody transfer via colostrum and suggest that neonatal calf serology reflects exposure to a MAP-positive dam and a high-risk periparturient environment rather than calf infection status. Measurement of MAP antibody titres in neonatal calves may therefore provide a biologically grounded indicator of early-life exposure that could complement existing JD surveillance and risk assessment approaches.

Keywords: Calf, paratuberculosis, exposure risk

Concomitant vaccination with inactivated EHDV-8 and BTV-3 vaccines is safe and does not impair immune response in cattle

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BACKGROUND and OBJECTIVE: Orbivirus infections have become increasingly frequent across Europe, causing severe outbreaks in cattle and sheep. Vaccination remains the most effective control measure; however, the growing number of circulating serotypes and the lack of multivalent vaccines complicate herd protection. To date, simultaneous administration of different Orbivirus vaccines has not been evaluated under controlled conditions in cattle. This study aimed to assess the feasibility and safety of concomitant vaccination, as well as the quality of the induced immune response.

MATERIALS and METHODS: A total of 125 EHDV-seronegative heifers from two herds were enrolled. Animals were vaccinated either with a single vaccine (n = 15) or concomitantly with two inactivated vaccines administered at distinct neck sites (n = 67). A non-vaccinated group served as control (n = 10). Prior exposure to BTV was recorded: some heifers had been vaccinated against BTV-4 and -8 (n = 53), or naturally infected with BTV-8 (n = 17), and carried BTV VP7-specific antibodies before inclusion. All animals tested negative for EHDV and BTV by qPCR (ID GENE BTV & EHDV Advantage Triplex, ID.Vet, Montpellier). The experiment was performed during the vector-free winter season (January–February). Inactivated vaccines against EHDV-8 (Hepizovac®, Ceva, France) and BTV-3 (Bluevac-3®, Melchior, France) were administered following the manufacturers' instructions. EHDV VP7 antibodies were detected using the ID Screen® EHDV Competition ELISA (ID.Vet, Montpellier) at d0, 21 and 42, and virus neutralization tests (VNT) against VP2 antigens were performed at day 42 (d42).

RESULTS: No adverse clinical reactions or local lesions were observed, confirming good vaccine safety. The inactivated Hepizovac® vaccine induced EHDV VP7 seroconversion in 61% of animals by day 21, increasing to 84% by d42. Neutralizing antibody

titers ≥ 3.32 (mean 4.1) were detected in 68% of the heifers. Interestingly, pre-existing BTV-8 seropositivity appeared to enhance early EHDV seroconversion (70% vs 42% at d21), though this effect was not maintained at d42. In contrast, the Bluevac-3® vaccine elicited limited neutralizing responses, with only 6% of vaccinated cattle seroconverting at d42. Concomitant administration of both vaccines did not alter immunogenicity profiles or safety outcomes.

CONCLUSIONS: The inactivated Hepizovac® EHDV-8 vaccine elicited a satisfactory antibody response, while the Bluevac-3® BTV-3 vaccine demonstrated low immunogenicity under the tested conditions. These results support the safety and practicality of concomitant Orbivirus vaccination in cattle, offering opportunities to optimize field vaccination protocols and reduce handling interventions.

Keywords: Orbivirus, vaccination, antibody response, BTV, EHDV, cattle

Occurrence and antimicrobial resistance of Salmonella enterica serovar Dublin from dairy farms in Türkiye

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BACKGROUND and OBJECTIVE: *Salmonella enterica* serovar *Dublin* (*S. Dublin*) is a cattle-adapted Salmonella serovar, it can affect cattle of all ages; unlike most other Salmonella serovars that causes enteric disease, it causes acute clinical signs of septicemia and respiratory disease in calves and abortion in cows. Meningoencephalitis and arthritis may also occur in calves. Adult cattle can be subclinical carrier and maintain the infection in the herd. Although host-adapted, *S. Dublin* may infect human and cause severe illness. *S. Dublin* infection is present in many countries worldwide. Prevalence of infection is not known in Türkiye. *S. Dublin* has increasingly been isolated from diagnostic samples in recent years in our laboratory. In this study, isolation and antimicrobial resistance profiles of *S. Dublin* were evaluated in samples submitted to MG Veterinary Diagnostic Laboratory.

MATERIALS and METHODS: Standard bacteriological culture, ISO 6579-1 salmonella culture, serotyping and real-time PCR methods were used for isolation and identification. Antimicrobial susceptibilities of 93 isolates were performed by disc diffusion method using 9 antimicrobial agents (ampicillin, amoxicillin-clavulanic a., ceftiofur, oxytetracycline, enrofloxacin, trimethoprim-sulfamethoxazole, gentamicin, spectinomycin and florfenicol).

RESULTS: A total of 157 *S. Dublin* were isolated from samples submitted between 2017 and March 2026 from 40 different dairy farms located different regions of Türkiye. *S. Dublin* were isolated from 96 internal organs of calves (including one brain sample), 19 aborted fetuses, 30 vaginal swabs of aborted cows, 7 calf faces, 4 internal organs of cattle older than 6 months and 1 milk sample of cow. *S. Dublin* was most frequently isolated from internal organs (heart, liver, lung, kidney and spleen) of young calves between 1 week and 3 months old, but less frequently from intestinal content or faces samples. In 65 (69.9%) of 93 isolates multi-drug resistance were detected. Seven isolates were only resistant two antimicrobials of oxytetracycline and florfenicol and, the highest resistance was detected against oxytetracycline (79.6%) and florfenicol (75.3%). All isolates were susceptible to enrofloxacin, gentamicin and spectinomycin.

CONCLUSIONS: The results highlight emergence of *S. Dublin* infection especially in large dairy farms in Türkiye. Because of common multidrug resistance of isolates, antimicrobial therapy should be based on antimicrobial susceptibility testing. Control and eradication of infection is difficult as the microorganism can be shed intermittently from asymptomatic carrier animals. Introduction of purchased carrier animals into a herd from other herds and countries is one of the important risk factors for the spread of infection.

Keywords: Salmonella enterica serovar Dublin, isolation, antimicrobial resistance, calf, abortion

Seroprevalence of Mycoplasmopsis bovis on UK farms from 2018 to 2025

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BACKGROUND and OBJECTIVE: *Mycoplasmopsis bovis* (formerly *Mycoplasma bovis*) causes a range of clinical conditions in both adult cattle and calves. *M.bovis* contributes to disease processes such as bronchopneumonia, arthritis and otitis in calves, as well as chronic mastitis in adult cattle. *M.bovis* infections are responsible for substantial economic and health problems worldwide. One of the challenges with *M.bovis* is that the clinical picture of disease diagnosed as *M.bovis* is not pathognomonic and often does not differ from clinical signs caused by infections with other bovine pathogens. This can lead to an underestimation of the impact of *M.bovis* at farm level. As part of a UK wide scheme to support disease surveillance on farm, serological testing for *M.bovis* was undertaken to gain an understanding of the role of *M.bovis* in the disease outbreaks and to raise awareness of it as a pathogen.

MATERIALS and METHODS: From 2018 to 2025 blood samples were submitted for serological testing from farms across the UK as part of a subsidised surveillance scheme. Samples were analysed by commercial laboratories using the ID Screen® *Mycoplasma bovis* Indirect ELISA. During 2025 bulk milk samples from dairy farms were also analysed for *M.bovis* antibodies.

RESULTS: Over the course of 8 years a total of 12813 blood samples were tested from herds across the UK. On an annual basis the percentage of samples testing positive for *M.bovis* ranged from 30% to 41%. Analysis of bulk milk tanks samples from 400 herds collected during 2025 found 65% of samples to be positive for *M.bovis* antibodies.

CONCLUSIONS: The results from this study highlight the importance of *M.bovis* as a potential pathogen on UK farms. It is important that farmers and veterinary surgeons are aware of the impact of the disease on animal health and productivity and take steps to minimise this. Veterinary surgeons should work with their clients to ensure they are able to rapidly and accurately identify the clinical presentations of *M.bovis*. The role of *M.bovis* in commonly occurring disease complexes such as bovine respiratory disease means that appropriate diagnostics should be utilised to understand the prevalence of *M.bovis* at an individual farm level. For herds where *M.bovis* is already present steps should be taken to minimise spread and reduce the impact of the disease. In herds where there is no evidence of infection the emphasis should be on preventing introduction through good biosecurity and stringent controls on purchasing stock.

Keywords: Mycoplasma Bovis, Mycoplasmopsis, serology, cattle, BRD, surveillance

HPAI monitoring in cattle in the Netherlands

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BACKGROUND and OBJECTIVE: In March 2024 highly pathogenic avian influenza (HPAI) A(H5N1), clade 2.3.3.3b, genotype B3.13 was found in U.S. dairy cattle. Despite having an effective monitoring and surveilance system in the Netherlands, concerns arose that infections could have occurred in Dutch cattle.

MATERIALS and METHODS: Archived cattle serum samples collected between 2022 and 2024 (n=1.062) were selected and tested for HPAI antibodies. To be able to timely detect the emergence of HPAI in Dutch cattle, we communicated with Dutch veterinarians and cattle farmers about possible symptoms of HPAI in cattle. Our experts at "Veekijker" (Livestock Watcher), a daily telephone helpdesk that practitioners can consult free of charge for support with veterinary cases or to report unusual symptoms, are extra alert to symptoms that could indicate cattle with HPAI infection. Furthermore, there is a weekly bulk milk syndrome surveillance in place. If a drop in milk production is found in a certain area, local practitioners are called and interviewed about any presentation of clinical signs of disease in herds in that area or if they have another plausible explanation for lower milk production during the period in question.

RESULTS: The results showed no indication of HPAI virus infection in Dutch cattle.

CONCLUSIONS: Between 2022 and 2024 there were no indications for HPAI virus infections in the Netherlands. Furthermore the ministry of Agriculture, Fisheries, Food Security and Nature designed and put in place a policy manual for HPAI disease control in case dairy cattle are present at the location of a HPAI outbreak on a poultry farm.

Keywords: HPAI, cattle, Netherlands, monitoring, surveillance, milk production

Anthrax-like sudden death in cattle caused by toxigenic Bacillus cereus: First European report in two decades

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BACKGROUND and OBJECTIVE: Bacillus cereus is a ubiquitous spore-forming bacterium belonging to the B. cereus group, commonly associated with foodborne disease in humans and mastitis in cattle. Although typically considered an opportunistic pathogen, certain strains possess virulence determinants capable of causing severe systemic infections. Over the past two decades, highly virulent B. cereus strains responsible for peracute disease and sudden death in livestock have been described mainly in Asia and Eastern Europe. These strains may share genetic features with Bacillus anthracis, complicating laboratory diagnosis and raising concerns for veterinary and public health surveillance. However, comparable cases of sudden death in adult cattle attributed to toxigenic B. cereus have not been documented in the European Union in the last 20 years. This report describes a confirmed case occurring in Western Europe.

MATERIALS and METHODS: Two adult cattle from a small extensive herd of 18 animals were found dead without premonitory clinical signs on a farm located in Marvão, southern Portugal, a sparsely populated region characterized by extensive livestock production. The area is classified as disadvantaged and lacks access to carcass collection and incineration services. Post-mortem examination revealed mild splenic congestion but no macroscopic lesions typically associated with anthrax. Given the peracute presentation and epidemiological context, laboratory investigation initially focused on excluding B. anthracis. Quantitative PCR assays targeting chromosomal and plasmid-associated anthrax markers (gene 183, pagA, capA/capC) produced scattered positive signals in spleen and lymph node samples. However, selective culture yielded hemolytic colonies, effectively excluding B. anthracis. Subsequent phenotypic and molecular characterization identified toxigenic B. cereus carrying genes encoding hemolysin BL (Hbl), non-hemolytic enterotoxin (Nhe), and cytotoxin K1 (CytK1).

RESULTS: The toxin profile detected, together with the peracute clinical presentation, is consistent with highly virulent B. cereus strains previously described in fatal infections in livestock outside the European Union. The partial amplification of anthrax-associated genetic markers highlights the well-recognized diagnostic challenges posed by the close genetic relationship within the B. cereus group. These findings reinforce the importance of combining molecular diagnostics with bacteriological culture and phenotypic characterization in suspected anthrax cases.

CONCLUSIONS: To our knowledge, this represents the first documented occurrence in Europe in the last two decades of sudden death in adult cattle caused by toxigenic B. cereus. Increased awareness of this pathogen is essential for accurate differential diagnosis with anthrax and for improving surveillance of emerging virulent members of the B. cereus group, particularly in remote rural regions where limited infrastructure may complicate carcass management and disease investigation.

Keywords: Anthrax-like infection, Bacillus cereus, cattle, Portugal, sudden death, toxigenic strains.

MLVA-16 typing of Brucella abortus Biovar 3 in Algeria: Two distinct clusters and epidemiological implications

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BACKGROUND and OBJECTIVE: Brucellosis is an infectious, contagious disease shared by numerous animal species and humans, remains endemic across most regions of the world. In Algeria, the prevalence of this zoonosis has remained persistently high despite the implementation of a national prophylaxis programme since 1995. In cattle, the infection rate was recorded at 2% in 2022, with Brucella abortus bv 3 as the predominant biovar. Epidemiological data on brucellosis in Algeria are primarily derived from serological surveys, with very few studies reporting phenotypic and molecular characterization of circulating Brucella strains. This critical knowledge gap hinders the development of targeted and effective control programmes. From an epidemiological standpoint, determining the species and biovars of Brucella in circulation, together with the analysis of their genetic fingerprints, constitute an essential prerequisite for the establishment of strategies for the control brucellosis. It is within this framework that the present study was undertaken, with the objectives of isolating and identifying Brucella strains responsible for bovine brucellosis in Algeria through phenotypic characterization, and performing molecular typing to compare them with international strains using the MLVA-16 method.

MATERIALS and METHODS: From October 2011 to May 2014, a total of 161 seropositive cattle from 13 wilayas representing the four regions of Algeria (Centre, West, East and South) were enrolled in the study. A total of 298 samples were collected, comprising 109 milk samples and 189 lymph node samples. Phenotypic characterization was performed in accordance with French standard AFNOR NF U47-105. Molecular analysis was subsequently conducted on 29 B. abortus bv 3 strains, including 28 bovine strains isolated and identified by bacteriological methods from various Algerian regions and sample types, along with one Algerian human strain obtained from the collection of the CNR/OIE/FAO/EU Brucellosis Reference Laboratory (ANSES). All strains were genotyped using the MLVA-16 technique. The resulting genotypic profiles were compared with those of previously published Algerian, African, Asian, South American, and European strains (n=247), and included the reference strains B. melitensis bv 1 16M and B. abortus bv 3 Tulya.

RESULTS: Molecular analyses revealed that Algerian strains were distributed into two distinct clusters: an African cluster and an Algerian-European cluster. This phylogeographic proximity between Algerian and European strains suggests that the origin of bovine brucellosis in Algeria may be linked to the importation of infected animals from Europe.

CONCLUSIONS: This study represents the first molecular investigation of B. abortus strains circulating in Algeria and provides meaningful insights into the epidemiological landscape of brucellosis in the country

Keywords: Brucella abortus, brucellosis, Algeria, MLVA-16

Tuberculin skin testing increases paratuberculosis milk antibody levels in both high-risk and low-risk dairy cows: implications for testing intervals

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BACKGROUND and OBJECTIVE: In the United Kingdom, dairy herds undergo routine testing for both bovine tuberculosis, using the single intradermal comparative cervical tuberculin (SICCT) test, and paratuberculosis, using a milk enzyme-linked immunosorbent assay (ELISA). Due to antigenic cross-reactivity between purified protein derivatives used in the SICCT test and Mycobacterium avium subspecies paratuberculosis (MAP), current guidance recommends a minimum interval of 42-60 days between the two tests. Recent

research suggested that this interference differed between cows classified as high risk of MAP shedding and controls, leading to advisory guidance that high-prevalence herds could shorten the interval to within 14 days to exploit a potential increase in sensitivity. The objective of this study was to evaluate whether the post-SICCT antibody response differed between cows at high and low risk of MAP shedding, and to quantify the impact of testing interval on ELISA diagnostic performance.

MATERIALS and METHODS: Individual cow milk ELISA records from 518 UK dairy herds (2012-2023; 1,226,709 tests from 340,839 cows) were matched to SICCT test records. Two case definitions were applied: an industry-based definition classifying cows as high risk after two or more positive results in four consecutive tests, and a more stringent progression-based definition requiring sustained positive or negative testing histories. Generalised additive models were fitted separately for case and control cows under each definition. Additional models were fitted for subsets of herds with high (≥10%) and low (≤2%) annual antibody prevalence. Apparent test performance characteristics were estimated across SICCT-ELISA intervals using bootstrap resampling.

RESULTS: Contrary to previous findings, both case and control cows showed a similar post-SICCT rise in antibody levels under both case definitions, peaking at approximately 18-22 days. In high-prevalence herds, this transient rise was evident in both cases and controls. A small post-SICCT antibody increase was observed in control cows in low-prevalence herds with approximately two years of consecutive negative results. Positive predictive value was reduced at shorter intervals across all prevalence levels. In high-prevalence herds, each additional true positive detected at the 0-14 day interval was accompanied by approximately 25 additional false positives, while in low- and medium-prevalence herds, there was no increase in true positives at this interval.

CONCLUSIONS: The post-SICCT antibody response occurred in both high- and low-risk cows with similar patterns, consistent with cross-reactive stimulation by shared mycobacterial antigens rather than an exclusively MAP-specific anamnestic effect that could be exploited diagnostically. Shortening the SICCT-ELISA testing interval increases false-positive classifications without a diagnostic benefit.

Keywords: Paratuberculosis, Bovine tuberculosis, Milk ELISA

Cost of epizootic haemorrhagic disease and lumpy skin disease in cow-calf French operations

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BACKGROUND and OBJECTIVE: Epizootic Haemorrhagic Disease (EHD) and Lumpy skin disease (LSD) are 2 high consequence diseases affecting cattle and recently introduced in France. Despite their impact, few economic studies have been conducted, particularly for cow–calf systems, highlighting the need to assess its consequences at the farm level. We aim to perform the economic assessment of the diseases in case of circulation in cow-calf farms.

MATERIALS and METHODS: A representative cow–calf herd stochastic model was developed with Monte Carlo simulation to assess the economic losses associated with EHD and LSD. The values of gross margins with or without the disease were compared for an average cow-calf operation.

RESULTS: EHD led to a 15% decrease in the average annual gross margin decrease (- €88 per adult cow). The losses arise mainly from a reduced number of weaned calves and extra adult cow mortality. The sensitivity analyse indicates that calf mortality, length of the calving interval, weaner-calf prices and disease severity are the most influential factors. Under the middle impact assumption, LSD total cost for a cow-calf farm [95%IC 95] were €132 [€115;€153] per cow-year. It reaches €360 [€308; €430] for high impact assumption.

CONCLUSIONS: This simple and transparent tool offers an operational basis for the rapid assessment of economic impacts in an epizootic or emergence context. For MHE, it provides a clear overview of the impact for farmers. For LSD, it highlights the potential impact for farmers in case no control measure is implemented. The evaluations are of high interest for a better bovine veterinary public policy management.

Keywords: Economics, cow-calf, Epizootic Haemorrhagic Diseas, Lumpy skin disease

Establishment of triple real time qPCR method for detection of infectious pathogens in bovine mastitis

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BACKGROUND and OBJECTIVE: Mastitis is a major disease affecting dairy industry. It has a negative impact on farm profitability and raises health and welfare concerns due to reduced milk production and treatment costs. The disease has many causes, with microbial infection being the most common causing mastitis, and the bacteria involved are classified into infectious and environmental pathogens. Controlling infectious pathogens is particularly important for preventing transmission of mastitis in dairy herds. Traditional methods based on pathogen isolation and cultivation are time-consuming and fail to meet the need for rapid and sensitive detection. Therefore, establishing a rapid method to detect infectious pathogens of mastitis is urgently needed, enabling early identification and isolation of infected cows, preventing further spread of the disease, and reducing economic losses.

MATERIALS and METHODS: Three major infectious pathogens of mastitis—*Staphylococcus aureus*, *Streptococcus agalactiae*, and *Mycoplasma bovis*—were selected as targets, and a triple fluorescence quantitative PCR (qPCR) method was developed using TaqMan probe technology. First, the detection genes for the three pathogens were identified through literature review. Corresponding conserved sequences were obtained from the NCBI website, based on which specific primers and fluorescent probes were designed according to standard primer and probe design principles. The specificity, sensitivity, and repeatability of the primers and probes were then validated. The probes were subsequently applied to detect clinical milk samples from cows with mastitis, and the results were compared with those of bacterial isolation and culture to assess the accuracy of the established method.

RESULTS: Specificity test showed that the designed primers successfully amplified the three target bacteria without cross-reacting with ten non-target bacterial species, indicating specificity of the method. The sensitivity test revealed that the minimum detection limit was 10^1 CFU/mL for *S. aureus* and 10^2 CFU/mL for both *S. agalactiae* and *M. bovis*, demonstrating high sensitivity. The repeatability test showed that the coefficients of variation for all three bacteria were below 10% in three parallel repeated tests, confirming good repeatability. In clinical testing, the coincidence rate between the established method and the traditional pathogen isolation and culture method exceeded 95%, indicating high accuracy.

CONCLUSIONS: The method developed in this experiment exhibits strong specificity, sensitivity, accuracy, and repeatability. It can be used for rapid diagnosis of mastitis caused by the three infectious pathogens—*S. aureus*, *S. agalactiae*, and *M. bovis*—in dairy herds. This allows timely detection, isolation, and treatment of affected cows, thereby cutting off the source of infection and facilitating the prevention and control of bovine mastitis.

Keywords: Bovine mastitis, Staphylococcus aureus, Streptococcus agalactiae, Mycoplasma bovis, Real-time quantitative PCR rapid diagnosis

INTERNAL MEDICINE AND TOXICOLOGY

Peracute herd-level mortality in dairy cattle: A stepwise clinical diagnostic approach

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BACKGROUND and OBJECTIVE: Peracute mortality in adult dairy cattle represents a critical emergency in field practice. When deaths occur within 24 hours of the onset of clinical signs, empirical treatment is often initiated before a definitive diagnosis is established. The objective of this report is to describe the structured clinical reasoning and stepwise diagnostic escalation undertaken during a high-mortality event in a commercial dairy herd and to demonstrate how systematic internal medicine assessment guided targeted herd-level intervention.

MATERIALS and METHODS: A large free-stall dairy herd requested urgent veterinary assistance after experiencing peracute deaths in adult cows, with affected animals deteriorating and dying within 24 hours or less from the onset of clinical signs. Over a two-week period, 30 adult cows died. Clinical findings included sudden reductions in milk yield, decreased rumination, weakness, and occasional neurological abnormalities. Clinical examination focused on respiratory pattern, rectal temperature, rumen motility, and hydration status. Abdominal breathing was observed in several cows, suggesting thoracic involvement. Blood samples were collected for hematological and biochemical analysis. Two complete necropsies were performed. Tissue samples were submitted for bacteriological culture and antimicrobial susceptibility testing. Differential diagnoses included clostridial toxemia, feed-related toxicosis, viral respiratory disease, and septicemic bacterial infection.

RESULTS: Clinical progression was peracute, with rapid deterioration and death. Hematological findings demonstrated a mixed inflammatory response: leukocytosis with neutrophilia in some cows and leukopenia with lymphopenia in others, consistent with severe systemic inflammation and endotoxemia. Biochemical alterations included increased concentrations of aspartate aminotransferase, gamma-glutamyl transferase, creatine kinase, and urea, indicating hepatic stress, muscular damage, and metabolic compromise. Necropsy revealed fibrinous pleuritis, hemorrhagic pneumonia, marked serosal congestion, and gastrointestinal mucosal alterations. Pasteurella multocida was isolated from multiple organs and blood, confirming fulminant septicemia. Targeted antimicrobial therapy based on susceptibility testing, combined with fluid and anti-inflammatory support, resulted in a progressive reduction in mortality over the following two weeks.

CONCLUSIONS: Peracute herd-level mortality requires immediate but structured clinical assessment. Early recognition of abnormal respiratory patterns, rapid laboratory diagnostics, and prompt necropsy were essential in differentiating septicemia from toxic or clostridial causes. This case illustrates that systematic internal medicine reasoning in emergency field conditions can transform herd-level panic into precision-guided management and significantly improve clinical outcomes.

Keywords: Peracute mortality, Septicemia, Pasteurella multocida, Dairy cattle, Clinical diagnostics, Herd-level management

Comprehensive discovery of hidden recessive harmful alleles in Swiss dairy cattle using large-scale life history records

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BACKGROUND and OBJECTIVE: Most cattle breeds have small effective populations, making them prone to recessive deleterious alleles. These are usually studied via forward genetics using data from affected and unaffected animals. However, traits like immune, reproductive or rearing, may be missed. We aimed to uncover cryptic recessive variants in Swiss dairy cattle affecting reproduction, milk yield, and rearing by leveraging large-scale life-history records.

MATERIALS and METHODS: Life history records for large genotyped pedigrees of Swiss Holstein (N=63,103), Simmental (N=10,881) and Brown Swiss (N=67,912) were available. We mined these datasets to identify 532 and 1029 cases of juvenile mortality in Holstein (≤90 days) and Brown Swiss (≤180 days) respectively. We also identified 2067 Brown Swiss cows culled at the age between 540 and 900 days (coinciding with the age at first insemination) and 498 Simmental cows culled at the age beween 900 and 1300 days (coinciding with the onset of production). Recessive haplotypes associated with these cases of juvenile death or culling of cows were identified using imputed genotypes for >600K genome-wide SNPs. Expectations for haplotype homozygosity in case and control group (individuals recorded as parents at least once in the pedigree) were estimated using genotyped parents and grandparents. Haplotypes showing a ≥25% enrichment for haplotype homozygosity in cases and ≤-25% enrichment in control group was considered candidate associations. Selected

homozygous animals for the newly identified haplotypes underwent long-read whole-genome and RNA sequencing. Clinical and pathological examination was performed in homozygous cases.

RESULTS: In the Holstein population, our approach identified the previously reported locus associated with cholesterol deficieny encompassing the APOB gene. The recovery of a known recessive locus added confidence to the applied methodology. In Brown Swiss and Simmental breeds, we identified three new loci responsible for an increased juvenile mortality or culling in the homozygous state and present a series of validations based on large-scale genotyping, clinical examination, and functional studies for candidate variants affecting the ASCC2, ALKBH8, and ALDOA genes. We clinically characterized disorders linked to ASCC2 and ALKBH8 variants. ASCC2-homozygous animals showed spastic paresis, ataxia, and subfertility, whereas ALKBH8-homozygous exhibited failure to thrive and immunodeficiency. The ALDOA-related allele affected milk production performance.

CONCLUSIONS: We revealed multiple previously undetected recessive loci associated with increased mortality in Swiss dairy cattle. Our haplotype-based approach is widely applicable to different life stages and to any population with extensive genomic resources, facilitating the management of inherited disorders to mitigate untreatable genetic conditions and their economic impact.

Keywords: Bovine spastic paresis, imunedeficiency, recessive disorders, rearing loss, failure to thrive

Inflammation of the forestomach mucosa in calves – A comparison of the effects of ruminal drinking and paromomycin overdosage

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BACKGROUND and OBJECTIVE: Inflammation of the forestomach mucosa is a well-known consequence of ruminal drinking in young calves, typically occurring secondary to primary diseases such as neonatal diarrhea or following repeated forced-feeding. Fermentation of milk in the rumen, particularly by lactic acid bacteria, leads to a marked decrease in ruminal pH, as well as to the production of D-lactate. The resulting acidosis causes inflammation of the forestomach mucosa with hyperkeratotic changes and even mucosal sloughing. In recent years, some calves presented to the Clinic for Ruminants (LMU Munich) showed severe diphtheroid-necrotizing inflammation of the forestomach mucosa without detectable milk residues in the rumen. According to the medical history, these calves had been treated orally against cryptosporidiosis with paromomycin for several days, often at excessive dosages.

MATERIALS and METHODS: The differences between forestomach inflammation caused by ruminal drinking and that associated with paromomycin administration are described based on clinical findings, ruminal fluid analysis, laboratory results, and necropsy findings.

RESULTS: Calves with ruminal drinking–associated acidosis typically exhibit varying degrees of depression and sometimes bruxism.The abdomen may be distended, with a plain or even bulging left paralumbar fossa. On percussion and succussion, pinging and/or splashing sounds are usually detectable on the left side. Metabolic acidosis with increased plasma D-lactate concentrations is common and affected calves exhibit alterations of the palpebral reflex as well as impaired ability to stand. Rumen contents are whitish and milky,

containing casein clots with a sour odor. The ruminal pH is markedly decreased. In severe cases, blood and mucosal debris may be present. In contrast, calves with paromomycin-associated forestomach lesions also show impaired general condition, often pronounced bruxism and empty chewing, and occasionally regurgitation of necrotic mucosa. Systemic acidosis is less frequent and less severe; D-lactate concentrations are not elevated. Eyelid closure is prompt and complete, and standing ability is not impaired. The abdomen is typically empty, without abnormal findings on percussion or succussion. Rumen contents lack significant milk residues; pH is not acidic but the fluid is foul-smelling, often hemorrhagic, and mixed with mucosal tissue. Necropsy findings confirm the absence of coagulated milk in paromomycin-treated calves and reveal severe hemorrhagic and diphtheroid-necrotizing inflammation of the forestomach mucosa. In contrast, ruminal drinking is more commonly associated with neutrophilic-(pustular) lesions in the superficial epithelium of apical rumen papillae.

CONCLUSIONS: In calves with suspected rumenitis a ruminal fluid analysis should always be performed and high dosages of paromomycin can cause severe alterations of the forestomach mucosa.

Keywords: calves, forestomach inflammation, ruminal drinking, paromomycin

Preliminary evaluation of milk per- and polyfluoroalkyl substances (PFAS) concentrations and somatic cell count in environmentally exposed dairy cows

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BACKGROUND and OBJECTIVE: Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants that may accumulate in food-producing animals chronically exposed under field conditions. Milk represents a route of elimination for these compounds, however their potential biological effects in dairy cows remain largely unexplored. PFAS are associated with immunotoxic effects in several species, suggesting that somatic cell count (SCC), a marker of intramammary inflammation, may provide an indicator of biological responses in dairy cows. This study provides the first preliminary evaluation of the association between bovine milk PFAS concentrations and SCC.

MATERIALS and METHODS: Forty-nine lactating dairy cows from a background-contaminated area were stratified by parity into primiparous (GP, n=22) and multiparous (GM, n=27), balanced according to days in milk (DIM). Individual milk samples, collected on the day of routine milk recording, were analyzed for twenty PFAS using a validated mass spectrometry method. Somatic cell count (SCC) for each cow was obtained from the corresponding Dairy Herd Improvement Association controls. Differences in PFAS detection frequencies between groups were evaluated using chi-square tests, while parity-related differences in concentrations were assessed using Mann-Whitney test. Linear regression models were used to determine the possible relationship between SCC and PFAS concentrations across groups. Significance was set at $p<0.05$.

RESULTS: Regardless of the group, a 100% detection frequency was observed for PFOA, FHxSA and 6: 2 FTS, whereas PFHpA, PFNA, PFDA, 4: 2 FTS, 8: 2 FTS, PFNS, and PFDS were never detected. Detection frequencies differed between groups for PFBA (45.5% GP; 7.4% GM; $p=0.002$) and NaDONA (59.1% GP; 7.4% GM; $p<0.001$), whereas PFBS was more frequently detected in GM (77.8%) than in GP (4.5%, $p<0.001$). Median concentrations of specific PFAS differed between groups, with higher values in GP for PFBA ($p=0.002$), NaDONA ($p<0.001$), FHxSA ($p=0.01$), whereas PFBS levels were higher in GM ($p<0.001$). Similar median concentrations for PFOS (5 pg/mL) and for 6: 2 FTS (25 pg/mL) were observed between groups. Interestingly, significant associations between SCC and specific PFAS occurred only in GP. In this group, each 1 pg/mL increase in PFOS concentration was associated with an increase of 117,118 cells/mL ($p=0.012$), and each 1 pg/mL increase in 6: 2 FTS concentration with an increase of 213,371 cells/mL ($p<0.001$).

CONCLUSIONS: These preliminary findings suggest parity-specific associations between selected PFAS

concentrations and SCC under environmental exposure conditions. Given the exploratory nature of this analysis, further studies in larger cohorts and with longitudinal designs are needed to confirm these observations and better characterize potential toxicological implications.

Keywords: Per- and polyfluoroalkyl substances (PFAS), dairy cows, somatic cell count, environmental exposure, inflammation, milk contamination

Determination of neutrophil-to-lymphocyte ratio and ischemia-modified albumin levels in calves with naturally occurring sepsis

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BACKGROUND and OBJECTIVE: This study assessed the neutrophil-to-lymphocyte ratio (NLR) and ischemia-modified albumin (IMA) concentrations in calves with naturally occurring sepsis and evaluated whether these parameters could serve as potential diagnostic biomarkers in septic calves.

MATERIALS and METHODS: Thirty-five calves were enrolled in the study, including 25 calves with naturally occurring sepsis (sepsis group) and 10 healthy calves (control group). From each calf, 10 mL of jugular venous blood was collected for hematological analysis (K3-EDTA tubes) and serum biochemistry (gel and clot activator tubes). Clinical examination was performed, and monitoring parameters, including oxygen saturation and non-invasive blood pressure, were assessed.

RESULTS: Compared with the control group, calves with sepsis exhibited significantly higher heart rate, respiratory rate, and capillary refill time (CRT) values ($P < 0.05$), whereas peripheral oxygen saturation (SpO_2) was significantly lower ($P < 0.05$). Serum ischemia-modified albumin (IMA) concentration was significantly increased in septic calves ($P < 0.05$), while total protein (TP) and globulin (GLB) concentrations were significantly decreased ($P < 0.05$). Hematological analysis revealed significantly higher eosinophil counts, neutrophil-to-lymphocyte ratio (NLR), mean corpuscular volume (MCV), and mean platelet volume (MPV) values ($P < 0.05$), together with significantly lower red blood cell (RBC) counts and hemoglobin (HGB) concentrations ($P < 0.05$), in calves with sepsis compared with healthy controls.

CONCLUSIONS: Elevated IMA concentrations in calves with sepsis may reflect ischemia-associated tissue injury, suggesting that IMA could serve as a potential biomarker for bovine neonatal sepsis. In addition, the NLR may provide supportive diagnostic value in the identification of sepsis in calves.

Keywords: Calf, sepsis, ischemia-modified albumin, neutrophil-to-lymphocyte ratio

The airway mycobiome and inflammation: First insights for neutrophilic and eosinophilic profiles in calves

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BACKGROUND and OBJECTIVE: Bovine respiratory disease (BRD) is a major economic and animal welfare problem and the main reason for antimicrobial usage. Airway inflammation can be induced by respiratory tract infection, non-infectious inflammatory factors, or a combination of both. Molds and yeasts are abundant in farming environments and their inhalation can contribute to neutrophilic as well as eosinophilic (type-2 skewed) inflammation in broncho-alveolar lavage fluid (BALF). Whereas neutrophilic cytological profiles are common in calves, eosinophilic airway inflammation is relatively rare (≈3%). In humans, fungi are known to contribute to chronic airway diseases such as asthma and may interact with the bacteriome, influencing infection risk and inflammatory tone. The respiratory mycobiome in cattle remains unexplored. The objective of this study was therefore to characterize the lower

respiratory tract mycobiome in calves and evaluate its association with healthy, neutrophilic or eosinophilic inflammatory profiles.

MATERIALS and METHODS: For this retrospective case-control study, 27 BALF samples were selected from a database of 87 samples collected from dairy and beef calves originating from 19 herds experiencing an acute BRD outbreak. Samples were selected based on cytological profile: healthy (n=10), neutrophilic (n=10) and eosinophilic (n=7). Differential cell counts were determined by light microscopy and manual counting. The airway mycobiome was characterized using Nanopore ITS-amplicon sequencing targeting the full ITS region (ITSF and ITS4 primers). Sequencing reads were analysed using MiniMap2 against the NCBI-ITS database. Alpha-diversity (Shannon and Simpson) and beta-diversity (Bray-Curtis) were analysed.

RESULTS: The study population consisted of 7 calves with upper respiratory tract infection, 9 calves with clinical pneumonia, 5 with subclinical pneumonia and 6 calves that were clinically and ultrasonographically normal. Fungal DNA was consistently detected in BALF samples. The airway mycobiome was dominated by Malassezia restricta (mean relative abundance 17%), followed by Candida albicans (8%) and Alternaria hordeicola (5%). Eosinophilic samples showed lower fungal α-diversity compared with neutrophilic and normal samples (mean Shannon index 1.57 vs. 1.89 and 1.96). A similar pattern was observed for Simpson diversity (0.66 vs. 0.78 and 0.77). In addition, Aspergillus versicolor was relatively enriched in eosinophilic samples compared with neutrophilic and normal samples. In neutrophilic samples, Wallemia muriae and Candida albicans were more present.

CONCLUSIONS: Fungi appear to be an integral component of the respiratory microbiome in calves during BRD outbreaks. Eosinophilic airway inflammation was associated with reduced fungal diversity and enrichment of A. versicolor, suggesting possible dysbiosis and a potential role of environmental molds in eosinophilic airway inflammation in calves.

Keywords: pneumonia, airway inflammation, molds, fungi, respiratory disease

NUTRITION AND METABOLIC DISEASES

Effects of probiotic and prebiotic supplementation derived from local isolates on performance, health, and serum parameters in pre-weaned calves

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BACKGROUND and OBJECTIVE: The inclusion of local probiotic and prebiotic microorganisms, isolated and characterized from local sources, into the rations of pre-weaned calves to enhance disease resistance is a significant strategy. This study aimed to determine the effects of a feed additive consisting of various bacterial species and yeast cultures derived from local isolates on growth performance, health status, and various blood parameters in calves.

MATERIALS and METHODS: Thirty female Holstein calves were studied for 75 days, assigned to control (n=15) or treatment (n=15) groups. All calves received 6 L/day of similar high-quality colostrum during the first three days. From day 4 onwards, milk and a total mixed ration (TMR) containing 10% wheat straw were provided ad libitum according to the farm program. The treatment group received an additional 5 g/day mixture of inactivated *Lactobacillus acidophilus*, *Lactobacillus helveticus*, *Lactobacillus fermentum*, *Lactobacillus plantarum*, and *Bacillus subtilis*, along with live *Saccharomyces cerevisiae* derived from local isolates, supplemented in the milk. Body weight (BW) was measured at 14-day intervals (with a 19-day final interval), and dry matter intake was recorded daily. Blood samples collected on days 21 and 63 were analyzed for non-esterified fatty acids (NEFA), cortisol, total protein,

bilirubin, albumin, interleukin 1-beta (IL-1β), interleukin 6 (IL-6), tumor necrosis factor-alpha (TNF-α), serum amyloid-A (SAA), and haptoglobin. Health status was monitored using the UC Davis respiratory scoring and a 4-point fecal scoring system.

RESULTS: On day 75, BW was significantly higher in the treatment group compared to the control group (92.31 kg vs. 86.61 kg; $P < 0.05$). Significant improvements were observed in average daily gain (ADG) (730 g vs. 654 g) and feed conversion ratio (FCR) (2.05 vs. 2.51) in favor of the treatment group ($P < 0.05$). Among blood parameters, serum albumin levels were higher in the treatment group, while IL-1β, IL-6, SAA, and haptoglobin levels were significantly lower ($P < 0.05$). Furthermore, decreased fecal scores and reduced diarrhea severity were observed in the treatment group, whereas no significant differences were found in respiratory health parameters between groups.

CONCLUSIONS: Local probiotic-prebiotic supplementation significantly improved BW, ADG, and FCR by stabilizing gut microbiota. Reduced levels of pro-inflammatory cytokines (IL-1β, IL-6) and acute-phase proteins (SAA, haptoglobin) indicate suppressed systemic inflammation and enhanced immunity. Additionally, improved fecal scores reflect better intestinal barrier protection. Consequently, incorporating local isolates into pre-weaned calf feeding strategies is recommended as an effective management method to maximize growth and health.

Keywords: Pre-weaned calf, Probiotic, Prebiotic, Immune system, Growth performance, Local isolates

Assessment of a novel 21-day sustained-release prepartum bolus on transition health and productivity in Holstein cows: Findings from a field study in Hungary

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BACKGROUND and OBJECTIVE: The transition from late gestation to early lactation is a physiologically demanding period for dairy cows. During the las three weeks before calving, dry matter intake typically declines while requirements for nutrients rise sharply to support fetal growth and colostrum synthesis. These shifts predispose cows to negative energy balance and increase the risk of metabolic and infectious disorders. Nutritional strategies that ensure consistent delivery of functional additives and micronutrients can help stabilize rumen fermentation, support immune and metabolic adaptation, and mitigate postpartum health challenges. A sustained-release bolus engineered to deliver yeast, B-vitamins, and trace minerals throughout the close-up period, maintains nutrient delivery even as dry matter intake declines in the weeks before calving. This study evaluated whether administering such a bolus 21 days before calving improves milk yield and reduces postpartum disease incidence in Holstein cows under commercial farm conditions.

MATERIALS and METHODS: A total of 413 close-up cows from a large commercial dairy farm in Hungary were enrolled and blocked by parity and previous lactation yield, then randomly assigned within each block to either the Treatment or Control group. Treated cows received a single sustained-release bolus containing live yeast, vitamins, and minerals 21 days before expected calving; controls received no bolus. All cows received 18 g/cow of the same yeast present in the bolus in the dry-cow and fresh cow TMR. Daily milk yield was recorded for the first 30 days in milk (DIM) in cows that completed at least 80% of the study period (Control: n = 178; Treatment: n = 205). Postpartum disorders (metritis, retained placenta, milk fever, displaced abomasum, mastitis) were monitored for 60 days in all animals enrolled. Milk yield was analyzed using one-way ANOVA and health outcomes were evaluated using chi-square

tests. Differences were declared significant at P < 0.05.

RESULTS: Cows receiving the bolus produced more milk during the first 30 DIM compared with controls (37.3 vs. 36.4 kg/day; +0.9 kg/day, +2.45%; P < 0.01). The incidence of postpartum disorders was lower in the treatment group (26% vs. 34%; −24% relative reduction). Metritis was significantly reduced (10% vs. 18%; −44%; P ≈ 0.02).

CONCLUSIONS: Administering a prepartum nutritional bolus 21 days before calving improved early lactation milk yield and reduced postpartum health disorders under commercial conditions. These findings support controlled-release nutrient delivery during the close-up period as a practical approach to enhancing transition cow performance and health.

Keywords: Transition, Sustained-release, Prepartum Bolus, Postpartum Disorders

Effects of dietary supplementation of inactive Saccharomyces cerevisiae fermentation product on performance, health, and certain blood parameters in suckling calves

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BACKGROUND and OBJECTIVE: This study determined the effects of dietary *Saccharomyces cerevisiae* fermentation product (SCFP) supplementation on growth performance, feed intake, health status, and blood parameters (cytokines and acute-phase proteins) in suckling calves.

MATERIALS and METHODS: The research was conducted on 40 female Holstein calves over 90 days. Following birth, calves were randomly assigned to control (*n*=20) and experimental (*n*=20) groups. Following the colostrum period (days 1–3), calves were fed according to the farm's milk program. From day 4, a total mixed ration (TMR) containing calf starter and 10% wheat straw was provided *ad-libitum*. The experimental group received 7 g/day of SCFP (3.5 g in milk and 3.5 g in a carrier ration) until weaning (day 75), and 7 g/day within the solid feed post-weaning (days 75-90). Body weight (BW) was recorded every 14 days, and dry matter intake (DMI) was monitored daily. On days 21, 63, and 90, metabolic profile [non-esterified fatty acids (NEFA), total protein (TP), albumin, and bilirubin], stress (cortisol), and immune response [interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), serum amyloid A (SAA), and haptoglobin] were analyzed. Health status was assessed via daily fecal scoring (on a 1–4 scale) and the UC Davis respiratory scoring system.

RESULTS: At the end of the 90-day trial, SCFP supplemented calves achieved a significantly higher average BW, reaching 6.4 kg more than the control group ($P < 0.05$). Consistent with this growth, both average daily gain (ADG) and daily DMI were significantly increased in the SCFP group compared to the control ($P < 0.05$), although feed efficiency ratios remained similar between groups ($P > 0.05$). Regarding metabolic and immune profiles, SCFP supplementation led to significantly lower serum concentrations of NEFA, total bilirubin, and the pro-inflammatory cytokine IL-6 ($P < 0.05$). Health outcomes improved markedly; the experimental group required only 8 medical interventions versus 22 in the control. Control calves exhibited higher average fecal and respiratory scores. Clinical duration of moderate/severe diarrhea (Scores 3–4) was reduced by 30% in the SCFP group.

CONCLUSIONS: In conclusion, the use of such biotechnological supplements in early-stage calf feeding serves as an effective management tool to mitigate weaning stress, minimize health risks, and maximize herd productivity by optimizing metabolic efficiency and enhancing systemic immune resilience during the critical pre- and post-weaning periods. These findings suggest that SCFP supplementation not only promotes physical growth but also supports the physiological robustness of calves, contributing to the long-term sustainability of dairy operations.

Keywords: Calf nutrition, growth performance, {Saccharomyces cerevisiae}, yeast fermentation product, postbiotic, immune system

Differential effects of Cu/Zn mineral forms on rumen fermentation kinetics and metabolic end-products in an in-vitro system

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BACKGROUND and OBJECTIVE: Copper (Cu) and zinc (Zn) play essential roles in rumen microbial metabolism and enzyme function; however, their effects depend strongly on mineral form and ruminal solubility. Highly soluble sulfate and organic complexes may release free ions rapidly, suppressing fermentation under high exposure. Hydroxy trace minerals, due to their lower solubility at rumen pH, may provide greater rumen stability. This in vitro study evaluated how Cu/Zn source and inclusion levels affect fermentation kinetics and metabolic end-products relevant to rumen health.

MATERIALS and METHODS: Six Cu/Zn mineral sources (sulfate, hydroxy (Excential SMART, Orffa Additives BV), two MHA-chelates, amino acid complex and organic source) were incubated in an in vitro batch culture system using grass silage substrate. Two inclusion levels were tested: low (40 mg Cu/kg; 100 mg Zn/kg) and high (40 mg Cu/g; 100 mg Zn/g). Fermentation was carried out for 72 h. Cumulative gas production (GP), triphasic kinetic parameters (including C-values and Rmax), total volatile fatty acids (VFA), individual VFA molar proportions, ammonia-N, and pH were measured.

RESULTS: At high inclusion, sulfate, chelated, and organic mineral forms caused marked reductions in GP (~28–38 mL/g OM) compared with the control (313.70 mL/g OM; $p < 0.0001$), whereas the hydroxy group maintained significantly higher GP (226.96 mL/g OM; $p < 0.05$). However, low inclusion levels produced no differences among mineral sources for GP, VFA, ammonia-N, or pH, indicating that physiological loads were insufficient to alter fermentation in the batch model. High-dose treatments significantly reduced the C_2 parameter for most sources, indicating collapse of Phase-2 (insoluble fraction) fermentation. In contrast, hydroxy maintained a stable C_2 and showed increased Rmax3 ($p < 0.05$), suggesting preserved late-phase microbial turnover. Total VFA decreased in all treatments ($p < 0.0001$), but the hydroxy source exhibited the smallest reduction. Hydroxy also retained a more physiological VFA profile, maintaining propionate ($p < 0.05$) and lowering the acetate-to-propionate ratio. Ammonia declined across treatments ($p < 0.05$), though values remained higher under hydroxy compared with other sources. pH increased sharply for most high-dose treatments ($p < 0.05$), whereas hydroxy maintained values close to control.

CONCLUSIONS: Hydroxy Cu/Zn consistently maintained fermentation kinetics and metabolic end-products than other mineral forms. The absence of differences at low levels reflects the limitations of a static in vitro batch system, where dilution and finite substrate can mask subtle source-related effects. Taken together, the findings indicate that the controlled-release behaviour of hydroxy interacts more compatibly with rumen fermentation than other Cu/Zn forms evaluated.

Keywords: in vitro, Copper, Zinc, hydroxy, rumen

Vitamin status and antioxidant capacity in transition dairy cows and calves of different ages: Effects of strategic vitamin supplementation

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BACKGROUND and OBJECTIVE: The transition period in dairy cows and critical developmental stages in calves, such as birth, weaning, and regrouping, are associated with increased metabolic demands and oxidative stress. Elevated oxidative stress may negatively affect health, disease susceptibility, and productive as well as reproductive performance. Adequate antioxidant

reserves are therefore essential to counteract these effects, with fat-soluble vitamins playing a key role in antioxidant defense. The aim of this study was to evaluate the dynamics of vitamin E, vitamin A, and β-carotene concentrations during these critical periods in dairy cows and calves, and to identify stages where strategic parenteral vitamin supplementation may be beneficial.

MATERIALS and METHODS: The study integrated data from three related experiments. First, 30 dairy cows were enrolled three weeks before calving and their calves were subsequently monitored after birth. Second, 30 calves were followed from 7 days to 2 months of age, including regrouping and housing changes. Third, the vitamin and antioxidant status of cows with clinical metritis was evaluated.Across all three integrated studies, control groups received no vitamin supplementation, whereas experimental groups were parenterally supplemented with β-carotene (Dalmavital), vitamins A, D_3 and E (ADE inj.), or vitamin E with selenium (Vita E Selen). Blood samples were analyzed for a basic biochemical profile, alpha-tocopherol and retinol, β-carotene, selenium, and glutathione peroxidase (GPx); malondialdehyde (MDA) was additionally determined in calves. No supplementation was applied in cows with metritis; this part of the study evaluated vitamin concentrations in clinically healthy cows and cows with clinical metritis. Analyses were performed using standard methods (spectrophotometry, HPLC, AAS). Data were evaluated using descriptive statistics and ANOVA.

RESULTS: Total vitamin concentrations in cows showed a decreasing trend during the transition period. Across the integrated studies, cows supplemented with vitamin preparations before calving maintained higher postpartum vitamin concentrations compared with controls ($P< 0.01$). The highest vitamin concentrations were observed in calves born to cows supplemented with β-carotene before calving. In the secon related experiment, supplementation with β-carotene and vitamin E on day 7 of life resulted in the most pronounced improvement in vitamin status ($P<0.001$, $P<0.01$,$P<0.05$). In the metritis study, cows with clinical metritis showed higher MDA concentrations ($P<0.001$) and lower vitamin A and vitamin E levels ($P< 0.01$) compared with healthy cows.

CONCLUSIONS: Events associated with increased oxidative stress (parturition, weaning, housing changes, and disease) were associated with marked reductions in antioxidant capacity and vitamin concentrations. Strategic vitamin supplementation may help preserve antioxidant status and support overall animal health.

Keywords: Vitamin supplementation, β-carotene, Antioxidant status

Markers of gastrointestinal tract maturation in dairy calves

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BACKGROUND and OBJECTIVE: Appropriate Gastrointestinal Tract (GIT) development is crucial to ensure optimal health, production and welfare of calves post-weaning. Knowledge regarding the changes occurring in the calf's GIT as it matures is limited, and current GIT assessment techniques are indirect or inconsistently implemented. We aim to describe how tissues and digesta within the calf GIT change as calves transition from a pseudo-monogastric to a functional ruminal digestive system by identifying gastrointestinal section-specific changes in tissue metabolism.

MATERIALS and METHODS: Fifteen Holstein-Friesan male calves, from a single unit, were housed in individual pens allowing nose-to-nose contact. The diet consisted in 3 L of milk powder fed at 150 g/L twice a day. Water, straw and concentrate feed were provided ad libitum. At 3 (pre-weaning), 6 (weaning) and 12 (post-weaning) weeks-old, 5 calves were euthanised and mucosa samples were collected from the GIT (rumen, abomasum, duodenum, jejunum and caecum). Total RNA was extracted and bulk Illumina RNA sequencing performed on all the samples, with transcripts counts analysed using the packages DeSeq2 and Mixomics on R software version 4.4.3.

RESULTS: The heatmap of the sample-to-sample distance showed high proximity between samples from the same organ, independent of the time of sampling, for the rumen, abomasum and caecum samples. Partial Least Squares Discriminant Analysis (PLS-DA) showed complete discrimination of the samples over the different time points in the rumen, abomasum and caecum. When abomasum

mucosa was compared between pre-weaning and post-weaning, 111 and 48 gene transcripts were found to be significantly downregulated and upregulated, respectively (false discovery rate < 5%). Over-representation analysis using Gene Ontology identified 12 significantly down regulated pathways between the two time points. A third (4/12) were linked to G-protein-coupled receptors (GPCRs), described in human as orchestrating digestion (secretion, motility, transport), controlling disease processes (diseases of motility, secretion, inflammation, pain), and regulating growth and repair. Other pathways include gastric acid secretion, serine hydrolase activity, and pathways associated with digestive obstructive disorders

CONCLUSIONS: The preliminary rumen, abomasum and caecum results identified candidate organ specific metabolic markers of weaning potential. The abomasum differential expression analysis was indicative of significant pathway changes, reflecting the profound modifications in calf digestive physiology when transitioning from a pseudo-monogastric to a ruminant digestion. Further studies will aim to characterise the digesta environment.

Keywords: Weaning, calf, nutrition, transcriptomics, metabolic markers

Effects of rumen protected amino acids on blood variables reflecting inflammation and liver function in fresh dairy cows

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BACKGROUND and OBJECTIVE: The goal of this experiment was to establish the effect of supplementation of the rumen protected amino acids (RPAA) Met and Lys (Adisseo) to the diet of fresh cows on blood variables reflecting inflammation and liver function.

MATERIALS and METHODS: Primiparous (n=40) and multiparous (n=58) Holstein cows were blocked by calving date and lactation number and within blocks assigned randomly to 1 of 4 treatments beginning at calving. During the far-off and close-up dry periods, all cows received the same diets. Treatments were as follows: 1) Control (CON), basal diet AA supply without RPAA, 45 g/d of Met and 137 g/d of Lys (3.06 Lys: Met), 2) MET, supplemental Met, 53 g/d Met and 137 g/d Lys (2.61 Lys: Met), 3) ML, supplemental Met and Lys, 53 g/d Met and 162 g/d Lys (3.04 Lys: Met), and 4) HML, high supplemental Met plus Lys, 62 g/d Met and 162 g/d Lys (2.61 Lys: Met). Cows were on experiment from 24 d before expected calving and treatments continued from calving until 63 d post-calving. Data were analyzed using a randomized block design with the MIXED procedure of SAS (SAS Institute Inc.). The statistical model included treatment, parity, day, and their interactions.

RESULTS: The DMI was 18.0, 19.1, 19.9, and 19.1 kg/d and energy-corrected milk was 34.7, 36.5, 38.6, and 35.7 kg for CON, M, ML, and HML respectively. Blood was sampled on d -10, +1, +7, +14, +28 and +63. Cows fed MET had higher glucose concentration than CON (3.79±0.06 vs. 3.61±0.06, respectively, P=0.03). MET had lower BHB, urea and creatinine concentrations than CON (0.69±0.05 vs. 0.89±0.05, P=0.004; 4.16±0.02 vs. 5.27±0.02, P<0.0001; 90±1.3 vs. 93.60±1.29, P=0.05; respectively). HML had lower NEFA, ROM and ceruloplasmin than CON (0.43±0.03 vs. 0.57±0.03, P=0.003; 16.99±0.38 vs. 18.13±0.39, P=0.02; 2.52±0.05 vs. 2.70±0.05, P=0.01; respectively). ML had higher albumin, cholesterol, paraoxonase, β-carotene, and tocopherol than CON (36.81±0.29 vs. 35.61±0.29, P=0.003; 4.49±0.09 vs. 3.71±0.09, P<0.0001; 73.70±1.37 vs. 68.74±0.1.37, P=0.01; 0.38±0.02 vs. 0.26±0.02, P<0.0001; 3.71±0.12 vs. 3.24±0.12, P=0.004 respectively). ML had the highest liver function index (LFI) than CON, MET, and HML (3.33±0.47 vs. 0.69±0.48, 1.37±0.48 and 1.67±0.47, P=0.001).

CONCLUSIONS: Addition of RPMet and RPLys to the fresh cow diet had a positive effect to prevent inflammation and support liver function.

Keywords: methionine, lysine, inflammation, transition cow

The effects of purified lignin supplementation on growth performance, inflammation, antioxidant status, and hematological parameters in pre-weaning simmental calves

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BACKGROUND and OBJECTIVE: The use of plant-derived polyphenolic compounds as functional feed additives has gained increasing attention due to their potential roles in modulating oxidative stress and inflammatory responses in livestock. Among these, lignin—a complex phenolic polymer—has emerged as a promising yet underexplored candidate in ruminant nutrition. This study aimed to comprehensively evaluate the effects of sulfur-free purified lignin supplementation on growth performance, antioxidant status, and inflammatory responses in pre-weaning Simmental calves. Sixty one-day-old calves were randomly assigned to two groups (n = 30 per group) and housed individually in hutches with ad libitum access to calf starter and water. From day 2 until weaning at day 63, calves in the treatment group received 10 g/head/day of purified lignin incorporated into the milk replacer, whereas the control group received no additive. Body weight was recorded weekly, and blood samples were collected on days 30 and 63 to assess hematological, antioxidant, and inflammatory parameters.

MATERIALS and METHODS: Data were tested for normality and analyzed using the PROC GLIMMIX procedure of SAS (version 9.4). The statistical model included treatment, sex, time, and their interactions as fixed effects, with calf nested within treatment included as a random effect. Statistical significance was declared at $P < 0.05$, and trends at $P < 0.10$. Results are presented as least squares means ± standard errors.

RESULTS: Lignin supplementation did not significantly affect growth performance parameters, average daily gain, feed intake, or feed conversion ratio ($P > 0.05$). Notably, however, male calves in the lignin group exhibited a consistent numerical increase in total body weight gain, suggesting a potential sex-specific response pattern. Most antioxidant indicators, including total antioxidant capacity, superoxide dismutase, and malondialdehyde, as well as interferon-gamma and heat shock protein 70, were not significantly influenced by treatment ($P > 0.05$). In contrast, lignin supplementation elicited clear biological responses, significantly reducing tumor necrosis factor-alpha (TNF-α) and cortisol concentrations while enhancing glutathione peroxidase (GSH-Px) activity by day 63 ($P < 0.05$).

CONCLUSIONS: In conclusion, purified lignin supplementation at 10 g/head/day did not significantly influence growth performance but demonstrated targeted physiological effects on key stress- and inflammation-related pathways. The concurrent reduction in TNF-α and cortisol, together with increased GSH-Px activity, highlights the potential of lignin to support redox balance and attenuate systemic stress in early-life calves. These findings position purified lignin as a novel and biologically active functional feed additive, warranting further investigation into its mechanisms of action and potential applications in calf nutrition.

Keywords: Purified lignin, Simental calves, Oxidative stress, Polyphenolic compounds

Comparison of an oral solanum glaucophyllum/calcium bolus or calcium boluses on periparturient calcium concentration, metabolic diseases, milk production, cull rate and rumination in dairy cows

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BACKGROUND and OBJECTIVE: Clinical hypocalcemia or milk fever has been greatly reduced by anionic or phosphorus binder prepartum diets. However, subclinical hypocalcemia (blood Ca < 2.0 mM), and dyscalcemia (blood Ca < 2.2 mM at 4 DIM) remain a concern. Solanum glaucophyllum (SG) contains β-glycosides of 1,25-dihydroxyvitamin D3 in its leaves. Rumen bacteria digest the glycosides

liberating bioactive 1,25-dihydroxyvitamin D3 activating transcellular intestinal Ca transport. The efficacy of oral boluses comprised of SG leaf and Ca salts in reducing hypocalcemia and health problems in cows was tested against a commercial oral Ca salt bolus.

MATERIALS and METHODS: Multiparous Holstein cows were matched by parity and previous milk yield. One cow from each of 211 pairings received two SG / calcium boluses (SG-bol) at calving. The second cow in the pair received three doses of a Ca bolus (Ca-bol), administered at calving, 12 - 24 and 24 - 36 hours after calving. Cows were fed an anionic diet. Blood was collected from a subset of 3rd parity cows to determine blood Ca concentrations at timed intervals after calving. Daily milk production, health events, and rumination monitor health alerts based on decreased rumination, were recorded. Treatment effects on plasma Ca concentrations and milk yield were evaluated using a mixed model for repeated measures. Chi-square (or Fisher's exact test) compared treatment effects on health data.

RESULTS: SG-bol cows had higher (P< 0.02) blood Ca concentrations at 36, 48 and 72 hours after calving. SG-bol cows plasma Ca concentration averaged 2.2 mM at 36 hours and 2.43 mM at 96 hrs. Ca-bol cows average plasma Ca concentration was < 2.0 mM at 48 hours and was 2.26 mM at 96 hrs. Rumination Alerts and ketosis incidence were significantly reduced (P< 0.05). SG-bol cows were less likely to die or be culled in the 1st 60 DIM, exhibited less diarrhea, and fewer cases of milk fever than in the Ca-bol cows (P<0.10). There was no significant treatment effect on milk production, the incidence of retained placenta and metritis in the cows.

CONCLUSIONS: Both treatment boluses supplied soluble Ca salts permitting rapid paracellular absorption of a portion of the administered Ca, improving plasma Ca for several hours. The SG leaf glycosides of 1,25-dihydroxyvitamin D3 also activate transcellular Ca absorption which is much more efficient than paracellular Ca absorption and is sustained for 96 hours. Improved blood Ca concentration in early lactation was likely responsible for improvements in health of the SG-Bol cows.

Keywords: Solanum glaucophyllum, hypocalcemia, 1,25-dihydroxyvitamin D, metabolic disease

Effects of rumen-protected branched-chain amino acid supplementation on performance and metabolic health in transition dairy cows

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BACKGROUND and OBJECTIVE: Transition dairy cows undergo profound metabolic adaptation, characterized by negative energy balance as dry matter intake (DMI) declines while lactational demands escalate, contributing to hepatic lipid accumulation, oxidative stress, and periparturient immunosuppression. These changes heighten disease risk and impair productivity. Rumen-protected branched-chain amino acids (RP-BCAA) may mitigate these perturbations by supporting anabolic signaling and improving antioxidant capacity. This study evaluates the effects of RP-BCAA supplementation on production performance, metabolic status, antioxidant capacity, and immune response in transition dairy cows.

MATERIALS and METHODS: A total of thirty-eight dairy cows and heifers of Holstein and Simmental breeds were randomly assigned to two treatment groups: one group received the control diet, and the other was supplemented with RP-BCAA during the transition period (3 weeks before and after parturition). The RP-BCAA group received 185 g/day of BCAA during the prepartum period, while postpartum cows were supplemented with 551 g/day, mixed with molasses. Blood samples were collected at three time points: three weeks before calving, on the day of calving, and three weeks postpartum. Milk samples were collected on the day of calving and on days 7, 14, and 21 post-calving. Metabolic profile, antioxidant biomarkers, and immune parameters were assessed in blood and milk samples. Feed intake, milk yield, and body condition score (BCS) were monitored throughout the study. Data were analyzed by three-way ANOVA including the effects of group, breed and parity.

RESULTS: RP-BCAA supplementation increased DMI ($P < 0.05$) both before and after calving while stabilizing BCS ($P < 0.05$). Milk yield did not differ between groups overall. However, a significant group × breed interaction indicated that milk yield increased in supplemented Simmental cows ($P < 0.05$) but not in Holstein cows. Supplemented cows exhibited a lower blood concentration of non-esterified fatty acids ($P < 0.05$). Supplementation also increased glutathione peroxidase activity and intestinal fatty acid-binding protein ($P < 0.05$) and reduced glucose-dependent insulinotropic polypeptide ($P < 0.05$) in blood postpartum. In milk, immunoglobulin A and lactoferrin were numerically higher, and lactate dehydrogenase was numerically lower in supplemented cows, but no milk marker differed significantly between groups.

CONCLUSIONS: RP-BCAA supplementation during the transition period improved feed intake, body condition and antioxidant capacity in dairy cows. Its effect on milk yield was breed-dependent, and no significant benefit was detected for immune or mammary markers in milk. Further research is necessary to determine whether the benefits extend beyond the transition period and influence lactation persistency and reproductive performance.

Keywords: transition cows, BCAA, dry matter intake, antioxidant capacity, metabolic status, breed

PARASITIC DISEASES

Nationwide herd-level seroprevalence of Neospora caninum in dairy herds in Türkiye based on bulk tank milk ELISA and identification of associated risk factors

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BACKGROUND and OBJECTIVE: *Neospora caninum* is a major infectious cause of bovine abortion, leading to substantial reproductive and economic losses in dairy production systems worldwide. This study aimed to determine the nationwide herd-level prevalence of *N. caninum* in Türkiye and to identify farm-level risk factors associated with infection.

MATERIALS and METHODS: Bulk-tank milk samples were collected from 466 dairy farms across 40 provinces representing seven geographical regions of Türkiye between June and November 2023. Antibodies against *N. caninum* were detected using a commercial competitive ELISA. Farm-level data on management practices, water sources, dog presence and feeding practices, pasture use, and abortion history were obtained through a structured questionnaire. Univariable and multivariable statistical analyses were performed to identify associations with herd-level seropositivity.

RESULTS: Overall herd-level seropositivity was 27.47% (128/466; 95% CI: 23.4– 31.7%), with significant regional variation ($p = 0.03394$). The highest prevalence was observed in the Marmara (39.2%) and Southeastern Anatolia (37.5%) regions, while the lowest prevalence occurred in the Mediterranean region (16.7%). Herd seropositivity was strongly associated with reported abortion history, with seropositive herds having markedly higher odds of abortion (OR = 29.4; 95% CI: 16.5–52.9; $p < 0.0001$). Herds using groundwater had a significantly higher likelihood of seropositivity compared with those using municipal water ($p = 0.002$), an association that remained significant after multivariable adjustment. Although dogs were present on all farms, feeding practices varied regionally, with most farms providing leftover or raw animal-derived feed, while commercial dog feed use was largely restricted to western Türkiye.

CONCLUSIONS: This study provides the first nationwide herd-level assessment of *N. caninum* exposure in Turkish dairy farms using bulk-tank milk ELISA and demonstrates widespread infection associated with key management and environmental risk factors. These findings support the implementation of targeted control strategies focusing on improved biosecurity, water hygiene, and routine herd-level monitoring to reduce vertical transmission and long-term infection pressure.

Keywords: abortion, bulk-tank milk ELISA, dairy cattle, herd-level prevalence, Neospora caninum, risk factors

Decrease in serum pepsinogen level after housing in heifers: Does this affect its value for the implementation of a targeted treatment?

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BACKGROUND and OBJECTIVE: Serum pepsinogen level (SPL) is often used to assess Ostertagia ostertagi larval hypobiosis infection in heifers at the end of their first grazing season, in autumn and winter. The temporal variation of SPL in untreated animals at this period is however poorly documented. A decrease of SPL after housing can yet be suspected, as larvae intake stops and the remaining ones enter hypobiosis. The measured SPL during housing could therefore underestimate the infection by O. ostertagi. The objectives of this project were therefore (i) to better describe the kinetic pattern of SPL in autumn and winter in the absence of treatment, and (ii) to assess whether measuring SPL at housing, the most convenient time for sampling, remains a pertinent targeted-treatment indicator.

MATERIALS and METHODS: Blood samples were collected regularly from 152 heifers across 10 farms (mid-altitude mountains, Central France) to measure SPL: monthly from late August until housing, at housing and then every 14 days for 2 months and again monthly until turnout. After pairing based on age and daily weight gain (DWG) on each farm, one heifer in each pair was randomly dewormed at housing (oxfendazole per os or ivermectin subcutaneously).
SPL variations in untreated heifers between the different sampling dates were evaluated using a first linear mixed model. A second linear mixed model was used to assess the link between SPL measured at housing and the effect of treatment on DWG by comparing treated and untreated heifers.

RESULTS: In untreated heifers, SPL changed significantly ($p < 0.0001$) over time between September and March, with four distinct phases: (1) a peak at the end of the grazing period, (2) a decline that could begin before housing and reach a minimum value one month after housing, (3) a winter stabilization phase at low levels, and (4) a progressive increase starting from late February. Treated heifers at housing showed significantly greater growth than untreated ones when SPL > 1.5Utyr ($p = 0,001$), but no difference when SPL < 1.5Utyr.

CONCLUSIONS: Early sampling at the end of the grazing period, or at the latest very shortly after housing, is key to measuring SPLs that reflect O. ostertagi-infection level in heifers. Despite the important decline that can begin before housing, SPL measured at housing remains the best predictor of the effect of treatment on growth, and thus a good criterion for targeted treatment.

Keywords: Ostertagia ostertagi, Integrated gastrointestinal nematode management, bovine parasitology, helminth infection

Occurrence of Cryptosporidium, Giardia and Eimeria in Selected Cattle farms of Northern and Central Italy

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BACKGROUND and OBJECTIVE: Cryptosporidium, Giardia and Eimeria are widespread diarrhoea-causing bovine protozoa. Furthermore, cattle-borne Cryptosporidium and Giardia can be zoonotic. This

study has investigated their occurrence and clinical impact in dairy farms of northern and central Italy, along with the presence of potentially zoonotic Cryptosporidium strains.

MATERIALS and METHODS: Overall, 200 faecal samples were collected from calves of ≤ 6 months, belonging to age group (AG) 1 (1 week-1 month; n. 67), AG2 (1-3 months; n. 66), and AG3 (3-6 months; n. 67). Samples were ranked according to a faecal score, i.e. compact (FS=1), pulpy (FS=2), or watery (FS=3) faeces, and tested with a commercial ELISA detecting coproantigens of Cryptosporidium and Giardia, direct immunofluorescence for Cryptosporidium and Giardia, Ziehl-Neelsen stain, sucrose stratification for the detection of Giardia cysts, and faecal flotation (coupled with McMaster's assay when positive) for Eimeria oocysts. Samples positive for Cryptosporidium in at least one test were subjected to PCRs-coupled sequencing specific for two target genes.

RESULTS: In total, 59% of the calves tested positive for Cryptosporidium, i.e. 57%, 16.5%, and 16.5% at the ELISA, immunofluorescence, and Ziehl-Neelsen, respectively. Cryptosporidium was found in 59.7%, 54.5% and 62.7% animals belonging to AG 1, 2, and 3 respectively. Giardia was detected in 47% of the animals, being 39%, 30.5%, and 14% positive by ELISA, immunofluorescence, and sucrose stratification. Eimeria was recorded in 19% of the samples, with oocyst per gram values from <50 to 16,050. Co-infections were detected in 40% of the animals, being the most frequent combination Cryptosporidium + Giardia (26%). Crypt osporidium was the most prevalent protozoon in diarrhoeic calves (FS 2 or 3), i.e. 66 animals had with enteric signs, of which 42 were positive also for Giardia and/or Eimeria. Of the 44 calves with a monospecific infection by Cryptosporidium, 54.5% had diarrhoea with FS 2 or 3 in 36.4% and 18.2% of the animals, respectively. Thirty-five of 118 molecularly examined samples were PCR-positive, with 33 amplicons successfully subjected to sequencing. Of them, 21 were identified as Cryptosporidium parvum with 14 subtypes belonging to the zoonotic subtype IIa.

CONCLUSIONS: Although the coproantigen test results should be interpreted with caution, as they may overestimate infection rates compared to immunofluorescence and PCR, these findings highlight the epidemiological and clinical relevance of enteric protozoa in cattle and confirm the frequent occurrence of zoonotic C. parvum. The control of intestinal protozoa should rely on strict hygiene, supported by effective antiparasitic treatment and vaccination against C. parvum.

Keywords: Cattle, protozoa, diarrhoea

Dynamics of Cryptosporidium parvum Gp40 antibodies in colostrum and transition milk of vaccinated dairy cows

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BACKGROUND and OBJECTIVE: Cryptosporidiosis is a major cause of neonatal calf diarrhea. Vaccinating pregnant cows and managing colostrum and transition milk enhance passive transfer of antibodies against the Gp40 *C. parvum* glycoprotein, improving immunity early in life. However, the dynamics of antibody concentration and total antibody availability across the first milkings, and the influence of factors such as parity, milking time, or revaccination to calving interval (RCI), are not fully understood. The objective of this study was to evaluate Gp40 antibody kinetics during the first six milkings and assess factors affecting antibody concentration, total antibody output, and their relationship with milk yield and Brix values.

MATERIALS and METHODS: The study included 109 cows vaccinated with a commercially available C. parvum vaccine (Bovilis® Cryptium®, MSD) according to standard protocol: 26 primiparous, 28 second parity, and 55 in third or later lactation. A total of 565 individual samples were collected from first to sixth milkings. Gp40-antibody concentration (log_2 ELISA), Brix percentage, and milk yield were measured at each milking. A composite variable ("antibodies × milk yield") was calculated to estimate total antibody availability. Because variables were non normally distributed, non parametric statistical analyses were applied (Kruskal Wallis, Wilcoxon, Spearman).

RESULTS: Gp40-antibody concentration showed a significant negative correlation with milk yield (ρ=−0.48; p<0.0001), indicating a dilution effect. Primiparous cows had significantly higher Gp40-antibody concentrations than multiparous cows (p<0.05), likely due to lower production, but no parity differences were found for the composite antibody yield variable (p=0.33). Milking time did not influence antibody concentration (p=0.52), but morning milkings produced more milk (10.4 vs. 6.9 L; p<0.0001), resulting in higher total antibody output (p<0.0001). A longer RCI had a strong positive effect, with cows revaccinated more than 29 days before calving showing the highest total antibody amounts (p<0.0001). In transition milk, total antibody output gradually declined across milkings but remained adequate until the fifth milking, independent of parity. Interestingly, when correcting for liters, only 18% of the Gp40-antibody concentration was present in colostrum, total gp40 Ab presence staying stable until sixth milking. Brix values were negatively correlated with yield (ρ=−0.52; p<0.0001) and positively associated with Gp40-antibody concentrations in transition milk.

CONCLUSIONS: Vaccination produced comparable total Gp40-antibody output across parities, validating its use in properly vaccinated primiparous cows. Transition milk from the second to fifth milking provides the highest antibody availability for feeding calves. Brix measurement appears to be a practical tool for rapid assessment of transition milk pool quality.

Keywords: Cryptosporidiosis, Gp40 antibodies, colostrum, transition milk, dairy cows

Galba schirazensis is an alternative intermediate host of {Calicophoron daubneyi}

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BACKGROUND and OBJECTIVE: Rumen fluke infection is an emerging parasitic disease in Europe. The predominant species, *Calicophoron (C.) daubneyi*, shares its intermediate host in most European countries with the liver fluke *Fasciola (F.) hepatica*, namely the mud snail *Galba (G.) truncatula*. Recent experimental infection studies have demonstrated that *G. schirazensis*, a snail species closely related to *G. truncatula*, may also serve as an intermediate host for *C. daubneyi*, but appears to play little to no role in the transmission of *F. hepatica*. The aim of the present study was to investigate whether *C. daubneyi* metacercariae derived from experimentally infected *G. schirazensis* are capable of establishing a patent infection in sheep.

MATERIALS and METHODS: Three helminth-free, indoor-reared White Bavarian Alpine ewe lambs aged 8,5-9 months and originating from the university's sheep flock were orally inoculated with 150 *C. daubneyi* metacercariae each. The metacercariae had been produced under laboratory conditions from experimentally infected *G. schirazensis*. From day 65 post inoculation (p.i.), fecal samples were collected every three days and examined for rumen fluke eggs using the Flukefinder® technique. All sheep were slaughtered on day 140 p.i., followed by examination of the forestomachs for adult flukes. All experimental procedures were approved by the Government of Upper Bavaria (reference number ROB55.2–2532.Vet_02–24-74).

RESULTS: All three sheep began shedding *C. daubneyi* eggs at 72, 78 and 96 days post inoculation, respectively. At slaughter, 13, 40, and 77 adult rumen flukes were recovered from the rumen of the three animals, respectively.

CONCLUSIONS: The results demonstrate that *G. schirazensis* is a competent intermediate host for *C. daubneyi*. In regions where this snail species occurs (e.g., France, Spain, Portugal), it may facilitate transmission of rumen fluke under warmer and less humid environmental conditions than *G. truncatula*. The presence of *G. schirazensis* may therefore have important implications for the epidemiology of *C. daubneyi* in Europe.

Keywords: Rumen fluke, Epidemiology, Paramphistomidosis, Fasciola hepatica

Quantitative and qualitative characteristics of stephanofilariasis lesions in the bovine udder

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BACKGROUND and OBJECTIVE: Stephanofilariasis, caused by nematodes of the genus Stephanofilaria spp., is characterized by ulcerative lesions on udder of lactating cows. Infrared thermography is a non-invasive tool used to detect pathophysiological changes, through thermal variations, caused by inflammation or infection. Somatic cell count (SCC) is widely used as an indicator of subclinical mastitis, with values above 200,000 cells/mL commonly considered suggestive of inflammatory processes. This study aimed to describe the quantitative characteristics of ulcerative udder lesions in cows with stephanofilariasis, including lesion morphometry (cm²), udder skin surface temperature, thermal asymmetry, and SCC. The study also characterized the qualitative macroscopic features of the lesions.

MATERIALS and METHODS: Twenty-nine lactating Holstein cows from a commercial farm, exhibiting ulcerative lesions on the midline of the udder and testing positive on direct sediment examination, were selected. Thermographic images of the left (TL) and right (TR) front quarters of the udder were captured, and maximum temperatures were subsequently corrected based on ambient temperature and relative humidity. SCC data was obtained from the farm's routine monitoring system, and morphometry was analysed using ImageJ. TL, TR and morphometry were summarized as mean ± standard deviation, whereas thermal asymmetry (TempDiff = |TL−TR|) and SCC were summarized as median and interquartile range (IQR).

RESULTS: Qualitatively, lesions showed typical features of stephanofilariasis: serosanguineous exudation (68.97%), irregular borders (68.97%), ulceration (96.55%), nodular form (3.45%), and crust formation (82.76%). Although all lesions were located along the midline of udder, their specific position varied: central (41.38%), left (34.48%) and right (24.4%). Due to the wide variation in lesion size (10.25 - 138.16 cm²), they were separated into two groups: medium (10-24 cm²) and large (>25 cm²). Most of the lesions were large (86.21%), with a mean SCC of 291,500 cells/mL. Udder skin temperatures remained within expected physiological limits. TL had a mean temperature of 37.93 ± 0.76 °C, while TR had a mean of 37.78 ± 0.66 °C. TempDiff had a median of 0.30 °C (IQR: 0.10–0.50), indicating localized inflammatory activity. Median SCC was 65,000 cells/mL and maximum values exceeding 1.9 million cells/mL, reflecting substantial variability in the subclinical inflammatory state among affected cows. It is important highlight that higher TempDiff it was observed in lesions with irregular borders (0.185 ± 0.129°C) those with regular borders.

CONCLUSIONS: It is possible to conclude that most large lesions positive for stephanofilariasis presenting exudate and irregular borders have higher SCC and TempDiff, probably because of the persisting inflammation process.

Keywords: Stephanofilaria spp., Udder lesions, Dairy cattle, SCC, Thermography, Ulceration

First identification of Cryptosporidium parvum virus 1 (CSpV1) in different subtypes of Cryptosporidium parvum from diarrheic calves, lambs and goat kids in France

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BACKGROUND and OBJECTIVE: Cryptosporidium spp. remain a major cause of waterborne diarrhea and illness in developing countries and represent a significant burden to farmers worldwide. Cryptosporidium parvum virus 1 (CSpV1), of the genus Cryspovirus, was first reported to be present in the cytoplasm of C. parvum in 1997. Full-length genome sequences have been obtained from C. parvum from Iowa (Iowa), Kansas (KSU) and China. We aimed at characterizing the genome of CSpV1 from France and used sequence analysis from Cryptosporidium isolates to explore whether CSpV1 genome diversity varies over time, with geographical sampling location, with C. parvum genetic diversity, or with ruminant host species.

MATERIALS and METHODS: A total of 123 fecal samples of cattle, sheep and goats were collected from 17 different French departments (57 diseased animal fecal samples and 66 healthy animal fecal samples). Samples were screened for Cryptosporidium spp. presence with immunofluorescence technique. Nested PCR of the small subunit ribosomal RNA (rRNA) gene, followed by restriction fragment length polymorphism (PCR-RFLP) and sequence analyses were used to identify Cryptosporidium species. Then, C. parvum was further subtyped by sequencing the highly polymorphic 60 kDa glycoprotein (gp60) gene. qPCR and sequence analysis were used to detect CSpV1 RNA virus fragments.

RESULTS: Subtyping analysis of the C. parvum isolates revealed the presence of two zoonotic subtypes families IIa and IId. Sequence analysis of CSpV1 revealed that all CSpV1 from France, regardless of the subtype of C. parvum (IIaA15G2R1, IIaA17G2R1 and IIdA18G1R1) are more closely related to CSpV1 from Turkey, and cluster on a distinct branch from CSpV1 collected from C. parvum subtype IIaA15G2R1 from Asia and North America. We also found that samples collected on a given year or successive years in given location are more likely to host the same subtype of C. parvum and the same CSpV1 strain. Yet, there is no distinct clustering of CSpV1 per French department or ruminants, probably due to trade, and transmission of C. parvum among host species.

CONCLUSIONS: All together, these results provide insightful information regarding CSpV1 evolution and suggest the virus might be used as an epidemiological tracer for C. parvum. Future studies need to investigate CSpV1 role in C. parvum virulence and on subtype ability to infect different species.

Keywords: Cryptosporidium parvum, cryspovirus, calves, lambs, kids, France

Molecular characterization and zoonotic risk assessment of Cryptosporidium spp. and Giardia duodenalis in ruminants and camels in Tunisia

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BACKGROUND and OBJECTIVE: *Cryptosporidium* spp. and *Giardia duodenalis* are protozoan parasites infecting both humans and animals. They are recognized as major causes of neonatal diarrhea in young ruminants and camels. In Tunisia, the epidemiology of these infections remains poorly documented.

MATERIALS and METHODS: The aim of this study was to analyze and characterize *Cryptosporidium* spp. and *Giardia duodenalis* in calves, lambs, goat kids and camels across different farms in Tunisia. Between October 2024 and February 2026, and during three sampling periods, a total of 348 fecal samples were collected from calves (N= 231), lambs (N= 63), goat kids (N= 34) and camels (N= 20). Fecal samples were screened for the presence of *Cryptosporidium* spp. oocysts and/or *Giardia duodenalis* cysts using direct immunofluorescence test (IFD). Samples that tested positives by IFD, were further screened by PCR targeting specific genes: 18S and gp60 for *Cryptosporidium* spp., and TPI for *Giardia duodenalis*. PCR products were analyzed by Sanger sequencing to determine Cryptosporidium species and the subtypes, as well as Giardia duodenalis assemblages.

RESULTS: The prevalence of infection by *Cryptosporidium* spp. was 43% (100/231) in calves, 38% (24/63) in lambs, 38% (13/34) in goat kids and 20% (4/20) in camels. Five species were identified, with *C. parvum*, as the most prevalent, followed by *C. bovis*, *C. meleagridis*, *C. xiaoi* and *C. ryanae*, mainly identified in goat kids and lambs. Subtypes of *C. parvum* belonged to two families IIa and IId, with IIa15G2R1 subtype as the most predominant. *Giardia duodenalis* was detected in calves (63/231; 27.3%), lambs (18/63; 28.6%), goat kids (7/34; 20%) and camels (2/20; 10%). Assemblage A, B and E were identified in both lambs and calves, whereas only the assemblage E was detected in camels and goat kids.

CONCLUSIONS: In conclusion, *Cryptosporidium parvum* (IIa15G2R1 subtype) is the main identified specie in neonatal diarrhea in farm animals in Tunisia. The high prevalence of infection in all animal species suggests that cryptosporidiosis is a significant health-threatening disease. Three assemblages of *Giardia duodenalis*, A, B and E, were identified in calves and lambs. Assemblage E is commonly associated with ruminants, whereas assemblage A and B are zoonotic. The detection of zoonotic *C. parvum* subtypes, particularly in calves and lambs, along with the presence of zoonotic *G. duodenalis* assemblage (A and B) highlights an important public health concern. These findings suggest that farm animals may serve as a source of direct human infection and/or environmental contamination.

Keywords: Cryptosporidium spp., Giardia duodenalis, ruminants, camels, PCR

Association of Neospora caninum seropositivity with reproductive failure in a sheep flock: results of an ongoing study

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BACKGROUND and OBJECTIVE: Ovine neosporosis is a documented cause of reproductive loss, and serology is frequently used for herd-level screening. However, clinical significance of seropositivity requires further characterization under field conditions. This study aimed to a) investigate the association between *Neospora caninum* seropositivity and reproductive failure in naturally infected sheep, and b) define the diagnostic agreement between a competitive ELISA (cELISA) and an Indirect Fluorescent Antibody Test (IFAT).

MATERIALS and METHODS: The study was conducted during the breeding season on a sheep farm with a history of abortions. *Neospora caninum* DNA was identified as the sole agent among ten abortifacient pathogens tested. Pregnancy diagnosis was performed using ultrasonography 30 days post-mating, with follow-up examinations at 60 and 90 days afterward. Serum

samples were collected from 86 ewes at three months post-mating and were tested for anti-*N. caninum* IgG antibodies using a commercial cELISA. A subset of 77 serum samples was also tested by IFAT for anti-*N. caninum* and anti-*Toxoplasma gondii* IgG antibodies (cut-off ≥1: 100). Statistical analysis was performed using Chi-squared, Z-tests ($p<0.05$), and Cohen's Kappa using MedCalc Statistical Software version 23.5.1.

RESULTS: A total of 22 ewes encountered reproductive failure, comprising embryonic deaths in 13 cases, abortions in five, and no pregnancy detection in four, while 64 ewes did not. High *N. caninum* seropositivity was recorded, with 80.2% (69/86; 95% CI: 70.6%-87.3%) of ewes positive by cELISA and 74.0% (57/77; 95% CI: 63.2%-82.5%) positive by IFAT (titres 1/50 to >1/3200). For the 77 samples tested with both methods, a substantial agreement was established (kappa=0.6; total agreement: 87.0%). Anti-*T. gondii* IgG antibodies were detected in 33.7% (26/77, 95% CI: 23.2%-44.3%) of ewes, though evidence of serological cross-reactivity was low (3.9%, 3/77, 95% CI: 1.3-10.8%). A statistically significant association was found between *N. caninum* seropositivity and reproductive failure (x^2 = 7.2835; p=0.007). Furthermore, significantly lower mean cELISA S/N% values, indicative of higher antibody levels, were observed in ewes with reproductive failure (mean: 8.81 ± 5.90) compared to those without (mean: 29.78 ± 28.60; Z = -2.764, p = 0.006).

CONCLUSIONS: Our findings demonstrate a significant association between *N. caninum* seropositivity and high antibody titres with reproductive failure in this naturally infected flock. This study supports the use of cELISA as a screening tool. IFAT should be employed to strengthen the cELISA results, mitigate potential cross-reactivity and determine specific antibody titres. This quantitative information is essential for informing targeted management decisions, such as selective culling or breeding strategies in sheep flocks.

Keywords: Neospora caninum, Sheep, reproduction failure, ELISA, IFAT

PHARMACOLOGY AND THERAPEUTICS

Quantitative and qualitative benchmarking of antiparasitic drugs in Dutch dairy cows

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BACKGROUND and OBJECTIVE: Cattle are frequently challenged by parasitic infections and infestations which results in a range of more or less severe clinical symptoms and may also impact carcass weight, milk production and thus considerable economic profitability. To avoid this, the use of antiparasitic drug treatments is deemed necessary to control these infections and infestations, in particular in areas with higher animal densities and in case preventive measures appear to be not effective. The use of anthelminthic drugs resulted in the selection of resistance in e.g. helminth populations, increasingly limiting the efficacy of these treatments. Treatments with these drugs may also result in the excretion of active substances or their active metabolites that exert undesired biocidal side-effects on non-target organisms. This is considered a challenge for an environmentally sustainable livestock sector. While all drugs used in food producing animals are subjected to an environmental impact assessment, farmers and veterinarians are usually unaware of the outcome and therefore not able to use this for treatment decisions. Transparent monitoring and benchmarking on antibiotic use decreased and improved the quality of antibiotic use in The Netherlands and we considered that a similar system would be effective for antiparasitic drugs. We decided to design this system and tested this with real data.

MATERIALS and METHODS: We used data on the sales of antiparasitic drugs of 273 cattle farms and calculated Daily Defined Dosages per farm and drug (DDDAF and DDDAS) corrected for age and herd composition. Furthermore, we characterized the environmental impact of all used drugs using a classification on percentage of bioavailability, compound and active metabolite excretion, their environmental impact, and their persistence.

RESULTS: Antiparasitic sales appears to fluctuate throughout the year. Many farms bought no or only low amounts of these drugs and others had a DDDAF above 200. Furthermore, the classification system indicates considerable differences in the environmental impact between antiparasitic drugs for similar indications that were in particular due to the route of administration (e.g. injections or pour-on) and the result of the drug metabolism.

CONCLUSIONS: The large variation in use between farms and the differences in environmental impact between drugs used for similar indications highlight the complexity of antiparasitic use in dairy cattle. Benchmarking combined with environmental classification may provide a basis for developing stewardship strategies, but further work is needed to understand drivers of use and how such information can support sustainable decision-making.

Keywords: antiparasitic drugs, sustainability, environment, benchmarking, dairy sector

From systemic circulation to male gametes: Comparative pharmacokinetics of ivermectin in plasma, seminal plasma, and spermatozoa of rams

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BACKGROUND and OBJECTIVE: Ivermectin is a lipophilic anthelmintic with high systemic efficacy. Understanding how it transitions from blood circulation into male reproductive compartments is essential for assessing its impact on reproductive health. This study aimed to compare the pharmacokinetic profiles of ivermectin in plasma, seminal plasma, and sperm cells of rams.

MATERIALS and METHODS: A single subcutaneous dose of ivermectin (0.2 mg/kg) was administered to seven rams. Serial blood and semen samples were collected over 30 days. Sperm cells were separated from seminal plasma. Ivermectin concentrations in plasma, seminal plasma, and sperm cells were analysed via HPLC-FLD.

RESULTS: Statistical analysis revealed significant differences ($P < 0.05$) among all three compartments for some pharmacokinetic parameters. The peak concentrations (Cmax) and systemic exposure (AUC0-∞) were reached in blood plasma, while notably higher values were observed in spermatozoa than in seminal plasma. The elimination and distribution parameters (Vz/F and Cl/F) were highest in seminal plasma, with the lowest values recorded in blood plasma ($P < 0.05$). The ivermectin's last observed time (Tlast) was longest in plasma and shortest in seminal plasma. Although not statistically significant ($P > 0.05$), MRT values followed a descending order from spermatozoa to seminal plasma and blood plasma.

CONCLUSIONS: This study demonstrates that ivermectin passes from systemic circulation into the semen of rams. Specifically, sperm cells were found to retain ivermectin at a higher rate than seminal plasma, which can be attributed to ivermectin's lipophilic nature and its affinity for sperm cell membrane structures. The data obtained provides a basis for studies evaluating the potential effects of ivermectin on reproductive health and semen quality.

Keywords: Ivermectin, ram, plasma, seminal plasma, sperm cell, pharmacokinetic

Synthetic mRNA delivered into the bovine lung or mammary gland induces expression of encoded protein: A proof-of-concept study towards the development of a novel therapeutic approach

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BACKGROUND and OBJECTIVE: Synthetic mRNA applied directly to tissues can induce expression of proteins (e.g., neutralizing antibodies or antimicrobial peptides) to prevent or treat disease at the site of infection. Synthetic mRNA has been successfully delivered via nebulization to the lungs of lab animals or through aerosol application to the female reproductive tract of sheep, but to date, a similar approach has not been reported in cattle. The objective of the current study was to deliver synthetic mRNA into the lungs and mammary glands of lactating dairy cows and confirm and quantify production of the encoded protein.

MATERIALS and METHODS: Healthy adult Holstein cows (n = 6) in late lactation were randomly assigned to receive either synthetic mRNA encoding the luminescent biomarker molecule NanoLuc® delivered via nebulization to lungs with a polymeric carrier (n = 5), or water (negative control, n = 1). Individual quarters of cows were assigned to receive intramammary infusion of synthetic NanoLuc mRNA via teat cannula with a lipid carrier (n = 10 quarters), or 0.9% NaCl (negative control, n = 9 quarters). Cows were monitored for adverse clinical signs, milk was collected, and a California Mastitis Test (CMT) was performed prior to and 24 h following treatment. Milk samples were frozen at -80°C until protein expression was measured using the NanoGlo® assay (Promega). Euthanasia was performed 24 h post-treatment; lung and mammary tissues were collected and snap frozen for luminescent imaging via an in vivo imaging system (IVIS), or formalin fixed for histological evaluation by a blinded pathologist.

RESULTS: Milk samples collected from mammary quarters treated with synthetic mRNA had relative light unit (RLU) values that ranged from 0.8 - 9.4 x 105, confirming the production of NanoLuc. Imaging of mRNA-treated lung and mammary tissues for luminescence revealed that the average radiance of treated tissue was 10-fold to 1000-fold higher than negative control. Overall, no adverse health outcomes were observed. Histological evaluation of both treated and untreated lung and mammary gland tissue is pending.

CONCLUSIONS: While the current work was limited in scope and these findings are preliminary, the results demonstrate that synthetic mRNA delivered into the lungs and mammary glands of lactating Holstein cows led to the production of the encoded protein within 24 hours after treatment. Administration of mRNA into the bovine lung or mammary gland could be used to induce local expression of proteins to prevent or treat a variety of diseases.

Keywords: dairy cows, udder, mastitis, respiratory

Caudal epidural anesthetic effect of lidocaine with thermo-responsive hydrogel in cattle

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BACKGROUND and OBJECTIVE: Caudal epidural anesthesia is a widely used anesthetic technique in cattle for procedures involving the perineal region. However, the duration of analgesia is often limited when conventional local anesthetics are used, which may be insufficient during prolonged procedure or unexpected intraoperative events. Thermo-responsive hydrogels have recently gained attention as controlled-release drug delivery systems, allowing sustained release of incorporated agents depending on temperature changes.

MATERIALS and METHODS: Ten Holstein cows were randomly assigned to two groups (n=5 per group), with one group receiving 2% lidocaine (Lido group) and the other receiving a thermo-responsive hydrogel containing 2% lidocaine (Gel group). Caudal epidural anesthesia was performed at the first intercoccygeal space (Cd1-2) at a dose of 1 mL per 100 kg body weight. Analgesia was evaluated by needle pin prick of 18 G hypodermic needle using a three-point scoring system (0 = normal response, 1 = reduced response, 2 = complete block) assessed separately on the left and right sides and averaged. Two regions (vulvar and perineal) were evaluated. Analgesic duration was defined as the time from marked analgesia (score ≥ 1.5) to partial recovery (score ≤ 1) and from analgesia onset (score ≥ 1) to complete recovery (score = 0). Statistical analysis was performed using independent samples t-tests.

RESULTS: The duration of analgesia at the vulvar region was significantly loner in the Gel group compared to the Lido group for both definitions of analgesic duration, with significant differences observed in both the duration from marked analgesia to partial recovery and from analgesia onset to complete recovery ($p < 0.01$). At the perineal region, no significant difference was observed between groups for the duration from marked analgesia to partial recovery ($p > 0.05$); however, the duration from analgesia onset to complete recovery was significantly longer in the Gel group compared to the Lido group ($p < 0.01$).

CONCLUSIONS: Thermo-responsive hydrogel significantly prolonged the duration of epidural anesthesia compared to lidocaine, particularly at the vulvar region, where both definitions of analgesic duration showed consistent effect. At the perineal site, no significant difference was observed in the duration from marked analgesia to partial recovery, likely due to the low incidence of complete sensory block. These findings suggest that the prolonged analgesic effect may help ensure animal welfare during procedures with extended duration, such as veterinary surgical training or unexpected intraoperative events.

Keywords: Cattle, Caudal epidural anesthesia, Thermo-responsive hydrogel, Analgesia duration

Pharmacokinetics of inhaled marbofloxacin in plasma, milk, and bronchoalveolar lavage fluid of sheep

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BACKGROUND and OBJECTIVE: Effective antimicrobial therapy depends on achieving adequate drug concentrations at the site of infection. Inhalation may enhance pulmonary drug delivery while reducing systemic exposure. Marbofloxacin is a broad-spectrum fluoroquinolone commonly used parenterally in respiratory infections. This study aimed to compare the plasma, milk, and bronchoalveolar lavage (BAL) pharmacokinetics of marbofloxacin following intravenous and inhalational administration in sheep.

MATERIALS and METHODS: The study was conducted on a total of 12 sheep. Marbofloxacin was administered intravenously at a dose of 10 mg/kg and inhalation at a dose of 2 mg/kg. Plasma, milk, and BAL samples were collected at specific times after marbofloxacin administration. Marbofloxacin analysis in plasma, milk, and BAL samples was performed using high-performance liquid chromatography, and pharmacokinetic parameters were calculated using non-compartmental analysis.

RESULTS: No local or systemic side effects were observed after IV and inhaler administration of marbofloxacin. Following IV administration, clearance and steady-state distribution volume were determined to be 305.91 mL/h/kg and 966.59 mL/kg,

respectively. The elimination half-life was 4.50 hours after IV administration and 0.80 hours after inhaler administration. The plasma area under the curve was determined to be 32.39 h*µg/mL and 0.24 h*µg/mL after IV and inhaler administration, respectively. Marbofloxacin was detected in milk samples up to 24 hours after IV administration and up to 6 hours after inhaler administration. The AUCmilk/AUCplasma ratio was determined to be 1.90 and 6.09 for IV and inhaler administration, respectively. Although marbofloxacin was detectable in BAL samples up to 12 hours after both administrations, the AUCmilk/AUCplasma ratio was higher after inhaler administration.

CONCLUSIONS: In conclusion, while marbofloxacin exposure to plasma and milk after inhaler administration was low, exposure to BAL was found to be high. Therefore, high marbofloxacin exposure in BAL after inhaler administration is quite important in terms of treatment efficacy, while low exposure in milk and plasma samples is quite important in terms of minimizing adverse effects. However, more detailed studies are needed to investigate the pharmacokinetics, therapeutic efficacy, and safety of marbofloxacin after inhaler administration in sick animals.

Keywords: Marbofloxacin, pharmacokinetics, sheep, intravenous, inhaler.

PUBLIC HEALTH FOOD SAFETY AND ANTIMICROBIAL RESISTANCE

Molecular characterization of multidrug-resistant streptococcus agalactiae from bovine mastitis: A rising superbug threat with vancomycin resistance

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BACKGROUND and OBJECTIVE: Streptococcus agalactiae (Group B Streptococcus, GBS) is an important pathogen in both veterinary and human medicine. In dairy cattle, it remains a major cause of contagious bovine mastitis, resulting in substantial economic losses. In humans, it can cause invasive disease in pregnant women, neonates, and immunocompromised individuals. The circulation of virulent and antimicrobial-resistant GBS strains in livestock presents a notable One Health concern, especially in regions with intensive antimicrobial use. In Egypt, most small- and medium-sized dairy farms do not routinely apply dry cow therapy, contributing to persistent intramammary infections and sustaining the transmission of contagious pathogens such as S. agalactiae. This study aimed to characterize key virulence determinants and antimicrobial resistance (AMR) genes in S. agalactiae isolated from bovine clinical mastitis in Gharbia Governorate, Egypt, and to report the emergence of vanA-mediated vancomycin resistance.

MATERIALS and METHODS: Milk samples were collected from cows with clinical mastitis in small- to medium-scale herds. Phenotypic identification of S. agalactiae was followed by molecular confirmation using 16S rRNA gene sequencing. Eight virulence and AMR-related genes—CAMP, cylE, rib, hyl, Pbp1A, tetO, aac (6') -aph (2''), and vanA—were screened by polymerase chain

reaction (PCR). Three vanA-positive isolates were sequenced and deposited in the NCBI GenBank database under accession numbers PQ317939 (TANTA1), PQ317940 (TANTA2), and PQ317941 (TANTA3). Gene prevalence and distribution were analyzed to assess virulence potential and multidrug resistance (MDR) patterns.

RESULTS: Sixteen isolates were initially identified as S. agalactiae, of which 14 (87.5%) were confirmed by 16S rRNA sequencing. All confirmed isolates carried CAMP, tetO, Pbp1A, and aac (6') -aph (2'') (100%). The prevalence of additional virulence genes was 92.9% for cylE, 85.7% for rib, and 78.6% for hyl. The vanA gene, associated with high-level vancomycin resistance, was detected in 28.6% of isolates. All vanA-positive isolates carried the full panel of tested virulence and AMR genes, indicating a combination of strong pathogenic potential and MDR features. This study provides the first molecular evidence of vancomycin-resistant S. agalactiae in Egypt, with publicly archived sequences.

CONCLUSIONS: The coexistence of multiple virulence factors and AMR genes in S. agalactiae from Egyptian dairy herds reflects the emergence of highly virulent, MDR GBS lineages. The detection of vanA-positive strains (TANTA1–TANTA3; PQ317939–PQ317941) highlights a significant threat to mastitis control, therapeutic success, and potential zoonotic transmission. Enhanced molecular surveillance, responsible antimicrobial use, and One Health-based control strategies are urgently required to limit the spread of these high-risk clones.

Keywords: Streptococcus agalactiae, Multidrug-resistant strains, Vancomycin resistance, Virulence factors

Extended-spectrum cephalosporin resistance in "Escherichia coli" in cows and calves after dry cow therapy with cephapirin or cloxacillin

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BACKGROUND and OBJECTIVE: Intramammary antimicrobial therapy at drying-off is a common procedure to cure and prevent mastitis in dairy cows, although its potential to select for antimicrobial resistance remains debated. Therefore, this randomized controlled field study investigated whether dry cow therapy with cephapirin, a first-generation cephalosporin, categorized as to be used with caution on the European Medicine Authority categorization list, selects for extended-spectrum cephalosporin-resistant (ESC-R) or extended-spectrum beta lactamase (ESBL) producing "Escherichia coli" in dairy cows and their neonatal calves compared to cloxacillin, a narrow-spectrum penicillin, categorized by EMA as prudent use.

MATERIALS and METHODS: To mimic a worst-case scenario, twenty-two dairy cows carrying ESC-R E. coli around 2 weeks before drying off, were selected from fifteen Dutch commercial dairy farms, served by the Utrecht University Farm Animal Practice, with a history of above Dutch average antimicrobial use and ESBL presence in pooled feces, were randomly assigned to intramammary cephapirin or cloxacillin dry cow therapy at drying off. Fecal and colostrum samples from cows and fecal samples of their offspring were collected longitudinally for presence and quantity of ESC-R and ESBL-producing "E. coli", as well as for genomic analysis and were analyzed at the Veterinary Microbiologic Diagnostic Laboratory of Utrecht University.

RESULTS: Cephapirin residues in colostrum were consistently below the maximum residue limit. No significant differences in ESC-R "E. coli" counts or prevalence were observed between treatment groups in cows or calves. All calves acquired ESC-R "E. coli" rapidly after birth, likely through horizontal (from the environment) rather than vertical (from the mother) transmission, and harbored diverse resistance genes and sequence types.

CONCLUSIONS: These findings indicate that in a worst-case scenario, first-generation cephalosporin dry cow therapy with cephapirin does not increase selection or transmission of ESC-R "E. coli" in the feces of treated cows nor in the feces of their offspring, compared to drying off with cloxacillin. This study also emphasizes the importance of environmental sources in early-life colonization of resistant "E. coli" in dairy calves.

Keywords: Dry cow therapy, antimicrobial resistance, first generation celalosporin, cephapirin

EU-JAMRAI2: Developing an antimicrobial stewardship program for food-producing animals within EU

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BACKGROUND and OBJECTIVE: Antimicrobial resistance (AMR) is one of the most relevant global health challenges, and it represents a serious social and economic burden, as it threatens human, animal, plant and the environmental health. As AMR threatens animal health, it also undermines the food supply chain. The European Joint Action on Antimicrobial Resistance and Healthcare-Associated Infections (EU-JAMRAI) represents Europe's largest coordinated effort to address AMR through a One Health approach, fostering cooperation across human, animal, and environmental health sectors. EU-JAMRAI2 brings together 30 countries and more than 120 partners and aims to support Member States in implementing concrete measures to combat AMR. A key objective is the development of an antimicrobial stewardship (AMS) Framework for food-producing animals (FPA), i.e. cattle, poultry and swine, as well as overcoming implementation barriers through behavioural change strategies.

MATERIALS and METHODS: A Framework is an organized structure with a set of core elements and guiding principles that support the development, implementation, and evaluation of AMS in animal health. AMS is defined as a coordinated set of strategies and actions designed to ensure the proper use of antimicrobial agents to improve treatment outcomes, reduce antimicrobial resistance, and ensure the continued effectiveness of these medicines, protecting animal, environmental and public health. The Framework is being developed by working groups of experts in the field based on their own expertise, the results of a workshop for AMS and infection prevention and control in animal health held in 2025; and on a comprehensive literature review conducted in collaboration with the Federation of Veterinarians of Europe (FVE).

RESULTS: A draft Framework for AMS in FPA has been developed. It includes a general overview of AMS programs and a specific part outlining core elements, identifying skills needed for professionals, and indicators for monitoring and assessing level of implementation.

CONCLUSIONS: The Framework can be used as practical tool for Member States to develop and implement AMS programs at national and animal sector level. The Framework can also be considered as a benchmarking scheme to allow harmonization and comparability of AMS programs in animals across EU countries. Through EU-JAMRAI2, Member States are supported in strengthening their National Action Plans on AMR by facilitating expert collaboration and developing digital and offline tools to support implementation.

Keywords: antimicrobial resistance, antimicrobial stewardship, framework, food-producing animals

The role of bovine-derived food products in the potential transmission of Klebsiellapneumoniae resistant to critical antimicrobials to humans: Preliminary results

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BACKGROUND and OBJECTIVE: *Klebsiella pneumoniae* (*Kp*), a major nosocomial pathogen, is increasingly associated with community-acquired infections. Recent community outbreaks in Asia involving highly lethal carbapenem-resistant *Kp*, lately also reported in Italy, raise concerns about the existence of reservoirs beyond hospital settings. This study investigates Bovine-Derived Food Products (BD-FPs) as potential sources of *Kp* and its resistance to critical antimicrobials (carbapenems, third-generation cephalosporins - 3GCs and colistin), assessing their circulation in the food chain.

MATERIALS and METHODS: Between September 2025 and March 2026, three BD-FP categories (meat, n=138; milk, n=48; soft cheese, n=13) were analysed at the IZSLER laboratories in Emilia-Romagna, Italy. Meat and soft cheese were collected within Regional and Self-Monitoring Food Plans or from post-slaughter carcasses. Milk samples were those evaluated within the Italian quality-based milk payment program. *Klebsiella* spp. was isolated using standard microbiological methods, while selective protocols were applied to specifically recover carbapenem- and 3GC-resistant strains. Additionally, 64 *Klebsiella* spp. isolated from individual milk samples during routine mastitis screening were considered. Presumptive *Klebsiella* isolates were identified by MALDI-TOF. Antimicrobial resistance (AMR) was assessed by broth microdilution method and results interpreted using CLSI clinical breakpoints. Strains resistant to carbapenems, 3GCs and colistin were screened for corresponding resistance genes by PCR.

RESULTS: *Kp* was isolated from bulk milk (6/48, 12.50%) and meat (13/138, 9.42%), but not from soft cheese, and represented 93.75% (60/64) of individual milk strains. Overall, 41/79 (51.90%) isolates were resistant to at least one antimicrobial, while 9/79 (11.39%) strains of individual milk (8) and meat (1) origin were multidrug-resistant (MDR). Resistance was highest to sulphamethoxazole (27/79, 34.18%), followed by trimethoprim (23/79, 29.11%) and tetracycline (16/79, 20.25%), with all other antimicrobials showing <20% resistance. Notably, one meat and one individual milk isolate were 3GC-resistant, carrying *bla*TEM/*bla*CTX and *bla*SHV genes, respectively. Colistin resistance was detected in three individual milk isolates, likely chromosomal, as *mcr* genes were not detected.

CONCLUSIONS: Similar *Kp* isolation rates were observed in bulk milk (12.50%) and meat (9.42%) samples, suggesting both BD-FPs may act as *Kp* sources. *Kp* was frequently associated with AMR, with over 50% of BD-FP-derived strains resistant to at least one antimicrobial. Notably, *Kp* from individual milk was mostly MDR and resistant to 3GCs and colistin. *Kp* subclinical mastitis poses an additional risk for bulk milk intended for human consumption, as milk from infected animals, without clinical signs or visible milk alterations, may be accidentally mixed into bulk milk, increasing the possibility of *Kp* transmission to consumers.

Keywords: Bovine-derived food products, Klebsiella pneumoniae, critical antimicrobials, One health

Cross-sectional study on risk factors of fecal shedding of ESBL- producing Enterobacterales in neonatal dairy calves from a high-density dairy production area in Northern Italy

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BACKGROUND and OBJECTIVE: Extended-spectrum β-lactamase (ESBL)-producing Enterobacterales are

recognized as critical antimicrobial-resistant pathogens of major public health concern. Dairy calves are frequently colonized early in life and may represent an important reservoir within livestock production systems. This study investigated risk factors at farm-level, seasonal variation associated with faecal shedding of ESBL-producing Enterobacterales in neonatal dairy calves from a high-density dairy production area in Northern Italy.

MATERIALS and METHODS: A cross-sectional study was performed on 18 dairy farms in Lombardy between June 2023 and February 2024, where faecal samples were collected in both summer and winter. A total of 392 Holstein-Friesian calves (<21 days old and no previous antimicrobial treatment) were enrolled. 0.1 g of faeces were enriched in Mueller-Hinton broth for 24 hours at 37°C, then 100 microliters were cultured on selective-chromogenic media for ESBL-producing bacteria. MALDI-TOF MS was used for species identification. ESBL production was confirmed with the double disc synergy test (DDST), and *bla*CTX-M, *bla*TEM and *bla*SHV genes detection by multiplex PCR. Farm management and biosecurity were assessed using a questionnaire. A multivariable Poisson regression model was used to identify factors associated with ESBL prevalence and seasonal variation was assessed with chi-square test.

RESULTS: All the presumptive ESBL-producing Enterobacterales were confirmed by the DDST. All farms had ESBL-producing Enterobacterales - positive calves during summer. Farm-level prevalence ranged from 37% to 100%, with an average of 84.9%. ESBL-producing Escherichia coli was the most bacterial species detected (88.9% of the ESBL-producing Enterobacterales). 86.9% of the studied isolates had one ESBL-encoding genes and blaTEM and blaCTX-M pattern was the most detected. In the multivariable model, cleaning of colostrum-feeding equipment (IRR 0.75, 95% CI 0.62–0.90) and selective dry cow therapy (IRR 0.73, 95% CI 0.60–0.88) were associated with reduced prevalence. Immediate cow–calf separation was also protective (IRR 0.91, 95% CI 0.83–0.99). Conversely, specific colostrum administration practices and use of non-cephalosporin antimicrobials for mastitis treatment were associated with increased prevalence. Waste milk feeding was not significantly associated with ESBL shedding. Seasonal sampling, conducted in 10 out of 18 farms, revealed lower prevalence in winter ($p < 0.01$) of ESBL-producing Enterobacterales in calves' faeces.

CONCLUSIONS: ESBL producingEnterobacterales were endemic in intensive dairy farms within this high-density production area. Farm management practices, particularly colostrum hygiene, were key determinants of early-life gut colonization. Additionally, environmental conditions, as low temperature, influence faecal shedding in calves. These findings highlight actionable biosecurity targets to mitigate antimicrobial resistance within a One Health framework.

Keywords: ESBL-producing Enterobacterales, E. coli, risk factors, calves

Janus Faces of Escherichia coli in dairy farms: antimicrobial resistance, virulence, and molecular diversity of isolates from cows and calves

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BACKGROUND and OBJECTIVE: The Arborea dairy district in Sardinia (Italy) is a geographically isolated, highly specialized enclave lacking external AMR pressure from swine production, urbanization, or heavy industry. This unique setting serves as a "natural laboratory" to study bacterial ecology under rigorous modern management. Participating farms operate under continuous veterinary supervision and structured risk-based monitoring (ClassyFarm). Following EU Regulation 2019/6, antimicrobial stewardship has tightened—especially regarding youngstock prophylaxis—and rapid on-farm diagnostics are routinely used to target calf diarrhea treatments (e.g., confirming cryptosporidiosis) and prevent unnecessary antimicrobial exposure. This highly controlled, isolated environment enabled us to characterize and compare virulence and

antimicrobial resistance (AMR) patterns of *Escherichia coli* across bovine mastitis cases, pre-weaned calves, and their environment, revealing strikingly divergent ecological roles within the same farms.

MATERIALS and METHODS: Twelve dairy farms with mastitis history were monitored for six months; six showed *E. coli* recurrent cases. Management questionnaires were administered and samples collected: quarter milk from mastitis cases, feces from pre-weaned calves (10/farm), and environmental samples. All *E. coli* isolates underwent sensitivity testing for critical antimicrobials, double-disk synergy testing for third-generation cephalosporin resistance (3GCR), and PCR for ESBL genes (*bla*TEM, *bla*CTX-M groups, *bla*SHV). Clonal relatedness was assessed by PFGE. One representative mastitis-associated *E. coli* (MAEC) and one multidrug resistant (MDR) calf underwent whole-genome sequencing (WGS). Culture-enriched Nanopore metagenomics characterized the calf resistome beyond single colonies.

RESULTS: Mastitis isolates (n=46) lacked MDR profiles, showing resistance primarily to penicillin (50%), and occasional tetracycline/ampicillin (6%). PFGE revealed high heterogeneity, consistent with opportunistic infections. Conversely, calf *E. coli* exhibited 3GCR in 60-90% of animals/farm. All 3GCR isolates carried *bla*CTX-M (100%, group 1 or 9, with farm-specific dominance), formed PFGE clonal clusters, and were MDR (except one). The sequenced ST6786 calf isolate possessed chromosomal *bla*CTX-M-14, multiple resistance genes, hemolysins and biofilm factors. Culture-enriched metagenomics from the single "non-MDR" calf isolate revealed a broader fecal resistome than culture alone. Crucially, the sequenced ST58 MAEC carried a large virulence plasmid containing resistance genes against trimethoprim-sulfamethoxazole, an antimicrobial used exclusively for treating calf diarrhea.

CONCLUSIONS: In this modern dairy setting, *E. coli* plays divergent roles: highly virulent but largely susceptible MAEC cause mastitis in cows, while clonal ESBL/MDR strains with chromosomal *bla*CTX-M colonize calves and the environment. Detecting calf-associated resistance genes in udder pathogens suggests environmental spillover from youngstock to the lactating herd, highlighting the need to pair antimicrobial stewardship with calf-focused biosecurity to disrupt stable resistance reservoirs.

Keywords: AMR, E. coli, ESBL, calves, mastitis

Genome-Scale comparative analysis of clinical Escherichia coli and Klebsiella pneumoniae reveals implications for one health surveillance

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BACKGROUND and OBJECTIVE: Antimicrobial resistance (AMR) is a critical global health challenge affecting humans, animals, and the environment within the One Health framework. *Escherichia coli* and *Klebsiella pneumoniae* are important opportunistic pathogens and members of the ESKAPE pathogens group, recognized as high-priority pathogens by the World Health Organization. These bacteria are major causes of urinary tract infections (UTIs) in humans and are also associated with septicemia and other infections in animals. Their detection in environmental sources further indicates fecal contamination and potential public health risks. The present study aimed to perform a comparative genomic analysis of multidrug-resistant (MDR) *E. coli* and *K. pneumoniae* isolates from human, animal, and environmental sectors and to assess phenotypic–genotypic concordance patterns of antibiotic resistance.

MATERIALS and METHODS: A total of 579 *E. coli* and 370 *K. pneumoniae* isolates obtained from human, animal, and environmental sectors were included in this study. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method following guidelines from the Clinical and Laboratory Standards Institute. Whole-genome sequencing (WGS) of the isolates was carried out using the Illumina NovaSeq platform. Antimicrobial resistance genes were identified using the Comprehensive Antibiotic Resistance Database (CARD), and plasmid profiling was performed using PlasmidFinder.

RESULTS: In *E. coli*, a total of 182 antimicrobial resistance genes were identified in human isolates, 96 in animal isolates, and 48 in environmental isolates using the Comprehensive Antibiotic Resistance Database (CARD). Plasmid analysis using PlasmidFinder detected 22 plasmids in human isolates, 17 in animal isolates, and 25 in environmental isolates. Similarly, in *K. pneumoniae*, a total of 175 antimicrobial resistance genes were detected in human isolates, 64 in animal isolates, and 92 in environmental isolates using the Comprehensive Antibiotic Resistance Database (CARD). Plasmid profiling using PlasmidFinder revealed 52 plasmids in human isolates, 34 in animal isolates, and 42 in environmental isolates. Overall the concordance was 77% in *E. coli* ad 79% in *K. pneumoniae*.

CONCLUSIONS: The comparative genomic analysis revealed a widespread distribution of antimicrobial resistance genes and plasmids across human, animal, and environmental sectors, highlighting the interconnected transmission dynamics of AMR within the One Health framework. These findings underscore the importance of integrated surveillance and the development of alternative antimicrobial strategies to mitigate the spread of resistance determinants across sectors.

Keywords: Antimicrobial resistance, Escherichia coli, Klebsiella pneumoniae, Whole genome sequencing

Mental health among veterinary students, veterinarians, and farmers in Austria

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BACKGROUND and OBJECTIVE: Mental health challenges and high suicide rates are increasingly recognized as important occupational health issues in veterinary medicine and agriculture worldwide. High study- and workload as well as societal and political pressure make these professional groups particularly vulnerable. Therefore, the objective of these studies was to describe the prevalence of mental health burdens and associated risk factors in the three cohort groups in Austria – veterinary students, veterinarians, and farmers – and to compare these findings with those of the general Austrian population.

MATERIALS and METHODS: Three online questionnaire surveys were conducted among Austrian veterinary students, veterinarians, and farmers using validated psychometric screening instruments, including the PHQ-9 (depression), GAD-7 (anxiety), ISI-2 (insomnia), PSS-4 (perceived stress), suicide ideation (item 9 of PHQ-9), and SBQ-R (suicide risk). Sociodemographic, occupational, and lifestyle factors were assessed to identify predictors of mental health outcomes. Data were analyzed using descriptive statistics and multivariable regression models.

RESULTS: Among Austrian veterinary students (n = 430; 29% of the registered students), a significantly higher mental health burden was observed compared with the general population. Overall, 55% exceeded the clinically relevant cut-off for depressive symptoms, 53% for anxiety, 21% for insomnia, 79% for high stress levels, 30% suicide

ideation, and 21% for suicide risk. Mental health symptoms were inter alia associated with low physical activity, high smartphone use, and loneliness. Similarly, Austrian veterinarians (n= 440, 10% of the registered veterinarians) showed significantly higher odds of clinically relevant depressive, anxiety, and insomnia symptoms (OR 1.35–2.70) compared with the general population, as well as a high prevalence of suicide ideation (14%) and suicide risk (13%). Risk factors included financial concerns, long working hours, lower professional experience, physical inactivity, and animal suffering. Mental health challenges were also evident in the farming community, which reported higher clinically relevant stress levels (67%), insomnia (15%), depression (28%), anxiety (31%), and suicidal ideation (20%) than the general Austrian population (51%, 9%, 21%, 15%, 14%, respectively), and a high prevalence of clinically relevant suicide risk (11%). Financial constraints, high workload, physical inactivity outside of work, and loneliness were identified as key stressors, while social support and opportunities for recovery were protective factors.

CONCLUSIONS: Mental health challenges affect both veterinary professionals and the farming communities they serve. The three study groups are closely interconnected. The high mental health burden among students appears to persist into professional life. In particular, large animal practitioners have close personal and professional relationships with farmers. Increasing awareness of occupational stressors and strengthening access to mental health support resources may contribute to improved well-being among these three vulnerable groups, which are essential for public health and food production.

Keywords: occupational risk factors, suicidality, depression, stress

Q fever awareness among European ruminant farmers, large animal veterinarians and human general practitioners

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BACKGROUND and OBJECTIVE: A European Q Fever Committee was established in 2024, bringing together veterinary and medical experts from multiple disciplines and countries. A large survey was conducted among ruminant farmers, large animal veterinarians, and human medical doctors in eight European countries to assess their awareness and level of knowledge of Q fever, caused by Coxiella burnetii (coxiellosis).

MATERIALS and METHODS: The survey was carried out in 2024 by an independent opinion-polling company. The selected countries were Germany, France, Spain, Italy, the United Kingdom, Belgium, the Netherlands, and Poland. Profession-specific questionnaires were developed in English and translated into local languages. More than 900 farmers were interviewed. Eighty veterinarians were surveyed in each country, except Belgium (60) and

the Netherlands (30). Fifty medical doctors were interviewed per country, except in Belgium and the Netherlands (30 each). Interviews were conducted using Computer-Assisted Telephone Interviewing technology, recorded, and lasted 20–25 minutes

RESULTS: Cattle (571), sheep (198), and goat (177) farmers were interviewed (only cattle and goat farmers in the Netherlands, only cattle and sheep farmers in the UK, only cattle farmers in Germany, Belgium, and Poland). Approximately 75% of farmers were either unaware of Q fever or only familiar with its name. Personal experience with the disease was limited (2–5%) in most countries, but higher in the Netherlands (12%) and France (13%). Around 80% of veterinarians in France and the Netherlands had encountered Q fever cases and demonstrated accurate knowledge. In Belgium, Germany, Italy, and Spain, fewer than two thirds had such experience, and knowledge was poorer; this dropped below 50% in the UK and Poland. Consequently, vaccination rates remain low: about 1%, except in France and the Netherlands. Among human doctors, awareness was generally high, personal experience with cases was frequent in Spain (74%) and France (60%) but rare in Poland and Italy. Human doctors are well aware of the disease. Poland and Italy seem to be the exception, with 70 and 64% of respondents having no experience with Q fever cases

CONCLUSIONS: This is the largest European survey assessing perceptions of Q fever among animal and human health professionals. While national differences exist, farmer awareness is consistently low, and professional knowledge remains heterogenous. These findings underscore the urgent need for coordinated, science-based, harmonized strategies for Q fever management within a One Health framework. The European Q Fever Committee aims to support these efforts

Keywords: Q fever, Coxiella burnetii, Zoonosis, Awareness

REPRODUCTION IN DAIRY AND BEEF CATTLE

Modifiable risk factors for delayed calving in seasonal dairy herds

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BACKGROUND and OBJECTIVE: This prospective cohort study aims to identify risk factors associated with the odds of pregnancy in the seasonal pastoral system. The study also examines risk factors associated with the odds of calving late in the subsequent calving season.

MATERIALS and METHODS: Two thousand, one hundred and three cows from seven moderate-yielding seasonal-calving dairy herds in Southern Ireland were enrolled. Farm and cow-level data were collected over an 18-month period. These were collated with reproductive indices available from the national database, Irish Cattle Breeding Federation (ICBF). Univariable analysis was performed to identify variables eligible ($P < 0.2$) for potential inclusion in the multivariable analysis. The variable 'Calving groups' was assigned as those animals calving in the first (early), second (mid), third (late) month and after (out of season) of the calving season. Calving group in Year 1 was a strong predictor of pregnancy and late calving in Year 2. Farm identity was also a strong predictor of odds of pregnancy and late calving. These factors (farm identity and calving date in Year 1) were included in all initial univariable analyses. Multivariable regression models were fitted using backward-stepwise methodology for selection of variables in the final model. Variables were excluded if $P > 0.02$ (likelihood ratio test) with the least significant being removed at each iteration until a final model was produced where all explanatory variables were significant at this level.

RESULTS: Calving date category in Year 1 ($P < 0.001$), endometritis ($P < 0.06$), body condition score (BCS) at breeding ($P = 0.05$), parity ($P<0.001$), and daily milk yield ($P < 0.001$) were associated with odds of pregnancy during the breeding season. Lameness (OR 0.68), endometritis (OR 0.68) and increasing parity (OR 0.84) were negatively correlated with pregnancy. Higher BCS at breeding (OR 1.76) and higher daily milk yield (at first milk recording after the start of the breeding season) (OR 1.06) were positively correlated with pregnancy outcome.

Calving date category in Year 1 (P < 0.001), twinning (P < 0.05), severe BCS loss (P < 0.05) and late embryonic loss (P < 0.001) were positively associated with calving late in Year 2.

CONCLUSIONS: The existing calving spread is a major factor in the timing of subsequent calving and the odds of pregnancy. Several other risk factors have been identified for both late calving and non-pregnancy. These modifiable risk factors can be targeted at farm management level to improve reproduction indices.

Keywords: Dairy, reproduction, seasonal production, risk factors

Validation of intrauterine propolis and honey (PropoMiel) treatment in postpartum endometritis in organic dairy cows in Quebec

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BACKGROUND and OBJECTIVE: Endometritis, defined as a superficial inflammation of the uterine mucosa occurring beyond 21 days postpartum, has a reported prevalence of approximately 50% in dairy herds in Quebec, Canada (Lefebvre 2021). Conventional treatment protocol involves intrauterine administration of antibiotics (Tison 2014). However, organic farms in Quebec operate under are strict regulations limiting antibiotic use, necessitating alternative therapeutic approaches. Natural products such as Propolis and honey (PropoMiel), known for their antibacterial properties, may represent a promising alternative. The objective of this study was to evaluate the efficacy of PropoMiel intrauterine treatment in postpartum cows diagnosed with clinical or subclinical endometritis by evaluating the effects on uterine health, endometrial cytology and fertility in organic dairy cows.

MATERIALS and METHODS: 1,495 cows from 26 organic farms were analyzed for two years. A genital exam was performed between 28 and 42 days postpartum using vaginoscopy and ultrasonography, followed by endometrial cytology. Cows were subsequently randomly assigned to control or treatment with PropoMiel 5%. A second visit was conducted after 14 days during the first year to assess treatment effects on vaginal discharge and neutrophils on endometrial cytology. Reproductive performance variables, including conception rate at first artificial insemination (AI), number of AIs per pregnancy, and days open, were evaluated at the end of the experiment.

RESULTS: The incidence of PVD, endometritis, and cervicitis was 6%, 12%, and 23%, respectively. No significant effect of treatment on reproductive performance was observed (p>0.05), including conception rate at first AI, number of AIs per pregnancy, days open, or overall pregnancy rates. Similarly, no significant differences were detected in days in milk or ovarian cyclicity, as assessed by the presence of a corpus luteum. A significant reduction (p<0.05) in the proportion of neutrophils on endometrial cytology was observed between the two examinations; however, improvements in reproductive performance were only suggested as a trend in cows in their third lactation. The relatively low incidence of uterine disease observed in postpartum cows from organic dairy farms may partially explain the absence of measurable treatment effects.

CONCLUSIONS: In conclusion, organic dairy farms exhibited a lower incidence of PVD and subclinical endometritis compared with conventional herds in Québec, although the incidence of cervicitis appeared comparable. Intrauterine administration of PropoMiel did not significantly improve reproductive performance, although no adverse effects were observed by farmers.

Keywords: Dairy cows, Endometritis, Organic farms, Quebec

Evaluation of a rapid resynchronization program for timed-rebreeding in Holstein and Simmental heifers

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BACKGROUND and OBJECTIVE: This study described the use of a resynchronization program using an intravaginal P4 implant (PRID) from D12 to D19 after previous timed artificial insemination (TAI) on percentage of heifers that become pregnant during a 41-day timed rebreeding program with no detection of estrus.

MATERIALS and METHODS: Holstein (n = 638) and Simmental (n = 692) heifers were submitted to resynchronization program (D12 to D19 PRID) and, on D21 after TAI, non-pregnant heifers were ascertained based on corpus luteum (CL) vascular perfusion using Doppler ultrasound. Heifers were designated as non-pregnant on D21 (ReBreed1 at D21) or D20 (Rebreed2 at D41) based on the absence of CL or a CL with <25% of total area with vascular perfusion. Holstein heifers were inseminated with sex-sorted semen and Simmental heifers received conventional semen. Non-pregnant heifers were immediately inseminated and given 100 ug of GnRH. To generate three potential TAIs within a 41d period, the 6-d rebreeding program was replicated 12 days after the second TAI. Heifers that had a CL with >25% vascular perfusion were considered to have a functional CL and were designated as potentially pregnant. Potentially pregnant heifers, were rechecked 33 days after TAI using ultrasound and, if found to be not pregnant on D33, the heifer was considered a false positive. False positive heifers and early ovulations were resynchronized using a 6-d Co-Synch protocol [d−8, GnRH + PRID; d-2, PGF2α remove PRID; d-1 second PGF2α; d0, GnRH + TAI] for TAI at D41.

RESULTS: The pregnancy/AI for the 1st TAI was 46.3% (616/1330), ReBreed1 (D21) 39.2% (177/450), and ReBreed2 (D41) 41.7% (71/170). The false positive rate at ReBreed1 (D21) was 27.3% (195/714) and the pregnancy/AI to the second CoSynch protocol (D41) was 43.9% (105/239). The total number of pregnant heifers at the end of the 41-day TAI period was 72.8% (969/1330). Holstein heifers had greater pregnancy rate than Simmental heifers at both Day 33 (76.3% vs. 69.6%; P = 0.008) and Day 63 (72.2% vs. 63.8%; P = 0.001), whereas embryonic loss was greater in Simmentals compared with Holsteins (10% vs. 5%; P = 0.005).

CONCLUSIONS: In conclusion, the rapid resynchronization strategy using a progesterone device after TAI combined with Doppler ultrasound pregnancy diagnosis on Day 21 enables efficient reinsemination within a shortened breeding interval, achieving satisfactory fertility. Comparison of results to other rebreeding programs are needed in future studies.
This study is supported by the Turkish Academy of Science (TUBITAK, TOVAG 223O469)

Keywords: heifer, rebreeding, fertility

Calving management practices among cattle veterinarians in 11 European countries

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BACKGROUND and OBJECTIVE: Appropriate calving management is critical for optimizing maternal and neonatal outcomes, yet variation in obstetrical decision-making persists among practitioners. This study aimed to characterize calving assistance practices among veterinarians in 11 European countries and to identify demographic and herd characteristics associated with intervention timing, hygiene protocols, cesarean section decision-making, and pharmaceutical use.

MATERIALS and METHODS: A survey was distributed to veterinarians between November 2023 and January 2025. A total of 1,190 valid responses were included. Questions addressed timing of cow movement to calving pen, waiting time before obstetrical intervention, hygiene measures, calving tools' use, cesarean section decision criteria, and pharmaceutical interventions during difficult calvings. Associations between each practice and demographic and herd characteristics were evaluated using multivariable logistic regression models. Multiple correspondence analysis (MCA) was used to identify patterns of calving practice among respondents.

RESULTS: Most respondents recommended moving cows to calving accommodation more than 6 h before calving (73%) and initiating assistance within 30–60 min after hoof appearance (70%). Dairy veterinarians waited longer before intervening than beef veterinarians when assisting both heifers and adult cows. When assisting adult cows, female veterinarians intervened earlier than male veterinarians. Calving jacks were widely used (81%), although rope-only extraction was also frequently reported (56%), particularly among dairy practitioners and female respondents. Hygiene practices were inconsistent: 80% reported using palpation gloves and 61% washing the vulva, but fewer disinfected the vulva (39%) or ropes/chains (46%). Excessive calf size (93%) and inability to correct dystocia (89%) were the primary indications for cesarean section, with 47% electing for surgery after 15–30 min of calving assistance without progress. MCA identified two principal gradients: one reflecting medication intensity (NSAIDs, calcium, antimicrobials, epidural use) and another representing hygiene-focused and early-intervention practices. Younger veterinarians, female respondents, and those working predominantly with dairy herds were more strongly associated with medication-intensive profiles. Pharmacological use peripartum was common, particularly NSAIDs (85%), calcium (69%), systemic antimicrobials (53%), and epidural anesthesia (42%). Among reported used drugs, meloxicam (61%) was the most frequently used NSAID, penicillins (76%) predominated for systemic antimicrobial therapy, and tetracyclines (71%) were the most commonly used intrauterine antimicrobials.

CONCLUSIONS: Substantial heterogeneity exists in calving management across Europe. Practice patterns were influenced by age, gender, herd type, and country. These findings underscore the development of consensus-based guidelines and continuing education to promote consistent, evidence-based obstetrical management. This work was supported by Fundação para a Ciência e a Tecnologia project UIDB/00276/2025 (CIISA) and LA/P/0059/2020 (AL4AnimalS).

Keywords: Survey, Calving, Management

Evaluation of the effect of natural prostaglandin administration postpartum on the reproductive performance of dairy cow

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BACKGROUND and OBJECTIVE: Post-partum in dairy cows significantly affects the animal's productive and reproductive efficiency; clinical monitoring during this phase is essential to detect and treat any reproductive tract diseases. In the early postpartum period, the uterus of dairy cows is invaded by various bacteria, both anaerobic and facultative anaerobic, which are naturally eliminated approximately within 28 days after calving. However, occasionally this process fails, predisposes cows to pathological conditions such as endometritis, negatively affecting subsequent reproductive performance. Prostaglandins F2α (PGF2α) are commonly used to improve uterine involution, promote luteolysis, contributing to the resolution of uterine infections in dairy cows, support ovarian activity recovery, and optimize fertility. The aim of the present study was to evaluate the effects of administering natural PGF2α to dairy cows between days 8 and 14 postpartum on the immediate postpartum period and subsequent reproductive performance.

MATERIALS and METHODS: A total of 300 Italian Holstein cows were recruited and randomized into two experimental groups based on days in milk (DIM) and parity (treated group and control group). Between the 8th and 14th day postpartum, the Treated group (n = 169) received an injection of natural prostaglandin (25 mg Dinoprost as Trometamol intramuscular administration (IM) per subject, equivalent to 5 ml in the semitendinosus muscle, Enzaprost®, Ceva Sante Animale, France), while Control group (n = 131), received an injection of saline solution (5 ml IM per animal). All the animals were inseminated for 1st artificial insemination (AI) using Double-Ovsynch starting at 55±3 days in milk.

RESULTS: The data obtained showed that a single administration of prostaglandin F2α between day 8 and day 14 postpartum increased the Conception rate (CR%) for 1st AI, with a higher number of pregnant cows in the treated group (54.8%) compared to the control group (39.1%) (p=0.001; OR=2.383, 95% CI: 1.420-3.998). The administration of PGF2α had a significant impact on the probability of not exceeding 250 DIM without pregnancy ("off cows") (p=0.003; 94% treated, 84,6% control). Specifically, the percentage of "off" cows in the control group (15.4%) was more than double that of the treated group (6%).

CONCLUSIONS: The data from this study, therefore, suggest that a single administration of PGF2α, between the 8th and 14th post-partum day, may have positive effects on the reproductive performance of dairy cows. In particular, the treated group showed a higher conception rate compared with the control group, along with a concomitant reduction in days open.

Keywords: Dairy cows, postpartum, prostaglandin F2α, reproductive performance

Impact of eCG administration during a 6-day Co-Synch protocol on pregnancy per AI and embryonic loss in Holstein heifers

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BACKGROUND and OBJECTIVE: This field study was conducted to evaluate whether eCG administration improves pregnancy outcomes in nulliparous Holstein heifers subjected to a 6-day timed AI synchronization protocol.

MATERIALS and METHODS: A total of 722 Holstein heifers from a single commercial herd were enrolled between October and November under moderate environmental conditions. Heifers were synchronized using a 6-day Co-Synch protocol either without eCG (Control; n = 342) or with eCG administration at the time of the first PGF2α injection (eCG; n = 380). The synchronization protocol included GnRH injection (50 µg of lesirelin)

and insertion of a progesterone releasing intravaginal device (PRID, 1.55 g P4) on Day 0. Prostaglandin F2α (150 µg of d-kloprostenol) was administered on Day 5. On Day 6, a second PGF2α dose was given and the PRID was removed. Timed artificial insemination was performed on Day 8 concurrently with a second GnRH injection. Heifers were inseminated with conventional semen from a single Holstein sires. Pregnancy per AI (P/AI) was determined by transrectal ultrasonography on Days 33 and 63 after TAI and embryonic loss rates were recorded. Data were analyzed using the chi-squared test.

RESULTS: Pregnancy per AI did not differ between control and eCG at either 30 or 60 days. At 30 days, pregnancy rates were 49.4% (168/342) for the control group and 50.2% (191/380) for the eCG group (P = 0.82). Similarly, 60-day pregnancy rates were 46.7% and 44.2%, respectively (P = 0.48). Embryonic loss was greater in the eCG group compared with controls 12.0% (23/191) vs 4.7% (8/168) (P = 0.017).

CONCLUSIONS: In conclusion, under commercial management conditions, eCG administration in 6-day Co-Synch protocol did not enhance pregnancy per AI. However, eCG administration was associated with a significantly greater incidence of embryonic loss. Further controlled studies are warranted to clarify the biological mechanisms underlying this response in Holstein heifers.

Keywords: heifer, Fertility, synchronization, timed AI

Apelin in the postpartum of healthy and subclinical endometritis cows

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BACKGROUND and OBJECTIVE: Apelin is an anti-inflammatory adipokine linked to regulation of leucocyte chemotaxis and macrophage polarization. Macrophages are involved in inflammation resolution and display the surface APJ receptor for Apelin. However, the role of Apelin in cow postpartum endometrial inflammation remains unknown. This study aimed to evaluate the endometrial presence of Apelin and its concentrations in plasma and uterine fluid in postpartum dairy cows, and relate them with uterine macrophage phenotype in the pathogenesis of subclinical endometritis (SCE).

MATERIALS and METHODS: Postpartum dairy cows (n=30) were enrolled into 3 groups with different endometrial health phenotypes, according to cytology at 21 and 42 days postpartum (DPP): i) Healthy (H, n=10); ii) Recovered SCE (SCE-R, n=10) and iii) Persistent SCE (SCE-P, n=10). A blood sample was collected at 10, 21 and 42 DPP, and uterine fluid was recovered at 21 DPP for measurement of Apelin concentrations by ELISA. The protein content of macrophage pro-inflammatory M1 (CD86) and anti-inflammatory M2 (CD163) phenotypes was quantified by Western Blot in the pellet of the uterine fluid sample. An endometrial biopsy was taken at 42 DPP for detection of Apelin by immunohistochemistry. Comparisons between groups were evaluated by linear mixed-effects models (PROC MIXED; repeated measures ANOVA) or by One-way ANOVA (PROC GLM), and Spearman correlations assessed associations between macrophage phenotypes and Apelin concentrations.

RESULTS: Compared to H cows, Apelin plasma concentrations were greater (P<0.05) in SCE-R cows at 10 DPP, and in SCE-P cows at 42 DPP (P<0.01). At 21 DPP, Apelin uterine fluid concentrations were greater in SCE-R than in H cows (P<0.05), and the abundance of CD163 was greater (P<0.05) in SCE-R than in H and SCE-P cows. The CD163 abundance was correlated with Apelin plasma concentrations at 10 DPP (r=0.453; P<0.05). Apelin was mainly detected in epithelial glandular cells, which SCE-R cows showed stronger staining in glandular and stromal cells than H cows.

CONCLUSIONS: Results evidenced that Apelin is produced in endometrial cells, and that Apelin concentrations are present in the plasma and uterine fluid. High concentrations in early postpartum plasma (10 DPP) and uterine fluid (21 DPP) were associated to the recovery from SCE, which was also associated to the high uterine fluid abundance of macrophage M2 protein. Apelin high

concentrations in late postpartum (42 DPP) were associated to persistence of SCE, suggesting a delayed anti-inflammatory action, facing the persistent inflammatory stimulus. In conclusion, Apelin may emerge as potential early postpartum non-invasive diagnostic marker of the recovery/persistence of SCE.

Keywords: endometritis, apelin, macrophage

Cow-level and management factors affecting reproductive performance in Hungarian dairy farms

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BACKGROUND and OBJECTIVE: Reproductive efficiency in high-producing dairy herds is influenced by multiple physiological and management-related factors. This study evaluated the effects of selected cow-level and management-related factors on reproductive performance and pregnancy loss in dairy cows synchronized with a Double-Ovsynch protocol.

MATERIALS and METHODS: A retrospective study was conducted using records from 2542 Holstein dairy cows from two commercial Hungarian dairy farms. All cows were synchronized using a Double-Ovsynch protocol prior to the first artificial insemination. Reproductive parameters evaluated included calving-to-first insemination interval, days open, calving interval, services per pregnancy and pregnancy loss. Cow-level factors (parity, milk yield at insemination) and management-related factors (previous dry period length, timing of synchronization protocol initiation, and semen type) were analyzed for their association with reproductive outcomes.

RESULTS: Parity did not significantly influence calving-to-first insemination interval, days open, or calving interval. However, multiparous cows had a lower probability of pregnancy within 300 days postpartum compared with primiparous cows (HR = 0.85; P < 0.001), a longer interval to pregnancy, and higher pregnancy loss (10.2% vs. 7.1%; P = 0.009). Cows in the fourth or higher lactation showed the lowest pregnancy probability (HR = 0.67; P < 0.001), longer days open and calving interval, and the highest number of services per pregnancy. Higher milk yield at insemination negatively affected fertility. Cows producing more than 45 kg had reduced pregnancy likelihood (HR = 0.83–0.86; P = 0.01), and those producing more than 55 kg had an increased risk of pregnancy loss (OR = 2.01; P = 0.002). Among management factors, previous dry period length significantly influenced fertility. Short dry periods (≤50 days) were associated with higher pregnancy probability and fewer services per pregnancy, whereas prolonged dry periods (>80 days) markedly reduced reproductive efficiency. Delayed initiation of the protocol (>55 days in milk) decreased pregnancy probability by approximately 25% (HR = 0.75; P = 0.001). The use of sex-sorted semen increased pregnancy likelihood (HR = 1.42; P < 0.001) and reduced the number of services per pregnancy compared with conventional semen, without affecting pregnancy loss.

CONCLUSIONS: Both cow-level and management-related factors significantly influenced reproductive performance in dairy cows in this current study. Higher parity, elevated milk yield at insemination, prolonged dry periods were associated with reduced fertility, whereas shorter dry periods and the use of sex-sorted semen improved reproductive outcomes. Optimizing these factors may contribute to improved reproductive efficiency and reduced pregnancy loss in high-producing dairy herds.

Keywords: Dairy cow, first service conception rate, Double Ovsynch, key performance indicators

Association of prepartum alerts detected by an ear-attached automated health monitoring system with health and production outcomes in early lactation of dairy cows

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BACKGROUND and OBJECTIVE: The objective of this observational study was to evaluate the association of prepartum alerts within 50 days prior to calving with health and production outcomes in Holstein cows, using an ear-attached automated health monitoring system (CowManager, AgisAutomatisering, NL).

MATERIALS and METHODS: A total of 3,380 cows (1,123 primiparous cows and 2,257 multiparous cows) from a commercial dairy farm were included in the statistical analyses. A prepartum alert (transition alert) was defined based on a proprietary algorithm. Cows were classified according to the number of transition alerts from d 50 until d 0 prior to calving into 3 categories: (1) no transition alert (Alert0); (2) one transition alert (Alert1), and (3) 2 or more transition alerts (Alert2). Generalized linear mixed models were used to morbidity, calving related events, and milk yield. Cox proportional hazard models were used for time to event data (i.e., culling within 60d).

RESULTS: In primiparous cows, 8.3% and 5.7% of cows were in group Alert1 and Alert2, respectively. In multiparous cows, 10.8% and 14.5% of cows were in group Alert1 and Alert2, respectively. Compared to cows in Alert0, cows in Alert1 (odds ratio [OR] = 1.74; 95% CI 1.32 – 2.27; P<0.01) and Alert 2 (OR = 1.50; 95% CI 1.15 – 1.96; P<0.01) had increased odds for a calving related event. Compared to cows in Alert0, cows in Alert1 (OR = 1.44; 95% CI 1.11 – 1.87; P<0.01) and Alert 2 (OR = 1.64; 95% CI 1.29 – 2.09; P<0.01) had increased odds for a health-related event. The occurrence of a transition alert was negatively associated with predicted 305 d milk yield at second test day (P=0.01). In multiparous cows (P= 0.01), cows in Alert1 (11,912 ± 107kg) and Alert2 (11,504 ± 99kg) produced less milk compared to Alert0 (12,326 ± 38kg). There was no significant difference in primiparous cows (P=0.90). Compared with Alert0 cows, cows in Alert1 (hazard risk [HR] = 1.81; 95% CI 1.27– 2.58; P<0.01) and Alert2 (HR = 2.79; 95%CI 2.10 – 3.70; P<0.01) had an increased hazard of culling within 60d.

CONCLUSIONS: In conclusion, there was an association between prepartum alerts detected by an ear-attached automated health monitoring system and postpartum health and production outcomes. Irrespective of parity, cows with a prepartum alert had greater risk for a calving or health related event and culling in early lactation. Multiparous cows with a prepartum alert also had reduced milk yield.

Keywords: Automated health monitoring, transition cow, prepartum alert

Timed embryo transfer success in lactating dairy cows using fresh in vitro–produced embryos fertilized with conventional or sex-sorted sperm

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BACKGROUND and OBJECTIVE: Efficiency of in vitro embryo production and subsequent embryo transfer outcomes in dairy cattle can be influenced by donor type, semen type, and recipient fertility. This study aimed to: (1) compare in vitro embryo production using conventional or sex sorted semen to fertilize oocytes collected from high genetic merit beef and dairy donors, and (2) evaluate pregnancy outcomes in lactating dairy cows following transfer of fresh embryos produced using conventional or sex sorted semen after timed embryo transfer.

MATERIALS and METHODS: Oocytes were collected weekly for four consecutive weeks from elite beef and dairy donors using ultrasound guided ovum

pickup. After in vitro maturation, oocytes underwent fertilization with frozen–thawed conventional or sex sorted semen from proven fertility sires and were cultured to the blastocyst stage. A total of 507 Grade 1 blastocysts, either fresh or previously frozen, were transferred to lactating dairy cows recipients synchronized with a PRID based protocol (PRID Delta®, Ceva Sante Animale, Libourne, France). Recipients were blocked by parity, days in milk, and genetic merit, then randomly assigned to receive an embryo derived from beef or dairy donors fertilized with conventional or sex sorted semen. Pregnancy diagnosis was conducted via transrectal ultrasonography on day 32 after synchronized estrus and confirmed on day 63. Pregnancy loss was defined as loss between the two examinations.

RESULTS: Beef donors yielded more oocytes per collection session, a higher proportion of blastocysts, and a greater number of blastocysts per fertilization session compared with dairy donors. Blastocyst development was similar between fertilizations performed with conventional or sex sorted semen, including comparisons using semen types from the same sire. Pregnancy rates at day 32 and day 63 did not differ between embryos originating from beef or dairy donors, nor between embryos produced using conventional or sex sorted semen. Pregnancy loss did not differ among groups. Serum progesterone concentration on day 7 was not associated with pregnancy status at either time point.

CONCLUSIONS: Beef donors demonstrated higher oocyte recovery and blastocyst production compared with dairy donors. Embryo development was comparable between fertilizations performed with conventional and sex sorted semen. Pregnancy outcomes following transfer of fresh embryos were similar regardless of donor type or semen type. These findings support the use of sex sorted semen for in vitro embryo production without compromising embryo development or pregnancy success in synchronized lactating dairy cows recipients.

Keywords: dairy-beef integration, in vitro fertilization, blastocyst, assisted reproductive technology

Non-antibiotic intrauterine therapy at 3 weeks postpartum in dairy cows: Effects of PVP-I and hypertonic glucose on uterine pathogens and reproductive performance

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BACKGROUND and OBJECTIVE: Endometritis impairs fertility, prolonging days open, reducing pregnancy rates, and increasing culling rate in dairy cows. With growing concerns about antimicrobial resistance, non-antibiotic uterine treatments are needed. Prior work shows polyvinylpyrrolidone-iodine (PVP-I) promotes endometrial regeneration and reduces bacterial counts; hypertonic glucose is another antibiotic-free option. The efficacy of PVP-I or 50% glucose given as early as 3 weeks postpartum (W3) for cows with retained fetal membranes (RFM) or endometritis remains unclear. This study aimed to clarify the effects on endometrial polymorphonuclear neutrophil percentage (PMN%), intrauterine pathogens, and subsequent reproductive performance.

MATERIALS and METHODS: Four hundred four lactating Holstein cows from 12 commercial farms were examined at W3 (22.5 ± 0.3 d) by vaginal discharge scoring and cytobrush sampling and classified as RFM (n=118), clinical endometritis (CEM, n=43), subclinical endometritis (SEM, n=95), or normal (NOR, n=148). After sampling, cows received an intrauterine infusion of 50 mL of 2% PVP-I (PVPI), 50% glucose solution (GLCS), or isotonic saline (ISCS), randomized within groups. PMN% and cultures for five pathogens (*Trueperella pyogenes*, *Escherichia coli*, *Fusobacterium necrophorum*, *Prevotella melaninogenica*, *Bacteroides pyogenes*) were assessed at W3 and W4 (29.5 ± 0.3 d). Estrus expression (60–80 d postpartum), pregnancy rate after AI/TAI, days open, and number of services were followed up to 300 d.

RESULTS: At W3, detection rates of *T. pyogenes* and *E. coli* were higher in the RFM group than in other groups ($P < 0.05$). PMN% decreased from W3 to W4 across all treatments, with no significant differences among infusion types. PVP-I significantly reduced the proportion of cows positive

for pathogens from W3 to W4 (P = 0.04) and increased the proportion with no detectable bacteria (P = 0.04). Both PVP-I and GLCS reduced rates of heavy bacterial growth versus ISCS (P < 0.05). In RFM cows, PVP-I increased the proportion showing estrus within 80 days postpartum compared with GLCS (63.6% vs. 22.6%, P = 0.002); no significant difference was observed between PVP-I and ISCS. No consistent differences in overall pregnancy rates or numbers of inseminations were observed among treatments.

CONCLUSIONS: Intrauterine infusion of PVP-I or 50% glucose at W3 reduced uterine bacterial load, though PMN% was unaffected. Notably, PVP-I improved estrus resumption in RFM cows, suggesting reproductive benefits primarily via antimicrobial action rather than measurable reduction in endometrial PMNs. Early non-antibiotic intrauterine therapy, particularly PVP-I, warrants further study to optimize timing, dosing, and impact on reproductive outcomes.

Keywords: Dairy cows, Intrauterine infusion, Polyvinylpyrrolidone-iodine, Postpartum uterine disease, Reproductive performance

Beyond the outbreak: Assessing the decline in bovine reproductive efficiency following epizootic hemorrhagic disease (EHD) infection

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BACKGROUND and OBJECTIVE: Epizootic Hemorrhagic Disease (EHD), caused by the EHD virus (EHDV) and transmitted by Culicoides midges, has become a consolidated threat to cattle in the Iberian Peninsula. While acute symptoms are documented, its chronic impact on long-term reproductive efficiency remains unclear. This study quantifies the reproductive "aftershock" of EHDV by comparing pre-infection, outbreak, and recovery periods.

MATERIALS and METHODS: A longitudinal, retrospective, epidemiological study was conducted on a cohort of cattle, over three seasons: S1 (2021-23, EHD absent), S2 (2023-24, outbreak), and S3 (2024-25, post-outbreak). The population showed a seroprevalence of 99.40%, necessitating intra-herd temporal comparisons. Parameters included Calving-to-Calving Interval (CI), Days Open (DO), pregnancy loss rate, and reproductive disorders prevalence by type of problem (abortions, stillbirths, neonatal deaths, and weak-born calves). Non-parametric tests, Chi-square tests with corrected residuals, and logistic regression (Odds Ratio [OR]; 95%CI) were used, with S1 as the reference.

RESULTS: (P<0.05). DO rose from 79 days (S1) to 81 days (S2) (P=0.002), reaching 97 days in S3 (P=0.112). The pregnancy loss rate doubled from 4.4% (S1) to 8.8% (S3) (OR=2.082; CI95%: 1.286–3.372; P=0.003). General reproductive problems jumped from 14.2% (S1) to 29.5% (S2) and 19.4% (S3;), with a significantly higher probability of occurrence in S3 vs. S1 (OR=1.454; CI95%: 1.063–1.988; P=0.019) and in S2 vs. S1 (OR=2.527; CI95%: 1.879–3.398; P=<0.001) were observed. Clinical patterns shifted: the likelihood of abortion was 1.919 times higher in S3 than in S1 (OR=1.919; CI95%: 1.061–3.474; P=0.031) and was the most prevalent problem in S3 (46.7% of all reproductive problems in S3); stillbirths were more probable in the season of outbreak (S2) than in S1 (OR=2.583; CI95%: 1.411–4.727; P=0.002) and the most prevalent problem in S2 (39.5%); neonatal death was less frequent post-outbreak(S3) than in S1, with EDH absent (OR=0.343; CI95%: 0.177–0.667; P=0.002), with it being the most prevalent problem in the time before EHD (S1, 40.4%). The prevalence of weak-born calves did not change with time. Geographic area and pregnancy status showed no significant correlation with CI nor with other reproductive parameters.

CONCLUSIONS: EHDV exerts a severe, prolonged negative impact on bovine reproduction that extends beyond the acute clinical phase. Veterinary management must incorporate long-term monitoring strategies to mitigate these persistent effects. Supported by the Agriculture Council of the Junta de Extremadura.

Keywords: Emerging disease, long-term effects, beef cattle

Temporal patterns of insulin resistance and NEFA in late pregnancy: Links to risk of dystocia in beef cows

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BACKGROUND and OBJECTIVE: Dystocia in cattle increases stillbirth, neonatal mortality, and postpartum disorders, resulting in economic losses for producers. Although maternal insulin resistance and hyperglycaemia are implicated in fetal macrosomia in humans, whether maternal metabolic adaptations during late gestation relate to dystocia in cattle remains unclear. This study aimed to clarify whether blood metabolic markers and insulin-resistance indices during late pregnancy associate with calf birth weight and dystocia risk in beef cows.

MATERIALS and METHODS: Forty-five Japanese Black cows were enrolled, and blood was sampled at six time points between 229 and 285 days of pregnancy. Serum glucose, insulin, NEFA, BHB, pregnancy-associated glycoproteins (PAGs), and oestrone sulphate (E1S) levels were quantified. Insulin-resistance indices—HOMA-IR (HR), RQUICKI (RQ), RQUICKI-BHB (RQBHB), and glucose: insulin ratio (G: I)—were calculated. Areas under the curve (AUCs) were computed for the late-pregnancy period and for two subperiods (229–257 days as first-stage and 258–285 days as second-stage) owing to a feeding change at day 258. Of the 45 cows, 16 experienced dystocia and 29 eutocia.

RESULTS: Serum insulin and BHB increased toward term; NEFA decreased at day 271, then rose by day 285. Among the insulin resistance indices, HR significantly increased at day 285, whereas RQ decreased. RQBHB and G: I significantly decreased from day 257 to 285. Calf birth weight was greater in the dystocia group ($P < 0.05$), and the calf: dam weight ratio tended to be higher ($P = 0.068$). No direct correlations were found between AUCs of metabolic indices and calf birth weight. PAGs and E1S AUCs correlated moderately negatively with HR and positively with RQ, RQBHB, and G: I. Compared with eutocia, the dystocia group exhibited a smaller insulin AUC and larger G: I AUC across 229–285 days ($P < 0.05$). Subperiod analyses showed lower insulin AUC in dystocia during the first stage and higher NEFA AUC during the second stage. Logistic regression found no independent predictors reaching significance. The AUCs for first-stage insulin, RQ, and G: I and second-stage NEFA indicated low–moderate predictive power; proposed cutoffs had high sensitivity or specificity but limited overall accuracy.

CONCLUSIONS: Maternal metabolic changes—lower insulin and altered RQ/G: I in the first stage of late gestation and elevated NEFA later—are associated with dystocia and higher calf birth weight. Insulin, RQ, and NEFA are candidate blood biomarkers for dystocia risk screening in cattle, but predictive performance is modest. Multicentre validation and refinement are required before field application.

Keywords: Beef cows, Calf birth weight, Dystocia, Insulin resistance, Late pregnancy, Metabolic indicators

Impact of ongoing versus regressing pre-existing corpus luteum on fertility in the Ovsynch protocol

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BACKGROUND and OBJECTIVE: This study aimed to determine whether conception rates are still lower in cyclic cows that respond to the first GnRH (GnRH1) of the Ovsynch protocol, and whether the status of the pre-existing corpus luteum at the start of Ovsynch (whether it persists or regresses between GnRH and $PGF_2\alpha$) affects the success of the protocol in these cows

MATERIALS and METHODS: The ovarian maps of 1,920 cyclic cows subjected to the Ovsynch protocol were evaluated retrospectively. During ovarian mapping, cows were examined at the first and

second GnRH, $PGF_2\alpha$, at the time of insemination, and 7 d after TAI. At each examination, the location of ovarian structures and the number of corpus luteum (CL) present were identified and recorded. The cows were initially divided into two groups based on their response to the GnRH1: responders (n=1104) and non-responders (n=816). Subsequently, among the responder, cows were reclassified according to whether the CL pre-existing at the initiation of the protocol persisted until the $PGF_2\alpha$. Thus, cows were categorized as either having a preexisting CL (CL+; n=967) or a regressing (CL-; n=137).

RESULTS: Cows responding to the GnRH1 showed higher (P<0.01) second GnRH (GnRH2) response (93% vs 90%), lower (P<0.0001) early ovulation before TAI (5.7% vs 10.7%) and embryonic loss (P<0.002; 5.6% vs 11.7%), and higher conception rates at 31 and 62 days (OR = 1.45, 95% CI: 1.19–1.77 and OR = 1.56, 95% CI: 1.22–1.97). No significant differences were observed in early AI before TAI or return rate. In addition, cows in the CL (−) group had lower conception rates and significantly lower odds of conception compared with CL (+) cows (OR = 0.55; 95% CI: 0.35–0.87; p = 0.0104), while no significant differences were observed in GnRH2 response, early ovulation and AI before TAI, or return rate.

CONCLUSIONS: This study demonstrates that both the ovarian response to GnRH1 and the fate of the pre-existing CL at the start of the Ovsynch protocol are critical determinants of fertility outcomes in cyclic dairy cows. A positive response to GnRH1 improved synchronization, optimized follicular development, and increased pregnancy rates while reducing embryonic loss. Moreover, the presence of a functional CL throughout the protocol increased conception rate by elevated progesterone, supporting better oocyte competence and early embryo development. Overall, maintaining a stable high-progesterone environment—either by ensuring GnRH1 ovulation or preventing premature CL regression—appears essential for maximizing reproductive success following TAI.

Keywords: Cow, ovsynch, ovarian activity, conception

Twinning risk factors and pregnancy loss according to pregnancy type in grazing dairy cows

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BACKGROUND and OBJECTIVE: Twinning is an important reproductive problem in high-producing dairy cows and may increase the risk of pregnancy loss. The objective was to identify risk factors associated with twin pregnancy and to evaluate pregnancy loss by to pregnancy type: singleton (PRE), unilateral twins (UNI) or bilateral twins (BI).

MATERIALS and METHODS: A retrospective cohort study was conducted in a commercial dairy farm in Argentina (~4,000 milking cows). Pregnant cows diagnosed in May–June 2024 (n=4,487) were enrolled; historical records were extracted and cows were followed to pregnancy outcome. Mean (±SD) BCS was 2.9±0.25, days in milk at conception 132±90, milk yield 29.5±8.3 L/day, and parity 2.3±1.3. Cows were inseminated by timed AI (n=2,059) or heat detection (n=2,428). Ultrasound diagnosis was performed at a median of 34 days post-AI and classified as PRE (singleton), UNI (twins in the same uterine horn), or BI (one twin per horn); recheck was at ~day 75. Data were analyzed using PROC GLIMMIX (SAS) for twinning rate (UNI+BI) and pregnancy loss, with insemination method, parity, season, BCS, days in milk, and MILK as predictors.

RESULTS: Overall twinning rate was 9.9% and was associated with insemination method (TAI 13.4% vs HD 7.0%; P<0.0001) and MILK (P=0.0052). For each 1-L/day increase in MILK, the odds of twinning increased by 1.9% (OR 1.019; 95% CI 1.006–1.033). A total of 607 cows did not present for pregnancy recheck. Pregnancy loss was 20.0% (776/3,880) and was associated with pregnancy type (P<0.0001), parity (P=0.0001), and breeding season (P=0.0041).

Pregnancy loss was 19.3% (678/3,508) for singletons, 29.7% (77/259) for unilateral twins, and 18.6% (21/112) for bilateral twins. Adjusted loss for pregnancy type was were lower for PRE vs UNI (OR 0.58, 95% CI 0.43–0.79; P=0.0014) and for BIL vs UNI (OR 0.49, 95% CI 0.27–0.91; P=0.059), while BIL vs PRE did not differ (OR 0.84, 95%CI 0.49–1.46; P=0.81).Pregnancy loss was higher for cows inseminated in summer (24.4% [132/540]) than in autumn (18.1% [249/1,373]), winter (18.7% [190/1,017]), or spring (21.6% [205/950]).

CONCLUSIONS: Twinning risk increased with timed AI and higher milk yield around the fertile insemination. Pregnancy loss was influenced by pregnancy type, parity, and season, with the highest losses observed in unilateral twin pregnancies. These findings highlight the importance of managing high-producing cows, particularly during summer, and closely monitoring twin pregnancies to reduce reproductive losses.

Keywords: Twinning, Pregnancy loss, Twin pregnancy type (unilateral vs bilateral), Dairy cows

Getting cows pregnant but failing the heifers: A reproduction problem to be addressed?

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BACKGROUND and OBJECTIVE: Dairy heifer fertility is a key determinant of reproductive efficiency and herd sustainability. Although dairy cow fertility is now considered acceptable after the decline of the last century, field observations suggest, heifer fertility may be lower than expected. Our objective was to clarify whether heifer fertility in dairy herds is unsatisfactory and to identify probable causes.

MATERIALS and METHODS: Data were collected by seven veterinarians from 45 Spanish dairy farms across 11 provinces, each contributing on average five consecutive years, totalling 459 farm_years (2004–2025). The experimental unit was the "farm_year". Information on reproductive and health management was not available for all farm_years, so each sub dataset was analyzed accordingly. The dataset included annual cow and heifer fertility and reproductive/health management data. A linear mixed effects model (REML) was performed with heifer fertility as the dependent variable, predictors (AI strategy, sexed semen use, age at first AI, disease control…) and farm as a random intercept, accounting for repeated measures across years.

RESULTS: Average cow and heifer fertility were 33.8±8.0% and 53.8±11.2%, respectively; first A_fertility was 36.5±9.5% and 56.5±11.5%. Cows' first_AI_fertility increased strongly over time (24.8±3.2% in 2004 vs. 39.1±1.4% in 2025), while heifer first_AI_fertility remained almost unchanged (52.55±14.0% vs. 55.1±11.6%). Regarding management, 52.4% (144/275) of farm_years used sexed semen; 98.4% (308/313) controlled BVD, 93.1% (285/306) IBR, 61.2% (178/291) neosporosis, and 1/101 Q_fever. Active BVD occurred in 2.6%, IBR in 2.4%, and neosporosis in 12.4% of reporting farm years; eight farm years reported Q_fever status, with four infected. Only 34.8% (105/302) synchronized heifers; the rest relied on observed estrus (70.3% inseminated), rising to 88.84% in farms using sensors. In synchronized farm years, 88.67% of heifers were inseminated after PG protocols and 48.2% with progesterone devices, reflecting consistent farm level strategies. In the mixed effects model, three predictors remained: fertility increased slightly over time (β=0.917; P=0.062); sexed semen showed a significant negative association (β=−0.116; P=0.010); and farms not controlling Neospora showed 9.4 percentage points lower fertility (P<0.001).

CONCLUSIONS: Heifer fertility improved only slightly over time and markedly less than cow fertility in the same farm-years. Management and health factors strongly influenced outcomes. Sexed semen reduced fertility, and farms not controlling Neospora showed poorer performance. Although these farms are voluntary collaborators and not

national averages, the findings raise concern that heifer fertility problems may be more widespread than assumed and highlight the need to optimize reproductive management and address health-related contributors to suboptimal heifer fertility.

Keywords: Heifer fertility, synchronization, sexed semen, vaccination status

Preliminary results on the use of next-generation sequencing for pathogen detection in bovine abortion cases

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BACKGROUND and OBJECTIVE: Abortion is a major fertility problem within cattle. At least 2% of the cattle herds in Flanders (Belgium) have more than 5% abortions. In Belgium, farmers are obliged to send all aborted foetuses to a laboratory for a standardized diagnostic protocol. Few tests are mandatory, and farmers and veterinarians have a wide range of additional tests available. Unfortunately, in only 20-50% of the cases a definitive diagnosis is made. This makes farmers and veterinarians reluctant to perform additional diagnostic tests. The objective of this study is to evaluate next-generation sequencing as an alternative to routinely used diagnostic methods.

MATERIALS and METHODS: Fourteen aborted foetuses and corresponding placentas were collected from farms with increased abortion rates. Autopsy was performed on all foetuses. Samples for bacteriology and virology were collected from macroscopic abnormal tissues. When no lesions were present, abomasal fluid samples were collected. Aerobic bacterial culture was performed together with selective media for Salmonella spp. and Listeria monocytogenes. The presence of Chlamydia spp., Leptospira spp., Anaplasma spp, Bluetonguevirus, Schmallenbergvirus and Coxiella burnetii were tested with PCR. Additionally, all samples were analysed with nanopore sequencing.

RESULTS: No viruses were detected with PCR. Escherichia coli, Listeria monocytogenes, Vibrio sp and Trueperella pyogenes were the only detected pathogens in clinically affected tissue. Nanopore sequencing was performed on collected samples of six foetuses, that contained E. coli. Vibrio sp. and T. pyogenes. No bacteria were detected with nanopore sequencing. The remaining samples will be run in the near future.

CONCLUSIONS: For the six aborted foetuses, no bacteria that were detected with routine diagnostics were found with nanopore sequencing. This is not surprising, due to the varying results in the detection of bacteria with nanopore sequencing. However, no clear conclusions can be made about the use of nanopore sequencing for the detection of pathogens in bovine abortion cases, as more samples still need to be analysed and no viruses were found. Additionally, 16S sequencing will be evaluated as an alternative sequencing method.

Keywords: Bovine, abortion, next-generation sequencing

SHEEP AND GOATS

Nation-wide control program of ovine footrot in Switzerland

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BACKGROUND and OBJECTIVE: Ovine footrot is a contagious, painful foot disease of sheep that occurs worldwide It is the most common cause of lameness and responsible for remarkable economical losses in the sheep industry. *Dichelobacter nodosus* is the primary etiological agent. Various footrot control interventions have been implemented in the past in several regions worldwide. They include phenotypic

or genotypic selection of less sensitive sheep, vaccination, antibiotic treatment, careful hoof trimming, repeated footbathing in disinfectant solutions and combinations thereof. The goals of this study are to (i) briefly describe the control program implemented in Switzerland and (ii) provide results after 6 months of its implementation.

MATERIALS and METHODS: All Swiss sheep flocks are subjected to the control program, based on the five pillars strategy: (i) education and training of personnel involved; (ii) yearly PCR-testing (*aprV2* gene expression, encoding for this key virulence factor of *Dichelobacter nodosus*), using four-feet interdigital cotton swab samples of a risk-based sample of sheep per flock; (iii) treatment of affected flocks; (iv) culling of severely diseased sheep, and (v) implementation of biosafety measures to prevent disease spread (no animal traffic of sheep from affected flocks except from transportation to the slaughter house). Treatment measures include careful and meticulous hoof trimming, repeated footbathing in a biodegradable disinfectant solution and systemic antimicrobial treatment in selected cases. Vaccination is not allowed. This testing&treatment period lasts 6 months, from Oct 1 to March 31 and is followed by a 6 months summer pasturing period. Commingling on summer pastures is only allowed for flocks tested negative. The program started on October 1, 2024 and lasts for a maximum of 5 years. The overall goal is to reduce the prevalence of ovine footrot at the flock level (flocks with at lest one sheep harbouring *D. nodosus* carrying the *aprV2 gene*) in Switzerland to <1%.

RESULTS: During the initial testing&treatment period, 12,432 flocks were examined. The overall prevalence of ovine footrot in Switzerland at the herd level at first PCR testing was found to be 20.4%. Implementation of the five pillars strategy of footrot control allowed to reduce the prevalence to 12.3% at the end of the first testing&treatment period.

CONCLUSIONS: The reduction of the number of affected flocks by 39.7% within 6 months of initiation of the control program is remarkable. Current research focuses on identification of risk factors for failure of complete elimination of the causative agent at the flock level and for re-infection during the summer pasturing period.

Keywords: ovine footrot, lameness, sheep, {Dichelobacter nodosus}, five pillars control program, biodegradable disinfectant

Udder health metrics in grazing dairy ewes

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BACKGROUND and OBJECTIVE: There is limited published research on milk quality and mastitis in grazing dairy ewes, leaving producers and their advisors in pasture-based systems reliant on extrapolation from bovine mastitis or overseas dairy sheep research. The objectives were to describe teat and udder morphology and pathology, estimate somatic cell counts (SCC), validate the California mastitis test (CMT), and determine an SCC threshold for diagnosing subclinical mastitis in grazing dairy ewes on New Zealand farms.

MATERIALS and METHODS: A repeated cross-sectional study was conducted on 20 commercial New Zealand dairy sheep farms during the 2022–2023 season. Approximately 15 randomly selected ewes per farm were examined in early, mid, and late lactation. Udder depth, suspension (ratio of width at abdominal attachment to height), gland separation, and teat placement were scored on a five-point scale. Teat length and width were measured, and the presence of supernumerary teats, asymmetry, visible teat or udder inflammation, lesions, and involuted glands was recorded. Teats and glands were palpated, and teat ends were scored for hyperkeratosis. Gland-level milk samples were collected for CMT, composite SCC, and bacterial culture. Subclinical mastitis was defined as ewes having one or two bacteriologically positive glands and SCC >500,000 cells/mL and/or CMT ≥1.

RESULTS: A total of 893 observations were recorded from 882 unique ewes. Mean teat length and width were 27.5 mm (95% CI = 27.2–27.7) and 15.8 mm (95% CI = 15.7–15.9), respectively. Udder depth, suspension, and teat placement had median scores of 4, 3, and 3, and varied across visits and age groups. Asymmetry was recorded in 39% (95% CI = 35.5–42.4%) of ewes and increased with age. Ewe-level prevalences of teat end hyperkeratosis, inflammation, lesions, palpable defects and involuted glands were all <6%. Geometric mean SCC was 169,039 (95% CI = 153,221–185,641) cells/mL and decreased across the season. Mean log10 SCC

increased linearly with CMT score with a moderate correlation (Kendall's tau = 0.47, 95% CI 0.43–0.52). An SCC threshold of approximately 400,000 cells/mL provided the greatest diagnostic accuracy for intramammary infection (IMI) using a single SCC measurement, but positive predictive value was low (0.367).

CONCLUSIONS: Teat and udder morphology in grazing dairy ewes was similar to that reported overseas and varied substantially between farms, while pathology was uncommon. The CMT is a valid screening tool. An SCC threshold of approximately 400,000 cells/mL maximised diagnostic accuracy for intramammary infection using a single measurement; however, follow-up testing is required due to low positive predictive value.

Keywords: sheep, udder, mastitis, somatic cell count, California mastitis test, diagnosis

Environmental heat stress indicators in semi-arid espinal silvopastoral systems: Effects of tree age and orientation relative to the trunk

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BACKGROUND and OBJECTIVE: Silvopastoral sheep production systems in the semi-arid drylands of central Chile represent a subsistence model in which cattle, sheep and goats are raised extensively with minimal investment in infrastructure. Under current climate change scenarios, increasing ambient temperatures have intensified environmental conditions that predispose grazing animals to heat stress. In this context, the microclimate generated beneath tree canopies has gained relevance as an ecosystem service that may mitigate thermal stress and improve animal welfare. However, canopy development and the daily dynamics of shade projection may strongly influence local microclimatic conditions. The objective of this study was to evaluate the effects of tree age and orientation relative to the trunk of espinal (Vachellia caven) trees on environmental heat stress indicators in a silvopastoral system

MATERIALS and METHODS: Thirty representative trees were selected within a 6-ha area and classified into three canopy area groups. Tree age (Young, Adult and Mature Adult) was determined by ring counting, and three trees per group were selected for detailed monitoring. Two thermal stress sensors (TM-188) were installed on each tree at north and south orientations in the intermediate canopy zone. Air temperature, relative humidity and black globe temperature were recorded every five minutes over a 24-h period. Five time-blocks representing contrasting thermal loads were subsequently defined. The heat stress indices Temperature–Humidity Index (THI), BGHI (Black Globe–Humidity Index) and Wet Bulb Globe Temperature (WBGT) were calculated and analyzed using ANOVA and multiple mean comparisons to evaluate the effects of tree age, orientation and time-block.

RESULTS: Time-block and orientation had significant effects on all heat stress indicators ($p<0.05$), while tree age significantly affected THI and BGHI. Northern orientations showed the highest thermal load values across all indices. Mature adult trees exhibited the lowest THI and BGHI values, indicating a greater capacity to moderate microclimatic conditions.

CONCLUSIONS: These results highlight the importance of tree development and spatial orientation in reducing environmental heat stress in grazing ruminants and support the role of silvopastoral systems as an effective strategy to enhance thermal comfort and animal welfare under semi-arid conditions.

Keywords: heat stress, animal welfare, silvopastoral

Effects of oral selenite triglycerides supplementation on blood selenium levels and bone marrow haematopoiesis in young Alpine-mix goat kids

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BACKGROUND and OBJECTIVE: Selenium deficiency poses a major health concern in small ruminants, adversely affecting growth and immune function. Selenite triglycerides, an organic form of Se, offer advantages over traditional injectable preparations. However, their efficacy has mainly been studied in cattle, pigs, and sheep. Investigating their effects in young goat kids may support a more effective, less invasive supplementation strategy, improving animal welfare and health. The aim of this study was to evaluate the effects of selenite triglycerides on bone marrow cytology, as well as blood morphology and biochemistry in Alpine-mix goat kids.

MATERIALS and METHODS: The study included 20 six-week-old Alpine-mix goat kids, randomly assigned to two groups: an experimental group (n = 10), which received an oral selenite triglycerides preparation during the 6. week of life, and a control group (n = 10), which received no selenium supplementation. To evaluate the supplement's impact on bone marrow haematopoiesis, bone marrow biopsies were performed at weeks 6 and 10 of the goat kid's life to prepare MGG-stained smears for cytological evaluation. Blood samples were collected weekly from weeks 6 to 10 to monitor changes in blood morphology and selenium levels. However, during the first week, samples for selenium concentration were taken at the beginning of the study and three days after supplementation started.

RESULTS: Cytological evaluation of bone marrow smears showed that one-week supplementation with selenite triglycerides positively influenced erythropoiesis and granulopoiesis in caprine bone marrow. This effect was particularly evident in the increased number of orthochromatic erythroblasts ($p < 0.05$) and eosinophils ($p < 0.05$), as well as earlier precursors of these cell lineages ($p < 0.05$). The bone marrow cells in the group receiving the supplement absorbed dyes faster and more intensively than the same cells in the control animals. Moreover, a significant increase in blood selenium levels was observed in the experimental group as early as the third day of the experiment ($p < 0.05$) and remained higher ($p < 0.05$) throughout the entire duration of the experiment.

CONCLUSIONS: Selenite triglycerides appear to be a promising alternative for selenium supplementation in young goats as they increase blood selenium levels and stimulate bone marrow haematopoiesis. Their low toxicity and oral administration make them a safer and less invasive option than injectable selenium preparations, while still allowing for controlled, individual supplementation. Acknowledgements: Funded by the Minister of Science under the Regional Initiative of Excellence Program.

Keywords: bone marrow, caprine haematopoiesis, goat kids, selenite triglycerides, selenium supplementation

Advancing sustainable control of Haemonchus contortus: Efficacy of a glycoengineered vaccine

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BACKGROUND and OBJECTIVE: *Haemonchus contortus*, the barber's pole worm, is considered the most pathogenic nematode of small ruminants. As a hematophagous parasite of the abomasum with high fecundity, it can cause severe anaemia, reduced performance, and, particularly in young animals,

mortality. Although traditionally associated with tropical and subtropical regions, its prevalence is increasing in temperate climates. Subclinical infections further contribute to economic losses through impaired productivity.
Current control approaches primarily target gastrointestinal nematodes in general and include anthelmintic treatment alongside management and monitoring strategies such as pasture management, selective breeding, FAMACHA scoring (FAMACHA; Onderstepoort Veterinary Institute, South Africa), body condition assessment, and faecal egg counts. However, the widespread emergence of anthelmintic resistance has intensified the need for sustainable alternatives. Integrating vaccination into parasite control programs may represent a promising long-term solution. Previous studies have identified "hidden antigens" (e.g., H11, H-gal-GP) capable of inducing protective immunity, yet recombinant vaccines have so far failed to achieve consistent efficacy. In this study, a glycoengineered recombinant anti-*Haemonchus* vaccine produced in Hi5 insect cells was evaluated *in vivo* (GZ: 2023-0.734.950).

MATERIALS and METHODS: Thirty-five male sheep were allocated to five groups: a negative control, a positive control (infection with 5,000 L3 larvae), and three infected and vaccinated groups receiving either the glycoengineered recombinant antigen (GEA), a non-glycoengineered recombinant antigen (NEA), or the commercial Barbervax (Wormvax Australia Pty Ltd, Australia) vaccine. Animals were monitored over 16 weeks for clinical, haematological, parasitological, and safety parameters, followed by determination of adult worm burdens.

RESULTS: Vaccination with the glycoengineered antigens resulted in measurable protective effects. Compared with non-vaccinated controls, GEA-immunised animals exhibited improved haematological parameters, including higher erythrocyte counts, haematocrit, and haemoglobin concentrations, consistent with a reduced severity of anaemia. FAMACHA scores were correspondingly lower. Parasitologically, GEA vaccination achieved a 25.36% reduction in worm burden and an 81.09% decrease in faecal egg excretion. In addition, differences observed between GEA and NEA groups suggest that glycan modifications substantially influenced the magnitude of the protective immune response.

CONCLUSIONS: These findings indicate that the novel vaccine can induce protective responses against *H. contortus*. Further studies are required to optimise dosage, vaccination schedules, and field applicability.

Keywords: vaccine trial, sheep, faecal egg count, antigen, immune reaction

Presence of wildlife mammals near sheep farms and associations with sheep health in the farms during a countrywide study in Greece

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BACKGROUND and OBJECTIVE: The objectives of the present study were i) the study of wildlife species near sheep farms in Greece, ii) the reporting of associations of presence of wildlife with climatic factors and iii) the evaluation of associations with parameters related to the health of the sheep on the farms.

MATERIALS and METHODS: A countrywide study was performed in 325 sheep flocks throughout Greece, which were visited by the researchers. Information about the health management applied on the farms and regarding the presence of wildlife mammals near the farms were collected through interviews of farmers, which were performed by using a structured questionnaire. Moreover, faecal samples were collected from the sheep on the farms.

RESULTS: Wildlife mammals were present near 300 (92.3%) farms: fox (74.5% of farms), wildboar (48.3%), grey wolf (39.7%), golden jackal (20.0%), hare (15.4%), roe-deer (14.5%), ferret (12.9%), badger (9.5%), brown bear (5.8%), red-deer (4.0%), jackrabbit (1.8%), mink (0.6%), otter (0.6% of farms). Median number of wildlife species near farms was 2 (interquartile range: 2). With regard to associations with climatic factors, presence of wildlife near farms was associated with seasonality of precipitation (fox, wildboar, grey-wolf, golden jackal, roe-deer, badger, brown bear, mink) or mean temperature (wilboar, grey wolf, golden jackal, roe-deer, badger, brown bear, red-deer, jackrabbit). Sheep losses by wildlife occurred in 29.9% of farms; wildlife involved in such losses were mostly wolves and jackals. Epg counts in faecal samples from sheep when co-grazing with wildlife ruminants occurred, were higher: 349±53 epg versus 212±14 epg in samples from sheep where this

did not occur; there was also a higher proportion of Haemonchus contortus in faecal samples from former animals: 32.7%±1.5% versus 29.0%±0.7%. Annual incidence of abortions and neonatal diarrhoea among farms in which co-grazing with wildlife ruminants occurred, was also higher: 1.6% and 8.2%, respectively, versus 0.0% and 1.7% among farms it did not take place.

CONCLUSIONS: The results provide a detailed mapping of interactions of wildlife with sheep and confirm that presence of wildlife near sheep farms can adversely influence health of farmed sheep.

Keywords: common grazing, goat, health management, sheep, transmission, wildlife

Machine-milking practices, animal welfare-related reactions and quality of milk produced in dairy sheep farms

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BACKGROUND and OBJECTIVE: The specific objectives of this study were i) the evaluation of the frequency of reactions potentially associated with the reduced welfare of dairy sheep during the milking process, ii) the identification of predictors associated with the presence of these reactions, and iii) the evaluation of associations with the quality of milk produced on a farm.

MATERIALS and METHODS: The study was conducted in 52 dairy sheep farms in Greece. The farms were visited, and the milking process was observed and monitored, and the reactions of ewes during the milking process were recorded; samples from the bulk-tank milk were collected for somatic cell and total bacterial counting. Univariable and multivariable analyses were performed.

RESULTS: The median duration of milking per row was 7.9 min, and the median duration of the milking process was 105 min. The reaction observed less often from ewes was vocalisation (in 9.6% of farms), and the one observed more frequently was 'spot stepping' (in 98.1% of farms). In total, eight different predictors were identified for the displaying of the various reactions observed and recorded; of these, two were related to the milking parlour and six were related to the milking process. Moreover, three different predictors were identified for the milk quality parameters; of these, one was related to the milking parlour and two were related to the milking process. Finally, a mild correlation was seen between the proportion of ewes that attempted to remove the milking cluster, and the somatic cell counts in the bulk-tank milk.

CONCLUSIONS: The results confirmed that incorrect practices during machine-milking can be stress factors for dairy sheep. In light of the present findings, procedures by milkers should be carefully carried out, which will contribute to minimising the nervousness and aggravation of ewes during milking. Stress factors can adversely affect the quality of milk produced on the farm. The findings underline that maintenance of animal welfare is linked to high product quality. This connection can further extend to consumer perceptions: the ethical treatment of dairy sheep is considered as an important facet of the overall food quality and sustainable production.

Keywords: goat, mastitis, milking routine, sheep, welfare

Urinary biomarkers and decision-making thresholds in ovine renal disease: Differentiating tubular and glomerular injury in selected clinical entities

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BACKGROUND and OBJECTIVE: Urinalysis is an important diagnostic tool in ruminants, providing information on renal function and general health. Proteinuria indicates glomerular and/or tubular damage and may represent an early sign of renal injury. In ruminants, proteinuria may be associated

with inflammatory, toxic, metabolic, or systemic disorders affecting renal integrity. Urinary γ-glutamyl transferase (GGT) is a sensitive marker of tubular epithelial injury and may increase before detectable serum biochemical changes. Thus, urinary biomarkers can identify renal damage prior to the development of azotemia. While reference intervals for urinary protein indices are established in cattle, validated values for sheep are limited, reducing diagnostic accuracy. The aim of this study was to establish reference intervals (RI) for the urine protein-to-creatinine ratio (UPC) and urinary GGT-to-creatinine ratio (UGGTC) in healthy ewes and to assess their application in selected clinical entities for defining thresholds differentiating tubular from glomerular injury.

MATERIALS and METHODS: Urine samples without active sediment from 126 mid-lactation ewes were used to determine reference intervals for the urine protein-to-creatinine ratio (UPC) and urinary γ-glutamyl transferase-to-creatinine ratio (UGGTC). Samples were collected from 150 clinically healthy animals during their first or second lactation. Animals had no medical issues since lambing, tested negative on the California mastitis test, and were seronegative for Maedi-Visna virus. All originated from five caseous lymphadenitis-free farms. RI were calculated using the 2.5th–97.5th percentiles (SPSS v29). Urine samples with UPC values above the upper reference limit were further evaluated for UGGTC. These included samples from 22 sheep with clinical bluetongue (BT), 11 lambs with kanamycin-induced renal injury, 8 ewes seropositive for *Anaplasma phagocytophilum*, and 6 ewes seropositive for *Borrelia burgdorferi* sensu lato.

RESULTS: Reference intervals were 0.08–0.20 for UPC and 0.15–0.66 for UGGTC. Among BT cases, 19/22 exceeded the UGGTC upper limit and 7/19 had cellular casts. All lambs with kanamycin toxicosis had elevated UGGTC values, with 8/11 showing cellular casts. All animals seropositive for *Anaplasma phagocytophilum* or *Borrelia burgdorferi* had UGGTC values within reference intervals.

CONCLUSIONS: This study establishes clinically applicable decision-making thresholds for UPC and UGGTC in sheep. Elevated UGGTC values were strongly associated with tubular injury in bluetongue and aminoglycoside nephrotoxicity whereas in *Anaplasma phagocytophilum* and *Borrelia burgdorferi* seropositive ewes, proteinuria occurred without increased UGGTC, suggesting a predominantly glomerular origin. The combined assessment of UPC and UGGTC enables early differentiation between tubular and glomerular injury before azotemia develops, thereby improving clinical decision-making in ovine renal disease.

Keywords: biomarkers, proteinuria, enzymuria, renal injury, sheep

Longitudinal study of antibiotic resistance of staphylococci from cases of subclinical mastitis in sheep in Greece: Incidence and risk factors

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BACKGROUND and OBJECTIVE: The objectives of the present study were i) the description of incidence of isolation of antibiotic-resistant staphylococci from mastitis in dairy sheep flocks and ii) the identification of relevant risk factors. This work extends previous field studies of subclinical mastitis in sheep and focuses on laboratory characteristics of staphylococcal isolates.

MATERIALS and METHODS: Staphylococcal isolates (n = 179) from cases of subclinical mastitis in 12 dairy sheep farms monitored throughout a lactation period in Greece, were evaluated for antibiotic resistance to 18 antibiotics by using the automated system BD-Phoenix™-M50. Multivariable analysis was performed to determine risk factors for the incidence risk of isolation of antibiotic-resistant staphylococci.

RESULTS: Antibiotic resistance was detected in 32% of isolates. It involved 11 antibiotics, most often ampicillin (63% of resistant isolates), penicillin (63%) and tetracycline (47%). Among these, 21 isolates were multidrug-resistant. The incidence risk of isolation of staphylococci resistant to at least one (any) antibiotic throughout the study period was 24% and the incidence risk of isolation of multidrug-resistant staphylococci throughout the study period was 9%. The omission of anti-staphylococcal mastitis vaccination of ewes emerged as a risk factor for the increased incidence risk of isolation of resistant staphylococci. The higher number of antibiotics used for treatment of mastitis and the younger age of

lambs taken away from their dam emerged as risk factors for the increased incidence risk of isolation of multidrug-resistant staphylococci. Biofilm-forming antibiotic-resistant staphylococci were recovered mostly from farms in which anti-mastitis vaccination was not applied: 56% versus 44% from farms in which anti-mastitis vaccination was applied.

CONCLUSIONS: The findings contribute to the effective treatment of mastitis in dairy sheep farms.

Keywords: antibiotic resistance, biofilm, mastitis, methicillin, risk factor, Staphylococcus

Health Impact of staphylococcal mastitis vaccination in a French dairy sheep flock

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BACKGROUND and OBJECTIVE: Staphylococci are the main pathogens responsible for mastitis in dairy sheep flocks. The mastitis control program is an essential step toward improving milk quality and preventing disease. One of the measures included in these control programs is vaccination. The objective of this study was to evaluate the technical impact of vaccinating against Staphylococcal mastitis compared to a period before vaccination.

MATERIALS and METHODS: The study was performed in a conventional managed dairy sheep Lacaune flock in the Roquefort Area (Aveyron, France). All animals were vaccinated against Staphylococcal mastitis according to the recommended administration program. The control group is a season without vaccination (N=471 animals, November 2021- April 2022).
First year of vaccination (N=483 between November 2022 and April 2023). Second Year of vaccination (N=486 between November 2023 and April 2024). Third year of Vaccination (N=529 between November 2024 and April 2025).
Several Parameters of milk quality were examined throughout the study, including:

•Bulk Tank Somatic Cell Count (BTSCC) K cell/ml; Somatic cell count by instantaneous fluoro-opto-electronic counting
•Staphylococci excretion SFU/ml -horizontal method for the enumeration and detection of coagulase-positive staphylococci by the MPN (most probable number) method
•Number of clinical mastitis cases.

RESULTS: The average BTSCC was 333,9 in the control year, first year of vaccination 273,9, second year of vaccination 286,5 and third year of vaccination 180,0. After Linear regression with group as factor, the P-values are:
•Control vs Vaccination 1 year=0.02,
•Control vs vaccination 2 year=0.106,
•Control vs Vaccination 3 year <0.001,
•Vaccination 1 year vs Vaccination 2 year = 0.929,
•Vaccination 1 year vs Vaccination 3 year <0.001,
•Vaccination 2-year vs Vaccination 3 year <0.001
Across the three years of vaccination, the staphylococci excretion remained stable and lower (10 SFU/ml) than during the control period (250 SFU/ml)
The clinical mastitis count was (logistic regression with group as factor):
• Control year: N=30 (6,36%)• Year Vaccination 1: N=12 (2,48%) P Value vs control =0,025
• Year Vaccination 2 N=10 (2,06%) P Value vs control =0,009
• Year Vaccination 3 N=2 (0,38%) P Value vs control =0,001

CONCLUSIONS: The vaccination with the inactivated Staphylococcus aureus vaccine contributed to decrease and control bulk tank Staphylococci and somatic cell counts and it helped to reduce clinical mastitis too. The results indicate that vaccination is an effective tool for controlling subclinical and clinical mastitis caused by Staphylococci on the farm, and it can be used alongside other preventive
measures to support disease prevention.

Keywords: sheep, vaccination, mastitis, milk quality, somatic cell count,

Optimizing laparoscopic artificial insemination success in Ile-De-France sheep: Influencing factors and field outcomes

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BACKGROUND and OBJECTIVE: Laparoscopic artificial insemination with high genetic value frozen sperm is a key factor in sheep industry. Several factors can influence the effectiveness of this technique (protocols, health and endocrine status of the ewes', sperm quality, environment, etc.). The season, the lunar phase, meteorological parameters and farm management factors were investigated to determine the most important ones affecting pregnancy rate.

MATERIALS and METHODS: A total of 250 Ile-de-France ewes were inseminated by laparoscopy in spring (March, April) and in autumn (October, November) in commercial sheep farms in Hungary with frozen sperm from an European Union licensed artificial insemination station in France. The age of the ewes was between 10 month and 10 years with a BCS between 2.5-3.5. The animals were kept in semi-intensive, deep-litter housing in a pen, with access to a pasture from spring to autumn. Hay and water was available ad libitum and they were given roughage once a day.

RESULTS: In the autumn season, only 20.0% of the inseminated ewes became pregnant while in spring, this was 51.3%. In the spring season, an extreme change in minimum temperature affected negatively the probability of conception (P = 0.0692), within the four days preceding insemination. Increased variance in the maximum and minimum temperature decreased the probability of conception (Tmax: P = 0.0472; Tmin: P = 0.033) within 40 days after insemination. Spring inseminations resulted in a significantly higher number of twin pregnancies (29.23%) compared with autumn inseminations (12.73%). The proportion of twin pregnancies was lower (21.90%) in the waning moon period compared to the waxing moon period (28.47%). None of the examined farm management factors had an effect on pregnancy. The lunar phase only affected the ovulation rate, with higher twin pregnancies during inseminations performed in the first and third quarters of the moon ($p<0.1$). The age of the mother had a significant influence on insemination results. Mothers, under 1000 days of age, were 60% more successful in laparoscopic artificial insemination than older mothers, as the probability of insemination decreases with age.

CONCLUSIONS: In the Ile de France breed, therefore, higher insemination results can be achieved in ewes under 1000 days of age and in the spring months (out of season). In our humid-temperate continental climate (in Hungary), spring laparoscopic inseminations were more effective in Ile-de-France sheep, with weather conditions and age of the ewes potentially influencing the outcome.

Keywords: sheep, artificial insemination, laparoscopy, meteorology

Greek oregano essential oil accelerates recovery in lambs with neonatal diarrhea syndrome

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BACKGROUND and OBJECTIVE: Neonatal Diarrhea Syndrome (NDS) is a major cause of morbidity and mortality in lambs under 20 days of age, commonly involving *Cryptosporidium* spp., rotavirus, coronavirus, and enteropathogenic strains of *Escherichia coli*. Oregano essential oil (OEO), rich in carvacrol and thymol, exhibits antibacterial, antiviral and anti-inflammatory properties and has been proven effective for controlling diarrhea in calves. Its proposed mechanisms include inhibition of enteropathogens, modulation of gut microbiota, and support of intestinal barrier integrity, suggesting a potential role in mitigating diarrhea severity. This study evaluated the effects of Greek OEO administration on the treatment of NDS in lambs.

MATERIALS and METHODS: A total of 50 diarrheic lambs were allocated into two equal groups. Lambs in oregano group (OG, n=25) received Greek OEO orally at 25mg/kg body weight from the onset of diarrhea until recovery and those of control group (CG, n=25) received no administration. Lambs were classified as diarrheic when exhibiting a fecal score≥1 on a three-point scale (0=normal, 1=intermediate, and 2=watery feces). All animals were clinically examined daily, and fecal scores were recorded. Based on these records, the number of days with diarrhea and the average fecal score were calculated. Diarrhea index (DI) was defined as the product of the number of diarrheic days and the average fecal score. On the first day of diarrhea, blood, fecal, and nasal swab samples were collected. Tests included viral RT-PCR and fecal parasitology. Plasma total protein was measured in samples obtained on day 2 of life to assess passive transfer. All diarrheic lambs received oral electrolytes until recovery. In cases presenting clinical signs suggestive of bacteremia, treatment with non-steroidal anti-inflammatory drugs (meloxicam) and antibacterials (ampicillin-dicloxacillin) was initiated. Antibacterials were also administered in cases of diarrhea lasting ≥5 days.

RESULTS: Rotavirus A was identified as the etiological agent of diarrhea in all cases. The percentage of lambs with adequate passive immunity transfer exceeded 80% and was similar between groups (P>0.05). The median duration of diarrhea was significantly shorter in the OG compared to the CG (2 vs 4 days; P<0.05). As for the average fecal score during diarrhea, it was significantly lower in the OG (1.38±0.31) than the CG (1.58±0.27). Similarly, D.I. was significantly lower in the OG in comparison with the CG (3.0 vs 6.0; P<0.05).

CONCLUSIONS: Greek OEO administration was associated with a significant reduction in the duration and clinical severity of diarrhea in lambs, supporting its potential use as an alternative therapeutic approach in NDS.

Keywords: Greek oregano essential oil, neonatal diarrhea syndrome, lambs, antimicrobial alternatives, small ruminant internal medicine

Betaine modulates lipid flux from rumen to muscle under mild dietary energy restriction in lambs

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BACKGROUND and OBJECTIVE: Mono-unsaturated fatty acids (MUFAs), particularly oleic acid, play a central role in lipid metabolism and membrane

function. Mild dietary energy restriction in intensive lamb production may alter rumen fermentation patterns and systemic lipid metabolism. Functional methyl donors such as betaine may enhance metabolic adaptation by modulating microbial activity and hepatic lipid metabolism. This study investigated whether betaine supplementation under mild dietary energy restriction improves glucogenic fermentation, hepatic lipid export, and intramuscular lipid composition toward a higher MUFA proportion.

MATERIALS and METHODS: A total of 28 male Akkaraman lambs were housed in individual pens and randomly assigned to a 2 × 2 factorial design with two metabolizable energy (ME) levels of the concentrate mixture (2750 vs. 2500 kcal/kg DM) and two betaine supplementation levels (0 or 4 g/day), with seven lambs per treatment group. The experiment lasted 70 days, including a 14-day adaptation period followed by a 56-day experimental period. Betaine (96%) was added to the concentrate feed in a top-dressed manner and mixed manually before feeding. Diets consisted of 85% concentrate mixture and 15% alfalfa hay (DM basis), and lambs had free access to fresh drinking water throughout the experiment. Rumen propionate proportion, serum triglyceride concentration, and the fatty acid profile and area of the Musculus longissimus dorsi were evaluated. Data were analyzed using a 2 × 2 factorial ANOVA with lambs as experimental units. Main and interaction effects were assessed, and significant interactions ($P < 0.05$) were further examined using one-way ANOVA after verifying normality and homogeneity of variances.

RESULTS: The area of the Musculus longissimus dorsi did not differ among groups. Betaine increased the ruminal propionate proportion with a significant interaction with metabolizable energy ($P = 0.009$), indicating enhanced glucogenic fermentation. Serum triglyceride concentrations were also increased by betaine with a significant interaction with energy level ($P = 0.035$), suggesting enhanced hepatic lipid export. At the tissue level, betaine increased intramuscular MUFA proportion ($P = 0.032$) while reducing α-linolenic acid ($P = 0.035$).

CONCLUSIONS: The coordinated increases in ruminal propionate, circulating triglycerides, and muscle MUFA suggest enhanced rumen–hepatic–muscle metabolic integration. Increased propionate may support hepatic gluconeogenesis, providing substrates for triglyceride synthesis and subsequent VLDL-mediated lipid export to muscle tissue, thereby promoting intramuscular MUFA enrichment. Overall, betaine supplementation under mild dietary energy restriction appears to act as a metabolic regulator linking rumen fermentation with hepatic lipid transport and intramuscular fatty acid remodeling, potentially improving the nutritional quality of lamb meat.

Keywords: Betaine, Energy restriction, Rumen fermentation, MUFA enrichment

Systematic review and meta-analysis of fertility outcome following {in vivo} insemination with sex-sorted semen in sheep

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BACKGROUND and OBJECTIVE: Sex-sorted semen (SS) offers economic benefits to sheep producers, but lower fertility outcome (FO), defined as probability of pregnancy, pregnancy rate or lambing rate, than that following conventional semen (CS) artificial insemination (AI) may limit its use. To systematically review the literature, and analyse factors associated with FO following AI in sheep using SS vs CS.

MATERIALS and METHODS: Available literature was searched using the PRISMA guidelines, resulting in 11 studies with 14 experiments that were reviewed. From these, information from 70 study cohorts representing 13 experiments was used to conduct a meta-analysis which confirmed that FOs for SS and CS AI were 37% (95% CI = 31–43%) and 52% (95% CI = 45–59%), respectively ($p < 0.01$).

RESULTS: FO improved over time, with FO following SS and CS AI improving at the same rate over the period investigated (1997–2022). In a subgroup analysis, five factors were identified that potentially decrease the FO after SS AI disproportionately. These were sperm dose ≤ 4 million spermatozoa, semen preservation (fresh vs frozen-thawed), estrus synchronization using an intravaginal sponge (vs a controlled internal drug release device), presence of gonadotropin-releasing hormone in the synchronization protocol, and absence of bio-

stimulation using a teaser ram. In a random-effects model analysis, an interaction between sperm dose and SS vs CS, and semen preservation were independent predictors of FO after adjusting for the effect of timing of insemination.

CONCLUSIONS: FO following insemination with SS is 15% lower than that of CS, which can be narrowed by increasing the semen dose for SS inseminations and improving the synchronisation of ovulation with the timing of insemination.

Keywords: sex-sorted, semen, sheep, systematic review, meta-analysis

Periparturient reproductive complications, survival and outcomes in hospitalized goats: A multicenter prospective study

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BACKGROUND and OBJECTIVE: Periparturient reproductive complications in does are increasingly encountered in referral hospitals; however, prospective multicenter data describing complication frequency, survival, and management practices remain limited. The objective of this study was to provide an integrated description of periparturient reproductive complications, factors associated with nonsurvival, and antimicrobial administration practices in hospitalized does.

MATERIALS and METHODS: A prospective, multicenter observational study was conducted at nine university veterinary teaching hospitals in the United States. The study population included 198 periparturient does hospitalized for parturition-related management from October 2021 to June 2022. Data were collected using standardized clinician-completed questionnaires, including signalment, obstetrical history, reproductive complications, interventions, survival to discharge, and antimicrobial use (drug class and indication). Data were analyzed using cross-sectional evaluation of reproductive complications, matched case–control analysis for nonsurvival, and descriptive and regression analyses of antimicrobial administration.

RESULTS: The most common periparturient reproductive complications were retained fetal membranes (26%), vaginal or perineal trauma (19%), uterine tears (18%), and metritis (13%). Overall survival to hospital discharge was 79%. Factors associated with nonsurvival included longer duration of labor before hospital admission, uterine tears, vaginal or perineal trauma, pregnancy toxemia (in does hospitalized ≥2 days), and cesarean section (in does hospitalized ≥2 days). Antimicrobial therapy was administered in 77% of cases and was strongly associated with cesarean section. Ceftiofur and oxytetracycline were the most commonly administered antimicrobials. Marked interhospital variability in antimicrobial use was observed.

CONCLUSIONS: Periparturient reproductive complications in hospitalized does are common and associated with substantial morbidity and risk of nonsurvival. Uterine tears represent a frequent and severe complication, particularly in small-breed does following dystocia and on-farm manipulation. Longer duration of labor before hospital admission is also associated with decreased survival. Antimicrobial administration is widespread and heterogeneous across institutions, highlighting the need for evidence-based guidance in periparturient goat care. Together, these findings underscore the importance of early veterinary involvement, appropriate obstetrical management, and antimicrobial stewardship to improve clinical outcomes in periparturient goats. These data provide a multicenter benchmark and inform strategies to optimize periparturient management.

Keywords: Periparturient, Dystocia, Uterine tear, Survival, Cesarean

Diagnostic performance of infrared nasal thermography for the detection of enzootic nasal adenocarcinoma in goats

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BACKGROUND and OBJECTIVE: Enzootic nasal adenocarcinoma (ENA) is a contagious epithelial tumour of goats associated with enzootic nasal tumour virus type 2 (ENTV-2). The disease is progressive and fatal, causing nasal obstruction, respiratory distress, weight loss, and secondary infections. Early diagnosis under field conditions is difficult because initial clinical signs are subtle and definitive confirmation requires post-mortem histopathology. The absence of a detectable humoral immune response further limits serological screening. Infrared thermography is a rapid, non-invasive imaging modality capable of detecting surface temperature changes associated with neoplastic and inflammatory processes and may represent a practical herd-level screening tool. The main objective of this study is to evaluate the diagnostic accuracy of infrared nasal thermography for antemortem detection of ENA in goats using histopathology as the reference standard.

MATERIALS and METHODS: An observational diagnostic accuracy study was conducted in 86 Murciano-Granadina goats from an intensive dairy herd with confirmed ENA presence. Thermographic images of the ethmoidal region were obtained one day prior to slaughter under standardised environmental conditions using a high-sensitivity infrared camera. Images were qualitatively assessed for unilateral thermal asymmetry or focal hyperthermia. After slaughter, all heads underwent systematic necropsy and bilateral histopathological examination of the ethmoidal turbinates. Sensitivity, specificity, predictive values, accuracy, and Cohen's kappa were calculated.

RESULTS: Histopathology confirmed ENA in 23 goats (26.7%). Infrared thermography showed a sensitivity of 82.6% and a specificity of 90.5%, with an overall diagnostic accuracy of 88.4%. Positive and negative predictive values were 76.0% and 93.4%, respectively. Agreement between thermography and histopathology was substantial ($\kappa = 0.711$; $p < 0.001$). Thermographic patterns in affected goats were characterised by unilateral focal hyperthermia and nasal thermal asymmetry (in cases of unilateral tumours), and bilateral hyperthermia (in cases of bilateral tumours), consistent with increased regional temperature and altered airflow dynamics caused by tumour growth.

CONCLUSIONS: Infrared nasal thermography demonstrates good diagnostic performance and substantial agreement with histopathology. Although it cannot replace laboratory confirmation, its rapid, non-invasive nature and high specificity support its use as a complementary antemortem screening tool for herd-level management of ENA in dairy goats.

Keywords: Goat, Enzootic nasal adenocarcinoma, nasal thermography

Epidemiology of persistent intramammary infections in primiparous dairy goats: A multi-herd longitudinal study

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BACKGROUND and OBJECTIVE: The epidemiology of intramammary infections (IMI) in dairy goats

remains complex, particularly regarding pathogen persistence in primiparous replacement stock. Non-*aureus* staphylococci (NAS) are frequently isolated, yet their clinical significance is often underestimated due to the limitations of single-point sampling. High-resolution diagnostic tools, such as MALDI-TOF MS, offer unprecedented opportunities to track species-level infection dynamics over time. This study aimed to move beyond static prevalence data by applying a longitudinal framework to model infectious episodes in primiparous goats, identifying which bacterial species act as transient opportunists and which establish chronic intramammary colonization.

MATERIALS and METHODS: A longitudinal study was conducted on 188 primiparous dairy goats from three herds over 8 months (April-November), by sampling twice a month (one week apart). Half-udder milk (n=2,716) was analyzed via bacteriological culture and MALDI-TOF MS. Polymicrobial and sterile samples were excluded to define true infectious episodes. A temporal grid was established for each half-udder/pathogen combination to distinguish intra-monthly (sporadic) from inter-monthly (persistent/chronic) consecutive isolations. A binary outcome ("LONG") was created by combining persistent (≥2 consecutive months) and chronic (>4 consecutive samples) IMI. To evaluate species associated with extended infection durations, 73 episodes were analyzed using a Generalized Estimating Equation (GEE) logit model, accounting for half-udders nested within goats as non-independent, with days in milk (DIM) at first isolation as a covariate, and setting *Staphylococcus caprae* as the reference species. **RESULTS:** Of 73 IMI episodes, 46 were classified as LONG. *S. caprae* emerged as the most persistent species. Grouped persistent NAS (*S. xylosus, S. simulans, S. gallinarum*) showed similar persistence behavior to *S. caprae* (OR=1.11; p=0.92). Conversely, *S.equorum* (OR=0.02; p=0.03) and the grouped "OTHER BACTERIA" category (OR=0.04; p=0.01) were significantly less likely to cause LONG infections compared to *S. caprae*. Interestingly, *S. aureus* demonstrated significantly lower odds of causing LONG infections than *S. caprae* (OR=0.07; p=0.03). Other NAS (*S. epidermidis, S. warneri*) showed a non-significant trend toward lower persistence (OR=0.14; p=0.13), as did *Corynebacterium* spp. (OR=0.40; p=0.36). The DIM at first isolation did not significantly influence IMI duration (p=0.77).

CONCLUSIONS: *S. caprae* and specific persistent CNS behave as dominant, long-lasting colonizers in primiparous dairy goats, challenging the assumption that non-*aureus* staphylococci are of minor significance for udder health in dairy goats. The significantly lower persistence of *S. aureus* compared to *S. caprae* highlights complex, species-specific dynamics in goat udders, in line with the intermittent shedding of this major pathogen. These findings emphasize the necessity of pathogen-specific control strategies for replacement stock.

Keywords: Goat, mastitis, Non-aureus staphylococci, primiparous goats, MALDI-TOF MS

Assessing the impact of colostrum quality on neonatal lamb behavior

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BACKGROUND and OBJECTIVE: Small ruminant production is vital for the agricultural economy; however, neonatal survival remains a major challenge. While the physiological benefits of colostrum are well known, its specific effects on early behavior need more research. The aim of this study was to examine the relationship between colostrum quality and lamb behaviour. The objective is to enhance their welfare and survival rates.

MATERIALS and METHODS: The study was conducted at the Animal Health and Animal Production Application and Research Center of the Faculty of Veterinary Medicine at Bursa Uludag University. Behavioral data were collected via a camera (Xiaomi CS300,Xiaomi Corporation,Beijing, China). Maternal-neonatal interactions were permitted for the first hour after birth to allow for natural nursing and suckling. However, in cases of inadequate maternal care or failure to suckle, colostrum was hand-milked and administered via a bottle. Colostrum quality was assessed immediately after parturition using a Brix refractometer. Lambs were categorized into three groups based on

maternal Brix percentages: Low Brix (20–25%),Medium Brix (25.1–30.0%),and High Brix (>30.1%).Observations were conducted four times daily (10: 00,14: 00,18: 00,and 22: 00) in 30-minute periods until 30 days of age. Parameters, including suckling frequency, suckling duration, and durations of standing, sleeping, and lying were recorded by pre-trained observers to ensure consistency(This study was supported by the TÜBİTAK under the 2209-A Program (Project No: 1919B012335508).

RESULTS: Statistical analysis revealed significant variations in nutritive behaviors across all independent variables. Regarding colostrum quality, high-brix groups exhibited enhanced suckling kinetics, while significant differences were also observed based on birth weight and type ($p<0.001$). Lambs with birth weights below the mean (<4.19) showed higher suckling frequency (0.78±1.35) and duration (17.59±33.44 seconds) compared to heavier lambs. Similarly, triple-born lambs demonstrated the highest suckling intensity (0.85±1.85 times; 18.04±40.10 seconds). Gender did not significantly influence suckling frequency ($p>0.05$), although males had longer suckling durations than females ($p<0.05$). Observational timing significantly modulated behavioral parameters ($p<0.001$), with peak suckling activity and standing duration occurring during the second observation interval (14: 00), while the fourth interval (22: 00) was characterized by maximum sleep duration (19.35±9.28 minutes). Non-nutritive behaviors, such as lying and standing, were predominantly influenced by temporal factors and birth weight rather than by the quality of colostrum alone.

CONCLUSIONS: This study establishes colostrum quality as a key factor of nutritive behaviors in neonatal lambs. High-quality colostrum enhances suckling frequency and duration, which are vital indicators of neonatal survival. While non-locomotor behaviors remain independent of Brix, the findings underscore the importance of colostrum management for early behavioral adaptation.

Keywords: behavior, brix, colostrum, lamb, welfare

Effect of ß-glucan from Euglena gracilis as an antioxidant on goat semen cryopreservation

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BACKGROUND and OBJECTIVE: Cryopreservation induces oxidative stress that can negatively affect sperm quality. β-glucan is a biologically active polysaccharide known for its antioxidant properties and potential protective effects against oxidative damage. However, the potential role of β-glucan supplementation in goat semen cryopreservation has not been sufficiently clarified. Therefore, this study aimed to investigate the effects of different doses of β-glucan on post-thaw sperm quality parameters, lipid peroxidation, and total antioxidant capacity in goat semen.

MATERIALS and METHODS: During the non-breeding season, semen samples were collected twice per week from five healthy Saanen bucks with proven fertility. The ejaculates were pooled to minimize individual variation and divided into four equal experimental groups consisting of β-glucan concentrations of 1 mM (βG1), 2 mM (βG2), and 4 mM (βG4), together with a control group without antioxidant supplementation. Each group was diluted using a two-step extender protocol prior to cryopreservation. Post-thaw semen samples were evaluated for sperm motility, plasma membrane functional integrity (HOST), acrosome integrity

(FITC-PSA), DNA fragmentation (TUNEL), mitochondrial membrane potential (JC-1), lipid peroxidation (MDA), and total antioxidant capacity (TAC).

RESULTS: Post-thaw sperm motility and plasma membrane integrity were significantly lower in the control group than in the β-glucan-supplemented groups ($P < 0.05$). Acrosome damage was significantly higher in the control group than in all β-glucan-treated groups, particularly in the βG1 and βG4 groups ($P < 0.05$). DNA fragmentation was significantly higher in the control group compared with the βG4 group, whereas no significant differences were detected among the control, βG1, and βG2 groups. No significant differences were detected across the groups in mitochondrial membrane potential, MDA levels, or total antioxidant capacity ($P > 0.05$).

CONCLUSIONS: In line with our results, supplementation of 1mM, 2 mM and 4 mM β-glucan to freezing extender improves the post-thaw spermatological characteristics of goat semen. In further studies, analysis such as antioxidant gene expression profile at the molecular level is required to clarify the question marks about the antioxidant mechanism of ß-glucan in sperm cryopreservation.

Keywords: Goat; ß-glucan, mitochondrial membrane potential, DNA fragmentation, antioxidant activity

Molecular evidence linking Treponema spp. to contagious ovine digital dermatitis occurrence at farm- and sheep- level

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BACKGROUND and OBJECTIVE: Contagious ovine digital dermatitis (CODD) causes severe lameness in sheep and poses significant welfare and economic challenges. Three *Treponema* species (*T. medium*, *T. phagedenis*, *T. pedis*) are considered primary pathogens, though *Dichelobacter nodosus* and *Fusobacterium necrophorum* are frequently co-isolated, and the exact disease aetiology remains unclear. This study aimed to investigate whether presence of these treponemes is essential for CODD occurrence.

MATERIALS and METHODS: Seventeen farms across Wales (n=8), England (n=3), and Ireland (n=6) were classified as CODD-positive (clinical CODD history, n=13) or CODD-negative (no history, n=4). Lame sheep chosen by the farmer (20-30/farm) were mobility scored on a 0-3 scale based on lameness severity and were enrolled if presenting with interdigital dermatitis (ID), footrot (FR), CODD, or mixed (ID/FR and CODD) lesions. CODD lesions were further graded by inflammatory stage (1-5 scale). Clinically healthy controls (up to 10/farm) were also

sampled. Swab samples were collected from lesions on one selected foot, or from the interdigital space in the case of controls. DNA extracted from swabs was analysed by quantitative PCR (qPCR) for the three *Treponema* species. Mobility and lesion scoring, sample collection, and molecular work was performed by the primary veterinary researcher following published methods. Statistical analysis of results was performed in RStudio.

RESULTS: Of 486 foot swab samples analysed, 390 were from CODD-positive and 96 from CODD-negative farms. Swab samples included ID (n=96), FR (n=98), CODD (n=109), mixed (n=25), and controls (n=157). Among CODD-positive farm samples, 76% of both CODD (83/109) and mixed (19/25) lesions were positive for at least one *Treponema spp.*, compared with 44% (27/62) of FR, 8% (6/76) of ID, and 3% (4/118) of control samples. All CODD-negative farm samples were negative for all treponemes. Farm CODD status and presence of foot lesions were significantly associated with treponeme detection (Chi-squared test, $p < 0.01$). Logistic regression showed significantly higher odds of treponeme detection in CODD (odds ratio [OR]=91.0, 95% confidence interval [CI] 34.2–318.1), mixed (OR=90.3, 95% CI 25.7–401.8), and FR lesions (OR=22.0, 95% CI 8.0–78.2) compared to controls; ID lesions were not significantly associated (OR=2.44, 95% CI 0.67–9.84).

CONCLUSIONS: Key *Treponema* species were strongly associated with CODD lesions and absent from farms without clinical CODD history. This supports a primary role for treponemes in CODD pathology and suggests they may be necessary for flock-level disease occurrence. Ongoing analyses of *D. nodosus* and *F. necrophorum* will further clarify the polymicrobial interactions involved in CODD development.

Keywords: Contagious ovine digital dermatitis, Footrot, Interdigital dermatitis, Lameness, qPCR, Treponema spp.

The seasonality of Coxiella burnetii induced abortions in small ruminant farms in Greece, 2024-2025

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BACKGROUND and OBJECTIVE: A common cause of abortions and reproductive disorders in sheep and goats is *Coxiella burnetii*, an obligatory intracellular bacterium that is distributed worldwide on farms of domestic ruminants and is a significant zoonotic agent that causes Q fever in human hosts. The objective of this study was to evaluate the seasonality of *Coxiella burnetii*-induced abortions in small ruminant samples obtained throughout Greece.

MATERIALS and METHODS: Vaginal swab and aborted foetus samples were obtained by veterinary practitioners between January 2024 and December 2025 from ovine and caprine farms throughout the mainland of Greece with reported abortive cases. The samples were obtained from the animals that aborted during the preceding week. Nucleic acid extraction was performed using a commercial spin column kit (INDICAL Bioscience) and an IDEAL™ 32 extraction robot (Innovative Diagnostics). Molecular investigation was performed using a novel commercial kit (ID Gene™ Ruminant Abortion Multiplex HRM, Innovative Diagnostics) according to the manufacturer's instructions on an MIC qPCR Cycler (Bio Molecular Systems). The kit is a test for the detection of the main pathogens causing abortion in ruminants based on PCR technology coupled with HRM. The results obtained were analyzed with the HRM data interpretation software, DISoft (Innovative Diagnostics).

RESULTS: A total of 1281 ovine and caprine samples were examined, and the results indicated a high prevalence of *Coxiella burnetii* in the investigated group, detected in approximately 25% of the animals. The results per trimester indicate a clear seasonality in the positivity rate of the *Coxiella burnetii*-induced abortions. Particularly, in the first trimester of 2024 83/148 (56.1%) were positive, in the second trimester

of 2024 19/40 (47.5%) were positive, in the third trimester of 2024 8/100 (8.0%) were positive, in the fourth trimester of 2024 32/227 (14.1%) were positive. In 2025, the positive PCR samples were 87/300 (29.0%), 17/59 (28.8%), 5/175 (2.8%) and 31/232 (13.4%) in the respective trimesters.

CONCLUSIONS: Our findings demonstrate a characteristic seasonal pattern in *Coxiella burnetii*-induced abortions, peaking during the first half of the year (first and second semester) in two consecutive years. Identifying this seasonality is critical and highlights the need of implementing preventive measures e.g. vaccination and biosecurity protocols in optimal timings (prior to peak transmission periods) in order to reduce economic losses and the zoonotic risk of Q fever to all livestock professionals.

Keywords: abortions, HRM, Coxiella burnetii, Qfever, OneHealth, Greece

Early-life faecal microbiota in lambs is shaped by time rather than neonatal interventions

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BACKGROUND and OBJECTIVE: Infectious disease is a major cause of neonatal lamb mortality. Consequently, antibiotics are frequently used in treatment, prophylaxis and metaphylaxis on sheep farms. Besides the need for antibiotic stewardship in livestock, antibiotic-induced perturbations in the host's intestinal microbiota have become increasingly acknowledged as being deleterious for animal health. Treatment with probiotics has been proposed as a method to improve the health of young animals by modifying their intestinal microbiota: However, whether the intervention meaningfully shapes the neonatal lamb microbiota remains unclear. Equally poorly understood is what a healthy microbial trajectory actually looks like in the first days of a lamb's life. This study aimed to characterize early-life faecal microbiota in lambs and to investigate how neonatal prophylactic antibiotic and probiotic treatments affect the microbiota from the perinatal period to grazing.

MATERIALS and METHODS: To achieve these aims, an experimental trial was conducted where sibling lambs were allocated to three parallel groups: antibiotic and probiotic treatment groups and a control group. The treatment was a single dose of sulfadiazine/trimethoprim or commercially available probiotic product administered *per os* shortly after birth. Faecal swabs were collected within two hours post-partum, 24 hours later, and at summer pasture. In total, 172 faecal swabs including samples collected from 16 antibiotic-treated, 11 probiotic-treated and 27 control lambs underwent 16S rRNA amplicon sequencing (V3-V4 hypervariable region).

RESULTS: Alpha diversity estimates, beta diversity and microbial composition of the microbiota significantly differed between each sampling time point. The alterations were particularly evident after the first 24 hours, where a major decline in alpha diversity occurred. At that time, the microbiota was dominated by genera *Escherichia-Shigella* and *Clostridium sensu stricto 1*. Whilst the reduction in alpha diversity within the first 24 hours was slightly more pronounced in the antibiotic treatment group, no other treatment effects were observed. At pasture, diversity levels were partially recovered showing a composition increasingly similar to the faecal microbiota previously described in adult sheep.

CONCLUSIONS: This study showed that faecal microbiota of lambs undergoes a major transition within the first 24 hours, making animals likely more susceptible to infection, and we recommend further investigations focusing on the role of early-life microbiota in lamb health. Our results indicate that instead of prophylactic antibiotic and probiotic treatments, identification of other efficient preventive measures in sheep farms should be considered to improve lamb health and farm economy.

Keywords: ovine, lamb, faecal, microbiota, antibiotics, probiotics

SMART FARMING AND ARTIFICIAL INTELLIGENCE

Integration of sensor-based behavior monitoring and metabolic indicators to predict health, welfare, and reproductive performance in dairy cows

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BACKGROUND and OBJECTIVE: Precision livestock farming technologies, including noseband sensor systems, enable continuous monitoring of rumination, feeding, drinking, and locomotion behaviors that are closely linked to dairy cow health, metabolic status, and reproductive performance. Previous studies have demonstrated that rumination time predicts feeding and locomotion patterns, reflects changes in milk lactose concentration during early lactation, and is altered during subclinical metabolic disorders and heat stress. The objective of this study was to evaluate whether integrated interpretation of sensor-derived behavioral indicators combined with metabolic and inflammatory biomarkers can serve as early-warning predictors of health disorders, welfare compromise, and reproductive impairment in dairy cows.

MATERIALS and METHODS: This prospective cohort study included 120 Holstein dairy cows monitored from 14 days prepartum to 60 days postpartum using noseband sensor technology. Sensors continuously recorded rumination, eating, drinking, chewing activity, and locomotion. Heat stress was identified using the temperature–humidity index calculated from on-farm temperature and relative humidity; periods with THI ≥ 68 were classified as heat stress and compared with thermoneutral conditions. Blood samples collected at −14, +7, +21, and +42 days relative to calving were analyzed for haptoglobin, serum amyloid A, NEFA, BHB, and glucose. Subclinical ketosis and acidosis were diagnosed using biochemical thresholds supported by clinical examination. Milk lactose concentration was assessed during early lactation. Reproductive outcomes included interval to first ovulation and conception rate at first insemination. Data were analyzed using mixed-effects models.

RESULTS: Consistent with earlier observations, cows that developed subclinical ketosis or acidosis exhibited significant declines in rumination, eating, and locomotion behavior beginning 5–7 days before clinical diagnosis ($p < 0.01$). Decreases in rumination and eating times were significantly associated with lower milk lactose concentrations during early lactation, supporting their utility as indirect metabolic indicators. During heat stress periods (THI ≥ 68), cows showed reduced rumination time and altered locomotion patterns compared with thermoneutral conditions, independent of feed availability. Elevated acute-phase proteins and NEFA concentrations were significantly correlated with behavioral disruptions and preceded clinical signs. Combined behavioral and biochemical indicators predicted delayed first ovulation and reduced conception rate at first insemination more accurately than single metrics alone (AUC > 0.85).

CONCLUSIONS: Integrating noseband sensor–derived behavior profiles with targeted metabolic biomarkers and environmental heat stress indicators enhances early detection of health disorders and identifies cows at risk for reproductive impairment. These integrated sensor–biomarker signatures provide actionable insights for precision monitoring, welfare assessment, and improved reproductive management in dairy herds, validating and extending insights from multiple behavioral and physiological studies.

Keywords: noseband sensors, rumination behavior, heat stress, metabolic health, dairy cow reproduction

Prepartal reticuloruminal motility as parameter for prediction of peripartal hypocalcemia risk in cattle

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BACKGROUND and OBJECTIVE: Hypocalcemia affects mostly dairy cows during the peripartal period. Most of the diet based prophylactic measures (e.g. anionic salt feeding) are suitable for larger herds, but they are less or not feasible for small herds as they are common in Austria. An individual risk prediction would allow customized prophylactic measures and targeted intervention. However, the current cow-level prediction methods of hypocalcemia risk which are typically based on laboratory parameters (e.g. AP, NABE) have only low to moderate predictive values. The objectives of the study were to assess associations between reticuloruminal motility patterns before calving and Ca concentration at calving and to explore whether prepartal reticuloruminal motility data are feasible to predict peripartal hypocalcemia risk.

MATERIALS and METHODS: A total of 89 calvings from 47 cows and 22 heifers were monitored using reticuloruminal bolus sensors (smaXtec) over 60 days prior to the expected calving and 60 days postpartum. The sensor continuously recorded rumination time, rumen motility and reticular temperature. Blood samples were taken several times before, during and after calving. Serum calcium concentrations were measured using automatic analyzer (tCa) and POC device (Horiba) for iCa. Principles of experimental animal care were carried out according to the national ethical law

RESULTS: Associations between total serum calcium concentrations at parturition and rumination time, reticular temperature as well as the rumen motility during the days before calving have been found indicating a potential predictive value of these latter parameters. Cows with low tCa (<1.8 mmol/l) or iCa (<0.8 mmol/l) a few days before parturition had reduced rumination times compared to cows with normal tCa levels (>2.2 mmol/l).
Using a rumination time (RT) threshold of 480 minutes/day one day before calving, hypocalcemia was predicted with 66.7% sensitivity and 96.8% specificity. Additionally, a deep learning model trained on external data, which incorporated rumen cycles, reticular contraction duration, and reticular temperature. The model achieved 83.2% sensitivity and 98.2% specificity for hypocalcemia risk prediction.

CONCLUSIONS: The results indicate that continuously measured reticuloruminal motility data before calving are generally feasible for peripartal hypocalcemia risk prediction in dairy cows. The deep learning model performed superior to the classic threshold model, highlighting the potential of multi-metric sensor analytics to improve early hypocalcemia risk detection. Multicentric field studies on larger sample sizes are necessary before application in veterinary practice.

Keywords: hypocalcemia, rumen motility, risk prediction, sensor data

Precision dairy farming in veterinary practice: From data collection to clinical decision-making

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BACKGROUND and OBJECTIVE: Precision dairy farming (PDF) systems enable continuous, individual-animal monitoring of behavioural and production parameters, supporting early disease detection and improved herd health management. These technologies provide valuable decision-support tools; however, their effective implementation still relies on veterinary expertise and clinical diagnostics. The aim of this study was to present, through the example of a large-scale dairy farm, how precision dairy farming integrates into routine veterinary practice and to highlight the

continuing importance of veterinary clinical assessment.

MATERIALS and METHODS: The study was conducted on a commercial dairy farm housing 970 lactating cows, with a total herd size of 1,570 animals including replacement stock. The farm utilized precision dairy farming system, which continuously monitors individual feeding behaviour, rumination activity, and inactivity. Between October 1, 2025 and January 31, 2026, alerts generated by the system were reviewed daily. Cows identified by the system were clinically examined by the herd veterinarian, and diagnoses were established based on clinical examination and complementary diagnostic procedures where necessary. New cases were recorded and categorized according to the confirmed diagnosis.

RESULTS: During the study period, 413 alerts were generated, resulting in the clinical examination of the respective cows. Of these, 276 were classified as new cases. In 63 cases (22.8%), no clinically relevant disorder was confirmed at the time of examination.The remaining cases (n=213) were diagnosed as follows: The confirmed diagnoses included mastitis (n = 50), subclinical ketosis (n = 75), clinical ketosis (n = 15), left displaced abomasum (n = 7), lameness (n = 30), pneumonia (n = 4), diarrhoea (n = 26; including 8 suspected SARA cases), fatty liver syndrome (n = 4) and metritis (n=2).

CONCLUSIONS: The precision dairy farming system proved to be an effective tool for identifying cows at risk of health and production disorders, particularly metabolic diseases. At the same time, the high proportion of cases without confirmed clinical disease highlights the importance of veterinary clinical examination in interpreting alerts and making appropriate decisions. The system supported early detection and timely intervention, but did not replace veterinary expertise. Accurate diagnosis, treatment decisions, and differentiation between true and false positive alerts remained dependent on the veterinarian's clinical skills.

Keywords: Precision dairy farming, cattle diseases, commercial dairy herd

Monitoring of calving using a tail accelerometer

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BACKGROUND and OBJECTIVE: Accurate monitoring tools are crucial for the successful detection of calving and, consequently, dystocia in both dairy and beef cattle farming to avoid economic loss. SmartVel® (RF-Track, Cesson Sevigne, France) is an accelerometer attached to the cow's tail that detects behavioural changes associated with the onset of labour and notifies the farmer that calving has begun. In this study, the efficiency of the SmartVel® device and the impact of early intervention on dystocic calvings on calf survival were evaluated.

MATERIALS and METHODS: Calving data were collected from three farms that were using SmartVel® in the Alsace region of France. Farm A produces beef (Black Angus), while Farms B and C are dairy (Montbeliarde and Holstein-Friesian, respectively). Calving data of 23, 68, and 24 animals, respectively, were recorded, including age of the dam, number of previous calvings, pregnancy length, duration of monitoring via SmartVel®, occurrence of twins and dystocia, interventions performed, and survival of dam and calf. Analysis was performed using R3.5.2.

RESULTS: In total, SmartVel® accurately predicted the onset of calving in 111 cases (positive predictive value: 96.5%). Results showed a 26.1%, 19.1%, and 12.5% dystocia rate in farms A, B, and C, and 66.7%, 81.8%, and 50.0% calf survival rate among dystocic calvings, respectively. Statistical analysis showed that SmartVel® was equally accurate independently of the cow's gestation time, number of previous calvings, breed, and monitoring duration.

CONCLUSIONS: SmartVel® appears to be a reliable, easy-to-use, and efficient tool that increases farm productivity and animal welfare and could become a powerful asset in precision livestock farming.

Keywords: cattle, dystocia, calf survival

Assessing observer reliability for computer vision-based assessment of abdominal contractions during calving

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BACKGROUND and OBJECTIVE: Effective calving monitoring is critical in dairy farming. Computer vision (CV) systems for calving monitoring use behavioural data from video, but robust algorithms require reliable ground truth from human annotations, an aspect often overlooked during development. Abdominal contractions are important candidate signs for automated calving monitoring, but definitions used for live human observation are hard to apply to video, highlighting the need for video-specific contraction ethograms that take camera perspective into account. We aimed to identify the ethogram–camera view combination that maximizes inter- and intra-observer reliability and is suitable as gold standard for CV.

MATERIALS and METHODS: We used two video-recorded calvings to assess contraction-scoring reliability across multiple ethograms. Calvings were recorded with two synchronized cameras (lateral and top view) and we used a 2-hour period from each where three observers annotated contractions using the software BORIS. We evaluated three ethograms for abdominal contractions: (1) individual point events; (2) continuous bouts with start and end time; (3) continuous bouts by grouping contractions separated <10 s as one bout. Observers were equipped with a definition manual and reference clips. We measured inter- and intra-observer reliability using Cohen's Kappa for each ethogram and camera view.

RESULTS: Ethogram 1 resulted in low intra-observer agreement (κ = 0.30–0.57) and very low inter-observer agreement (<0.10). Marking the exact beginning of contractions proved difficult and unreliable for annotation. Ethogram 2 resulted in high intra-observer reliability (κ = 0.85–0.94), but inter-observer reliability remained moderate (κ = 0.60), likely due to the lack of a clear definition of when contractions belonged to the same bout. Ethogram 3 was associated with high intra-observer reliability (κ > 0.9). Defining time interval bouts improved inter-observer reliability (κ = 0.71). The two camera views showed a similar reliability pattern with slightly lower Kappa values for lateral view. Top view offered full abdominal visibility and clearer abdominal wall excursion, improving scoring. Clinically, key calving indicators are the onset of rhythmic contractions and intervals between bouts. Based on reliability results, annotating contractions as continuous bouts is feasible for human observers and suitable for automated detection. Future work should test these ethograms across breeds and assess whether counting contractions per bout improves model performance.

CONCLUSIONS: Abdominal contractions in cattle are best annotated as continuous bouts rather than point events. Top-view cameras can improve visualization and scoring reliability. Video-specific ethograms may help bridge clinical and computer vision needs.

Keywords: Calving, Computer vision, Ethogram, Observer reliability, Precision livestock farming

Development of a wearable sensor system for fetal electrocardiography in cattle

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BACKGROUND and OBJECTIVE: Dystocia frequently results in severe complications for both cow and calf, with significant implications for animal welfare and productivity. Early identification of fetal distress may allow timely obstetrical interventions, such as assisted delivery or caesarean section. In contrast to human obstetrics, where continuous fetal monitoring is standard practice, reliable and practical prenatal monitoring systems for cattle are not yet widely available. The objective of this study is to develop and evaluate a low-cost, non-invasive monitoring system for assessing fetal heart activity in late-gestation dairy cows using wearable ECG technology.

MATERIALS and METHODS: The prototype system was based on two Polar H10 Equine heart rate sensors, which are commercially available wearable ECG devices adapted for simultaneous thoracic and abdominal recordings. System performance and usability for fetal ECG acquisition were evaluated by comparison with a Televet 100 ECG system serving as the reference method. Both systems recorded thoracic and abdominal ECG signals simultaneously. Two consecutive 10-minute measurements were conducted in 13 cows within three weeks prior to calving. Raw signals from both systems were resampled to 500 Hz to ensure comparability and processed using a 4th-order digital Butterworth bandpass filter (1-45 Hz) to reduce baseline wander, respiratory interference, and motion artifacts. Signal quality was assessed using predefined spectral, morphological, and physiological criteria. Overall, 51.5% of the recorded data met the quality criteria and were included in the analysis. Based on these recordings, RR intervals, heart rate, and waveform features were calculated. Agreement between methods was evaluated using Bland-Altman analysis.

RESULTS: Bland–Altman analysis showed good overall agreement between the H10-based system and the reference method. The mean bias was −0.98 bpm (SD = 1.17) for chest ECG measurements and 2.46 bpm (SD = 7.15) for estimated fetal heart rates, suggesting a small systematic overestimation of fetal heart rate by the H10-based system. Agreement decreased at higher mean heart rates, with greater dispersion and several more pronounced negative deviations. A small proportion of observations exceeded the limits of agreement.

CONCLUSIONS: The results indicate that low-cost wearable ECG sensors show promise for fetal heart rate monitoring in late-gestation cows. The prototype demonstrated practical feasibility and may represent a suitable approach for on-farm application. Nevertheless, signal quality was influenced by electrode placement and fetal position, with more reliable recordings obtained in advanced gestation. Further studies are required to improve robustness, optimize sensor positioning, and validate clinical applicability under field conditions.

Keywords: Cattle, fetal electrocardiography, wearable ECG, fetal heart rate

ICAR IDF initiative on sensor data for functional traits: reference standards and guidelines to quantify rumination for health and animal welfare recording

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BACKGROUND and OBJECTIVE: The increasing adoption of sensor technologies and precision farming tools in the dairy cattle sector is revolutionizing the continuous, real-time monitoring of animal health, welfare, and productivity. These digital advancements provide high-frequency, objective, and large-scale data that is essential for proactive herd management. Despite technological proliferation, a significant scientific gap remains in the validation of these commercial sensors. Specifically, a lack of standardized and rigorous protocols often leaves the accuracy and reliability of the generated data undetermined. The overall aim of the joint ICAR IDF initiative is to develop guidelines to support the adoption of novel technologies and use of sensor data for improving animal health and welfare.

MATERIALS and METHODS: The joint initiative establishes a common agreement on definitions and terminology for health conditions and behaviors measured with sensor systems. In accordance and in collaboration with other working groups, the project develops standards to facilitate the exchange of data and information across different farms and sensor technologies. Making guidelines based on best practices for data collection, handling, and analysis for different use, e.g. genetics, health and welfare monitoring. Generating recommendations and protocols for testing the performance of sensor systems. Sensor-based rumination data was

selected as the primary use case due to its high adoption rate and critical value as a health indicator. Despite its popularity, the field currently lacks standardized definitions and independent validation, often leaving the true impact on animal welfare and production unverified.

RESULTS: Preliminary results and recommendations on use of rumination data for health and welfare assessment, sensor key performance indicators, and preferred reference standards will be presented.

CONCLUSIONS: Irrespective of the use of sensor data, validation and standardization are key to ensure that sensor data from diverse sources remain reliable and comparable, building the trust necessary for data-driven health and welfare decisions. Close collaboration between ICAR IDF with relevant stakeholders and manufacturers leads to the development of unified guidelines, which will promote global adoption of the use of sensor data in herd management, animal health, and welfare assessment.

Keywords: Precision livestock farming, rumination behavior, guidelines for validation, health and welfare

Impact of automatic monitoring versus an intensive health control system in replacement holstein calves and heifers on survival, reproduction, and productive lifespan

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BACKGROUND and OBJECTIVE: Automated monitoring systems (AMS) for youngstock offer a promising approach to improve early disease detection and optimize labor through prioritized clinical examination. This study aimed to evaluate whether an AMS (Sensehub™ Dairy Youngstock) could match or surpass short-term health and growth performance, as well as long-term survival, reproductive efficiency, and first lactation milk yield achieved through an intensive veterinarian-based monitoring.

MATERIALS and METHODS: 805 Holstein calves were enrolled at birth and followed through the completion of their first lactation. Calves were assigned via stratified randomization to: (1) an AMS (SenseHub™ Dairy Youngstock, SHY; n = 397), in which only calves flagged by a Health Index < 86 were clinically examined according to farm SOP; or (2) an intensive health control group (Control; n = 408): the farm veterinarian followed the farm's protocols and SOPs for each rearing stage, performing scored clinical assessments to detect sick animals. Recorded parameters included: health (morbidity from pneumonia, diarrhea, lung ultrasound scores), growth (birth weight, weaning weight, 8month weight), survival (mortality and culling), reproductive data (AI date and conception AI), and first lactation milk yield. Statistical comparisons were performed using tests or chisquare tests depending on data distribution, and a multivariable model was used for milk production.

RESULTS: Health & Growth. SHY calves showed significantly improved lung health at weaning ($p < 0.001$), reducing lung lesions (11.9% vs. 20.9%, $p = 0.0007$) and improving lung scores ($p = 0.0005$). Treatment patterns indicated earlier detection in SHY calves. Growth performance did not differ between groups. Survival. Non survival from birth to first lactation in the SHY group showed a non significant but favorable trend (15.1% vs 18.9%, $p = 0.19$). In addition, SHY reduced the risk of removal during the first lactation (10.3% vs 15.9%, $p = 0.03$). Reproduction SHY heifers showed higher pregnancy rates by the end of the first lactation (90.8% vs. 88.0%, $p = 0.21$) and reduced culling due to reproductive failure, resulting in improved reproductive success (80.5% vs. 72.1%). Milk Production. SHY heifers tended to produce more milk in the first lactation (+278 kg at 305 d; +262 kg ECM). The response was primarily indirect, as improved survival increased days in milk, with each additional day contributing 17.33 kg of ECM.

CONCLUSIONS: SHYS group achieved health and performance outcomes comparable to, and in some aspects exceeding, intensive veterinarian based monitoring systems, while reducing labor demands. Its benefits in lung health, survival, reproductive success, and first lactation performance highlight AMS as an efficient and cost effective strategy for dairy youngstock management.

Keywords: Automated monitoring, lung health, survival, reproductive success, milk yield

Animal factors dictate learning strategy: Evaluating cattle adaptation to a virtual fencing system

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BACKGROUND and OBJECTIVE: Grazing system is a widely adopted management practice in livestock production. However, physical fencing can be costly and inflexible for the system. Virtual fencing (VF) offers a smart alternative for managing grazing systems. This study, part of the DaVaSus project, aimed to evaluate the learning process of cattle during the training for a virtual fence system.

MATERIALS and METHODS: The study was conducted in April 2024 at the regenerative and ecological farm Gut&Bösel in eastern Brandenburg, Germany. The experiment consisted of a twelve-day training period (divided into six stages) where the entire herd (n=171 animals) comprising Angus breed and Saler cattle with different ages was habituated to the VF within a single paddock. The VF boundary was repositioned daily to prevent the animals from associating the fence with a fixed location. The training aimed to teach the cattle to associate an auditory warning signal with an avoidable electric pulse, both delivered via collars from the eShepherd® virtual fencing system (Gallagher Group Limited, Hamilton, New Zealand). The success ratio was calculated by dividing the number of successful acoustic signals (i.e., those not followed by an electric pulse) by the total number of acoustic signals issued. We hypothesized that the trend success ratio over time would differ based on animal characteristics (i.e., breed, sex and age). Additionally, behavioral reactions to the VF were recorded by a drone and categorized using a reaction scoring table. Analysis using a linear mixed-effects model was carried out with JMP Statistical Software (18.2.1; SAS Institute Inc., Cary, NC, USA).

RESULTS: A significant interaction was found between the training stage and animal age class ($p<0.001$). Younger cattle demonstrated a significant initial adaptation, achieving an 85% of success ratio in the first stage. In contrast, the adults showed a rather gradual learning curve but attained the most consistent success ratio by the final training stage. Significant variation in learning was also revealed among individual animals ($p<0.001$), while breed and sex had no significant effect on the success ratio.

CONCLUSIONS: These results suggest that age is a possible predictor of the learning strategy in VF systems. Implementing training protocols to account for specific learning patterns could enhance the efficiency and welfare outcomes of virtual implementation. This research is funded by the German Federal Ministry of Agriculture, Food and Regional Identity (BMLEH) based on a decision of the Parliament of the Federal Republic of Germany, granted by the Federal Office for Agriculture and Food (BLE).

Keywords: Beef Cattle, Grazing Management, Smart Farming, Virtual Fencing, Animal Behavior

The added Value of Sensor-Based Monitoring of Activity and Drinking Behavior After an Experimental Infection with Bluetongue Serotype 3 in Sheep

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BACKGROUND and OBJECTIVE: Bluetongue is a vector-borne disease causing endothelial wall damage resulting in a variety of symptoms including fever, lethargy, edema, mucosal lesions and lameness. In infection experiments, wearable sensors can be used for automated and continuous real-time monitoring of behavioural disease parameters without disturbing the animal. They are a promising tool for reduction and refinement by providing more quantitative and objective data compared to traditional intermittent and semi-quantitative clinical observations. In this vaccine efficacy experiment, we explored the added value of sensor-based monitoring of activity and drinking

behaviour in sheep after an experimental infection with Bluetongue virus serotype 3.

MATERIALS and METHODS: Sheep (n=8 per group) had received either a mock-vaccine (T01) or two different experimental BTV-3 vaccines (T02 or T03) at four weeks prior to experimental challenge. After challenge, temperature measurements and (bi-)daily clinical observations were performed to assess vaccine efficacy on clinical disease. In addition to these traditional disease parameters, sheep were equipped with an ear-based non-invasive accelerometer. From this sensor, activity data were broadcasted enabling real-time visualization of individual activity patterns over time. One pen housing n=4 animals of each group was also equipped with a RFID system to detect the number and duration of drinking visits.

RESULTS: BTV-3 challenge resulted in typical and prominent symptoms in all groups including fever starting from day 4 and lethargy and lameness starting from day 5 or 6 onwards. The overall trends observed in mean clinical scores and activity levels seemed similar and showed a reduction of clinical disease in BTV-3 vaccinated animals in T02. However, the drop in animal activity detected by the sensor tended to start approximately 1 day earlier compared to the clinical data. A comparison of both data sets at individual animal level is still ongoing. Despite the development of mucosal lesions, the challenge had a limited impact on appetite and based on preliminary RIFD data analyses, also drinking behavior seemed to be not (strongly) affected. Clinical symptoms were peaking between day 9 and 14 and severity and/or duration of symptoms ultimately resulted in pre-termination of n=6 mock-vaccinated sheep (T01) and n=2 (T02) and n=7 (T03) BTV-3 vaccinated sheep.

CONCLUSIONS: Experimental vaccine trials can benefit from sensor-based monitoring providing additional insights into behavioural patterns and disease symptoms. Compared to snap-shot clinical observations, continuous sensor data provide a higher resolution and sensitivity allowing a more detailed assessment of differences between treatment groups, especially during to the onset and recovery phase of the disease.

Keywords: sensor-based monitoring, disease parameters, bluetongue virus, experimental infection, vaccine efficacy, sheep

Determination of behavioural change thresholds using accelerometer-based sensor data for health monitoring in transition dairy cows

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BACKGROUND and OBJECTIVE: Accelerometer-based sensors have strong potential for early detection of health disorders in dairy cattle by identifying behavioral deviations prior to clinical signs, facilitating timely intervention and improving animal welfare. This study aimed to characterize changes in rumination and physical activity during the transition period in Holstein cows and to establish data-driven thresholds for early detection of health disorders

MATERIALS and METHODS: A total of 180 cows were enrolled, of which 156 were included in the final analysis. Three-axis accelerometer sensors were attached to the ear tag 30–35 days before expected calving and remained in place until 21 days postpartum. Health disorders, including hypocalcemia, ketosis, lameness, displaced abomasum, puerperal metritis, and mastitis, were identified through clinical examination, blood analyses, and standardized scoring systems. Daily rumination time (min/day) and physical activity (unit/day) were calculated across defined prepartum and postpartum intervals. Changes observed on the day of calving were expressed relative to the individual baseline (−5 to −1 days prepartum). Statistical analyses included Mann–Whitney U test, receiver operating characteristic analysis, and Youden index.

RESULTS: Of the 156 cows, 52.6% (n=82) remained clinically healthy and were classified as controls, whereas 47.4% (n=74) developed at least one health disorder. Among affected cows, 66.2% (n=49) exhibited a single disorder, while 33.8% (n=25) presented with multiple disorders. Rumination time decreased by approximately 35% on the day of calving relative to the preceding 5-day period. During postpartum days 1–5, mean rumination time was significantly lower in diseased cows compared with controls (371.6 ± 102.95 vs 437.7 ± 59.84 min/day; $p<0.001$). Physical activity was also reduced (456.9 ±

69.81 vs 488 ± 65.89; p<0.05). Notably, cows that developed health disorders exhibited significantly lower rumination during the prepartum period (454.8 ± 89.11 vs 506.8 ± 38.72 min/day; p<0.001). Changes in rumination demonstrated strong discriminatory performance (area under the curve = 0.875; 95% confidence interval: 0.82–0.93), whereas activity changes showed limited predictive value. The optimal cut-off for rumination decline was 30.5%, yielding 74.3% sensitivity and 89% specificity. The magnitude of rumination decline differed significantly between healthy and diseased cows (p<0.001) and was particularly pronounced in cases of subclinical ketosis (p<0.001).

CONCLUSIONS: Rumination dynamics are a robust indicator for early identification of health disorders during the transition period. A reduction of approximately 30% relative to individual baseline values provides a practical threshold for early warning. These findings support integration of accelerometer-based technologies into automated health monitoring systems to improve decision-making and management efficiency in dairy herds.

Keywords: Accelerometer, Dairy cow, Health monitoring, Rumination, Transition period

SURGERY AND LAMENESS

Utility of a freehand frameless navigation system in computed tomography-assisted ventral bulla osteotomy for otitis media in calves

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BACKGROUND and OBJECTIVE: Ventral bulla osteotomy (VBO) has not been widely adopted for treating otitis media in calves due to its prolonged surgical duration (>1 hour) and high surgical invasiveness. This study aimed to evaluate the utility of a freehand frameless navigation system for computed tomography (CT)-assisted ventral bulla osteotomy in 21 calves with otitis media.

MATERIALS and METHODS: Twenty-one calves had poor therapeutic effects for involvements of otitis media (unilateral and bilateral affections in 6 and 15 animals, respectively), followed by treatments with VBO at an average of 33.9 days after initial medical treatment. The present VBO technique was performed by following two steps: (1) Preparation procedure, which included, 1) CT scanning for the head and cranial neck region secured to a surgical table in dorsally recumbent, anesthetized animals, 2) creating reconstructed CT images on a navigation computer, and 3) registration for integration of real and image structures; and (2) Surgical procedure, which included 1) introducing a trocar fixed by a securing device with three reflective spheres through approximately a 1-cm skin incision made on the ventral surface of the cranial neck, 2) inserting a trocar into the affected tympanic bulla while looking at the trajectory image corresponding to its tip on a computer screen, 3) lavage in the tympanic bulla's cavity using a plastic catheter inserted via the trocar, and 4) intradermal suturing for the skin incision.

RESULTS: The time required for the preparation procedure ranged between 18 and 73 minutes. The time required for surgical procedure for the 36 affected ears of the 21 animals ranged between 7 and 26 minutes per ear. The median total time, as calculated by the sum of the preparation and surgical times, was 49.0 minutes (range, 41–57 minutes) for the six animals with unilateral otitis media, and 66.0 minutes (range, 47–106 minutes) for the 14 animals with bilateral otitis media. In 13 of the 21 treated animals, otitis media-associated clinical signs improved or disappeared suddenly or gradually. Postoperative complications included generalized convulsive seizures and swollen mandibles in one and three animals, respectively.

CONCLUSIONS: Multiple factors contributing to the successful implementation of the present VBO technique include: 1) the adequate size of the

tympanic bulla compensating for larger targeting errors; 2) applicability of manual trocar operation for both insertion and perforation of the tympanic bulla; and 3) reduced surgical invasiveness, resulting in reduced surgical time. This knowledge can contribute to further developments in imaging-assisted surgery in the veterinary field

Keywords: calf, computed tomography, frameless navigation system, otitis media, ventral bulla osteotomy

Surgical decision-making for atypical urachal abscesses in cattle: Clinical application of the pre-ınguinal approach and colpotomy

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BACKGROUND and OBJECTIVE: Urachal abscesses in cattle are commonly treated by ventral midline or paramedian laparotomy, particularly in calves. However, in older calves and adult cattle, surgical access to the urinary bladder apex is often difficult because of increased body size, anatomical distance to the pelvic cavity, pregnancy, and severe intra-abdominal adhesions, necessitating alternative surgical strategies in selected cases. Therefore, this study aimed to describe practical surgical decision-making for atypical urachal abscesses in cattle and to illustrate indications, advantages, and limitations of the pre-inguinal approach and of colpotomy.

MATERIALS and METHODS: Japanese Black cattle with urachal abscesses involving the urinary bladder apex were retrospectively evaluated considering clinical records, rectal palpation, and ultrasonography. In four cattle older than 18 months of age, a pre-inguinal approach was used to achieve direct visualization, abscess resection, and partial cystectomy when indicated. In one adult cow with an urachal abscess and severe adhesions, located close to the entrance of the pelvic cavity, colpotomy was selected. Using a standing vaginal approach, the urinary bladder apex with the abscess were exteriorized, resected, and the urinary bladder sutured under direct visualization. Surgical accessibility of the urachal abscesses and the urinary bladder apex, intraoperative findings, complications and postoperative outcomes of the five cases were evaluated.

RESULTS: When lesions were confined to the urinary bladder apex, adhesions were limited, and the abscess could be pushed toward the inguinal region on rectal examination, the pre-inguinal approach provided effective surgical access with acceptable operative time. In contrast, severe adhesions and location close to the pelvic entrance restricted safe manipulation via this route. Vaginal incision, however, allowed complete manual release of the adhesions and exteriorization of the lesion through the vulva without the need for general anesthesia, enabling reliable excision of the abscess without opening the abdominal wall. Except for one animal in which severe adhesions prevented lesion removal by pre-inguinal approach, the remaining four animals recovered uneventfully and returned to productive or reproductive function.

CONCLUSIONS: The pre-inguinal approach and colpotomy are complementary surgical options for atypical urachal abscesses in cattle. Careful selection of the surgical approach to the abdominal cavity based on lesion location, adhesion severity, body size, and reproductive status improves operative safety and clinical outcomes. When conventional intraperitoneal approaches are impractical, these alternative techniques provide effective solutions.

Keywords: cattle, urachal abscess, pre-inguinal approach, colpotomy, surgical management

Survey on the effect of salicylic acid on different hooves lesions causing lameness in dairy cattle

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BACKGROUND and OBJECTIVE: This controlled field trial compared topical Salicylic Acid (SA) with systemic ceftiofur hydrochloride for treating hoof lesions causing lameness in lactating dairy cows, assessing whether local therapy could reduce antibiotic use without compromising outcomes

MATERIALS and METHODS: Thirty cows were divided into two equal treatment groups and evaluated on Days 1, 7, 14, and 28 for lesion progress (healed vs improved), pain score, locomotion score, and body condition score (BCS). Standardized scoring systems (Sprecher 1–5, hoof-tester 0–3, Edmonson 1–5) were used across all assessments. SA bandaging was reapplied based on lesion response, while the antibiotic group received a 5-day systemic ceftiofur course.

RESULTS: SA resulted in faster clinical improvement: pain resolved sooner ($p=0.0025$), locomotion recovered more rapidly (ANOVA $F(2,56)=4.9863$; $p=0.0102$), and lesions transitioned more often to "healed" by Day 14 and Day 28. BCS increased under SA (≈2.4→2.8) but not under antibiotics (≈2.55→2.5) with a significant treatment effect ($F(1,28)=13.176$; $p\approx0.0011$). Ceftiofur remained more suitable for interdigital phlegmon, whereas SA performed better for digital dermatitis, supporting treatment selection by lesion type as a strategy to reduce herd-level antibiotic use.

CONCLUSIONS: Limitations include single-farm setting, small sample size, mixed lesion types, non-blinded scoring, and 28-day follow-up. Nevertheless, consistent improvements across pain, gait, lesion resolution, and BCS indicate that SA combined with standard hoof care may reduce reliance on systemic antibiotics while improving short-term animal welfare and recovery.

Keywords: BCS, Ceftiofur, Digital dermatitis, Lesion, SA

Axial horn fissure: A new threat for cattle in eastern France?

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BACKGROUND and OBJECTIVE: Axial horn fissure (AHF) is widely considered a minor cause of lameness of cattle, due to its reportedly low prevalence. However, in recent years, hoof trimmers in eastern France - where the Montbeliarde breed predominates - have observed an increasing prevalence of this lesion. This retrospective observational study aimed to estimate the incidence of AHF in eastern France.

MATERIALS and METHODS: Hoof-trimming records from two organizations operating across six French administrative departments were analyzed, covering data from 21,222 cows in 309 herds over a four-year period (2021-2024). Apparent incidence rates were calculated using two definitions of new claw disorder cases (first case in a cow only vs. first and recurrent cases) and cows at risk (cows trimmed vs. lactating cows). Rates were estimated annually and monthly, at animal, within-herd and between-herd levels, for both AHF and "alarm" lesions (as defined by Kofler et al.).

RESULTS: Incidence rate estimates differed between herds where the whole herd was trimmed (≥ 90% of lactating cows trimmed, 97 herds) and those where only part of the herd was trimmed (< 90% of lactating cows trimmed, 212 herds). In the latter group, estimates were higher when only trimmed cows were considered at risk, reflecting the targeted

selection of lame cows for trimming, and lower when all lactating cows were considered at risk, suggesting under-detection of lame cows by farmers. In herds undergoing whole-herd trimming, AHF (levels 2 and 3) was detected annually in 6.2% of trimmed cows and in 90% of the herds. The annual incidence rates for AHF were comparable to or exceeded those of alarm lesions: 7.6% of cows were affected by sole ulcer, 7.4% by M2-stage digital dermatitis, 4.5% by level 3 white line disease, and 1% by toe necrosis. A marked seasonal pattern was observed, with incidence rates of AHF peaking during summer months (frequently exceeding 8% in August or September) compared to winter months (range: 1.5% - 6%). The median within-herd incidence rate for AHF was 5%, but with substantial variability (range: 0 - 27%).

CONCLUSIONS: AHF should be considered as a major cause of lameness, at least in certain regions. The variability of incidence rates by months and within herds suggests that risk factors related to animal, herd management, or climatic conditions may exist. Further exploration of these factors would be valuable to design measures for better prevention of this lesion, which is challenging to cure.

Keywords: claw disorders, cows, lameness, trimming, epidemiology

General anaesthesia in cattle: A retrospective study of bovine patients at the University of Veterinary Medicine Vienna from 2003 to 2024

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BACKGROUND and OBJECTIVE: Many surgical procedures in cattle are performed in the standing position using a combination of sedation and local anaesthesia. However, for certain indications, such as navel disease, umbilical or abdominal hernias, general anaesthesia is required. In ruminants, general anaesthesia is associated with additional risks due to their specific anatomical and physiological characteristics. The aim of this study was to evaluate the anaesthetic protocols used in cattle at the Clinical centre for Ruminants, Camelids and Herd health management, University of Veterinary Medicine Vienna, between 2003 and 2024 in cattle undergoing surgical procedures under general anaesthesia. Specifically, the study aimed to assess the indications for anaesthesia, the anaesthetic agents used and their safety, as well as complications occurring during the procedures.

MATERIALS and METHODS: Data were extracted from the anaesthesia records of cattle that underwent general anaesthesia at the Clinical centre for Ruminants, Camelids and Herd health management, University of Veterinary Medicine Vienna, between 2003 and 2024 using the hospital information system. The following parameters were collected from the anaesthesia records: indication, ASA classification, duration of surgery, duration of anaesthesia maintenance, patient positioning, and drugs used for premedication, induction and maintenance. In addition, information on the ventilation system used, local anaesthesia techniques, and monitoring modalities (electrocardiography, blood pressure, pulse oximetry, capnography, core body temperature, nerve stimulation) was extracted. Furthermore, recorded complications and recovery characteristics were documented and analysed.

RESULTS: A total of 193 bovine patients underwent surgical procedures under general anaesthesia during the 22-year study period. Most of the animals were classified as ASA 2 (44%). The most common indications were umbilical disorders (28%) and umbilical hernia (19.2%). Pulse oximetry was the most frequently used monitoring modality, applied in 93.8% of cattle. The most common complications during anaesthesia maintenance were hypotension (19.2%), hypercapnia (5.7%), and hypoxemia (5.2%). Complications during premedication, induction, and the recovery phase were rare.

CONCLUSIONS: Overall, the anaesthesia protocols used at the University of Veterinary Medicine Vienna were associated with a low incidence of complications and can therefore be considered safe.

Keywords: surgery, general anaesthesia, anaesthesia protocols

Assessment of the Intra- and ınter-observer reliability of beef cattle mobility scoring performed by UK veterinarians and beef farmers

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BACKGROUND and OBJECTIVE: Lameness in cattle negatively affects welfare and productivity. The lameness prevalence of beef cattle in the United Kingdom (UK) is reported at 8.3% (range 2.0 to 21.2%) in finishing cattle, increasing to 14.2% (range 0.0 to 43.2%) in suckler cows. This figure varies depending on geographical location and production system, with lower lameness levels reported in feedlot cattle in North America (2.1% to 4.5%) [5,6]. Early identification of lameness allows for prompt treatment, and mobility scoring allows for herd-level prevalence data to be monitored. The objective of this study was to assess the intra- and inter-reliability of a four-point mobility scoring system when used by both beef farmers and veterinary surgeons on commercial beef cattle in the UK. The hypothesis was that both beef farmers and veterinary surgeons would have a reliability of over 0.70, with veterinary surgeons having higher intra- and inter-reliability of their scores than beef farmers.

MATERIALS and METHODS: An online questionnaire that contained forty video clips of beef cattle was created for mobility scoring performed by farmers and vets.

RESULTS: The Fleiss kappa coefficient for inter-observer agreement across all 81 respondents and all videos was 0.34, which showed fair agreement. Beef farmers generally had lower agreement than vets (0.29 vs. 0.38). Vets had significantly higher inter-observer reliability compared to beef farmers ($p = 0.035$). Overall, Cohen's kappa coefficient for intra-observer agreement across all respondents varied from 0.085 (slight agreement) to 0.871 (almost perfect agreement). A comparison of inter-observer reliability between farmers and vets was tested, which returned a significant value of $p = 0.035$. The mean Cohen's kappa value for farmers was 0.33, which is fair agreement, and for the vets, this was 0.40, which is moderate agreement. This tells us that veterinary surgeons are significantly more reliable at the application of a four-point mobility scoring system than beef farmers; however, both groups still demonstrate poorer reliability than the accepted level of 0.70.

CONCLUSIONS: The application of a four-point mobility scoring system for beef cattle had fair inter-observer reliability and a wide range of intra-observer reliability, but this is poorer than previously reported. This presents a challenge for the identification of lame beef cattle at both the individual and herd levels. This apparent unreliability of mobility scoring beef cattle could be a barrier in identifying lameness and the subsequent consequences of both welfare and efficiency because of this.

Keywords: beef, lameness, mobility scoring, reliability, welfare

A five-year clinical audit of caesarean sections in dairy and beef cattle in the United Kingdom: 952 surgeries

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BACKGROUND and OBJECTIVE: Although a common surgical procedure, there is currently a limited evidence base for the outcomes of cattle undergoing caesarean and what risk factors can affect them. The aim of this large-scale clinical audit in UK farm animal practice was to define the short-term calf and cow outcomes when undergoing surgery and describe variations in surgical techniques and medications given.

MATERIALS and METHODS: Data were collected across a five-year period via an online questionnaire, recording the techniques used and medications given for caesareans. QR codes linking to the survey were used to prompt completion after each caesarean. The vet was contacted 60 days post-caesarean to determine dam survival at 7 and 60 days and calf survival at 7 days. Data were

transferred into Microsoft Excel for processing and analysis.

RESULTS: Data were collected for 952 caesareans, with partial or complete follow-up data for 625. Where recorded, 75% (676/902) dams were described as beef and 25% (226/902) as dairy. Dam survival at 60 days was high (88%; 548/623) (beef 90% (394/437); dairy 82% (122/148)). Calf survival at 7 days was 69% (320/461) (beef 75% (247/331); dairy 55% (67/122)). Overall, 38% (348/928) of dams had significant assistance from the vet or farmer prior to caesar (beef 28% (189/668); dairy 63% (142/224)), with dam survival at 60 days dropping to 83% (188/227) in these cows and calf survival at 7 days to 54% (93/171). Overall, 86% (819/947) (beef 89% (599/675); dairy 79% (176/223)) were standing during the procedure with 9% (85/947) recumbent and 5% a mixture of both. Dams who were standing during the procedure had higher survival rates for both dams (91%; 479/527) and calves (72%; 280/388), than dams who were recumbent (67% for dams (43/64); 47% for calves (22/47)). Epidurals were used in 57.9% (536/926) of caesareans. Line blocks were the most commonly used local anesthetic block (477/948; 50.3%), followed by inverted L blocks (401/948; 42.3%). Virtually all dams were given NSAIDs (934/943; 99.0%) and systemic antibiotics (941/943; 99.8%).

CONCLUSIONS: Dam and calf survival were higher for beef dams; where the dam was standing; and where there was no significant prior assistance before the procedure. This clinical audit provides baseline data on how cattle caesareans are carried out in farm animal practice and is the first step in quality improvement work to identify interventions in practice that could improve outcomes as well support decision making with individual farmers.

Keywords: Caesarean, Audit, Cattle

The role of energy metabolism, hormones and inflammation on the development of hoof horn lesions

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BACKGROUND and OBJECTIVE: Evidence suggests involvement of energy metabolism and periparturient inflammation in the aetiopathogenesis of hoof horn lesions (HHLs), the role of hormones remains unexplored. The study objective was to examine the association of the inflammatory, hormonal, and metabolic status of cows in early lactation with the development of HHLs

MATERIALS and METHODS: We prospectively enrolled a cohort of 363 cows from two UK dairy farms. Cows were assessed at three timepoints: within one week post-calving (T1-Calving-1), one to two weeks post-calving (T2-Calving-2), and during early lactation (T3-Early). The presence of HHLs and BCS was assessed, alongside ultrasonographic measurements of digital cushion and backfat thickness. Plasma and serum samples were taken. Comprehensive lab analysis was conducted on 200 of the 363 cohort; all eligible primiparous animals (60) and 140 randomly selected multiparous animals. Serum concentrations of insulin, relaxin, β-hydroxybutyrate (BHB), interleukin-1α (IL-1α), non-esterified fatty acid (NEFA), and interleukin-10 (IL-10) were measured alongside plasma LPS concentrations. BHB was standardised and analysed as a continuous variable. Relaxin, insulin, and IL-10 were binned into quartiles, IL-6 was binned into terciles, and IL-1α and LPS were binned into deciles. Three multivariable logistic regression models were fitted to separately examine the relationship between explanatory variables at each timepoint

and the development of sole lesions at T3-Early (outcome variable).

RESULTS: A one standard deviation increase in serum BHB concentration at T1-Calving-1 was associated with a reduced likelihood of developing a sole lesion in T3-Early (OR: 0.29, 95%CI: 0.09–0.83, P= 0.029). Animals with a serum insulin concentration in the second (OR: 0.33, 95%CI: 0.12–0.91, P= 0.034), third (OR: 0.47, 95% CI: 0.16 – 1.33, P = 0.16) or fourth (OR: 0.28, 95%CI: 0.08–0.91, P= 0.039) quartile at T1-Calving-1 had a reduced odds of developing a HHL compared to the lowest quartile. At T2-Calving-2, an increased serum IL-6 concentration was associated with an increased odds of developing a HHL in T3-Early (second tercile; OR: 2.83, 95%CI: 1.19–6.96, P= 0.020, third tercile; OR: 2.03, 95%CI: 0.89–4.75, P= 0.095). At T3-Early, cows within the highest quartile of serum IL-10 concentration were more likely to develop a HHL compared to the lowest quartile (OR: 3.75, 95%CI: 1.36–10.81, P= 0.012). Serum relaxin concentration was not associated with HHLs at any timepoint.

CONCLUSIONS: These results further our understanding of the association between inflammation and energy metabolism shortly after calving and the development of HHLs, however the role of relaxin requires further investigation.

Keywords: Lameness, Dairy Cattle, BHB, Relaxin, Inflammation, Hoof Lesion

Prognosis associated with papillary canal ruptures with inversion in dairy cows: A retrospective study of 28 cases

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BACKGROUND and OBJECTIVE: Papillary canal lesions are frequent teat lesions in lactating dairy cows, causing economic losses. Among these lesions are rupture and inversion of the papillary canal which have not yet been specifically investigated. The objective of this study was to determine the outcome of this condition following surgery at a veterinary teaching hospital. We hypothesize that short-term prognosis is good but that mid to long term prognosis is guarded.

MATERIALS and METHODS: This retrospective study examined the files of 28 dairy cows treated with thelioscopy for a papillary canal rupture with inversion between January 2020 and December 2025. Informations on signalment, teat investigation and diagnosis, and treatments were collected using the medical records. Theloscopic images were reviewed for detailed diagnostic. Long-term outcome was determined using the Canadian Dairy Network Lactanet and by telephone interview or email investigation of the owner.

RESULTS: The study population consisted of 82.1% Holstein cows (23/28) and 17.9% Jersey cows (5/28). The cows were presented at a mean age of 3.6 years [2–9.3 years] and a mean day in milk of 88 [2–276 days]. Lactation numbers at presentation ranged from 1 to 3, with a mean of 2. All four quarters were represented, with a predominance of Q4 (46.4% (13/28)). At admission, 67.9% (19/28) had a positive bacteriological milk culture from the affected quarter. All the teats were treated by lateral theloscopy. The lesions were debrided by inserting an instrument by the damaged papillary canal. Following surgery, passive milking (rest) was used for a mean time of 15.8 days [5–40 days]. A combination of silicone, wax or indwelling teat catheter was used during this period. The treatment was adapted to each cow according to owner ability to provide them at the farm. Major postoperative complication was mastitis, affecting 25% (7/28) of the cows. Following hospitalization, 15.4% of cows (4/26) were culled because the teat never regained functionality despite treatment and 13.6% (3/22) had a productive life but had their treated teat permanently dried off (three-quarter cow). Overall, 68.2% (15/22) of cows recovered from the affected teat following treatment and 64.7% (11/17) underwent at least one subsequent lactation with four functional quarters.

CONCLUSIONS: Management by theloscopy combined with a postoperative passive milking protocol appears to be effective in achieving complete recovery of teat function. The long term prognosis for the cows in this study was favorable.

Keywords: Teat health, prognosis, surgery, dairy cattle

A randomized controlled trial on the effect of pre-calving formalin footbaths on the foot skin microbiome and presence of digital dermatitis lesions post-calving

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BACKGROUND and OBJECTIVE: Digital dermatitis (DD) is an infectious disease with a significant impact on cattle welfare and production. Footbaths are widely used on dairy farms as a prevention measure despite sparse evidence supporting their efficacy and the associated environmental cost. We aimed to investigate the impact of footbathing during the last month before calving on presence of DD lesions post-calving and on the foot skin microbiome.

MATERIALS and METHODS: The dry cow (close to calving) pen on one farm was split into two pens, with automatic footbathing and feeding stations installed on each side. The footbath in the control pen remained empty while the treatment pen footbath was filled with 1% formalin. We enrolled treatment and control animals weekly, approximately 30 days before the expected calving date, and randomly allocated animals to each pen. Animals were scored using the M-stage system at enrolment, a week post-calving, and five months in lactation. Hind feet skin swabs were collected at enrolment and within 24 hours post-calving for a subset of animals, and the extracted DNA was submitted for 16S rRNA gene amplicon sequencing. Chi-squared tests were used to investigate the effect of footbathing on the frequency of different categories of DD lesions post-calving: any, active (i.e., M1, M2, and M4.1), chronic (i.e., M3, M4), and M2. Two multivariable logistic regression models were also employed for data analysis. The 16S rRNA gene amplicon sequences were subjected to quality control and analysis using QIIME2 and R.

RESULTS: In total, 178 animals were enrolled in the trial. Animals in the control group were 2.38 (95% CI: 1.28-4.43), 2.69 (95% CI: 1.29-5.63), and 8.44 (95% CI: 1.01-65) times more likely to have any, active, or M2 DD lesions, respectively, compared to treatment group animals post-calving. Alpha diversity analysis identified no significant difference between samples at enrolment and post-calving or between treatment and control samples post-calving. Evaluating post-calving samples specifically, Unifrac distances differed between control and treatment samples. The post-calving skin microbiome in the treatment group was characterized by a significant decrease in the relative abundance of Lentimicrobium, Dichelobacter, and Mobiluncus, as well as three genera from the Peptostreptococcaceae family. Rothia, Globicatella, and Dermatophilus genera were more abundant in treatment samples post-calving.

CONCLUSIONS: Our study shows that formalin footbaths during the dry period can reduce DD lesions and DD-associated pathogens post-calving.

Keywords: Digital Dermatitis, Footbaths, Microbiome, Lameness

Randomized controlled trial evaluating an automated imaging system for early detection and control of lameness in dairy cows

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BACKGROUND and OBJECTIVE: Automated mobility monitoring offers the potential for continuous lameness detection and earlier intervention in dairy

herds. This study evaluated the effect of integrating a fully automated 2-dimensional imaging system into a lameness management protocol based on early detection and prompt treatment.

MATERIALS and METHODS: A randomized controlled trial was conducted on a large commercial dairy farm. A total of 419 multiparous Holstein cows ≤ 30 days-in-milk (DIM) were randomly allocated to a control group (CON; n=208) managed under standard farm practice, or an intervention group (AUTO; n=211) additionally monitored using weekly average automated mobility scores. AUTO cows exceeding predefined thresholds were selected for trimming weekly. Outcomes included monthly lameness prevalence based on human mobility scoring, severe and chronic lameness, trimming events and hoof block applications, foot lesion prevalence and severity at 180 DIM, reproductive performance and weekly milk yield (WMY). Differences in proportions were assessed using Chi-square or Fisher's exact tests. Counts of events were analysed using Poisson regression models. Time-to-event and binary outcomes were analysed using Cox proportional hazards regressions and binary logistic regressions, respectively. Repeated measures outcomes were analysed using linear mixed effects models.

RESULTS: AUTO group had a lower proportion of cows with severe lameness (2.0% vs. 7.9%; P = 0.006) and chronic lameness (3.9% vs. 9.8%; P = 0.030) compared with CON. AUTO cows underwent more trimming events (2.67 vs. 1.83 per cow; P < 0.001). At 180 DIM, AUTO group had a higher proportion of lesion-free cows (22.4% vs. 12.0%; P = 0.015) and a lower proportion with moderate lesions (16.0% vs. 25.3%; P = 0.042). Cows in the AUTO group had higher odds of conception to 1st artificial insemination (Odds Ratio = 7.6, 95% Confidence interval: 1.6–35.6) and greater hazard of conception by 150 DIM (Hazard Ratio = 3.1, 95% Confidence Interval: 1.7–5.7); however, due to a parity × group interaction, these effects were observed only in the small number of 2nd parity cows and should be interpreted cautiously. No differences were observed in WMY or culling hazard.

CONCLUSIONS: Integration of automated mobility monitoring into a lameness management protocol reduced severe and chronic lameness and improved mid-lactation foot health. Positive associations with reproductive performance were observed but should be interpreted cautiously due to the small sample size of 2nd parity cows. Overall, automated monitoring can enhance lameness control and welfare in commercial herds.

Keywords: automated monitoring, dairy cattle, lameness, machine learning, mobility scoring

Preliminary results from a randomised controlled trial evaluating the effect of pre-calving hoof trimming technique on the risk of claw horn lesions in early lactation

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BACKGROUND and OBJECTIVE: Preventive hoof trimming has a role to play in the prevention of lameness in dairy cattle and is a common part of lameness management on dairy farms worldwide. Despite this, there is very little peer reviewed literature in this subject area. The objectives of this study were to determine the risk of claw horn lesions and abnormal mobility in early lactation, between heifers receiving no foot trim before first calving, those trimmed with a conventional (small, narrow) model and those trimmed with a wide, deep model.

MATERIALS and METHODS: A total of 1,214 heifers were recruited from 6 farms between October 2021 and February 2023 and allocated to one of three treatment groups using stratified randomisation: • Negative Control (not trimmed) • Treatment 1: Five step functional trim with a conventional narrow, shallow model for Step 3 • Treatment 2: Five step functional trim with a deep, wide model being incorporated for Step 3 Heifers were enrolled at two-week intervals, 50-70 days prior to their predicted calving date. Exclusion criteria were applied including exclusion due to a history of lameness and/or lameness at recruitment. A total of 936 heifers were enrolled. Hoof trimming was undertaken by qualified hoof trimmers, supervised by the lead author. The size of the model on the hind lateral claws was standardised for each intervention group. Following enrolment heifers were mobility scored fortnightly. At 60-90 days in milk heifers were re-presented for examination ('early lactation inspection'). Multivariable logistic regression

models were used to test the following hypothesis: There is no difference in risk of abnormal mobility during the first 60 days in milk, or in prevalence of lesions at early lactation inspection, between heifers receiving no foot trim before first calving, those trimmed with a conventional (small, narrow) model and those trimmed with a wide, deep model.

RESULTS: Initial results indicate trimming pre-calving heifers with a large model significantly ($p<0.05$) reduces the risk of moderate and severe claw horn lesions at early lactation inspection, compared to heifers receiving either a conventional (small model) trim or those receiving no trim. There was no significant association between the risk of an abnormal mobility score at any stage in the first 60 days in milk and treatment group.

CONCLUSIONS: Trimming heifers pre-calving using a deep, wide model significantly reduces the risk of moderate and severe claw horn lesions at early lactation inspection when compared to using a conventional (small, narrow) model or not being trimmed.

Keywords: Dairy, Lameness, Hoof trimming, Claw horn lesions

UDDER HEALTH AND MASTITIS

Can we further reduce antimicrobial usage at dry-off?: Comparing a simplified quarter specific treatment protocol to systematic treatment

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BACKGROUND and OBJECTIVE: Selective antibiotic dry cow therapy (SDCT) is a proven method to reduce antimicrobial usage (AMU) at dry-off. Further AMU reduction may be achievable through stricter cow or quarter selection, namely by combining a SCC-based selection with a subsequent bacteriology. This study aims to evaluate, on various udder health criteria, a two-step in-series selection protocol—SCC screening followed by a quarter-specific on-farm rapid bacteriology—compared to systematic antibiotic treatment of all quarters (BDCT). We suppose that this method would reduce AMU by 90% while not negatively affecting udder health.

MATERIALS and METHODS: A randomized control trial was conducted to compare our method and BDCT on five criteria representing udder health (and their respective clinical difference margin). We recruited 773 cows from 9 different farms. Cows were randomized to either: BDCT group (A-CTRL): all quarters of all cows received antimicrobials and teat sealant; Selective group (B-SDCT): only bacteriology positive quarters (Petrifilm®, 3M) from cows ≥200 000 cells/ml at last measurement received an antimicrobial, and all quarters of all cows received a teat sealant. After treatment, we followed cows for 120 days. Criteria and AMU were used as outcomes in mixed models, with treatment group as a fixed effect, farm as a random effect, milk production at dry-off and lactation rank at dry-off were tested as interaction factors and kept in the model if significant ($p<0.1$). Estimated Marginal Means were then computed to allow a better understanding of the results.

RESULTS: Cows in group B-SDCT received significantly less antimicrobial due to dry-cow therapy (15% were treated), and quarter received even less antimicrobials (9% treated). Daily milk production, clinical mastitis incidence and subclinical mastitis (SCM) cure rate did not differ between the two groups, while somatic cell score was 0.6 points higher in group B-SDCT (1.8 vs. 2.5, $p<.01$) and new SCM rate was 11% higher in group B-SDCT (10% vs. 21% new IMI/100 quarters, $p<.01$). Cows in group A-CTRL presented slightly less positive bacteriology compared to B-SDCT at pre (50% vs. 60% respectively) and post-calving samples (58% vs. 64% respectively). These differences were mostly attributable to a higher prevalence of non-aureus staphylococci infections in the B-SDCT group.

CONCLUSIONS: These preliminary results suggest that our method of selective dry cow therapy massively reduces AMU at dry-off at the cost of a slight increase in SCC. Final analysis, including full bacteriology data, will clarify these findings and inform best practices for SDCT implementation.

Keywords: antimicrobial usage, selective dry-cow therapy, udder health

What wet testing really shows us: Pulsator dysfunction as the missing link between machine and mastitis

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BACKGROUND and OBJECTIVE: Milking machine performance plays a central role in teat health, udder defence, and milking efficiency. At a 182-cow dairy herd, rising mastitis incidence combined with farmer concerns about cow behaviour prompted a wet-milking evaluation. This assessment revealed multiple management and machine-related issues, but most notably a striking pulsator dysfunction characterised by shortened B-phases and prolonged A and D-phases. These abnormal pulsation patterns suggested a mechanical link between machine performance, teat-end stress, and mastitis vulnerability.

MATERIALS and METHODS: The herd is milked in a 2×12 Fullwood side-by-side parlour at 60 pulsations/min with a 64: 36 pulsation ratio and a system vacuum of 44.3 kPa. During each evaluation, fifteen cows were assessed for milking time, milkspeed, vacuum under the teat, short-milk-tube vacuum, blind milking time, and presence of bimodal flow. Additional assessments included cow behaviour, milker routine, hygiene of legs, teats and udders, and teat-end condition scoring.

RESULTS: Results showed that, mastitis incidence reached 30% annually, with a seasonal winter peak. Machine performance indicators highlighted high teat-end vacuum (20.3 kPa) and prolonged blind milking (2: 31 minutes on average, ranging up to 6: 11 minutes). Bimodality occurred in 13% of cows. Hygiene scores exceeding recommended thresholds, especially leg and teat-end hygiene (34.9% of legs and 35.7 % of teat ends had bad score). Teat-end condition was poor, with 33.3% of teats scoring 3–4, suggesting mechanical stress. Critically, pulsation curves demonstrated consistently shortened B-phases and prolonged D-phases in both measuring devices and also prolonged A-phases in device 2, indicating malfunctioning pulsators failing to fully open and close the liners.
Following pulsator maintenance, a new evaluation in November showed clear improvements: teat-end vacuum normalised to 11.4 kPa, blind milking reduced to 1: 06 minutes with far less variation, and milking time decreased from 6: 41 to 6: 08 minutes. Bimodality dropped to 6.7%, and pulsation deviations were reduced in severity (B and D) and turned to normal for phase A.

CONCLUSIONS: Through wet-milking testing we revealed multiple contributing factors to udder health challenges, including hygiene deficits, excessive vacuum exposure, and prolonged blind milking. However, pulsator malfunction emerged as the key mechanical driver linking poor vacuum stability, teat-end load, and mastitis risk. After corrective maintenance, substantial improvements were observed, underscoring the necessity of routine pulsator inspection, pulsation-curve evaluation, and accurate take-off calibration. For practicing bovine veterinarians, this case demonstrates how wet-milking assessments can uncover hidden mechanical faults that have significant clinical consequences.

Keywords: Wet testing, Pulsator, Mastitis

Impact of a risk assessment and management plan on adoption of udder health management practices in Canadian dairy herds

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BACKGROUND and OBJECTIVE: Best management practices (BMPs) for udder health are well known, yet mastitis remains a challenge in dairy production. Producer compliance with veterinary recommendations can be inconsistent, and veterinarians may not always initiate discussions regarding herd health management. A structured risk assessment and management plan (RAMP) can support personalized communication and tailored mastitis control. This study evaluated the effect of an udder health RAMP on adoption of veterinary recommendations in Canadian dairy herds.

MATERIALS and METHODS: This study was approved by the University of Saskatchewan Behavioral Research Ethics Board (Beh-REB 3337). Best management practices were identified through a literature review and from existing udder health programs, then categorized and ranked by an expert panel. Highest-priority practices were incorporated into a prototype RAMP. Each management practice was given a score from 1-5, with 5 representing a practice resulting in a high risk of mastitis on the respondent's dairy. Pre-testing on five dairy farms informed the final RAMP. Canadian dairy herds were eligible for recruitment by their veterinarian if they participated in milk recording and intended to continue production throughout the study. Herd veterinarians administered the RAMP and provided recommendations based on identified risks. Herd veterinarians followed up six months later to determine recommendation implementation and to document reasons for implementation or non- implementation. Herd demographics were summarized using descriptive statistics. Factors associated with implementation were evaluated using the Chi-square test.

RESULTS: Ninety BMPs were initially identified, with 36 included in the final RAMP across eight management categories: biosecurity, mastitis diagnosis, dry cow management, heifer management, housing management, milking management, treatment/prevention and udder health. Forty RAMPs were completed: Québec (n=26), Alberta (n=8), Ontario (n=4), and Manitoba (n=2). Median milking herd size was 74 cows (range 21-264) and most herds milked Holsteins (37/40). Median risk scores were lowest for milking management (2.46/5) and highest for housing (4.83/5). Veterinarians provided a median of three recommendations (range 1–6), of which 64% aligned with high-risk areas. Recommendations most often addressed treatment and prevention. Overall implementation of recommendations was 54%, and implementation was not associated with management category (p=0.6). The most frequent reason for non-implementation was the recommendation being of low priority (24%) to the producer.

CONCLUSIONS: Recommendation compliance in this study was consistent with previous reports. The RAMP appeared to support discussions about udder health. Our results highlight the complexity of aligning herd health recommendations with high-risk management practices and producer goals.

Keywords: Udder health, Best management practices, Risk assessment

Outcomes following clinical mastitis in dairy herds of Australia

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BACKGROUND and OBJECTIVE: Clinical mastitis remains a major health and economic challenge in dairy herds. This study evaluated outcomes following clinical mastitis episodes and associated risk factors in dairy herds in Australia. Understanding these outcomes can support evidence-based treatment decisions, optimise culling strategies, and improve overall herd health and productivity.

MATERIALS and METHODS: A 14-month repeated cross-sectional study followed 2,162 cows across 23 farms, documenting 3,334 clinical mastitis episodes. Outcomes were: 1) natural death or culling before the next calving and 2) the occurrence of subsequent clinical mastitis episodes within the same lactation. Potential risk factors included treatment type, the sequential number of the clinical mastitis episodes within the lactation, and the interval between the second-last and last clinical mastitis episodes. Multilevel competing risks time-to-event models were used, and potential confounders were identified using directed acyclic graphs (DAGs).

RESULTS: The hazard ratio (HR) and subdistribution hazard ratio (SHR) of 'death or culling' did not differ significantly by treatment type. Compared with antimicrobials alone, combined antimicrobial and nonsteroidal anti-inflammatory drug (NSAID) therapy had HR = 1.33 (95% confidence interval (CI): 0.95-1.88) and SHR = 1.32 (95% CI: 0.94-1.86), while no treatment had HR = 0.94 (95% CI: 0.71-1.25) and SHR = 0.93 (95% CI: 0.70-1.23). Adding NSAIDs to antimicrobials did not significantly reduce the HR and SHR of 'subsequent clinical mastitis episodes', with HR = 0.96 (95% CI: 0.77-1.20); and SHR = 0.90 (95% CI: 0.72-1.13). Cows receiving no treatment had a lower SHR for a subsequent episode (SHR = 0.82 (95% CI: 0.67-0.99)). Both the hazard of 'death or culling' and the hazard of 'subsequent clinical mastitis episodes' increased with the mastitis episode number within the lactation. For 'death or culling', HRs were 1.55 (95% CI: 1.22-1.97), 2.29 (95% CI: 1.60-3.29), and 2.99 (95% CI: 1.92-4.65) for second, third, and

fourth episodes within the lactation, respectively. For 'subsequent clinical mastitis episodes', HRs were 1.39 (95% CI: 1.18-1.63), 1.68 (95% CI: 1.33-2.14), and 1.93 (95% CI: 1.44-2.59) for second, third, and fourth episodes, respectively. The SHR for both outcomes also increased with episode number.

CONCLUSIONS: Both, natural death or culling before the next calving, and the occurrence of a subsequent clinical mastitis episode within the same lactation in cows with clinical mastitis were more strongly associated with number of previously experienced mastitis episodes rather than with treatment type. This indicates the need for targeted management strategies, appropriate antimicrobial use, supportive therapies, and close monitoring of cows with recurrent mastitis.

Keywords: Subtropical dairy, clinical mastitis, survival analysis, antimicrobials, nonsteroidal anti-inflammatory drugs, udder health

Development of predictive models to support selective dry cow therapy in dairy cattle

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BACKGROUND and OBJECTIVE: In dairy cattle, antimicrobial use is largely related to the management of intramammary infections. To reduce antimicrobial use and limit the development of antimicrobial resistance, selective dry cow therapy has been proposed as a strategy; however, this approach requires reliable identification of animals at risk of infection. The aim of this study was to develop a predictive model of the individual risk of intramammary infection during dry off using multiple indirect indicators available on farm.

MATERIALS and METHODS: A total of 302 multiparous cows from a commercial dairy farm were enrolled. Individual production and health data were obtained from official milk recording systems and herd management software. Information collected included milk yield before dry off, somatic cell count during the previous lactation, clinical mastitis history, teat end condition, parity, and genetic values related to udder health. Quarter milk samples were collected 0 to 7 days before dry off and cultured to identify causative bacteria. Cows were classified as positive when at least one quarter was infected by major pathogens (*S. aureus*, streptococci, or coliform bacteria), and negative otherwise. A generalized linear logistic regression model was developed to classify animals as positive, and therefore candidates for antibiotic treatment at dry off, or negative and not requiring treatment. The dataset was randomly divided into training (75%) and validation (25%) subsets, and variables were selected through a stepwise procedure based on model fit criteria

RESULTS: The best performing model for predicting intramammary infection at dry off included the last and third to last Linear Score measurements and teat condition. Higher Linear Score values significantly increased the odds of infection, (odds ratio ≈2.4 to 3.5) whether teat alterations were negatively associated with infection probability. The model achieved 72% accuracy with 63% sensitivity and 80% specificity in predicting infection at drying off. A second model using a categorized somatic cell count indicator and number of mastitis showed similar performances, with 69% accuracy, 63% sensitivity and 76% specificity.

CONCLUSIONS: The outcomes of the study highlighted the importance of the use of predicting model to assess the probability of infection at dry-off based on the previous data collected during lactation. The implementation of data analysis tools based on statistical models could allow improving the use of selective dry cow therapy in dairy herds with high level of sensitivity and specificity without the use of milk culture from each cow. Research funded by the Italian Ministry of Health, project number RC IZSVE 01/22

Keywords: Selective dry cow therapy, intramammary infection, antimicrobial stewardship, predictive models

Evaluation of an on-farm mastitis diagnostic test to optimise antibiotic use under New Zealand pastoral conditions

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BACKGROUND and OBJECTIVE: Clinical mastitis caused by gram-positive bacteria has been shown to have a positive response to antibiotic therapy, while other aetiologies do not require or do not respond to antibiotic therapy. A reduction in antibiotic use can be achieved by identifying those cases that do not require therapy. Under New Zealand pastoral systems gram-positive bacterial mastitis dominates with more than 80% of cases attributed to these bacteria. This study aimed to evaluate prudent antibiotic use by implementing a novel cow-side mastitis test, detecting gram-positive bacteria in milk, under New Zealand field conditions.

MATERIALS and METHODS: The study design was reviewed and approved by the Animal Ethics Committee – Ruakura of AgResearch (#2381). A multicentric convenience sample of twenty-two pasture-based, seasonal-calving herds, each milking 160–800 cows, were enrolled during the 2024–25 season. Farm staff were trained in the use of the diagnostic test (Vetscan Mastigram+, Zoetis) and asked to enrol cases of farmer diagnosed mild to moderate clinical mastitis. Cows were assigned to one of two groups. TG1 received conventional treatment: immediate intramammary cloxacillin and systemic carprofen, without diagnostic testing. Cows in TG2 were tested using the diagnostic kit. Test-negative cases were treated immediately with carprofen, and test-positive cases received intramammary cloxacillin and systemic carprofen. Cows enrolled in TG2 were only treated with antibiotics if the test was positive or the farm staff determined the case was not resolving. Farms were instructed to with-hold abnormal milk from supply.

RESULTS: Seven-hundred and eighty-two cases were enrolled in the study. Of the tested samples 85.1% were positive, indicating a gram-positive aetiology. There was no difference in the retreatment rate within 60 days of diagnosis by assigned group. The retreatment rate between the two groups was modelled with generalised linear models. The results of this modelling were passed into simulations for antibiotic reduction. The median reduction in antibiotic use by implementing the testing system under New Zealand field conditions was 10.7% and a positive ROI was predicted in 83% of the simulations.

CONCLUSIONS: New Zealand pastoral systems likely represent the worst-case scenario for clinical mastitis antibiotic optimisation as >80% of clinical mastitis cases are attributed to gram-positive bacteria. Even under these conditions, testing and selective treatment resulted in a reduction in antibiotic use and a positive return on investment.

Keywords: Mastitis, Diagnostics, Antibiotic Optimisation, Precision Agriculture

Effect of treatment of clinical and subclinical mastitis with a novel intramammary anti- inflammatory and at 12 or 24-hour treatment intervals

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BACKGROUND and OBJECTIVE: Bovine mastitis remains common with negative effects on animal welfare, production and financial returns. Animal ethics approval was obtained prior to commencement of this randomised controlled study which evaluated outcomes of mastitis treatments with 1 g of intramammary penicillin on 3 occasions, with or without inclusion of a novel steroid anti-inflammatory (HCA).

MATERIALS and METHODS: Cows with clinical (n=120 cows; 134 quarters) and subclinical (i.e. cows with a cow-composite SCC>200,000 cells/mL; n=410 cows, 715 quarters) mastitis were enrolled. All clinical cases were treated with penicillin on 3 occasions at 24 hourly intervals, half the cows also received HCA. Subclinical cases were treated with HCA (or not) and treated at 12 or 24 hourly intervals with penicillin, with cows randomly allocated in a 2 x 2 factorial design within herd. Examination of the milk for presence of clots (on a 0, 1, 2 scale) and

presence or absence of heat or swelling of the udder, and quarter-level milk samples were collected prior to treatment, and at 7, 14 and 21 days after treatment. Bacteriological cure was defined as non-isolation of the bacterial species present pre-treatment in any of the post-treatment samples. Cure rate was analysed using multivariable binary logistic regression, and quarter-level log 10 SCC was analysed using a GLMM, with HCA treatment, treatment interval (for the subclinical cases), age, and mastitis pathogen as main effects.

RESULTS: For the clinical cases, the bacteriological cure rate was 65.1% (95%CI 49.2-78.2%) vs 66.7% (95% CI51.6-79.0%) for control vs HCA treated cases, respectively. Treatment with the penicillin and HCA resulted in a lower overall log 10 SCC compared with penicillin alone treatment (3.02 (SE=0.07) vs 3.15 (SE=0.07)) and lower log 10 SCC at Days 14 and 21. HCA treated quarters had lower heat/swelling scores than the control quarters at 12, 24, and 48 hours after commencement of treatment, and tended ($p = 0.1$) to have lower clot scores. For the subclinical cases, the bacteriological cure rate was 65.2% (95%CI 58.8-72.9%) vs 64.9% (95%CI 57.4-71.8%) for penicillin alone vs penicillin + HCA treated cases, respectively, and was 64.6% (95% CI 57.2-71.4%) vs 66.5% (95% CI 59.1-73.3%) for the 12 vs 24-hourly frequency of treatment, respectively.

CONCLUSIONS: It was concluded that inclusion of HCA reduced SCC and inflammation in clinical mastitis cases, without reducing bacteriological cure rate in either the clinical or sub-clinical mastitis. There was no difference in bacteriological cure rate between treatments at 12 or 24 hourly intervals.

Keywords: anti-inflammatory, steroid, intramammary, penicillin, inflammation, mastitis

Ketoprofen as the sole initial treatment for non-severe bovine mastitis: Efficacy and antibiotic reduction

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BACKGROUND and OBJECTIVE: Mastitis in dairy cows is a major health issue traditionally treated with antibiotics. However, growing concerns about antimicrobial resistance have prompted exploration of alternative therapies. This study aimed to evaluate the efficacy of a non-steroidal anti-inflammatory drug (NSAID)-based treatment concept using ketoprofen (KE) compared to conventional antibiotic therapy (AB) for mild and moderate clinical mastitis (CM). The primary objective was to assess clinical cure at day 5, bacteriological cure at days 14 and 21, recurrence within 60 days, and new infection risk, while highlighting the potential reduction in antibiotic usage.

MATERIALS and METHODS: A randomized, multi-herd, non-inferiority trial was conducted on three dairy farms in Northern Germany between August 2022 and November 2023. A total of 222 lactating Holstein-Friesian cows with mild or moderate CM were enrolled. Animals were randomly assigned to two groups: AB group received local intramammary antibiotics (cefalexin and kanamycin, Ubrolexin® Boehringer Ingelheim) 2 injections 24 hours apart, while KE group received systemic ketoprofen (Romefen® PR 10%/Ketofen® 10% (Ceva Santé Animale) for three days, with antibiotics added only if no improvement was observed by day 3. Clinical examinations were performed every 12 hours for six days, and milk samples were collected for microbiological analysis.

RESULTS: Of 222 cases, 118 were treated with antibiotics (AB) and 104 with ketoprofen (KE). Clinical cure at day 5 was 91.2% for AB group and 67.8% for KE group ($P < 0.001$). Bacteriological cure

rates were 78.6% for AB group and 59.6% for KE group (P = 0.026). Recurrence within 60 days was 5.8% for AB group and 14.0% for KE group (P = 0.046), while new infection risk did not differ significantly between groups. Fourteen cows in the KE group required subsequent antibiotic therapy. The KE group achieved an 86% reduction in antibiotic doses compared to AB (P < 0.001). Because of the absence of need to discard milk after ketoprofen treatment, there was 35.9% less milk wasted in the KE group compared to the AB group (milk has been commercialized again as soon as its appearance returned to normal).

CONCLUSIONS: The NSAID-based treatment concept substantially reduced antibiotic usage and waste milk, contributing to antimicrobial stewardship and sustainability. However, lower clinical and bacteriological cure rates in the KE group indicate that sole NSAID therapy cannot replace antibiotics in all mastitis cases. Nevertheless, this approach represents a promising step toward reducing antibiotic consumption in dairy farming without compromising animal welfare.

Keywords: Mastitis, NSAID, Welfare, Sustainability.

Intramammary platelet lysate administration at dry-off as a potential alternative to antimicrobial therapy in dairy cows

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BACKGROUND and OBJECTIVE: Reducing antimicrobial use in dairy production is a priority within the One Health framework. In dairy cattle the majority of antimicrobial consumption is associated with mastitis treatment during lactation and at dry-off. Identifying alternative strategies capable of maintaining udder health while reducing antimicrobial use is therefore essential. Platelet-derived products, containing bioactive molecules involved in tissue repair and inflammatory modulation, may represent a promising therapeutic option. The aim of this study was to evaluate the feasibility and effectiveness of intramammary platelet lysate administration at dry-off.

MATERIALS and METHODS: A field trial was conducted on 17 dairy farms in northern Italy. Whole blood from donor cows was processed through sequential centrifugation to obtain platelet concentrate, followed by repeated freeze–thaw cycles to produce platelet lysate for intramammary use. Quarter milk samples were collected seven days before dry-off for bacteriological examination and somatic cell count (SCC). SCC values were converted into linear score (LS), an indicator associated with the risk of developing mastitis. Quarters were classified as healthy (LS ≤ 4), low risk (4 < LS ≤ 5), or high risk (LS > 5). Healthy quarters received sealant only, platelet lysate plus sealant, or antimicrobial plus sealant, whereas low- and high-risk quarters received platelet lysate plus sealant or antimicrobial plus sealant. Milk samples were collected again after calving and the difference in linear score (ΔLS) between dry-off and post-calving measurements was calculated.

RESULTS: A total of 605 quarters were included in the study: 446 healthy, 63 low risk, and 96 high risk at dry-off. In healthy quarters, no statistically significant differences in ΔLS were observed among treatments. Similarly, in low- and high-risk quarters, no significant differences in ΔLS were detected between platelet lysate and antimicrobial treatments. In high-risk quarters, antimicrobial treatment showed a trend towards a higher proportion of quarters returning to a healthy LS category after calving, although this difference was not statistically significant. No cases of clinical mastitis were recorded during the first 30 days after calving.

CONCLUSIONS: Intramammary platelet lysate administration at dry-off proved technically feasible under field conditions and achieved outcomes comparable to antimicrobial therapy in quarters with elevated somatic cell counts (LS > 4). Platelet lysate may represent a potential antimicrobial-sparing strategy within selective dry cow therapy programs, particularly in quarters with moderate somatic cell counts at dry-off (4 < LS ≤ 5). Further studies are needed to confirm efficacy and optimise administration protocols. Research funded by Regione Veneto (DGR 814/2022)

Keywords: dairy cow, dry-off, platelet lysate, somatic cell count, selective dry cow therapy, antimicrobial stewardship

Validation of a Streptococcus uberis mastitis model in late lactation

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BACKGROUND and OBJECTIVE: *Streptococcus uberis* (SUB) causes mastitis in dairy cows and is a significant threat to dairy herd health. Although SUB is renowned for causing persistent mastitis shortly after calving, problems also occur during late lactation. The objective of this study was to validate a SUB mastitis model in late lactating dairy cows evaluating two different strains of SUB that were applied using two different challenge methods.

MATERIALS and METHODS: In this within-cow comparison, two experimental groups of six cows were allocated to either study group A or B. Per cow, three quarters with low somatic cell count (SCC) and negative bacteriological culture were challenged and the fourth quarter was left unchallenged. One of both front quarters of each cow was challenged with approximately 200 cfu of SUB-strain A or B, where the other front quarter was not challenged. In both groups, one of the hindquarters was challenged with a teatdip containing 108 cfu/ml SUB-strain A, the other hindquarter with 108 cfu/ml SUB-strain B. Animals, quarters and milk were observed twice daily for clinical signs and SCC and SUB-counts were recorded. The infection model was considered successful if 60% of the quarters that were challenged with the teat dip method, or 70% that were challenged with the intramammary method, had at least two milk samples post-challenge with high-SCC and SUB-positive culture.

RESULTS: The teatdip challenge with strain A and B persistently infected 6/12 quarters (50%) and 9/12 quarters (75%), respectively. In the unchallenged group, 5/12 quarters (41.2%) developed a persistent infection. The log transformed SUB-counts showed that 37 out of 48 quarters had more than one milk sample with at least one log CFU of SUB. Successfully infected quarters showed a distinctive pattern of shedding (quick rise and persistent high level of SUB). In challenged quarters the percentage of abnormal observations was higher (IMM A 14%, teat dip A 8%, IMM B 10%, teat dip B 12%) as compared to the non-challenged quarters (6%).

CONCLUSIONS: Both the teat dip method with SUB-strain B and the intramammary method with SUB-strain A were successful (i.e. 60% of the quarters) in inducing a persistent SUB-infection in late lactating dairy cows. The teat dip challenge with strain A and B persistently infected 50%, respectively 75% of quarters.

Keywords: Mastitis infection model, Streptococcus uberis, intramammary

Prediction and decision of escherichia coli-induced mastitis using the feature importance explainable artificial intelligence tool (fiXAIt) framework

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BACKGROUND and OBJECTIVE: This study aimed to predict Escherichia coli (E. coli)–associated mastitis using milk composition and physicochemical parameters through an explainable machine learning approach.

MATERIALS and METHODS: A total of 372 milk samples were analyzed, including 177 healthy (class = 0) and 195 E. coli–positive samples (class = 1). Input variables included fat (%), nonfat solids (%), protein (%), lactose (%), mineral matter (%), electrical conductivity (mS/cm), specific gravity (kg/m³), and somatic cell count (SCC) mL/cell. The main objective was to evaluate the Feature Importance eXplainable Artificial Intelligence tool (fiXAIt), which enables both prediction and interpretation of model decisions. In the first stage, collinearity analysis was performed to detect variables carrying highly similar information. When multiple measurements reflect

the same physiological condition, the model may interpret the same signal repeatedly, complicating identification of the true decision drivers. Therefore, highly collinear variables were removed to simplify the model and improve the clarity and reliability of explanations. In the second stage, model selection was conducted. Model performance was evaluated not only by accuracy but also by the consistency between training and test results. Models with high training accuracy but lower test performance were considered prone to overfitting. Therefore, models with high test accuracy and minimal train–test differences were prioritized. Comparative analyses showed that the CatBoost algorithm provided the most balanced performance. In the third stage, the Self-Consistency metric of the fiXAIt framework was evaluated. This metric measures how well the generated explanations align with the model's internal decision logic. The highest Self-Consistency score was obtained with the CatBoost model.

RESULTS: For interpretation, two outputs of fiXAIt were used: Feature Existence Impact (FEI) and Feature Value Impact (FVI). Fat formed the structural backbone of the decision process (FEI: 1.000; FVI: 2.471). SCC showed the strongest influence on the prediction score (FEI: 0.568; FVI: 3.047). Electrical conductivity had lower structural and value impacts (FEI: 0.362; FVI: 1.254) and mainly acted as a supportive indicator. Other variables were not found to be significant predictors of E. coli mastitis.

CONCLUSIONS: Overall, the results suggest that the fiXAIt framework can enhance both predictive performance and decision transparency in the detection of E. coli-associated mastitis.

Keywords: Escherichia coli, Mastitis, Artificial Intelligence

Effect of pre-milking routines using brushes on bacterial counts in teat skin

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BACKGROUND and OBJECTIVE: Effective teat preparation before milking is critical in mastitis prevention, as it reduces bacterial contamination of the teat skin. Mechanical brush systems have been introduced as an alternative to conventional cleaning routines, combining rotating brushes with the application of disinfectant and subsequent drying. Despite their increasing adoption, evidence regarding their effectiveness compared with conventional methods remains limited. This study aimed to evaluate the efficacy of mechanical brushes in reducing bacterial load on teat skin under commercial dairy farm conditions. Secondary objectives included assessing the effect of combining brush cleaning with pre-dipping and evaluating potential differences in hygiene effectiveness among milkers.

MATERIALS and METHODS: The study was conducted in 3 dairy farms located in Portugal. In farms A and B, teat preparation consisted of pre-dipping with an iodine-based disinfectant followed by use of mechanical brushes, whereas in farm C teat cleaning was performed exclusively using the brush system. In each farm, teat skin swabs were collected from 20 cows before and after the cleaning procedure. Animals were divided into 2 groups: Test group, in which the farm's routine protocol was applied, and Control group, in which teat preparation consisted of iodine-based disinfection followed by manual drying with towel. Microbiological analysis included total bacterial counts on Tryptic Soy Agar and Enterobacteriaceae counts on MacConkey Agar. Bacterial reduction was assessed through logarithmic decrease in CFU/ml after 24 and 48 hours of incubation.

RESULTS: A significant reduction in bacterial load between pre- and post-cleaning samples was observed in all groups. However, differences between protocols varied among farms. In farm A,

the mechanical brush protocol resulted in a significantly greater reduction in total bacterial counts compared with the conventional method. In farms B and C, no significant differences between protocols were detected for total bacterial counts. For Enterobacteriaceae, the mechanical brush protocol showed a significantly greater reduction in farms A and B, while no significant differences were observed in farm C. Additionally, variability analysis indicated no significant improvement in the homogeneity of results between Test and Control groups.

CONCLUSIONS: Overall, results suggest that the combination of pre-dipping with brush cleaning may enhance teat hygiene, whereas the isolated use of brushes does not appear to provide clear advantages over conventional protocols. The considerable operational variability observed among farms highlights the need for further research to better establish the role of this technology in dairy milking hygiene protocols. Work supported by Fundação para Ciência e Tecnologia project UIDB/00276/2025 (CIISA) and LA/P/0059/2020 (AL4AnimalS).

Keywords: Teat hygiene, milking routine, mechanical brushes, bacterial load, mastitis prevention

Impact of milking systems and hygiene protocols on the herd-prevalence of major mastitis pathogens (S.aureus, St.agalactiae, St.uberis Mycoplasma spp.) in bulk tank milk in Spain

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BACKGROUND and OBJECTIVE: Bovine mastitis is the most economically impactful pathology in the dairy sector, involving major pathogens (S.aureus, St.agalactiae, St.uberis and Mycoplasma spp.). Transitions to Automatic-Milking-Systems (AMS) and selective dry-cow therapy (SDCT) have modified infection dynamics and hygiene needs. This study determined herd-prevalence and evaluated associations between routines and presence.

MATERIALS and METHODS: Bulk tank milk samples were collected by veterinarians for real-time-PCR (ALLIC-Laboratory, Barcelona, Spain). Data included milking systems (Parlor or CP, AMS, tie-stall), hygiene protocols (pre-dipping, sealing, liner-disinfection), performance metrics (herd size, yield, fat and protein, SCC). Statistical analysis (IBM SPSS v.30.0) used Chi-square, logistic regression and decision tree models.

RESULTS: Herd-prevalence with at least one pathogen was 352/464 (75.86%), with 121/352 (34.38%) mixed infections (2 St.uberis+S.aureus+St.agalactiae; 3 St.uberis+Mycoplasma; 10 St.uberis+St.agalactiae; 106 St.uberis+S.aureus). S.aureus prevalence was 143/418 (34.2%); 15/463 (3.2%) for St.agalactiae; 314/411 (76.4%) for St.uberis; and 4/464 (0.9%) for Mycoplasma spp. Milk yield was lower in farms with S.aureus (35.67±5.58 vs. 32.88±6.85 l/d; P<0.001), as was fat (3.98±0.28 vs. 4.03±0.29%; P=0.007). In BTM-positive farms, St. agalactiae and St.uberis RCS was higher (196.23±83.58 vs. 264.60±125.75; P=0.027 for St.agalactiae; 183.36±99.66 vs. 206.27±80.76; P=0.002 for St.uberis). No farm-differences were observed for Mycoplasma spp. S.aureus increased with SDCT vs. global dry cow therapy (38.6 vs. 26.5%; P=0.014), while milking system had no effect (P=0.991). In CP farms, S. aureus infection was higher when using individual towels vs. individual papers (58.3 vs. 30.9%; OR=2.889, 95%IC: 1.399–5.967; P=0.004). In AMS farms, sealing reduced S.aureus (50.0 vs. 30.9%; OR=0.263, 95%IC: 0.79–0.871; P=0.029), a clear recommendation for AMS. St.uberis showed higher prevalence in farms with AMS (82.6 vs.75.2 vs.56.3% for AMS vs. parlor vs. tie barns; P=0.046). In CP farms, infection probability was higher with individual towels than with papers for pre dipping (58.3 vs.30.9%; OR=4.537, 95%CI: 1.347–15.283; P=0.015). No factor was identified for AMS. St.agalactiae prevalence was reduced in SDCT-farms (1.3 vs. 6.7%; P=0.004). AMS farms showed higher prevalence (6.1 vs. 2.1%; P=0.041). In CP farms, St. agalactiae infection was greater with common paper vs. individual papers (12.5 vs. 1.9%; OR=13.85, 95%IC: 1.205–159.132; P=0.035), and liners-disinfection was associated with more St.agalactiae (1.5 vs. 8.9%; OR=9.260, 95%IC: 1.734–49.463; P=0.009). No specific factor was identified for AMS. No factor significantly affected Mycoplasma spp. prevalence.

CONCLUSIONS: Herd prevalence of St.uberis and S.aureus was high, with Selective Dry Cow Therapy, hygiene routines, and sealing (in Automatic Milking Systems) influencing pathogen presence differently. Acknowledgment to the BIAHE advisory group and collaborating veterinarians.

Keywords: Milking system, milk yield, SCC, prevention

MALDI-TOF MS associated with machine learning models to predict antimicrobial resistance in Staphylococcus aureus and Klebsiella pneumoniae isolated from bovine mastitis

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BACKGROUND and OBJECTIVE: Bovine mastitis is a prevalent disease in dairy herds, strongly associated with antimicrobial use and the emergence of antimicrobial resistance (AMR). Conventional susceptibility testing is time-consuming, limiting rapid decision-making. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) combined with machine learning (ML) has emerged as a promising for rapid AMR prediction. This study aimed to evaluate ML models with MALDI-TOF MS spectra to predict AMR in Staphylococcus aureus and Klebsiella pneumoniae isolates from bovine mastitis.

MATERIALS and METHODS: A total of 624 S. aureus and 642 K. pneumoniae MALDI-TOF MS spectra were transformed into 6,000-feature vectors and used to train and evaluate ML models. Antimicrobial susceptibility was determined by disk diffusion and converted into binary classes (susceptible vs. resistant). Five supervised ML algorithms were evaluated: Balanced Random Forest (RF), Support Vector Classifier, Light Gradient Boosting Machine (LightGBM), Extreme Gradient Boosting (XGBoost), and Multilayer Perceptron. Models were trained independently for each antimicrobial–species combination. The performance of ML models was evaluated using Area Under the Receiver Operating Characteristic curve (AUROC), Area Under the Precision–Recall Curve (AUPRC), and F1-score.

RESULTS: For Staphylococcus aureus, ML models showed AUROC values ranging from 0.61 to 0.95 and AUPRC values from 0.17 to 0.82. Ensemble-based models achieved the best results. LightGBM showed superior precision–recall balance for erythromycin (F1 = 0.67) and clindamycin (F1 = 0.69), while XGBoost presented the best performance for gentamicin (F1 = 0.63) and cefoxitin (F1 = 0.53). RF demonstrated similar results, particularly for penicillin (F1 = 0.69). Lower performance was observed for antimicrobials with high class imbalance, such as tetracycline and levofloxacin (F1-scores > 0.35). For Klebsiella pneumoniae, models showed AUROC values ranging from 0.68 to 0.97 and AUPRC values from 0.40 to 0.83. LightGBM presented the highest F1-scores for imipenem (F1 = 0.67) and trimethoprim–sulfamethoxazole (F1 = 0.62), while XGBoost showed high F1 values for cefotaxime and tetracycline (F1 = 0.64). RF also achieved robust results, including imipenem (F1 = 0.67). Performance was higher for carbapenems and more variable for antimicrobials with class imbalance, such as gentamicin and levofloxacin (F1-scores < 0.30).

CONCLUSIONS: ML models associated to MALDI-TOF MS spectra can predict AMR in bovine mastitis pathogens, with performance varying by species and antimicrobial. LightGBM and XGBoost consistently provided the best balance between discrimination and minority-class detection. This approach represents a promising complementary tool for rapid AMR evaluation in bovine mastitis pathogens.

Keywords: Bovine mastitis, Mass spectra, Rapid diagnostics, Supervised classification.

Evaluation of on-farm microbiological culture and California Mastitis Test as criteria for selective dry cow therapy at the cow and mammary quarter levels

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BACKGROUND and OBJECTIVE: The dry period is critical for mastitis control, offering the opportunity to eliminate existing intramammary infections (IMI) while carrying the risk of new IMI (NIMI) acquisition. Dry cow therapy (DCT), involving intramammary antimicrobials at dry-off, effectively addresses both goals. However, blanket DCT (BDCT), where all cows are treated irrespective of infection status, contributes to increased antimicrobial use (AMU). With antimicrobial resistance increasingly linked to livestock AMU, strategies to reduce AMU without compromising animal health are needed. Selective dry cow therapy (SDCT), which treats only cows or mammary quarters (MQ) that meet specific criteria, is a promising alternative. This study assessed the use of on-farm culture (OFC) combined with the California Mastitis Test (CMT) to guide SDCT at both MQ and cow levels, comparing AMU and NIMI risk.

MATERIALS and METHODS: A total of 1,386 MQ from 355 cows in five Brazilian herds were randomly assigned to three groups: a) BDCT (control), b) SDCT guided by OFC and CMT at the MQ level (MQ-SDCT), and c) SDCT guided by OFC and CMT at the cow level (C-SDCT). All MQ received an internal teat sealant at dry-off. Milk samples were collected 1–3 days before dry-off and at 7 and 14 days postpartum. Pre-dry-off milk samples from both SDCT groups underwent OFC and CMT to guide antimicrobial treatment, which was administered according to group-specific criteria (only infected MQ were treated in the MQ-SDCT, or all MQ were treated in the C-SDCT). Microbiological identification was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Bacteriological cure and the occurrence of NIMI were determined by comparing pre- and postpartum isolates. Differences in AMU among groups were evaluated with Pearson's chi-squared tests, and associations between treatment group and NIMI risk were analyzed using generalized linear mixed models (GLMM), adjusting for cow-level covariates including parity, days in milk at dry-off, breed, somatic cell count (SCC), daily milk yield, and clinical mastitis history during the current lactation.

RESULTS: Compared with BDCT, AMU was reduced by 53.0% in the MQ-SDCT group and by 30.5% in the C-SDCT group. Although higher SCC was associated with an increased risk of NIMI (odds ratio = 1.1), neither SDCT approach increased NIMI risk compared with BDCT.

CONCLUSIONS: Using OFC combined with CMT provides a practical approach for selecting cows or MQ for SDCT. This strategy enables the judicious AMU without adversely affecting udder health in early lactation, particularly in herds with limited cow-level data.

Keywords: selective dry cow therapy, on-farm culture, California Mastitis Test, antimicrobial stewardship

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BIOTECHNOLOGY & SUSTAINABILITY

Effects of Nano-Ozone Solution Supplementation on Cryopreserved Ram Sperm Quality and Post-Thaw Longevity

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BACKGROUND and OBJECTIVE: Oxidative stress during semen cryopreservation impairs sperm membrane function, mitochondrial activity, and DNA integrity, reducing post-thaw lifespan. Medical ozone can induce endogenous antioxidant defenses. This study evaluated whether nano-ozone solution (NOS) added to a ram semen freezing extender improves post-thaw sperm quality and 6-hour incubation resilience.

MATERIALS and METHODS: Semen was collected from six Merino rams (five collections per ram at ≥3-day intervals) by electroejaculation during the breeding season and pooled after meeting quality thresholds. Samples were diluted in a TRIS–citric acid–egg yolk extender; NOS was added to the glycerolated fraction at 0.5, 1, or 2 mg/mL (control: no NOS) before freezing in 0.25 mL straws using a programmable protocol and storage in liquid nitrogen. Straws were thawed at 37°C for 30 s and evaluated at post-thaw (0 h) and after incubation for 6 h at 39°C, 5% CO_2. Motility and hypo-osmotic swelling test (HOST) were assessed by phase-contrast microscopy; sperm kinematics were evaluated at 0 h by computer-assisted semen analysis (CASA). Acrosomal integrity by fluorescein isothiocyanate–conjugated Pisum sativum agglutinin (PSA-FITC) and DNA fragmentation by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) were analyzed via fluorescent microscopy. Mitochondrial membrane potential (MMP) was determined by JC-1/propidium iodide via flow cytometry. Malondialdehyde (MDA) and reduced glutathione (GSH) were quantified spectrophotometrically.

RESULTS: Compared with control, 0.5 mg/mL NOS improved motility (47 vs 43%), membrane integrity (77.6 vs 68.6%), acrosomal integrity (77.8 vs 70.8%), and DNA integrity (94.0 vs 92.6%) at 0 h ($p<0.05$), and maintained higher motility after 6 h (31 vs 23%) with better membrane (70.6 vs 60.2%) and acrosomal integrity (66.2 vs 55.4%). CASA kinematics at 0 h indicated a straighter, more progressive trajectory in NOS-supplemented groups in terms of straight-line distance/velocity (DSL/VSL), straightness (STR), and lower lateral head displacement (ALH) parameters versus control ($p<0.05$). High MMP increased at 0 h with 1 and 2 mg/mL NOS (63.9% and 59.9% vs 47.1%); after 6 h, the 2 mg/mL group showed the highest high-MMP proportion (70.3% vs 51.2%). GSH increased at 0 h in NOS groups (173.13, 183.18 and 192.10 respectively vs 161.85 mg/g), whereas MDA did not differ.

CONCLUSIONS: Low-dose NOS supplementation, particularly 0.5 mg/mL, enhanced post-thaw ram sperm functional quality and 6-hour survival, consistent with improved antioxidant status. Low NOS concentrations possibly have an effect of inducing moderate oxidative stress and activating antioxidant pathways. These properties can make NOS a powerful medical tool for sperm cryopreservation.

Keywords: CASA, cryopreservation, flow cytometer, nano-ozone, ram semen

Stress Indicators in Ewes Subjected to Different Embryo Transfer Methods

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BACKGROUND and OBJECTIVE: Assisted reproductive technologies are frequently used in the sheep industry to ensure genetic improvement. For embryo transfer (ET), semi-laparoscopic (SL) or laparoscopic (L) techniques are used primarily under field conditions. Veterinarians are responsible for selecting appropriate interventions and managing pain from an animal welfare perspective. Collecting saliva samples for measuring cortisol levels and using accelerometers for investigating the behavioural and physiological distress of sheep are validated proceedings.

MATERIALS and METHODS: Our study aimed to compare the effects of L and SL ET techniques on saliva cortisol concentrations and lying behavior as non-invasive approaches to stress in sheep. 40 ewes were synchronized for ET and placed randomly into individual pens 2 d before the operations. On the day of the operations, laparoscopic (n = 15) and semi-laparoscopic (n = 10) ET were performed according to standard veterinary protocols. Control (C) animals (n = 15) were placed into the laparoscopic cradle for 6 min without an ET procedure. Monitoring of standing and lying behaviors started 24 h before surgery and lasted until 24 h after the procedures were completed. Saliva samples were taken 4 times during the experiment.

RESULTS: In all groups, cortisol levels (ng/ml) were numerically, but not statistically significantly, higher 1 h after surgery (C: 4.8 ± 0.5; L: 5.1 ± 0.5; SL: 4.2 ± 0.7). The highest concentrations were measured at the 2 h post-surgery sampling in all treatments (C: 5.5 ± 0.5; L: 5.9 ± 0.5; SL: 4.8 ± 0.7); however, only the values of the control and laparoscopic groups differed significantly from baseline (C: 3.8 ± 0.5; L: 3.7 ± 0.5; SL: 3.1 ± 0.7). No differences were found across groups, and the saliva cortisol concentration returned to the initial level 24 h after the procedures (C: 3.4 ± 0.5; L: 4.1 ± 0.5; SL: 3.5 ± 0.7), showing that stress was eliminated within 1 day. Although total lying time did not differ across groups before or after the treatment in our study, the mean length of lying bouts was longer in all groups during the 24-h post-surgery period compared to the pre-surgery day.

CONCLUSIONS: Applying proper anesthesia and analgesia, L and SL ET procedures do not impose significant stress on ewes. Both techniques are appropriate for embryo transfer when performed lege artis, in accordance with veterinary standards. They align with animal welfare principles and are valuable tools for advancing breeding objectives and reducing genetic intervals.

Keywords: sheep, cortisol, activity, embryo transfer

Effect of 3-Nitrooxypropanol Supplementation to Sainfoin Hay on *in vitro* Methane Production and Rumen Fermentation Parameters

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BACKGROUND and OBJECTIVE: The aim of this study was to determine the effects of increasing doses of 3-nitrooxypropanol (3-NOP) added to drought-tolerant sainfoin hay on rumen fermentation and methane production using the in vitro gas production technique.

MATERIALS and METHODS: The in vitro total gas production method was conducted using glass syringes. In the experiment, 3-nitrooxypropanol was supplemented to sainfoin hay at different levels: control (0.0 mg/kg DM), K-NOP-60 (60 mg/kg DM), K-NOP-90 (90 mg/kg DM), K-NOP-120 (120 mg/kg DM), and K-NOP-150 (150 mg/kg DM). The effects on total gas production, methane production, metabolizable energy (ME), organic matter digestibility (OMD), pH, and ammonia nitrogen (NH_3-N) were evaluated.

RESULTS: The supplementation of sainfoin hay with different doses of 3-NOP resulted in a statistically significant reduction in in vitro methane production ($P < 0.05$). However, no significant differences were observed among the treatments for total gas production, metabolizable energy (ME), net energy for lactation (NEL), organic matter digestibility (OMD), ammonia nitrogen (NH_3-N), and pH values ($P > 0.05$). In vitro methane production remained unchanged in the K-NOP-60, K-NOP-90, and K-NOP-120 groups., whereas a decrease was observed in the K-NOP-150 group. The addition of 3-NOP at different levels did not significantly affect total gas production, ME, NEL, or OMD, nor did it influence ruminal pH or ammonia nitrogen concentrations.

CONCLUSIONS: In conclusion, the supplementation of 150 mg 3-NOP to sainfoin hay may reduce methane emissions without negatively affecting rumen fermentation parameters.

Keywords: 3-nitrooxypropanol (3-NOP), Sainfoin hay, In vitro gas production, Methane mitigation, Rumen fermentation

Integrated Assessment of Recycled Manure Solids Supplementation with Biochar for Dairy Cow Bedding

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BACKGROUND and OBJECTIVE: Recycled manure solids (RMS) are increasingly being used as bedding material for dairy cows since they support circular manure management and may contribute to reduce bedding costs. However, concerns remain regarding the use of RMS as bedding material and pathogen exposure, antimicrobial resistance dissemination, and greenhouse gas emissions during storage. This study performed an integrated assessment of RMS supplementation with pine biochar, examining the combined effects of such supplementation on microbiological safety and environmental sustainability.

MATERIALS and METHODS: Fresh RMS from a commercial dairy farm were evaluated in complementary storage trials under naturally ventilated conditions. For microbiological assessment, non-supplemented material and material supplemented with pine biochar at 2.5%, 5%, or 10% (weight/weight) were monitored over 30 days in two seasons. Microbiome dynamics were characterized by complete 16S rDNA gene sequencing using Nanopore next-generation sequencing, and culture-based analyses were used to quantify Enterobacteriaceae and enterococci and to characterize Escherichia coli and Enterococcus isolates regarding antimicrobial susceptibility and virulence-associated profiles. For environmental assessment, untreated RMS, acidified material, and RMS supplemented with 2.5% or 10% biochar were compared regarding physicochemical traits and greenhouse gas emissions during one month of storage.

RESULTS: Biochar supplementation altered the microbiome of RMS, with more favourable shifts being observed after 30 days than at shorter storage times. Supplementations at 2.5% and 5% were associated with lower relative abundance of several mastitis-associated bacterial groups, including Enterobacteriaceae, streptococci, enterococci, and staphylococci, as well as lower levels of Salmonella spp. However, Brucella spp. increased under some conditions. Culture-based analyses showed no significant reduction in Enterobacteriaceae or enterococci counts and no consistent decrease in

antimicrobial resistance or virulence profiles of indicator isolates were detected. In contrast, supplementation of RMS with 10% biochar significantly reduced cumulative carbon dioxide and nitrous oxide emissions, resulting in a 32% reduction in global warming potential when compared with untreated RMS.

CONCLUSIONS: Biochar supplementation of RMS produced dose-dependent and endpoint-specific effects, and no single inclusion rate optimized all outcomes simultaneously. Lower supplementation rates favoured potentially beneficial microbiome shifts, whereas the highest rate provided the greatest environmental benefit. These findings support biochar as a promising but not yet fully optimized strategy for improving the overall safety-sustainability profile of RMS used as dairy cow bedding.

Keywords: Recycled manure solids, biochar, microbiome, antimicrobial resistance, emissions

BUFFALO AND CAMELIDS

Anti-Trypsin Activity in First Colostrum of Water Buffaloes and Its Progression Over 5 Days Postpartum

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BACKGROUND and OBJECTIVE: Neonatal calves rely on the intake of colostrum to acquire essential nutrients, bioactive substances and immunoglobulins. In several species a known characteristic of colostrum is a trypsin-inhibitory activity, which is believed to protect bioactive compounds from proteolytic digestion, thereby facilitating their effective absorption. The aim of this study was to determine whether anti-trypsin activity is present in the first colostrum of water buffaloes, to describe its progression during the first five days postpartum, and to investigate whether it correlates with colostral Immunoglobulin G (IgG) content.

MATERIALS and METHODS: For this purpose, first colostrum from 12 buffalo cows was collected immediately after calving. To assess the temporal progression, colostrum was further collected every 24 hours (h) over five days postpartum from 14 cows. Anti-trypsin activity was determined photometrically and expressed as mg of inhibited trypsin per mL of colostrum. The IgG content was determined by ELISA.

RESULTS: Anti-trypsin activity was detected in the first colostrum (mean 1.01 ± 0.13 mg/mL). There was no correlation between anti-trypsin activity and IgG content at this time point. The mean inhibitory activity decreased progressively: 24 h (0.62 ± 0.33), 48 h (0.24 ± 0.19), 72 h (0.24 ± 0.18), 96 h (0.19 ± 0.22), and 120 h (0.13 ± 0.11) postpartum. Pairwise comparisons showed that activities at birth and 24 h later were significantly higher than at all later time points ($p < 0.0001$). No significant differences were detected among the measurements at 48 h, 72 h, 96 h, and 120 h after birth. A moderate correlation between anti-trypsin activity and IgG content was found only at 120 h postpartum ($r_s = 0.56$, $p = 0.04$).

CONCLUSIONS: The results of the study show that the first colostrum of water buffaloes has high anti-trypsin activity, which decreases significantly within the first 24 hours postpartum. This underlines the importance of obtaining first colostrum early and feeding it to calves to achieve an optimal supply of immunoglobulins.

Keywords: colostrum, immunology, calf health

Study on Ultrasonographic Measurements of Muscle and Subcutaneous Fat Thickness to Assess the Nutritional Status of Alpacas

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BACKGROUND and OBJECTIVE: Body condition scoring is a helpful and important tool in herd health management to either detect chronic diseases at an early stage or to determine dietary adjustments to a feeding program. The determination of the

nutritional status of alpacas is routinely performed via the inspection and palpation of different body regions following a body condition scoring system. However, visual appraisal can be misleading caused by the long fleece in alpacas. As body condition scoring via visual appraisal and palpation is still a subjective method, ultrasonographic measurement of back fat thickness is routinely used in cattle for the objective determination of nutritional status. This method has not been described for alpacas yet. The aim of this study was to evaluate the ultrasonographic measurement of the subcutaneous fat and muscle layers at two different body locations as an objective tool to determine the nutritional status of alpacas.

MATERIALS and METHODS: In total, 160 alpacas were included in the study. Body condition score (BCS) was estimated by systematic palpation; body weight was measured using a scale. Ultrasonography was performed at two boy locations: first at the lumbar region, positioning a linear probe (8 MHz) between the second and third lumbar vertebrae perpendicular to the spinal column, and second, in the gluteal region, measuring the distance between skin, the subcutaneous fat layer, and the muscle layer. The results of ultrasonographic measurement were related to body weight, and body condition score. Various factors like gender (female/male) and different reproductive statuses (castrated/intact males, pregnant, and early or non-pregnant females) were considered.

RESULTS: The results showed that the gender and pregnancy status of females had a significant influence on the ultrasonographic measurements at both localizations. Significant associations were found between body weight and BCS. The BCS and the soft-tissue thicknesses at both localizations were significantly associated in male animals and early or non-pregnant females. The associations to females in later pregnancy were much weaker. According to these results, ultrasonography can be recommended as an objective method with which to determine the nutritional status of alpacas.

CONCLUSIONS: The present study identified this ultrasonography as a reliable tool for the determination of nutritional status in male and early or non-pregnant female alpacas. The fast and reliable method can be recommended as an objective assessment method.

Keywords: alpacas, body condition score, ultrasonography

Impact Of Parenteral Mineral Supplementation on Ovarian Follicular Dynamics and Reproductive Performance in Water Buffaloes Subjected to A Synchronized Insemination Protocol

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BACKGROUND and OBJECTIVE: Reproductive efficiency is the primary determinant of economic sustainability in buffalo production, yet it is frequently compromised by mineral deficiencies in tropical forages. These imbalances disrupt gonadotropin secretion and are linked to significant subfertility. While oral supplementation is common, its efficacy is often negated by mineral antagonism and low bioavailability in grazing environments. This study evaluated the effect of an injectable mineral complex (Fosfosan®, Virbac Colombia) on the fertility and ovarian response of female water buffaloes (Bubalus bubalis) undergoing a Fixed-Time Artificial Insemination (FTAI) protocol.

MATERIALS and METHODS: The study was approved by the institutional ethics committee and conducted in three distinct tropical regions of Colombia: Pueblo Nuevo (Cordoba), Pelaya (Cesar), and Puerto Boyaca (Boyaca). A total of 272 Mediterranean and crossbred female buffaloes with a minimum weight of 350 kg were evaluated. The cohort was categorized into 32 heifers (11.76%), 48 primiparous buffaloes (17.64%), and 192 multiparous buffaloes (70.6%). Animals were maintained on permanent pasture (Panicum maximum) with ad libitum water. Females were randomly assigned to a control group or a treatment group receiving 10 ml of injectable mineral complex on days -30 and 0 of the FTAI protocol. Ovarian structures were monitored via transrectal ultrasonography on days -30, 0, and 12. Pregnancy was confirmed 30 days post-insemination. Data were analyzed using analysis of variance (ANOVA) and Chi-square tests ($p < 0.05$).

RESULTS: A significant effect of the location and the animal category was observed during the study. The primary finding was a highly significant increase in the diameter of the left dominant follicle (LDF) at the time of insemination (day 12) in supplemented females (13.27 ± 2.17 mm) compared to the control group (11.76 ± 2.52 mm; p = 0.001). No significant differences were observed in body weight or body condition score at the onset of the protocol. The supplemented group exhibited a higher pregnancy rate (52%) compared to the control group (48%), representing a positive numerical trend (p > 0.05).

CONCLUSIONS: By significantly increasing the diameter of the dominant follicle at insemination, parenteral administration promotes a superior preovulatory environment. This method effectively bypasses the limitations of oral intake in tropical grazing systems, serving as a potent tool for enhancing the success of FTAI programs across different age categories and locations.

Keywords: buffalo, trace elements, follicular dynamics, FTAI

Bulk Tank Milk Surveillance for Coxiellosis in the Mediterranean Water Buffalo (bubalus Bubalis) in Southern Italy

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BACKGROUND and OBJECTIVE: Domestic ruminants are the main reservoirs of Coxiella burnetii (C.burnetii), the causative agent of human Q fever and a major public health issue. Although included in the category of ruminant, there is a lack of knowledge about the role of water buffalo (Bubalus bubalis) in the epidemiological cycle of C. burnetii. The purpose of this investigation was to determine the molecular and serological prevalences of C. burnetii in bulk tank milk (BTM) from water buffalo in Southern Italy. The study also aimed to determine whether any differences in milk's chemical-physical parameters were associated with C. burnetii-positive results.

MATERIALS and METHODS: A total of 94 BTM samples were collected and evaluated for the presence of specific target genes (IS1111) using two distinct real-time PCR protocols, a commercial kit (Adiavet, BioX diagnostics) and an in-house real-time PCR. The same samples were also analyzed for the presence of specific antibodies using a commercial ELISA (ID Screen® Q Fever Indirect Multispecie, IdVet) that is commonly employed for ruminant serological analysis.

RESULTS: The overall molecular prevalence was 10.6% (10/94) but varied depending on the region: 28.5% in Basilicata (2/7), 9.5% in Campania (8/84) and 0% in Sicily (0/3). The cycle thresholds (Ct) found were between 33.6-37.3 (out of a total of 40 cycles), indicating a strong diluting effect even for water buffalo. The ELISA results also confirmed the widespread distribution of C. burnetii in the buffalo population of southern Italy. There were minor changes in several chemical-physical parameters (MilkoScan™ 7 RM), including differential somatic cell count (DSCC), pH, BhB (beta-hydroxybutyrate), K20 min (curd-firming time), and urea. Despite this, statistical analysis revealed no significant changes in the characteristics tested (30) between positive and negative farms. When the milk pools from seven farms in the Basilicata region were analyzed individually (five samples were collected from each farm), significant variations were observed in the percentages of total fat (especially MCFA, SCFA, SFA, and palmitic acid) and ST%.

CONCLUSIONS: This study found C. burnetii in the buffalo population in Southern Italy, with a slightly lower prevalence compared to other reservoir species. Positive herds showed no significant changes in BTM characteristics; however individual fat percentages varied significantly. Although there is not enough evidence that C.burnetii causes mastitis, evidence of milk changes in Coxiella-positive animals was shown in this study.

Keywords: Coxiella burnetii, water buffalo, BTM surveillance, PCR, ELISA

Health Management Practices in Austrian Llama and Alpaca Herds – Results from a Nationwide Survey Among Owners

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BACKGROUND and OBJECTIVE: Llamas and alpacas have been kept in Austria for several decades. Consequently, the population size has steadily increased. However, there is still a lack of data regarding the current number of llamas and alpacas as well as health management measures in Austria. Therefore, a survey-based study was conducted to collect data on various aspects of South American camelids (SAC) management, including husbandry, work organization, animal movement, purpose of use, and human-animal interaction, as well as health management practices in Austria.

MATERIALS and METHODS: A survey comprising 61 questions covering the aforementioned topics was developed and distributed among owners of SAC in Austria. The study was conducted between 11 January and 8 June 2024, and a total of 450 fully or partially completed questionnaires were included in the analysis. This analysis specifically focuses on the health management of SAC in Austria. The survey data were analyzed using Microsoft Excel software.

RESULTS: One third of the surveyed animal owners (32.7 %) are members of the Austrian Animal Health Service, while the majority of farms (88.6 %) document health data, either digitally or analog. Most respondents (66.4 %) spend 1–2 hours per day caring for their animals. Disorders of the digestive system were most frequently reported by animal owners as the primary cause of illness (32.9 %) and death (20.3 %). In contrast, death due to old age ranked second, which may reflect the long-term care and value attributed by their owners. Proper vaccination management also plays an important role in the prevention of major infectious diseases within herd management. The majority of vaccines administered targeted various Clostridium species, with a total of 212 of 360 animal owners (96.8 %) reporting vaccination against this group of pathogens.

CONCLUSIONS: Veterinarians are increasingly confronted with questions regarding specific aspects of husbandry, feeding and breeding of SAC as the SAC population increases each year. In addition, topics such as animal health and herd management are becoming increasingly prominent. The results of this study provide an overview of the current state of SAC husbandry in Austria and offer insights into opportunities for optimizing herd and health management. The survey results identified also the most common herd-related issues from the perspective of animal owners, thereby emphasizing the need for future research to focus on improved health monitoring and the development of preventive measures.

Keywords: disease prevalence, South American camelids, health management, preventive measures, questionnaire

Thyroid-Associated Cyst in An Alpaca – A Case Report

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BACKGROUND and OBJECTIVE: The objective of this case report was to describe the diagnostic process and pathological findings in a case of a thyroid-associated cyst in a 5-year-old alpaca stallion. The presentation highlights the diagnostic methods

including advanced diagnostic imaging in detecting a thyroid-associated cyst.

MATERIALS and METHODS: A 5-year-old alpaca was presented with a double-fist-sized enlargement in the right laryngeal region. Clinical examination revealed a 10x8x5 cm sized non-painful, soft-elastic swelling near the thyroid gland on the right side. Diagnostic procedures included cytology, ultrasound and computed tomography to evaluate soft tissue structures. Postoperative histopathological and microbiological analyses were performed.

RESULTS: CT imaging revealed an extensively growing, right-sided thyroid-associated cyst proximally that was well-demarcated from surrounding tissue. After surgical excision the histopathological examination diagnosed a papillary cyst adenoma of follicular thyroid cells. The patient was discharged from the hospital seven days after surgery. Six months after surgery no recurrence occurred.

CONCLUSIONS: This entity is described rarely in literature. The presented case shows the importance of clinical examination as well as CT imaging and further laboratory diagnostics to achieve a final diagnosis and provide a cause-based prognosis for the individual patient.

Keywords: alpaca, computed tomography, histopathology, papillary cyst adenoma, South American camelids, thyroid cyst

CALF HEALTH

The Ultrasonographıc Evaluation of Caudal Vena Cava Diameter Before and After Fluid Replacement in Neonatal Dehydrated Calves with Diarrhea

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BACKGROUND and OBJECTIVE: In calves with diarrhea, it is critical to accurately determine the severity of dehydration and provide adequate fluid therapy. The aim of this study is to compare caudal vena cava maximum diameter with expiration (CVCmax), caudal vena cava minimum diameter with inspiration (CVCmin), and caudal vena cava collapsibility index (CVC-CI) measurements before and after fluid therapy and to establish cut-off values for distinguishing between moderately and severely dehydrated calves.

MATERIALS and METHODS: Twenty-four calves, with their degree of dehydration assessed based on enophthalmos and skin elasticity duration, were divided into two equal groups. Group I: consisted of 12 calves with an estimated degree of dehydration of 8-10% and were considered moderately dehydrated. Group II: consisted of 12 calves with an estimated degree of dehydration 10-12% and were considered severely dehydrated. Clinical examination, complete blood count and blood gas analysis, hemodynamic parameters, systolic blood pressure, diastolic blood pressure, mean arterial pressure, and ultrasonographic examinations were performed for 48 hours: before treatment (hour 0), immediately after the first fluid bolus, and at hours 8, 24, and 48 after the first fluid bolus. The Friedman test was used for within-group comparisons over time, and the Mann-Whitney U test was used for between-group comparisons at different time points. Categorical data were analysed using the chi-squared test, and Fisher's exact test was used when expected cell counts were less than 5. Receiver operating characteristic (ROC) analysis was performed to determine the sensitivity, specificity, and cut-off (lower limit) of CVC diameter and CVC-CI (%) compared with selected parameters (SBP, DBP,

MAP, and L-lactate) to discriminate between moderate and severe dehydration. Statistical significance was set at P<0.05.

RESULTS: CVCmax and CVCmin increased significantly after treatment in diarrheic calves (P<0.05). Additionally, a significant decrease in CVC-CI (%) was observed in the treated diarrheic calves. Receiver operating characteristic (ROC) analysis showed that the area under the curve (AUC) of CVCmax was 0.885 (95% CI: 0.823-0.946; P<0.001), with 82% sensitivity and 85% specificity at the intercept point of 1.05, the AUC of CVCmin was 0.913 (95% CI: 0.861-0.964; P<0.001), with 89% sensitivity and 84% specificity at the intercept point of 0.66, and were the most reliable parameters in differentiating between moderate and severe dehydration.

CONCLUSIONS: A significant increase in CVCmax and CVCmin diameters, along with a significant decrease in CVC-CI, was observed with fluid therapy. The CVCmax and CVCmin diameters can provide valuable information for distinguishing between moderately and severely dehydrated calves.

Keywords: Calf, Dehydration, Neonatal diarrhea, Caudal vena cava, Caudal vena cava collapsibility index

A Holistic Approach to Colostrum Management: Enrichment of Maternal Colostrum Combined with Extended Colostrum Feeding as Control Measures for Bovine Rotavirus-Associated Neonatal Calf Diarrhoea

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BACKGROUND and OBJECTIVE: Calf Bovine Rotavirus A (BRV-A) infections are endemic worldwide. The disease is responsible for substantial economic loss, affecting future productivity through mortality and reduced liveweight gain in the preweaning period. In this case study, BRV-A genotype G6P11 was identified as a cause of neonatal calf diarrhoea (NCD) in a 600-cow autumn block calving herd. Preweaning calf morbidity was farmer reported at 100% and mortality at 6%. A holistic review of colostrum management was undertaken to manage high calf morbidity and mortality.

MATERIALS and METHODS: Calf faecal sample (n = 6) microbiology from calves 8–21 days of age identified BRV-A. Colostrum sample analysis for protective antibodies to BRV-A demonstrated optimal dam vaccine efficacy. Sixteen clinically healthy 1–4-day-old calves were blood sampled for serum total protein analysis, revealing sub-optimal concentrations (less than 5.2 g/dL) in seven of 16 calves (43.8%). A holistic approach to changes to colostrum management included: a reduction in calves' time to first feed post-calving (by utilisation of a frozen colostrum store); improvement in colostrum quality to 30% Brix by enrichment with whole bovine colostrum powder and implementation of extended colostrum feeding using this powder to supplement the commercial milk replacer ration for the first 10 days of life.

RESULTS: These changes effectively reduced the incidence of NCD in this herd from 100% (n = 430/430) measured over 10 weeks of calving to 8.6% (n = 6/70) measured over the last 4 weeks of calving, with mortality in calves less than 21 days of age reduced from 6% to 3% in the last 4 weeks of the calving block.

CONCLUSIONS: After management changes, there were substantial improvements to calf passively-derived immunity. On this farm, colostrum was generally well managed and the addition of a powdered colostrum product was made with this in mind. Each farm should be approached as a unique management system and recommendations to improve colostrum and calf serum immunoglobulin concentration should take into consideration all aforementioned aspects of colostrum management. Powdered colostrum products may offer veterinary clincians an additional preventative option (together with other management strategies such as vaccination and stringent hygiene) to manage neonatal calf disease.

Keywords: calf, colostrum, rotavirus

Effect of Synbiotic Supplementation on Blood Parameters in Holstein Heifer Calves During the Pre-Weaning Period

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BACKGROUND and OBJECTIVE: Early-life modulation of the intestinal microbiota using probiotics, prebiotics and synbiotics is a promising strategy to support health and performance in dairy calves. This study evaluated the effect of graded levels of a commercial synbiotic (Diproplus™, containing multiple probiotic strains and mannan-oligosaccharide) on blood parameters of Holstein heifer calves from birth to weaning.

MATERIALS and METHODS: Thirty-eight Holstein heifer calves were enrolled from birth to 73 d of age in a completely randomized design with five treatments. Calves were housed individually under uniform management and health protocols. Treatments consisted of a control group (no synbiotic) and four synbiotic groups receiving 0.5, 1.0, 1.5 or 2.0 g/head/day of Diproplus™ mixed into whole milk. All calves received 4.5 kg of milk daily in three meals, and had ad libitum access to starter concentrate and water. The synbiotic product contained Bacillus licheniformis, Bacillus subtilis, Pediococcus acidilactici, Lactobacillus brevis, Lactiplantibacillus plantarum, mannan-oligosaccharide and maltodextrin as carrier. Blood samples were collected at predetermined ages to determine hematological and biochemical parameters. Data were analyzed using ANOVA appropriate for a completely randomized design.

RESULTS: Serum glucose, cholesterol, triglycerides, β-hydroxybutyrate (BHBA) and urea at the end of the trial ranged from 86.9 to 103.9 mg/dL, 72.0 to 83.6 mg/dL, 20.6 to 29.7 mg/dL, 0.24 to 0.28 mmol/L and 24.7 to 29.0 mg/dL, respectively, and did not differ among treatments (P>0.05), indicating no detectable effect of synbiotic supplementation on basic carbohydrate, lipid or protein metabolism. Activities of ALT (15.0–18.4 U/L) were also unaffected (P>0.05). In contrast, AST activity showed a significant treatment effect, with the 2 g/day group (≈81.7 U/L) differing from the 0.5 g/day group (P<0.05). Total antioxidant capacity (TAC) was markedly improved in calves receiving 1.5 g/day of the synbiotic, with a mean value of 0.7788 mmol/L, which was significantly higher than in the control group (P<0.05). Serum albumin and total protein were also enhanced at this level: calves fed 1.5 g/day reached 3.050 g/dL albumin and 8.063 g/dL total protein, values significantly greater than those of calves receiving 0.5 g/day (albumin) and 0.5–1 g/day (total protein) (P<0.05). Serum malondialdehyde (MDA), as an index of lipid peroxidation, ranged from 1.229 to 2.513 nmol/mL and was not affected by treatment (P>0.05).

CONCLUSIONS: Under the conditions of this study, dietary supplementation with the Diproplus™ synbiotic during the pre-weaning period influenced blood parameters in Holstein heifer calves. Moderate inclusion levels appeared most promising and may contribute to better health and physiological status in early life.

Keywords: Holstein calves, Synbiotic, Blood parameters, Antioxidant capacity

Effect of Nutraceutical Administration at Birth on First Colostrum Intake

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Raizup Nutrition Care

BACKGROUND and OBJECTIVE: Ungulate species, including calves, are born without antibodies. Their only source of antibodies is maternal colostrum, which also contains numerous bioactive components essential for neonatal development and survival. Some calves may exhibit signs of weakness at birth, reducing their physiological and physical ability to quickly suckle colostrum and adequately absorb these components, including antibodies. Consequently, delayed colostrum intake is associated with an increased risk of failure of passive immune transfer, thereby elevating the likelihood of early morbidity. Recognizing this challenge, the present experimental study aimed to evaluate the administration of a nutraceutical, designed to stimulate calves' metabolism, and its effect on the timing of first colostrum consumption. Although the nutraceutical is intended to support

weak newborns, this study assessed its effect in healthy calves. Using healthy animals allows evaluation of the nutraceutical's impact on colostrum intake under controlled conditions, without confounding factors associated with perinatal weakness or disease.

MATERIALS and METHODS: Twenty healthy female calves were enrolled in this study immediately after parturition from healthy cows. Before first colostrum intake, each calf was placed in an individual pen. Half of them received the nutraceutical 15 minutes after birth. Colostrum was then provided in a bucket within each pen, and both the timing and quantity of consumption were recorded.

RESULTS: The group that received the nutraceutical consumed colostrum on average 41 minutes earlier than the control group. First colostrum intake was significantly faster in the nutraceutical group (p< 0,01). In this group, 40% of calves consumed colostrum within 1 hour, compared with 0% in the control group. After 2 hours, 100% of calves in the nutraceutical group had consumed 2 kg of colostrum, compared with 60% in the control group. These results highlight a markedly faster colostrum intake in calves receiving the nutraceutical.

CONCLUSIONS: All calves in these studies were healthy and had the same likelihood at birth of consuming colostrum quickly. However, the nutraceutical administration improved both the time to first colostrum intake and the volume consumed. The nutraceutical is designed to stimulate the calves' metabolism at birth, which can be impaired in weak newborns. Even in healthy calves, metabolic activation may require support, which is essential for rapid colostrum intake and the long-term benefits it provides. This support may also contribute to the absorption of all bioactive components from maternal colostrum.

Keywords: Newborn calves, Colostrum intake, Nutraceutical

Correlation of Serum IgG and Indirect Measures of Passive Immunity in Dairy and Dairy-Cross Calves Given a Colostrum Replacer: A Diagnostic Accuracy Study

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BACKGROUND and OBJECTIVE: The objective of this cross-sectional diagnostic accuracy study was to evaluate the correlation between RID-determined IgG and indirect measures of passive immunity in calves fed a colostrum replacer (CR). Further, we aimed to determine optimal diagnostic cutpoints for failed transfer of passive immunity (FTPI), defined as serum IgG < 10 g/L.

MATERIALS and METHODS: The study was conducted in 2024 on a 550-cow, spring-calving dairy herd in Pembrokeshire, West Wales, United Kingdom. Calves were removed from their dams when identified and received 700 g of whole bovine spray-dried colostrum containing a minimum of 100g IgG (Calf's Choice Total 100, Saskatoon Colostrum Company Ltd.) within 2 hrs of birth, followed by a pooled, pasteurized mix of excess dam's colostrum (that not suckled by the calves) and transition milk, defined as milkings 2-6 after calving (Godden, 2019). A total of 252 blood samples were collected between 24 and 36 hrs of age from replacement heifer and beef-cross calves. Samples were centrifuged within 1 hour and serum was stored at -20°C before analysis. IgG concentrations were determined using RID. Brix % and STP were measured using a digital refractometer, GGT and STP were analyzed using a wet chemistry system, and ZST was determined by SRUC Veterinary Services. Samples were also analyzed for direct measurement of IgG using a laboratory ELISA test (BIO-X K420, BIO-X Diagnostics, Rochefort, Belgium). Simple linear regression models were used to evaluate correlations between RID and each test, while receiver operating characteristic (ROC) curve analysis was used to determine optimal cutpoints for classifying FTPI.

RESULTS: Strong correlations were found between RID-determined IgG and STP by wet chemistry (adjusted R^2 = 0.91), ZST (adjusted R^2 = 0.89), STP measured by refractometer (adjusted R^2 = 0.83), Brix % (adjusted R^2 = 0.80), and laboratory ELISA (adjusted R^2 = 0.90). In contrast, weaker correlation was found with GGT (adjusted R^2 = 0.18). Optimal thresholds for determining FTPI were 4.73 g/dL using STP determined by wet chemistry, 14.5 units for ZST, 5.05 g/L for STP using a refractometer, 8.15% for Brix %, 1,312 IU/L for GGT, and 5.29 g/L for the laboratory ELISA.

CONCLUSIONS: These findings suggest that Brix %, STP, and ZST are effective indirect measures of serum IgG in calves fed a CR, with STP using wet chemistry demonstrating the strongest correlation. The laboratory ELISA performed well as a direct measure of serum IgG when compared to RID.

Keywords: calf, passive immunity, IgG

Analysis of Risk Factors for Neonatal Diarrhea and Growth Perfomance of Calves Regarding the Use of a Vaccine Containing the Glycoprotein 40 of Cryptosporidium Parvum in a French Limousin Farm

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BACKGROUND and OBJECTIVE: Cryptosporidiosis is a major cause of neonatal diarrhea in calves leading to reduced growth, occasional mortality, and economic losses. This pilot field study aimed to evaluate, in a French Limousin suckler herd, the effect of maternal vaccination containing glycoprotein 40 of Cryptosporidium parvum (GP40) on the incidence of neonatal diarrhea and on calf growth performance.

MATERIALS and METHODS: This prospective observational study was conducted on a herd of cows and their calves from November to April 2025 according to the national ethical law. Two groups were formed: one group of pregnant cows receiving standard vaccination against neonatal diarrhea (Rotavirus, Coronavirus, E. coli F5 and F41 (Bovilis® Rotavec Corona®); Control group), and a second group receiving this standard vaccination and a vaccination targeting GP40 (Bovilis® Cryptium®)(Test group). All vaccination was done in accordance with the product information. Allocation was randomized and balanced according to parity and cows' location in the barn. Calves were monitored from birth until their turnout to pasture on April 2025. They were weighed at birth and every 15 days thereafter, plasma measurements of total immunoglobulins (IgG) and anti-GP40 IgG and monitoring of diarrhea. Statistical analyses included comparison tests (Student's t-test, Chi²/Fisher), logistic regression (odds ratio, 95% CI), survival analyses (Kaplan-Meier, Logrank), and linear regressions.

RESULTS: A total of 116 calves (58 per group) were included. The prevalence of diarrhea between 3 and 30 days was 3.5% in the Test group versus 13.8% in the Control group (p = 0.094; Fisher's test). Maternal vaccination targeting GP40 was associated with an increased likelihood of absence of diarrhea in their calves (odds ratio 13.0; p = 0.015; logistic regression). Sick calves had lower plasma IgG concentrations (mean 9.8 mg/ml) than healthy calves (15.3 mg/ml; p = 0.021). Plasma anti-GP40 IgG titers were significantly higher in calves from the Test group (mean 15.6log2) than in controls (13.0log2; p < 0.001). The average daily gain over the first two months was numerically higher in the Test group (1067g/day) compared to the Control group (998g/day; p = 0.091).

CONCLUSIONS: Under suckler herd conditions, maternal vaccination targeting GP40 increases specific passive immunity in calves and is associated with a reduction in neonatal diarrhea, with a tendency to improve average daily gain. The transfer of passive immunity is a major determinant of protection. Despite the overall low incidence of diarrhea, warranting cautious interpretation, these results support the interest of this vaccination strategy and colostral management.

Keywords: Cryptosporidium, calf, cattle, diarrhea, vaccination, glycoprotein 40

Occurrence of C. Parvum Infections in Beef and Dairy Calves in Poland During the First Month of Life

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BACKGROUND and OBJECTIVE: Calf diarrhoea is one of the most common diseases affecting calves and important cause of morbidity and mortality in this group of animals. This disease is responsible for significant economic losses in dairy and beef cattle production worldwide. The aetiology of calf diarrhoea is complex and includes dietary, environmental factors, and bacterial, viral and parasitic pathogens. Cryptosporidium spp. are considered one of major infectious threats for calves in first month of their life. In Poland, previous research showed high incidence4 of this pathogen in healthy animals. The current study aimed to determine if there is an age component related to Cryptosporidiosis shedding, and if a difference in shedding can be seen between diseased & healthy faecal samples in dairy and beef calves caused by C. parvum.

MATERIALS and METHODS: For this study, samples from 942 untreated calves were taken. 565 samples came from healthy calves, and 374 from diarrhoeic calves from 151 farms (119 dairy and 32 beef farms). Fecal samples were collected from the animals and examined using an immunoenzymatic test (BioX, Belgium).

RESULTS: Fecal presence of C. parvum was different in dairy and beef calves. In dairy calves, the prevalence of infection was 18.5%, while in beef cattle it was 41.7%. Among dairy calves showing symptoms of diarrhea, the rate was 24.2%, compared to 14.9% in healthy individuals. In beef calves, the corresponding rates were 53.2% and 33.3%, respectively. In case of beef calves, prevalence gradually increased from 7.9% in first week of life to 66.0% in fourth week of life, while in dairy calves, prevalence increased in second week of live from 6.2% to 25.4% and after that remained at a similar level (20.6% in 3rd week and 22.1% 4th week of life).

CONCLUSIONS: Cryptosporidium parvum is a major cause of diarrhoea in calves during their first month of life in Poland, with higher infection rates in beef calves compared to dairy calves. Prevalence rises with age, particularly in beef herds, emphasizing the importance of early monitoring, maintaining uniform age groups of calves, implementing biosecurity measures such as all-in/all-out methods, and initiating protective vaccinations.

Keywords: calves, neonatal, cryptosporidiosis, Poland, prevalence

Assessing the Impact of Improved Navel Hygiene on Calf Health

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BACKGROUND and OBJECTIVE: Omphalitis is an often under-reported condition in calves, it is known that infection may remain external, only affecting the umbilical stump, or it may spread internally to vessels and organs. Recent work estimates the prevalence in calves to be 30 %, and calves whose navels are not disinfected after birth are over 2.3 times more likely to develop omphalitis. Omphalitis has been shown to increase the odds ratio for calf mortality by a factor of 2.5 and to reduce daily liveweight gain by 96 grammes. In 2023 a novel navel barrier solution (NBS) comprising alcohol, shellac and transition metal ions was developed as an alternative to iodine. This project aimed to assess the impact on antimicrobial usage and the prevalence of navel infections in a Midlands-based beef suckler unit when the traditional use of 10 % strong iodine was replaced by NBS which had been proven efficacious in other livestock species.

MATERIALS and METHODS: NBS was introduced as the standard protocol for the 2025 calving season, with no other changes made to housing, management, application frequency or calf management were made. Records from the previous four years were collected (when iodine was used) to allow retrospective analysis and comparison with the 2025 records (using NBS). Attention was given to recording the number of times a navel product was applied, number of navel swellings, number of calves

receiving treatment for omphalitis, and the total number of treatments administered for omphalitis.

RESULTS: A 91.5 % reduction in the total number of omphalitis treatments was observed in 2025 compared with 2024 (16 % of calves treated for omphalitis in 2024 v 1.56 % in 2025). The average number of uses of navel product was 1.02 compared with 2.0 in the previous four years. The farmer reported a perceived increase in calf vigour and reduced navel dry time in the first 48 hours of life.

CONCLUSIONS: The introduction of NBS as the standard product for use on calves' navels after birth provided rapid desiccation and improved protection of the navel compared with approaches used in previous years. On this farm, a 91.6 % reduction in omphalitis incidence was observed through this single change in navel management – highlighting the benefit of picking the right navel hygiene product and applying it rapidly after birth.

Keywords: calf health, omphalitis, omphalophlebitis, bovine

The Effect of Vaccinating Cows Against Cryptosporidosis on the Frequency of Therapeutic Interventions in Calves

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BACKGROUND and OBJECTIVE: Cryptosporidiosis (Cryptosporidium parvum) is the leading protozoan diarrhea problem in calves. The prevention of severe clinical disease has recently become achievable through the use of immunoprophylaxis by vaccinating pregnant heifers and cows during the pre-calving period. The aim of this study was to evaluate the protective effect of the vaccine (Bovilis® Cryptium, MSD animal health) on the frequency of therapeutic interventions during calf diseases.

MATERIALS and METHODS: Pregnant females of Holstein-Friesian (HF) were randomly divided into two groups: ST3 (n=19) the vaccinated group, and ST1 (n=15) the control group. The vaccine was applied following the manufacturer's instructions. Calves born from ST3 and ST1 dams, after supervised colostrum feeding, were fed transition milk until day 3 of life and milk replacer and calf concentrate from day 4. All calves were monitored daily for the first 15 days of life. Clinical assessment of the calves' health, including the potential need for treatment for diarrhea, and a blood draw were conducted on days 3, 5, 7, 9, and 12 after calving. Each diarrheal event was verified using a commercial plate tests (E. coli K99, coronavirus, rotavirus, C. parvum). Additionally the total IgG globulin levels (Brix scale) were determined in the colostrum/milk and calves serum. Analyses were conducted at a 5% significance level using STATISTICA, version 13 by TIBCO Software Inc. (2017).

RESULTS: A statistically significant difference was found in the frequency of required medical interventions between the ST1 and ST3 calves ($p = 0.023$). They were twice as frequent in the control calves when compared to those born from vaccinated dams. No significant differences were found between the means of colostral/milk IgG in ST1 and ST3 groups ($p= 0,947$). No statistically significant differences were noted between the mean immunoglobulin levels in serum of ST1 and ST3 calves on individual study days (d.3, 5, 7, 9 12 $p = 0.388$; 0.186; 0.319; 0.464; 0.698 respectively).

CONCLUSIONS: Supervised colostrum and transitional milk feeding in calves seems essential for guaranteeing their protection during the first weeks of life. Administering vaccines to mothers prior to calving did not enhance the total detectable colostrum/milk and serum globulin concentrations; however, employing immunoprophylaxis notably decreased the frequency of medical interventions and treatments needed in the calves from these dams. A limitation of the described studies is the lack of detailed data on the concentration of antibodies specific for Cryptosporidium parvum in the colostrum, milk and serum of the studied calves.

Keywords: cryptosporidosis, calves, immunoprophylaxis, prevention, neonatal disease

Thoracic Ultrasound in Beef Suckler Calves- Is It Useful or Even Possible?!

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BACKGROUND and OBJECTIVE: Bovine respiratory disease (BRD) is common in youngstock on UK beef suckler farms, but there is limited published work assessing the level of subclinical damage and its production significance. This study aimed to investigate the level of subclinical lung consolidation in pre-weaned suckler calves using Thoracic Ultrasound (TUS). It also aimed to compare TUS detection rates vs farmer treatment of respiratory disease, as well as investigating the practicality of TUS for the veterinary practitioner. Phase 2 of the study used body weights for each calf to allow Average Daily Live Weight Gain (ADLWG) to be calculated, to assess whether early lung consolidation could be production limiting.

MATERIALS and METHODS: TUS examinations of 204 calves (21-61 days old) were conducted by a single veterinarian between March and June 2022 on 8 Scottish Beef Suckler farms and compared to BRD treatment rates. Each calf was given a TUS score on a 0–5 scale based on a standardised lesion identification and scoring system. A normal TUS score was defined as ≤1 and abnormal scores as >1. 100 of these calves were followed through to Autumn 2023 when their weights were recorded and ADLWG calculated.

RESULTS: Of 204 ultrasonographic examinations, 66 (32%) were classified as abnormal and 138 (68%) as normal. The range of abnormal TUS % by individual farm was 16.7% to 56%. Of the 66 calves classified as abnormal, none were treated by the farmer. Due to time constraints, Phase 2 collection of final weights was limited to only 101 of the calves. Of these, the 36 calves with damaged lungs (Score >1) took an average of 25 days longer to reach sale weight and an ALDWG of 25g less, than the 65 without damage (Score ≤1).

CONCLUSIONS: TUS is more challenging in suckler calves than dairy calves, but several useful lessons were learned: - Scheduling is important; time scanning for when other handling is due to take place. - Restraint is more difficult than with dairy calves, but feasible. - Scanning of the right apical lobes was more challenging due to increased muscle mass, however scanning by reaching down from above helped. Larger volumes of transducing agent are required due to increased thickness of coats. The results indicated farmers were underdiagnosing BRD in youngstock. Use of TUS on suckler farms may improve diagnostic rates and accuracy, and demonstrate the value of preventive healthcare programmes.

Keywords: Thoracic ultrasound, beef, suckler

Reducing Topical Antimicrobial Use During the Routine Disbudding of Calves in the Uk

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BACKGROUND and OBJECTIVE: Disbudding of calves is a routine husbandry procedure, which takes place to reduce the risk of injury to other stock and improve safety of stockpersons. There are several techniques available for disbudding, with thermocautery (use of a hot iron) under local anaesthetic the most effective and preferred option in the UK. In 2025 a topical zinc gel-barrier product (ZGB) was developed which combined a natural bioresin complexed with zinc salts. The non-antibiotic, hydrophobic and non-colonizable ZGB product was thought to have potential benefit when used following routine disbudding of calves. This project aimed to assess the impact on antimicrobial usage and infections post-disbudding when the aerosolized oxytetracycline typically used following disbudding was replaced with topical ZGB.

MATERIALS and METHODS: A group of 40 calves at a calf-rearing unit were disbudded following sedation with 0.2 mg/kg xylazine administered by intramuscular injection in the neck, and administration of cornual nerve blocks bilaterally with three millilitres of procaine hydrochloride 40 mg/ml and epinephrine tartrate 0.02 mg/ml. Subcutaneous injections of 0.5 mg/kg meloxicam

were also administered. Routine disbudding was carried out with topical ZGB then applied to the disbudding site, in place of aerosolized oxytetracycline. The calves were re-examined seven days later and the degree of healing of the disbudding sites assessed using a published scoring system.

RESULTS: Seven days after disbudding 93 % of the calf disbud sites were found to be healing well, with 57 % of the sites fully healed. No calves were identified as displaying signs of abnormal healing or clinical signs of systemic infection following disbudding. Calf weights recorded at the time of disbudding and in the following week showed neither disbudding or the use of ZGB negatively impacted growth rates.

CONCLUSIONS: Using a topical zinc gel barrier (ZGB) instead of aerosolized oxytetracycline following disbudding reduced this farm's antibiotic usage by 1.43 mg/PCU - 9 % of the average antibiotic usage on UK dairies. This one change provided an effective, antibiotic-free option for wound care following disbudding whilst maintaining a hydrophobic layer over the site that prevents bacterial ingress.

Keywords: disbudding, gel barrier, antibiotic reduction, topical antimicrobial

Practical Implications for Improving the Quality of Bovine Colostrum Using Dried Colostrum Replacers

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BACKGROUND and OBJECTIVE: This study examined how much dried bovine colostrum replacer (CR) can be added to maternal colostrum without compromising transfer of passive immunity in calves. Because immunoglobulins cannot cross the cow's epitheliochorial placenta, calves rely on ingesting sufficient high-quality colostrum soon after birth. Supplementing inadequate maternal colostrum with CR is a common strategy to increase IgG content, but mixing CR with fresh colostrum may reduce the apparent efficiency of absorption (AEA) of IgG, potentially lowering calf serum IgG compared with unenriched colostrum.

MATERIALS and METHODS: Data came from a longitudinal cohort on a commercial Holstein-Friesian farm in the Netherlands (July 2024–January 2025). The dataset included colostrum Brix(%), volume fed, timing of administration, amount of CR added (IgG grams), and calf serum IgG (g/l) measured 2–7 days after birth. AEA% was calculated as [(50 kg)x(0.07)x(Serum IgG,g/l)/(Total IgG Delivered,g)] x 100. Correlation and linear regression assessed the relationship between added IgG and AEA%. A theoretical model used the median AEA% of non-supplemented colostrum as a reference to estimate the maximum enhancement level without reducing calf serum IgG. The regression equation predicted how added IgG alters AEA%, which was then used to estimate expected serum IgG in a 50 kg calf consuming 3 liters of supplemented colostrum.

RESULTS: Sixty-seven calves were analyzed: 37 received non-supplemented maternal colostrum and 30 received maternal colostrum supplemented with dried colostrum powder mixed in. AEA% was significantly lower in supplemented versus non-supplemented calves (median 18.3%, range 7.4–46.5% vs. median 24.9%, range 7.0–64.9%; p=0.04). AEA% correlated negatively with the amount of IgG added (r=-0.27; p=0.03) and showed a negative linear association with supplemented IgG (Beta=-0.39; 95% CI -0.74 to -0.07; p=0.03). Modeling indicated that increasing colostrum Brix from 18% to 22% raises expected serum IgG in a 50 kg calf fed 3 liters from 8.5 g/l to 10.4 g/l. However, increasing Brix from 18% to 25% yields no further serum IgG gain, and raising to 28% reduces expected serum IgG due to a marked decline in AEA%.

CONCLUSIONS: In summary, while CR can improve measured colostrum quality, adding larger amounts to fresh maternal colostrum can reduce IgG absorption efficiency and undermine serum IgG outcomes. Modest enrichment (e.g., toward Brix~22%) may be beneficial for very low-quality colostrum, but aggressive enhancement toward higher Brix targets (≥25–28%) appears counterproductive unless strategies are employed to mitigate AEA loss. Further work should clarify mechanisms (e.g., osmolality, viscosity, timing), define safe enrichment thresholds, and optimize feeding protocols to maximize passive immunity.

Keywords: Apparent efficiency of absorption, colostrum, passive transfer of immunity, colostrum replacers, lyophilized colostrum

The Impact of Feeding Neonatal Calves 3 or 5 Days of Transition Milk

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BACKGROUND and OBJECTIVE: Transition milk (TM), the milk collected from the second to sixth milking after calving, still contains antibodies. These antibodies cannot be resorbed in the calves' gut after the first 24 hours of life, but they can induce a local protection, especially when the cow has been vaccinated against neonatal diarrhea. Common practice in Portugal is to give TM or colostrum for the first three days of life. However, current advice is to provide TM minimum five days after vaccination. This study was set up to see if a difference could be seen between calves having received three (TM3) or five (TM5) days of TM.

MATERIALS and METHODS: Twenty-five animals were enrolled. All cows were vaccinated (following manufacturer's instructions) with both a commercial vaccine against Rota- & Coronavirus and E coli, and a vaccine against Cryptosporidiosis. Calves were separated into two treatment groups after colostrum provision. TM3 calves were fed TM up to day 3 of life, whereas TM 5 calves received an additional two days of TM before transitioning to milk replacer. All calves were monitored daily for 15 days: clinical signs of disease (Sx) and diarrhea intensity (Fx) were scored using the Wisconsin-Madison scoring system. Average daily growth between week 1 and 2 was estimated using thoracic circumference measurements to estimate body weight. Colostrum quality and serum IgG levels were estimated by using a BRIX refractometer.

RESULTS: Of the 25 calves, 13 were allocated to TM3, of which 2 were lost to follow up. Twelve calves were put in TM5, of which 1 was lost to follow up. No difference was observed in colostrum quality and immunity transfer between groups. The estimated mean weight of calves in TM3 was 45,9 kg in week one, compared to 45 kg in TM5. By week 2, calves in TM3 had gained 2,9 kg, to a mean of 48,8 kg, whereas calves in TM5 gained 4,2 kg, to a mean of 49,2 kg. TM3 calves showed higher Sx and Fx from days 8 to 13, suggesting more prolonged or severe symptoms. TM5 calves had lower scores and better fecal consistency during the same period.

CONCLUSIONS: The absence of a negative control group limits the ability to compare the advantage of TM3 to no TM feeding. Furthermore, this pilot study lacked sample size to allow statistical comparison between treatment groups. Nonetheless, TM5 showed better clinical outcomes, particularly in reducing the severity and duration of diarrhea, compared to TM3.

Keywords: Transition Milk, Cryptosporidium, Calf dhiarrea

Enhancing Dairy Calf Health in Automated Milk Feeder Systems: Insights from Farmers' Experiences

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BACKGROUND and OBJECTIVE: Restricted milk feeding hampers the welfare and health of dairy calves. Our previous work identified social constraints outside work among farmers and farm staff as a key barrier to adopting more biologically appropriate milk allowances. Automated milk feeders (AMF) may help overcome these constraints by enabling more frequent milk meals with reduced labor, however, few use them and they have been linked to increased risk of respiratory disease. Recommendations to improve AMF outcomes have rarely incorporated farmers' perspectives, which may reduce practical uptake. The aim of this qualitative study was therefore to explore AMF farmers' experiences of best practices and common pitfalls in adopting AMF for successful dairy calf rearing.

MATERIALS and METHODS: We conducted one focus group interview and 11 individual semi-

structured interviews with owners or staff from 14 Swedish AMF dairy farms. Participants were selected through purposive sampling to ensure variation in gender, age, farm role, herd size, AMF type, and manufacturer. Herd sizes ranged from <70 to >250 cows and the number of calves per AMF pen from 8 to 17. Interviews focused on work routines for calves and AMF, as well as on factors perceived as essential for successful calf rearing and good working conditions. Interviews were recorded, transcribed, and analyzed by aggregating meaningful components related to management practices and farm conditions (codes) into major processes and five phases (from pre-AMF to post-weaning) influenced by the temporal bracketing strategy.

RESULTS: Participants described extensive on-farm learning about the conditions required to successfully rear group-housed calves. Through exploration and continuous adaptation, they emphasized the importance of high-quality housing facilities that ensure good air quality, maintain small, closed calf groups, and support efficient hygiene and biosecurity routines. A well-distributed calving pattern throughout the year was also considered central for calf health and workload management. Participants emphasized giving calves a strong start before AMF introduction, optimizing their condition at entry, and ensuring a safe, smooth onboarding process. They also stressed the importance of maintaining and monitoring the AMF itself, drawing on earlier technical challenges to minimize future disruptions.

CONCLUSIONS: By integrating key lessons learned through participants' situated learning with findings from the scientific literature, we suggest that preventive investments in quality housing, attentive management—particularly of the youngest calves before and during AMF onboarding—and consistent AMF care are beneficial for enhancing calf health and performance, reducing workload, and improving experience of work and engagement with the calves.

Keywords: automated milk feeders, farmer experiences, calf health

Effects of Clostridium Butyricum supplementation on Gut Microbiota, Intestinal and Ruminal Integrity, and Growth Performance in Dairy Calves

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BACKGROUND and OBJECTIVE: The use of *Clostridium butyricum* in dairy calves has emerged as an alternative to antibiotics due to its probiotic, symbiotic, and immunobiotic properties. The bacterium supports intestinal microbial stability by promoting beneficial bacterial populations while suppressing pathogenic organisms. It enhances metabolic efficiency and growth performance and modulates the intestinal immune environment, thereby reducing tissue inflammation. Histologically, supplementation has been associated with increased expression of tight junction proteins, improved intestinal barrier integrity and overall gut health. The present study aimed to evaluate the effects of *C.butyricum* supplementation on intestinal microbiota composition, intestinal and ruminal tissue integrity, and general health in dairy calves from birth to 120 days of age, alongside growth performance, starter intake, passive immunity, and disease incidence.

MATERIALS and METHODS: A total of 150 calves were randomly assigned at birth to two groups: a control group (C; 72 females/3 males) receiving pasteurised milk and starter concentrate, and a treatment group (Cb; 72 females/3 males) receiving the same diet supplemented with 4g of *C. butyricum* in milk. After 28 days, intestinal and ruminal samples were collected from the male calves for microbiome and histopathological analyses. Passive immunity, growth parameters, faecal enteropathogens, and clinical health were monitored throughout the study. Growth, health, and tissue parameters were analysed using linear mixed-effects models with treatment, pathogen

interaction; intestinal and rumen cell parameters as fixed effects, and repeated measures for growth. Microbiome diversity was evaluated using alpha and beta metrics, including Chao-1 and Shannon analyses.

RESULTS: Calves supplemented with *C. butyricum* exhibited significantly greater colonic species richness, with higher observed and Chao-1 index compared with controls ($p \leq 0.05$), along with reduced *Escherichia coli*/*Shigella* and increased Lactobacillaceae, Firmicutes, and Actinobacteria. Histopathology revealed longer villi, deeper crypts, fewer mitotic figures and eosinophils, indicating reduced intestinal inflammation. Rumen papillae were longer and more abundant, with reduced parakeratosis in the Cb group. Growth performance was similar at birth, whereas at 90- and 120-days supplemented calves were significantly heavier and taller ($p<0.05$) with higher average daily gains at 30 and 90 days. The Cb group also showed fewer disease diagnoses (65 vs 80), delayed clinical onset, reduced pneumonia incidence, and lower treatment costs (€643.61 vs €823.90).

CONCLUSIONS: These findings demonstrate that *C. butyricum* supplementation improves intestinal microbial diversity, enhances mucosal and ruminal integrity, reduces inflammatory responses, supports superior growth, and decreases disease incidence and treatment costs. The results support its use as a viable non-antibiotic strategy to promote overall health and productivity in dairy calves.

Keywords: {C. butyricum}, calves, health, microbiota, immunomodulation, growth

Paromomycin Sulphate for Early Control of Cryptosporidiosis in Dairy Calves: Efficacy Across Diverse Production Systems

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BACKGROUND and OBJECTIVE: Cryptosporidiosis, primarily caused by *Cryptosporidium parvum*, is a major enteric disease in neonatal dairy calves, leading to diarrhoea, impaired growth, and economic losses. Transmission occurs via faecal–oral route, and oocysts persist in the environment. Control relies on passive immunity, hygiene, supportive care, and targeted prophylaxis. Paromomycin, an aminoglycoside from *Streptomyces rimosus* var. *paromomycinus*, inhibits aminoacylation of mRNA in the 30S ribosomal-subunit, disrupting parasite protein synthesis and preventing completion of the intestinal life cycle. The objective of the field trials was to prevent cryptosporidiosis prior to its clinical presentation during the second-week of life, thereby increasing overall health and growth retardation under varied rearing systems.

MATERIALS and METHODS: A total of 120 newborn heifer calves from three private dairy operations in northern (S1), central (S2), and western (S3) Mexico were enrolled. Calves were housed individually and randomly assigned at birth to treatment groups: farm-standard scours control, commercial prophylactic treatments (halofuginone lactate [HL] or nitazoxanide [N]), and paromomycin sulphate at 50 mg/kg body weight for seven days (PS). Passive immunity was assessed by Brix refractometry at 48 hours, and diarrhoea scoring was recorded daily for 60 days. Faecal samples collected at 7 and 14 days were analysed via acid-fast staining, with oocyst quantification performed in S2. Growth parameters were recorded from birth up to 120 days depending on the site.

RESULTS: Passive immunity levels were comparable across groups. At peak clinical cryptosporidiosis (day 14), paromomycin reduced prevalence in S3 (14%), nitazoxanide achieved moderate reduction in S2 (28.1%), while halofuginone in S3 showed an increased prevalence (33%). Quantitative oocyst counts in S2 revealed significantly lower shedding in the PS group compared with controls and N ($P<0.0014$). Cumulative diarrhoea scores during the second and third weeks were consistently lower in paromomycin-treated calves across all sites. Growth performance improved: S1 calves were 13.6kg heavier than controls at 90 days, S2 calves 16.8kg heavier than N-treated calves at 120 days, and S3 calves 6.9kg heavier than HL-treated, likely reflecting reduced milk allowance and passive immunity.

CONCLUSIONS: Overall, paromomycin sulphate reduced oocyst shedding by 34–50% during peak infection, decreased clinical signs and appetite loss, and was associated with fewer secondary respiratory cases. Superior weight gains at 60, 90,

and 120 days were observed compared with prophylactic alternatives. Effective passive immunity, high milk feeding planes, close clinical monitoring, and robust biosecurity enhanced disease control. These findings support paromomycin sulphate as an effective early intervention for cryptosporidiosis in dairy calves across diverse production systems.

Keywords: Cryptosporidiosis, neonates, paromomycin sulphate, oocyst shedding, growth performance

Evaluation of Passive Immunity Transferred by Colostrum in a Dairy Cattle Production in Mexico

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BACKGROUND and OBJECTIVE: Background. Colostrum is defined as the milk secretion produced by the female in the first few minutes after birth. Its components have nutritional and laxative functions, and above all, it is responsible for providing passive immunity to the newborn, protecting it against infectious diseases. It is essential that the calf receives colostrum in the first hours of life, with a high concentration of immunoglobulins (>50 g/L) and an adequate volume of colostrum (8-10% of its body weight). The objective is to determine the transfer of passive immunity through colostrum in a dairy cattle production unit in Mexico.

MATERIALS and METHODS: Materials and methods. The study was conducted on dairy cattle housed in an intensive production system with a total inventory of four thousand animals. Samples were taken from 50 cattle, pre and post colostrum, in them blood was obtained by coccygeal venipuncture to obtain serum and perform serological test of indirect and competitive immunoenzymatic assay to identify specific antibodies against Bovine Varicellovirus (Variv), Deltaretrovirus (Delv) and Pestivirus (Pestiv).

RESULTS: The fifty calves sampled during the pre-colostrum period were negative for the presence of specific antibodies against Variv and Pestiv, while 38% were positive for the presence of antibodies against Delv. During the post-colostrum period, 100% were positive for all three viruses.

CONCLUSION: The results obtained demonstrate that passive immunity transmitted to calves through colostrum is effective against Variv, Delv, and Pestiv. Regarding Variv and Pestiv, this immunity is associated with vaccination as part of preventive medicine programs in the production unit, but in the case of Delv, immunity in the female is conferred by natural infection with the virus.

Keywords: immunity, Colostrum, Cattle

The Role of Viral Enteropathogens (RVA, BCoV, BEV, BToV and BVDV) and the Frequency of Co-infections in Neonatal Calf Diarrhea

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BACKGROUND and OBJECTIVE: Neonatal calf diarrhea (NCD) is a major cause of morbidity and mortality in dairy and beef farms worldwide, resulting in significant economic losses. Viral enteropathogens play a key role in its etiology, with Rotavirus A (RVA) and Bovine coronavirus (BCoV) being the most frequently reported agents, while Bovine viral diarrhea virus (BVDV), Bovine torovirus (BToV), and Bovine enterovirus (BEV) may also contribute as single or co-infections. Accurate identification of these pathogens is essential for effective diagnosis, epidemiological surveillance, and disease control. Although studies have been conducted to detect viral enteropathogens in NCD

cases, data on diarrhea cases caused by simultaneous viral infections are limited. This study investigated the occurrence of simultaneous infections caused by important enteric viral agents that cause diarrhea in the neonatal period.

MATERIALS and METHODS: A total of 206 fecal samples from neonatal calves with diarrhea, collected from 14 different provinces of Turkiye, were included in the study. All fecal samples were homogenized with 10% (w/v) phosphate-buffered saline (PBS) and then analyzed using commercial Ag-ELISA kits to detect BVDV, RVA, and BCoV antigens. To detect BToV and BEV, nucleic acid extraction was performed from all samples, followed by complementary DNA (cDNA) synthesis, followed by PCR with the relevant specific primers.

RESULTS: Among tested 206 stool samples, 89 (43.2%) had singular infections, while 35 (17.0%) had co-infections. Single RVA infection was the most frequently detected (n=52; 25.2%), followed by BEV (n=25; 12.1%), BToV (n=6; 2.9%), BCoV (n=5; 2.4%), and BVDV (n=1; 0.5%). Most co-infections were binary combinations (n=30; 14.6%), whereas triple (n=4; 1.9%) and quadruple (n=1; 0.5%) viral infections were rare. The most frequent co-infection was included RVA and BEV (n=10; 4.9%), and the remaining co-infection patterns occurred at low frequencies (n≤4).

CONCLUSIONS: This study has demonstrated a high prevalence of viral agents in neonatal calf diarrhea, with RVA identified as the predominant viral pathogen. The considerable prevalence of BEV further emphasizes its potential clinical relevance and the need to include it in routine diagnostic screening. Although most infections occurred as single infections, co-infections were also detected at a notable rate, predominantly as dual combinations. These findings underline the importance of comprehensive diagnostic approaches and effective control strategies in herd health management.

Keywords: Neonatal Diarrhea, Calf, Viral enteropathogens, Prevalence, Co-infections

Randomized Field Trial on the Effects of on-Arrival Intranasal Vaccination Against Bovine Coronavirus on Ultrasound Confirmed Pneumonia and Average Daily Gain in Beef-on-Dairy Veal Calves

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BACKGROUND and OBJECTIVE: This randomized clinical trial evaluated the effect of upon-arrival intranasal vaccination against bovine coronavirus (BCoV) on pneumonia risk, lung ultrasonographic cure, clinical bovine respiratory disease (BRD), average daily gain (ADG), and cold carcass weight (CCW) in a beef-on-dairy veal calf farm.

MATERIALS and METHODS: Belgian blue beef x dairy calves (n= 219; age 14-21days) from multiple dairy farms were fattened in a single veal calf facility. On arrival, Immunoglobulin G (IgG) and serostatus for respiratory pathogens were determined, and calves were randomized by weight and lung consolidation (LC) ≥1cm status into a placebo control group (NC) and a group vaccinated against BCoV (IN). Clinical scoring and quick thoracic ultrasonography (qTUS) were performed on arrival (T1), day 12 (T2), 22 (T3) and 70 (T4). ADG was calculated from arrival to day 70. CCW was collected in the abattoir. Bacterial culture and nanosequencing were performed on non-endoscopic broncho-alveolar lavage (nBAL) samples from NC calves. Multivariable logistic regression and linear mixed models assessed vaccination effects on risk for LC, ultrasonographic cure, clinical BRD, ADG and CCW.

RESULTS: On arrival, 95.4% (209/219) were seropositive for BCoV, 13.7% (30/219) of the calves had IgG < 7.5 g/L, 1.4% (3/219) had clinical BRD and 8.7% (19/219) had LC ≥1 cm. By T2, clinical BRD was 2.7% (6/219), while 43.4% (95/219) had LC ≥1 cm. Circulation of BCoV was confirmed. No differences in clinical BRD or LC were found between IN and NC calves at any time point. After treatment, both short- (44.7%) and long-term cure (73.6%) were not affected by vaccination. Short-term cure decreased significantly with increasing lung consolidation depth. Lung consolidation at arrival was positively associated with LC at all subsequent observation points ($p < 0.001$). Mean ADG during the first 10 weeks was significantly higher in vaccinated calves (+0.039 kg/day, 95% CI: 0.002–0.075). Vaccination was associated with a higher week-10 live weight (+2.86kg, 95%CI: 0.14–5.57) and a numerical increase in CCW (+3.4kg; 95%CI: -3.4; 9.9). Both week-10 ADG and severe pneumonia at T4 were associated with lower CCW.

CONCLUSIONS: This is the first study evidencing a direct positive effect of upon-arrival vaccination on ADG in veal calves. Under the conditions of the present study, the high serostatus for BCoV, the low prevalence of failure of transfer of passive immunity and higher arrival body weights may have masked the underlying effects of vaccination on LC and ultrasonographic cure.

Keywords: Bovine respiratory disease, Immunity, Lung consolidation, Growth

Identifying Predisposing Factors for Neonatal Calf Diarrhea in Family-run Farms: Insights from a Novel Checklist

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BACKGROUND and OBJECTIVE: Neonatal Calf Diarrhea (NCD) represents a major health challenge in the livestock industry. Identifying critical points in animal health management is essential for its prevention. This cross-sectional, observational, study aimed to apply a novel checklist designed to identify management-related predisposing factors for NCD in calves.

MATERIALS and METHODS: Thirty bovine family run farms (19 intensive farming system, 11 semi-intensive farming system), located in Southern Italy (Campania Region) [median herd size: 42 cows; interquartile range (IQR): 33–58;] were investigated between September and December 2025. The checklist included 91 questions grouped into seven domains (D, 13 question/D): mothers/close-up (D1); calving area/calving event (D2); calf care/colostrum intake (D3); colostrum, milk, and milk replacer management (D4); calf management up to 28 days of age (D5); biosecurity (D6); and health monitoring program and veterinarian involvement (D7). Each item was scored from 0 (minimal predisposition) to 2 (strong predisposition). Farms were selected by convenience sampling. Differences among domain scores were assessed using the Friedman test followed by Wilcoxon signed-rank tests with Bonferroni correction ($p<0.0083$). Correlations among domains were evaluated using Spearman's rank correlation, and internal consistency was assessed via Cronbach's alpha. The study population was stratified into intensive and semi-intensive farming systems, and differences between these groups were analyzed using the same statistical approach.

RESULTS: At the time of the survey, clinical NCD was observed at the farm level in 90% of cases. The highest median scores were observed in D1 (Me=17.00/26.00, IQR=13.00–18.25), D6 (Me=14.00/26.00, IQR=11.75–18.00), and D7 (Me=14.00/26.00, IQR=13.00–14.00), indicating the greatest predisposing factors for NCD. Lower median scores were observed in D2 (Me=10.50/26.00, IQR=6.75–13.00), D5 (Me=7.50/26.00, IQR=6.00–9.00), D3 (Me=5.00/26.00, IQR=3.75–7.00), and D4 (Me=4.00/26.00, IQR=3.00–6.00). Significant differences were detected among most domains, except for D1 vs. D6, D3 vs. D4, and D6 vs. D7. A very good positive correlation was found between D1 and D2 ($rs=0.67$, $p<0.001$), and a good positive correlation between D5 and D6 ($rs=0.50$, $p<0.01$). No dominant responses emerged within domains, and no significant differences were observed between the two farming systems.

CONCLUSIONS: Overall, the checklist proved to be a reliable tool to identify NCD-related predisposing factors, highlighting that mothers/close-up (D1), biosecurity (D6), and health monitoring program and veterinarian involvement (D7) may require the greatest attention for effective prevention of neonatal calf diarrhea in family-run farms. Furthermore, this tool could be applied for pre- and post-assessments to evaluate the effectiveness of corrective actions implemented on the farms.

Keywords: Neonatal calf diarrhea, Calf Health, Farm management, Biosecurity, Welfare

Using Individual Lung Ultrasonography at the Group Level to Define Treatment Threshold in Large Groups of Calves for Tailoring Antimicrobial Use: A Simulation Approach

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BACKGROUND and OBJECTIVE: Bronchopneumonia is a major reason of antimicrobial use in groups of comingled calves. The clinical diagnosis may be challenging, and lung ultrasonography (LUS) has emerged as performant diagnostic test. However, little is known on the applicability of this test at the group-level to decide whether or not a group of calves should be treated. The objective of this study was to use simulation-based assessment of practical use of LUS to detect bronchopneumonia in a group of calves, and to determine the optimal combination for deciding when to treat a group based on a random sample of calves for LUS examination.

MATERIALS and METHODS: We simulate different scenario and assessed the performance of LUS as a group test when assessing a random number of calves (N=12, 20 and 30) and defining the group positive test based on the number of calves with lung consolidation (k; from 1 to 6), the true prevalence of bronchopneumonia in the tested population (TP; from 0.05 to 0.3) and prevalence of positive herds (HTP; from 0.1 to 0.7). Accuracy of LUS at the individual level (sensitivity (Se) and specificity (Sp)) were based on literature review using beta distribution. Two thousand iterations of Se and Sp were obtained, accounting for their negative correlation. Then, group accuracy parameters (HSe, HSp) were obtained based on Se, Sp, k, N and TP. The misclassification cost term analysis, which accounts from different plausible costs ratio (r) between false negative and false positive groups were also obtained to assess the robustness of the findings. Finally, the group positive and negative predictive values (HPPV, HNPV) were also calculated. For each parameter, the median, 5th and 95th percentile of obtained distributions were determined.

RESULTS: The HSe decreased as N or TP decreased, or as k increased, whereas the opposite pattern was observed for HSp. The HPPV and HNPV were highly impacted by their context of use (mainly interaction between TP, HTP, N and k). Interestingly, when TP ≤ 0.15, HNPV increased and HPPV decreased as k increased, whereas the opposite pattern was observed when TP ≥ 0.20. The MCT decreased as TP increased when k=1 or 2 and when N=30. For N=12, and k>3 MCT increased as TP increased and for N=20 when k>4, showing the importance of context of application of the test.

CONCLUSIONS: This study shows the importance of context of application when using LUS as a group-level test to tailor antimicrobial use in groups of calves.

Keywords: bronchopneumonia, stochastic, infectious disease, diagnosis

Health Status of Water Buffaloes in San Miguel Mecatepec, Veracruz: Hematological, Parasitological, and Urinary Evaluation, and Viral Diagnosis by PCR

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BACKGROUND and OBJECTIVE: Buffalo production in Veracruz is key for obtaining milk and meat and for improving the rural economy. Therefore, their health impacts productivity and public health, making it extremely important to conduct screenings to determine the health status of herds in Mexico.

OBJECTIVE: To determine health status through laboratory testing.

MATERIALS and METHODS: Thirteen animals (males and females) with the lowest body condition score in the entire herd (3) were randomly sampled. Blood samples were obtained using EDTH tubes via coccygeal vein puncture to determine hematocrit. Fecal matter (10 g) was collected for McMaster tests and gastrointestinal nematode egg counts. Urine was collected, and urine specific gravity was measured using a refractometer. Nasal swabs were taken for viral PCR testing for the main viral agents present in cattle (1.001–1.005).

RESULTS: Hematocrit levels were normal; fecal analysis was negative for nematodes; urine specific gravity ranged from 1.001 to 1.005, indicating no signs of kidney damage; and PCR was positive for bovine herpesvirus, bovine viral diarrhea virus, and respiratory syncytial virus.

CONCLUSIONS: There is significant viral circulation despite the absence of parasitosis; epidemiological surveillance, vaccination programs, biosecurity measures, and clinical studies are recommended to evaluate the productive impact and to make more measurements of urine density to establish the normal reference value in this species in Mexico.

Keywords: buffalo, hematocrit,BVDV,BHV-1,BRSV

Bridging The Gap: Key Findings of a Two Year Survey on Colostrum Management in Latvia and Lithuania

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BACKGROUND and OBJECTIVE: Colostrum is the fundamental source of passive immunity for neonatal mammals. Its role is particularly critical in cattle, as calves are born agammaglobulinemic and rely entirely on the successful transfer of maternal immunoglobulins. While the principles of colostrum management are well-established, their practical implementation in the field is often inconsistent. In Latvia and Lithuania, there is a notable paucity of data regarding both the biological quality of colostrum and the specific conditions under which neonates are fed.

MATERIALS and METHODS: Between 2024 and 2025, ten dairy farms in Latvia and Lithuania participated in the 'Day 1' voluntary monitoring programme. This initiative allowed farm managers to record data concerning maternal health-including vaccination protocols, antiparasitic treatments, and mineral supplementation-alongside neonatal feeding management (timing, volume, and hygiene). Colostrum density was assessed at the first milking using Brix refractometry (°B). In return, participants received bespoke reports to identify opportunities for management improvements. Over the two-year period, 1,739 measurements were collected from 923 animals, including both heifers and multiparous cows. Categorical variables were analysed using chi-square tests.

RESULTS: Overall colostrum quality was satisfactory, with a median of 25°B (IQR [23;28°B]). Samples were classified as good or excellent (60.9%) or poor (21.7%). Multiparous cows produced significantly ($p<0.01$) higher-quality colostrum than heifers or second-parity cows; notably, the probability of a younger cow yielding average or poor-quality colostrum was double that of an adult cow. The majority of calves (81.5%) received between 3 and 4 L of colostrum. Unexpectedly, these calves were fed significantly higher-quality colostrum ($p<0.01$) than those receiving smaller volumes. Furthermore, vaccination against neonatal calf diarrhoea (NCD) and the administration of mineral

supplements-specifically selenium-were highly significantly correlated ($p<0.01$) with superior colostrum quality.

CONCLUSIONS: Comprehensive studies on colostrum quality remain scarce within the Baltic region. The Day 1 programme serves as a valuable diagnostic tool for assessing current husbandry practices and physiological trends. A lot remains to do to link these findings with epidemiological features. The data suggests that targeted NCD vaccination and robust mineral supplementation are advisable interventions to optimise colostrum quality and, by extension, neonatal calf health.

Keywords: colostrum, refractometry, Day 1 program, selenium

Coliform Contamination Compromises Apparent Absorption Efficacy of Colostrum Immunoglobulins in Neonatal Calves - A French Field Study

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BACKGROUND and OBJECTIVE: While management protocols regarding immunoglobulin (IgG) concentration and timing for colostrum intake are well-established, the specific impact of its bacteriological quality on passive transfer of immunity (PTI) efficacy remains under-investigated within the French context. This study aimed to evaluate the bacteriological quality of colostrum across French dairy and beef herds, and assess its impact on PTI.

MATERIALS and METHODS: Ten commercial farms (six dairy, four cow-calf) were recruited. 287 dam-calf pairs met inclusion criteria: clinically healthy calves from uncomplicated calvings or caesareans, separated from dams <1 hour postpartum to prevent natural suckling. Colostrum was harvested and administered via the farm's standard feeding equipment (bottle, bucket, or tube) within 4 hours of birth. To evaluate hygiene at the point of consumption, a colostrum sample was collected directly from the feeding vessel immediately prior to administration. Bacteriological quality was assessed by enumerating total mesophilic flora (TPC) and coliforms. Calf serum samples were obtained between 2 and 7 days of age. Apparent Absorption Efficiency (AAE) was calculated as Total serum IgG (estimated, x100) to Ingested IgG (calculated).

RESULTS: Data from 225 compliant dam-calf pairs were analysed. Mean colostrum intake was 2.95±0.96 and 2.24±0.75L, for dairy and beef calves, respectively. Beef colostrum demonstrated higher mean IgG concentrations (108.0 64.3 g/L) compared to dairy colostrum (67.0±40.8 g/L), resulting in a higher total IgG mass ingested by beef calves (234.5 g vs 180.6 g). TPC exceeded 10^5 CFU/mL in 70.0% of dairy and 61.2% of beef samples. Conversely, coliform counts were generally controlled, with many samples below 10^4 CFU/mL. Mean serum IgG concentrations were comparable between dairy (15.4±8.5 g/L) and beef calves (16.8±8.4 g/L). AAE 30.6±21.2% and 32.7±26.0%, respectively. Crucially, tests demonstrated no significant association ($p>0.05$) between TPC and either serum IgG concentration or AAE. Nevertheless, coliform concentration was significantly ($p<0.05$) associated with impaired AAE.

CONCLUSIONS: This study highlights a high prevalence of bacterial contamination in colostrum administered on French farms. However, contrary to expectations derived from previous literature, elevated bacterial loads did not statistically impair the PTI in this cohort. This lack of interference may be attributed to the relatively controlled coliform levels observed, as coliforms are often implicated in blocking IgG absorption, which has been confirmed in this study. Nevertheless, strict hygiene protocols remain paramount to minimise pathogen exposure and prevent neonatal pathology.

Keywords: colostrum, immunoglobulins absorption, hygiene, coliforms

Mycotic Rumenitis Secondary to Ruminal Acidosis in a Nursing Calf

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BACKGROUND and OBJECTIVE: This report describes a case of a 21-day-old Holstein Friesian heifer with recurrent abdominal distension, from a dairy farm in Hidalgo, Mexico. The farm manager reported a 21-day-old heifer with a 15-day history of mild, recurrent left-sided abdominal distension. On physical examination, the animal was alert, with a heart rate of 110 bpm and a respiratory rate of 65 breaths per minute. Yellowish watery feces, mild abdominal distension, and a tympanic sound on percussion-auscultation were noted.

MATERIALS and METHODS: Treatment consisted of restricting pre-starter concentrate, performing an orogruminal probe to relieve tympany, and administering magnesium hydroxide reconstituted in warm water orally at 100 g every 8 hours for two doses. Additionally, a commercial medication containing gentamicin (2.2 g/L), nalidixic acid (1.0 g/L), trimethoprim (1.2 g/L), cobalt sulfate (0.25 g/L), copper oxide (0.5 g/L), and activated charcoal (20.0 g/L) was administered orally at 40 mL twice daily for 7 days. Kaolin-pectin was also given at 90 mL twice daily for 3 days. Although the heifer showed initial improvement, it was not discharged due to persistent mild abdominal distension and hyporexia. Ten days after treatment initiation, the heifer was found dead.

RESULTS: Hematoxylin and eosin-stained rumen sections revealed ortho- and parakeratotic hyperkeratosis of the mucosa, vacuolar degeneration of keratinocytes, and foci of neutrophils and pus cells in the ruminal epithelium. Extensive ulcers infiltrated by neutrophils, pus cells, and macrophages were observed, with branched hyphae embedded in the lesions. Grocott's methenamine silver stain confirmed the presence of fungal structures.

CONCLUSIONS: Based on the clinical presentation, postmortem morphological diagnosis, histopathology findings, and identification of fungal structures via Grocott's methenamine silver stain, fungal rumenitis secondary to opportunistic fungal colonization and invasion of the ruminal epithelium—resulting from ruminal acidosis—was confirmed. Fungal rumenitis is a secondary condition arising from physicochemical and metabolic disorders, typically linked to improper feeding practices, with ruminal acidosis being the most common cause. It may also stem from prolonged antibiotic use and inadequate calf management during lactation, primarily affecting dairy cattle.

Keywords: fungal rumenitis, ruminal acidosis, Holstein heifer, histopathology, dairy calf, opportunistic fungi

Postnatal Factors Influencing Calf Morbidity and Mortality in Dairy Cattle: A Systematic Review of the Literature (2000–2024)

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BACKGROUND and OBJECTIVE: Profitability and productivity of dairy farms depends directly on calf health. Moreover, calf mortality is an indicator of

animal welfare. The aim of the study was to conduct a systematic evaluation of literature from the last 24 years concerning postnatal factors affecting calf morbidity and mortality.

MATERIALS and METHODS: The factors selected were colostrum management (timing, amount of colostrum, second colostrum feeding), navel disinfection, prophylactic supplements (trace minerals and / or vitamins), monitoring and housing of the newborn calves for the first 3 days. A comprehensive search was performed in PubMed, CAB Abstracts, and Web of Science for studies published between 2000 and 2024 using defined keywords. Studies, which met predefined inclusion criteria, were evaluated.

RESULTS: In total, 4981 publications were screened and 86 publications were evaluated and analyzed for the postnatal period. The number of publications was distributed as follows: colostrum management (n=34), navel disinfection (n=10), prophylactic supplements (n=9), monitoring (n=3) and housing (n=30). An earlier colostrum feeding influenced the odds ratio (OR) of calf mortality (OR 0.77 – 0.91). A second colostrum feeding reduced morbidity (OR 0.21 – 0.51). Navel disinfection was related in some studies to reduced incidence of umbilical infections (OR 0.26 – 0.8). Supplementation of calves with trace minerals and / or vitamins reduced in some studies the risk of mortality (OR 0.41 – 0.68), while one study showed a higher relative risk of mortality of 1.48 (95% CI 1.09 – 2.01). The application of trace minerals and / or vitamins led in some studies to a reduced risk of morbidity (OR 0.44 - 0.74). Calf mortality was reduced in one study if more than two persons took care of the calves (relative risk 0.44; 95% CI 0.27 – 0.72) and morbidity was reduced if female or mixed teams of calf caretakers (OR 0.2 – 0.4) were responsible for monitoring. Housing of the calves showed an influence on morbidity. The main factors to reduce the risk of morbidity were good ventilation (OR 0.22 – 0.45), reduction of dust (hazard ratio 0.58; 95% CI 0.39 - 0.85), clean and dry bedding (OR 0.42 – 0.77) and a small number of calves per pen (OR 0.42 – 0.51).

CONCLUSIONS: The literature review shows that good management of dairy calves in the first 3 days can reduce morbidity and mortality. The success of individual measures may depend on the operational structure. This review can serve as motivation to improve calf management.

Keywords: calf health, colostrum, supplements, housing, monitoring, navel disinfection

Effects Of *Weizmannia Faecalis* DSM 32016 Supplementation on Growth, Gut Microbiota, And Health Outcomes in Holstein Friesian Calves

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BACKGROUND and OBJECTIVE: Calf health during the pre-weaning period critically influences lifetime productivity in dairy herds, yet effective strategies to support gut health and immune development remain limited. Probiotic interventions based on *Weizmannia faecalis* (formerly *Bacillus coagulans*) DSM 32016 (WF-32016) have shown gut-health benefits in monogastric species but are understudied in calves. This study aimed to evaluate the effects of dietary WF-32016 supplementation on growth performance, fecal consistency, immune status, and gut microbiota dynamics in dairy calves under commercial rearing conditions.

MATERIALS and METHODS: Twenty female Italian Holstein Friesian calves were enrolled at birth, randomly allocated to either control (CTR) or probiotic (PRO) groups, and individually housed for a 56-day trial. The PRO group received WF-32016 at 1×10^9 CFU/kg feed included in milk replacer and starter feed. Body weight was measured weekly, and fecal consistency scores were recorded daily during the first two weeks. Blood samples were collected at baseline and day 56 to assess immunoglobulin G (IgG) concentrations and serum total antioxidant capacity (T-AOC). Fecal samples were collected at multiple time points for 16S rRNA gene sequencing to examine changes in gut microbial community structure and predicted functional profiles.

RESULTS: At day 56, WF-32016 supplemented calves exhibited significantly greater body weight compared with controls (PRO: 75.55 ± 4.55 kg vs. CTR: 67.90 ± 6.09 kg; $p \leq 0.05$), representing an approximate 11% increase in final body weight. During the critical early rearing period, mean fecal scores were lower in the PRO group (0.49 ± 0.84) than CTR (1.06 ± 1.14; $p \leq 0.01$), indicating reduced diarrheal severity. At day 56, serum IgG and T-AOC levels were also significantly higher in PRO calves ($p \leq 0.01$), consistent with enhanced systemic immunity and antioxidant status. Microbiota analysis showed significant beta-diversity differences between groups ($p \leq 0.05$) with increased relative abundance of beneficial families including *Lactobacillaceae*, *Bifidobacteria*, and *Lachnospiraceae* in PRO calves. Predicted functional analysis revealed enrichment in carbohydrate metabolism pathways, particularly the pentose phosphate pathway ($p \leq 0.05$).

CONCLUSIONS: Dietary inclusion of *Weizmannia faecalis* DSM 32016 positively influenced growth performance, gut health, immune markers, and microbiota composition in Holstein Friesian calves during the first eight weeks of life. These findings support the probiotic's potential as a natural tool to enhance calf robustness and reduce reliance on antimicrobial usage in early life. This work contributes practical insights for improving calf rearing strategies within modern dairy production systems.

Keywords: Probiotic, gut health, antibiotic reduction

Bacterial Contamination of Bovine Colostrum Across Different Collection Methods: Hand Milking Remains the Gold Standard

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BACKGROUND and OBJECTIVE: Ingestion of excellent quality colostrum is an essential step for neonatal calves. The absorption of immunoglobulins from the colostrum by the calf can however be hampered by a high bacterial load of the colostrum. The aim of the present study was to evaluate whether the use of different collection methods could influence the degree of bacterial contamination of bovine colostrum.

MATERIALS and METHODS: Colostrum aliquots were collected from a convenience sample of 22 Holstein-Friesian holdings selected by a single veterinary practice in The Netherlands. All samples were stored at -20°C until shipment to the Dutch reference laboratory (Royal GD, Deventer, The Netherlands) for determination of the Total Bacterial Cell Count (TBCC; cfu/ml). For each farm, the colostrum collection method - hand milking, parlor milking, robotic milking, or a portable cow milker - was recorded. TBCC values were log-transformed prior to analysis. Mixed linear regression, with farm included as a random effect, was used to evaluate the impact of the collection method on colostrum TBCC. Statistical analysis was performed in R (R Core Team 2025).

RESULTS: Hand-milked colostrum samples had the lowest median TBCC (8.2×10^2 cfu/ml; range 1.8×10^2–9.1×10^3 cfu/ml), compared with samples obtained via parlor milking (2.0×10^4 cfu/ml; range 3.0×10^3–2.1×10^6 cfu/ml), robotic milking (1.5×10^6 cfu/ml; range 2.2×10^4–3.0×10^6 cfu/ml), and portable milking (3.0×10^6 cfu/ml; range 3.5×10^6 –3.0×10^6 cfu/ml). All hand-milked colostrum samples complied with the bacteriological standard (<100.000 cfu/ml). In contrast, only 69.2%, 50.0%, and 33.3% of the colostrum samples collected via parlor, robotic, and

portable milking, respectively, had a total bacterial count below 100.000 cfu/ml. Parlor milking was associated with a significant increase in colostrum TBCC compared with hand milking (beta = 1.5 log10 cfu/ml; 95% CI 0.3–2.8 log10 cfu/ml; p = 0.020). Robotic milking also significantly increased colostrum TBCC relative to hand milking (beta = 2.5 log10 cfu/ml; 95% CI 1.1–4.0 log10 cfu/ml; p < 0.001), as did portable milking (beta = 2.8 log10 cfu/ml; 95% CI 1.2–4.4 log10 cfu/ml; p < 0.001).

CONCLUSIONS: Hand milking was associated with significantly lower bacterial contamination of bovine colostrum than parlor, robotic, or portable milking systems, confirming it as the "gold standard" collection method in this study. These findings suggest that the implementation of stringent hygiene and equipment management protocols for mechanized systems should be prioritized and, where feasible, hand milking should be implemented in particular for the 1st colostrum milking to minimize colostrum bacterial load and support optimal passive transfer in neonatal calves.

Keywords: colostrum, Transfer of Passive Immunity, TPI, robot milking

Molecular Detection and Genetic Characterization of Emerging Bovine Rotavirus G10 Associated with Calf Diarrhea in Dairy Calves

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BACKGROUND and OBJECTIVE: Bovine Rotavirus A (BRVA) is one of the primary etiological agents of neonatal calf diarrhea, causing substantial economic losses in the livestock industry. While commercial vaccines predominantly target the G6 genotype, emerging evidence suggests an increasing prevalence of the G10 strain, highlighting the need for comprehensive investigation into its epidemiology and impact. This study aimed to determine the prevalence of BRVA in diarrheic calves in Saudi Arabia, with a particular emphasis on the molecular detection and genetic characterization of the G10 genotype.

MATERIALS and METHODS: Fecal samples were collected from ten Holstein calves with diarrhea across one farm in their first week of age in the Northern region of Saudi Arabia. Samples were initially screened using a rapid antigen test. Samples testing positive for BRVA were subsequently confirmed by real-time PCR and subjected to sequencing. Phylogenetic analyses were performed to elucidate the genetic relationships of the detected strains.

RESULTS: Nine out of ten samples tested positive for BRVA. Sequencing confirmed that all positive samples belonged to the G10 genotype. Phylogenetic analysis revealed close clustering of these G10 strains with globally reported isolates, underscoring their epidemiological relevance. Notably, the observed prevalence of G10 was higher than reported in previous studies, indicating an epidemiological shift.

CONCLUSIONS: The high prevalence of the G10 strain in calves with diarrhea suggests that existing vaccines that only target the strain G6 may not sufficiently prevent rotavirus infections, particularly in areas where G10 prevalence is increasing. Inclusion of the G10 genotype in commercial vaccines, such as Scourguard 4K®, may improve protection against BRVA infections and reduce associated economic losses in the livestock industry.

Keywords: Bovine Rotavirus A, neonatal calf diarrhea, G10 strain molecular detection genetic characterization

Viral Pathogen-Specific Prevalences During Outbreaks of Bovine Respiratory Disease in Calf Rearing Operations & Dairy Calves in Northwestern Germany

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BACKGROUND and OBJECTIVE: Bovine respiratory disease (BRD) is a major health problem, especially in the calf rearing industry (CRI) due to the high degree of commingling and accumulation of stressors during and after transport. However, also in conventional dairy farms (D), pneumonia & BRD cause significant losses. Viral pathogens are often involved, as they clear the way for bacteria. The objective of this study was to obtain further insights into the prevalence of different viral pathogens involved during outbreaks of BRD, and to compare between different production systems.

MATERIALS and METHODS: A cross-sectional study was performed from December 2018 until December 2025. The target population consisted of calves from both CRI and dairy herds in Northwestern Germany with a current acute outbreak of BRD. Respiratory samples, consisting of either deep nasal swabs (DNS) or nonendoscopic broncho-alveolar lavage (nBAL) liquid were collected from affected calves younger than 4 months and submitted for pathogen detection. Pathogen detection was performed (LUFA Nordwest lab, Oldenburg, Germany) using semiquantitative real-time PCR tests targeting three bovine respiratory pathogens: bovine respiratory syncytial virus (BRSV), bovine parainfluenzavirus type 3 (BPI3V) and bovine coronavirus (BCoV).

RESULTS: In total, 145 outbreaks of BRD were assessed, 58 in CRI calves, 87 in dairy herds. Of these farms, a total of 120 were sampled using swabs (CRI: 51, D: 69), and 25 by use of nBAL (CRI: 7, D: 18). At least one pathogen was detected in 73% of those outbreaks. BCoV was the most frequently identified virus (70%). BRSV and BPI3V were detected in 24,8% (95%CI: 18.03-32.7) and 22,7% (95%CI: 16.2-30.5) of all outbreaks respectively. BPI3V was never detected alone. A significantly higher presence of BRSV, BPI3V and BCoV genome was detected in samples from CRI.

CONCLUSIONS: In this study, bovine coronavirus was shown to be the most prevalent viral agent in samples from calves with clinical BRD. These findings suggest that BCoV should be consistently incorporated into respiratory diagnostic protocols as part of routine practice. Furthermore, BRSV & BPI3V were significantly more present in BRD outbreaks in specialized rearing units, highlighting the need for a multivalent vaccination at entry.

Keywords: bovine respiratory disease, bovine coronavirus, prevalence, calf rearing, production systems

Effects of Neonatal Nutritional Intervention on Clinical Parameters and Long-term Productivity in Dairy Calves

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BACKGROUND and OBJECTIVE: Neonatal calf diarrhea (NCD) and bovine respiratory disease (BRD) are major health challenges causing substantial economic losses in dairy production. While NCD induces acute metabolic disturbances during the neonatal period, its long-term effects on biological potential and lifetime productivity are often underestimated. This study evaluated the effects of a milk-based feed additive administered during the neonatal period on metabolic stability and assessed the long-term impact of neonatal health status and early nutritional intervention on reproductive performance and first-lactation productivity.

MATERIALS and METHODS: The study was conducted over 33 months in two integrated phases

in a Holstein-Friesian × Montbéliarde herd. In the neonatal phase, 40 calves (22 females, 18 males) were randomly allocated to an experimental group (E, n = 20; 11 females, 9 males) receiving 100 g/day of a commercial feed additive (Diakur® Plus, Boehringer Ingelheim, Germany) mixed into whole milk, or to a control group (C, n = 20; 11 females, 9 males) receiving whole milk only. Clinical observations, fecal scoring, diarrhea incidence, average daily gain (ADG), and blood gas and metabolic parameters were monitored. In the long-term phase, 81 female calves were followed to first lactation, including 11 experimental and 70 control animals. Evaluated parameters included age at insemination resulting in pregnancy (AIRP), pre-pregnancy body weight (PPBW), number of inseminations per pregnancy (NIPP), initial calving body weight (ICBW), age at first calving (AFC), and daily milk yield (MY).

RESULTS: Neonatal supplementation significantly improved fecal scores and stabilized blood pH, pCO_2, $cHCO_3^-$, potassium, hemoglobin, and plasma glucose levels ($p < 0.05$), indicating mitigation of metabolic acidosis. Diarrhea incidence and ADG were not significantly affected ($p > 0.05$). Rotavirus was the predominant enteric pathogen. In the long-term evaluation, 36 calves experienced neonatal disease (control n = 33; experimental n = 3), which was negatively associated with first-lactation milk yield ($p < 0.001$; $\beta = -2.144$). Calves receiving neonatal nutritional support achieved higher ICBW and significantly greater first-lactation MY compared to controls ($p < 0.05$), whereas AIRP, PPBW, AFC, and NIPP were not significantly affected. Calves previously infected with *Cryptosporidium parvum* subsequently developed BRD and failed to reach the herd's average milk yield.

CONCLUSIONS: Early-life nutritional intervention enhances metabolic resilience and provides measurable long-term productivity benefits. Improvements in calving body weight and first-lactation milk yield demonstrate that preventing neonatal setbacks preserves biological potential and supports sustainable herd productivity. Strategic early-stage support remains a vital pillar for optimizing future performance and ensuring overall livestock welfare.

Keywords: Neonatal calves, Nutritional supplement, Growth performance, First-calf heifers, Milk yield

Evaluation of Cryptosporidiosis Management Practices on British Cattle Farms

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BACKGROUND and OBJECTIVE: Cryptosporidium parvum is the most common cause of calf scour in Great Britian (GB), accounting for 33% of cases diagnosed. The parasite is highly resistant to cleaning and disinfection, with a rapid life cycle, making prevention challenging. Previously, only antibiotic or antiparasitic treatments were available, which are hard to administer and do not comply with current GB guidelines on blanket livestock antibiotic use. In 2025, a new vaccine for cryptosporidiosis (Bovilis® Cryptium®, MSD) was licenced in GB. The vaccine is administered to the dam during the final trimester of pregnancy, and it is recommended calves are fed colostrum and transition milk for 5 days to help ensure adequate passive immunity is conferred. The aim of this study was to gather data on farms' current practices for managing cryptosporidiosis and provide recommendations to ensure successful vaccination protocols.

MATERIALS and METHODS: The questionnaire was sent to veterinary practices in GB that sold or showed interest in the cryptosporidiosis vaccine. Participating vets were advised to complete the questionnaire with the farmer to ensure honest responses. The questionnaire consisted of 5 sections, each containing 5 questions. The 5 sections were: Diagnosis, Colostrum, Environment and Management, Prevention and Treatment. It focused on key aspects of cryptosporidiosis management, aiming to highlight each farm's practices and areas for improvement.

RESULTS: Eighty-four farms completed the questionnaire during October 2025-January 2026. Environment & Management was the lowest scoring section across the farms. Key FINDINGS: • 83% had diagnosed the cause of calf scour • Only 25% were regularly undertaking total proteins on calf bloods to assess passive transfer • 39% were testing quality of colostrum from cows regularly • Only 48% were

regularly mucking out, cleaning and leaving pens to rest and dry between batches • 51% were using a disinfectant proven against C. parvum • Only 27% were isolating sick calves • 61% were using a dam vaccine for rotavirus, coronavirus and E. coli.

CONCLUSIONS: Cryptosporidiosis management varies widely on GB farms, with few farmers testing calves for failure of passive transfer. As dam vaccines for calf scour rely on successful passive transfer, vets could improve vaccine outcomes by encouraging testing. Hygiene and disinfection are also lacking, yet are crucial for controlling cryptosporidiosis, as oocysts rapidly accumulate and cause disease. Many farmers may not realise the impact of good hygiene on calf health. This study highlights key challenges in managing calf scour caused by C. parvum and could guide future prevention strategies.

Keywords: cryptosporidiosis, calves, colostrum, passive transfer

Prevalence of Cryptosporidiosis in Hungarian Dairy Farms and Its Association with Calf-Rearing Technology, and Risk Control Strategies

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BACKGROUND and OBJECTIVE: Cryptosporidium parvum is a major cause of neonatal diarrhea in dairy calves and has a potential zoonotic risk. While many management strategies are used to prevent transmission, the specific effects of calf-rearing technologies, feeding practices, and hygiene protocols on the prevalence of cryptosporidiosis vary across farming systems. Understanding these associations is critical for developing effective control measures on large-scale dairy farms. The objective of this study was to determine the prevalence of Cryptosporidium infection in preweaned calves on large-scale Hungarian dairy farms and to identify key management, nutrition, and hygiene risk factors associated with the pathogen.

MATERIALS and METHODS: A cross-sectional study was conducted on 45 large-scale dairy herds (average herd size: 970 cows), representing 30% of large-scale dairy farms in Hungary. Data on management, housing, and feeding technology were collected via questionnaire, and individual faecal samples from 518 calves aged 4 to 21 days (10-20 samples / farm) were screened for C. parvum using real-time PCR. Statistical analysis consisted of Mann-Whitney U tests comparing factors between C. Parvum positive and negative farms, and Spearman correlations for prevalence associations and were performed in R. Statistical significance was set at $p<0.05$.

RESULTS: The parasite was detected on 82.2% (n=37) of farms, with an animal-level prevalence of 28.8%. Farms with positive Cryptosporidium results had significantly higher morbidity rates; mortality and diarrhea incidence did not differ significantly. Random forest classification and generalized linear mixed models identified whole milk feeding practices and halofuginone use as the strongest predictors of infection. Feeding unpasteurized whole milk increased the odds of infection (odds ratio = 4.67; 95% CI: 1.98–12.08) compared with not feeding whole milk. Equally, the absence of halofuginone treatment increased the risk (odds ratio = 2.09; 95% CI: 1.01–4.44). No significant associations were found with total hygiene scores or transition milk feeding.

CONCLUSIONS: Cryptosporidiosis is widespread in Hungarian dairy herds and significantly contributes to calf morbidity. Nutrition factors (whole milk, milk replacer) can influence the prevalence of Cryptosporidium on the farm. Specifically, feeding unpasteurized whole milk is a major risk factor, whereas pasteurization serves as an effective hygiene control point. The use of halofuginone further reduces the prevalence of Cryptosporidium parvum infection.

Keywords: Cryptosporidium parvum, management, hygiene, calves

Transplacental Bluetongue Virus Serotype 3 Infection Resulting in Anencephaly in a Twin Charolais Calf

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BACKGROUND and OBJECTIVE: Bluetongue virus (BTV) is an Orbivirus that affects domestic and wild ruminants and is transmitted by Culicoides biting midges. Transplacental infection during critical stages of gestation may result in reproductive failure and congenital central nervous system (CNS) malformations. Recently, BTV serotype 3 has emerged in several European regions, raising concerns regarding its reproductive impact and herd productivity.

MATERIALS and METHODS: A twin pregnancy in a Charolais cow in south Portugal, resulted in one live-born calf and one stillborn, mummified calf. The live-born calf presented severe neurological abnormalities, including bilateral horizontal nystagmus, persistent neck extension, absent pupillary reflex, and lack of proprioception and deep pain perception in the pelvic limbs. The co-twin exhibited limb deformities consistent with arthrogryposis, absence of hair coat, brownish skin discoloration and marked subcutaneous edema. Considering the findings in both calves, a congenital infectious etiology was suspected. Due to the severity of neurological deficits and incompatibility with life, the live-born calf was euthanized. Heparinized blood, cerebrospinal fluid (CSF), and tissue samples were collected from the euthanized calf for laboratory investigation. During necropsy, the cranial cavity was opened, revealing large blood clots and only a small rudimentary neural structure, which was submitted for histopathological examination. No additional congenital malformations were observed. No samples were collected and no post-mortem examination was performed on the mummified co-twin.

RESULTS: Pathological analysis confirmed the absence of cerebral hemispheres, excluding microencephaly and supporting a diagnosis of anencephaly. Real-time PCR testing was positive for BTV serotype 3.

CONCLUSIONS: This case supports the teratogenic potential of BTV serotype 3 in bovines following in utero infection, particularly during the critical gestational window (approximately 75–150 days), when CNS development is highly susceptible to viral damage. The occurrence of anencephaly in one twin and arthrogryposis in the mummified co-twin further reinforces the role of transplacental BTV infection in congenital abnormalities. Although this case appeared to be isolated within this herd, several other cases of neurologic signs in newborn calves were reported by practitioners in southern Portugal. These findings highlight the importance of surveillance, reproductive monitoring, and vector control strategies in regions where BTV serotype 3 is circulating.

Keywords: Blue Tongue virus, teratogenic defect, anencephaly, nervous system

Phytobiotic Supplementation as a Sustainable Strategy to Enhance Growth and Resilience Against "cryptosporidium Parvum" in Neonatal Calves

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BACKGROUND and OBJECTIVE: Phytobiotic compounds are promising alternatives in livestock production. They enhance intestinal health by supporting epithelial barrier integrity, modulating inflammatory responses, and reducing pathogen dissemination, acting as adjuvants against enteric diseases like cryptosporidiosis. This study evaluated the effects of a specific phytobiotic mixture on the growth performance and incidence of protozoan-associated diarrhea in female dairy calves.

MATERIALS and METHODS: Newborn calves were randomly allocated in two experimental groups. All received unpasteurized colostrum at 10% of their body weight, and were housed individually after a 24–48-hour transition. Feeding consisted of 6 L/day of pasteurized whole milk, divided into two daily feedings of 3 liters each. All calves had ad libitum access to a mash starter feed and fresh water throughout the study. Control group (n=15) received

the standard basal diet without supplementation; and Trial group (n=15) received the basal diet supplemented with 5 g/day of the phytobiotic compound, administered as a top-dressing in the morning milk feeding. Fecal samples were analyzed via Kinyoun staining for “Cryptosporidium Parvum”. Body weight and morphometric measurements were recorded at 30 and 60 days.

RESULTS: All Control calves tested positive for “Cryptosporidium Parvum”, with 87.5% requiring pharmacological treatment for first-pass diarrhea (day 6), and 12.5% for secondary infections (day 15). While the Trial group tested positive on day 16, 80% of calves presenting diarrhea resolved the condition without pharmacological intervention, with no recurrences. The Trial group showed lower pneumonia incidence (9% vs. 12.5%) and reduced mortality (12.5% vs. 20%), representing a 37.5% reduction in total losses and a significant improvement in the survival rate. Supplementation significantly improved growth performance (P<0.01). At 30 days, the Trial calves showed a higher Average Daily Gain (ADG) (0.489 kg vs. 0.273 kg; P=0.0017). By day 60, the Trial group reached an ADG of 0.713 kg vs. 0.346 kg in Control group (P<0.0001) and a total height gain of 10.18 cm vs. 7.66 cm in the Control group (P=0.0085).

CONCLUSIONS: Dietary supplementation with this phytobiotic mixture significantly enhances growth performance and skeletal development in neonatal calves. It demonstrates a robust "antibiotic-sparing" effect by modulating the immune response, allowing animals to resolve enteric challenges such as “Cryptosporidium Parvum” independently. These findings suggest that this phytobiotic blend is a highly effective and sustainable alternative to traditional pharmacological treatments, optimizing animal health and economic efficiency in dairy production.

Keywords: "Cryptosporidium Parvum", Phytobiotic, Intestinal health

Mastering The First Milk: How Day 1 is Revolutionizing Calf Health in France with the World's Largest Colostrum Database

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BACKGROUND and OBJECTIVE: Colostrum, the first milk produced by a cow after calving, is rich in essential antibodies that strengthen the calf's immune system and protect it against diseases. Providing high-quality colostrum is vital for the calf's survival and growth, and it also serves as a reliable indicator of the cow's health and proper calving preparation. This report describes the implementation of DAY 1 in France.

MATERIALS and METHODS: DAY 1 is a digital tool developed by VIRBAC. It has been purposely designed to help farmers optimize the quality of colostrum administered to calves immediately after birth. Data has been collected in France through the DAY 1 application. The tool operates using two main components. The "My Monitoring" (*Mon Suivi*) feature allows individual farmers to record specific calving data—such as colostrum quality (Brix value), cow breed, vaccinations, and feed—monitor their herd's evolution, and generate customized reports to receive targeted veterinary advice. Additionally, the "Observatory" (*Observatoire*) features pooled data from numerous farms into a national database. This enables farmers to benchmark their performance against peers, identify the most effective best practices, and better understand risk factors that negatively impact colostrum quality.

RESULTS: The field deployment of Day 1 has been a significant technological and human success. By transforming raw data into clear visual indicators, the tool has strengthened the veterinarian’s role as a technical advisor. Over the past five years, DAY 1 has enabled the collection of a significant body of data: 13,800 carefully linked colostrum data points to 13 rearing variables, 392 breeders are now registered and convinced of the benefits of the Day 1 service, 130 veterinary clinics are promoting Day 1 to breeders and leveraging the information in their daily practice and a fair representation of national livestock farming with an equivalent amount of data from dairy and beef cattle farming.

CONCLUSIONS: To make a long story short, DAY 1 offers a personalized yet collective approach, enabling farmers to better understand the importance of colostrum, improve calf health, and optimize production. DAY 1 represents a true revolution in the field of cattle farming, placing health of the newborn calf at the heart of concerns from its very first hours of life.

Keywords: colostrum management, calf health, benchmarking, herd health monitoring

Colostrum as the Ultimate Mirror of Herd Management: Elevating Preventive Herd Health Through the 'Day 1' Digital Tool

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BACKGROUND and OBJECTIVE: The passive transfer of immunity (PTI) remains critical to neonatal calf health and subsequent lifetime productivity. Whereas the monitoring of reproductive performance and milk quality is standard veterinary consultancy, colostrum management is frequently overlooked. This lack of interest is particularly acute in the suckler cow sector, where colostrum quality is one of the few objective and quantifiable indicators for assessing the effectiveness of dry cow management (encompassing nutrition, body condition assessment, anthelmintic strategies and vaccination schedules). Efficacious monitoring must transcend isolated quality assessments (such as Brix refractometry) to incorporate a holistic paradigm that includes volume, harvest timing, and pre-calving husbandry practices. Furthermore, the lack of a reference benchmark measurement frequently hinders producers' awareness and motivation to improve their management protocols.

MATERIALS and METHODS: To overcome this deficit, Virbac has engineered Day 1, a digital application devised to structure and streamline colostrum auditing. This clinical tool enables veterinary surgeons and producers to aggregate and centralise their farm data, specifically pertaining to colostrum quality and administration protocols, as well as essential management variables (including antiparasitic treatments, trace element supplementation, and environmental hygiene). The software computationally generates correlation analyses to elucidate distinct success factors or limiting constraints specific to cattle operation.

RESULTS: The field implementation of Day 1 has proven highly efficacious from both a technological and practical standpoint. By translating raw data into coherent visual metrics, the application has promoted the veterinary surgeon's capacity as a strategic consultant. Users are afforded a robust benchmarking functionality, permitting the comparison of individual herd performance against regional or national aggregates. This comparative framework acts as a motivational driver, repositioning colostrum management as a collaborative performance objective between the veterinary practitioner and the producer. First appraisals indicate a substantial enhancement in adherence to pre-calving protocols once the direct correlation with colostral quality is quantified and visualised.

CONCLUSIONS: Colostrum quality serves as the pre-eminent indicator of pre-partum preparation: an optimally nourished, appropriately housed, and adequately immunised dam invariably yields superior colostrum. Through Day 1, Virbac offers a digital intervention that transitions colostrum monitoring from an observational afterthought to an action plan of preventive herd health, analogous to reproductive monitoring. We anticipate expanding the accessibility of this reliable tool to a broader demographic of veterinary professionals and producers in Europe, thereby striving to standardise precision health management across both the dairy and beef sectors.

Keywords: colostrum management, calf health, benchmarking, herd health monitoring

Pathogen Profiles of Bovine Respiratory Disease in Great Britain

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BACKGROUND and OBJECTIVE: Bovine Respiratory Disease (BRD) is a multifactorial condition which remains a leading cause of calf morbidity and mortality in Great Britain. Improved understanding of contributing pathogens at herd level is essential for developing effective preventative health strategies. Polymerase Chain Reaction (PCR) testing detects nucleic acids of pathogens present in acute cases, while retrospective pathogen exposure can be assessed using serological testing. This study aimed to evaluate changes in the proportional contribution of BRD pathogens in British cattle herds since 2020-2022 and to review additional patterns.

MATERIALS and METHODS: From January 2023 to December 2025, 1406 calves from 279 farms across Great Britain were sampled. Five blood samples were collected per farm from unvaccinated calves aged 3–6 months not acutely affected with BRD. Commercial Enzyme Linked Immunosorbent Assay (ELISA) testing was undertaken for antibodies against Bovine Coronavirus (BCoV), Bovine Respiratory Syncytial Virus (BRSV), *Mannheimia haemolytica (M. haemolytica)*, *Mycoplasma bovis (M. bovis)* and Parainfluenza Virus 3 (PI3). Between August 2024 and December 2025, PCR tests were performed on pooled (3-5 samples/pool) nasopharyngeal (NP) swabs from acutely affected animals on 64 farms to detect BCoV, BRSV, *M. haemolytica*, *M. bovis*, PI3, *Histophilus somni (H. Somni)*, Bovine Herpesvirus 1 (BoHV-1) and *Pasteurella multocida (P. multocida)*.

RESULTS: Serological prevalence data was similar to previous years. BCoV had the highest virus seroprevalence (71.6%), followed by PI3 at 67.8% and BRSV at 21.6%. Antibody responses were detected in 69.7% of calves for *M.haemolytica* and 19.7% for *M.bovis*. BCoV was also the most frequently detected virus by PCR (43.8%), followed by BRSV (29.7%), PI3 (12.5%) and BoHV-1 (10.9%). Bacterial detection rates were *M. bovis* (32.8%), *H. somni* (50%), *M.haemolytica* (65.6%) and *P. multocida* (96.9%). Three or more pathogens were detected by PCR in 78.2% of samples, comparable to serological findings where 77.8% of farms showed antibody responses to three or more pathogens. Of the samples where BCoV was detected by PCR, in 59.3% of cases it was the only virus detected. Out of all the PCR tests, in 25% BCoV was the sole virus detected. *M. bovis* co-occurred in 33.3% of BCoV - positive PCR samples, with 43.3% serological co-positivity.

CONCLUSIONS: Pathogen profiles associated with BRD in Great Britain remain consistent with historical data. Notably, BCoV was the sole viral pathogen detected (by PCR on NP swabs) in a quarter of acute cases, highlighting its importance within the BRD complex.

Keywords: BRD, pathogen, prevalence

The Impact of Colostrum Quality, Passive Transfer on Pre- and Post Weaning Brdc Susceptibility in Dairy Calves Based on Thoracic Ultrasound Assesment

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BACKGROUND and OBJECTIVE: Bovine Respiratory Disease Complex (BRDC) causes significant economic losses on dairy farms, leading to reduced weight gain and increased mortality. While colostrum intake is critical for neonatal calf immunity against various pathogens, early diagnosis of respiratory issues remains challenging. Thoracic (lung) ultrasonography (TUS) outperforms traditional diagnostic methods such as auscultation (6% sensitivity) and Wisconsin scoring (63%), with 79.4% sensitivity and 93.9% specificity. Our research aimed to investigate the correlation between colostrum quality and passive transfer efficiency, and to assess their combined role in the development of BRDC both before and after weaning.

MATERIALS and METHODS: Thirty Holstein Friesian heifer calves from a large Hungarian dairy farm were enrolled in the study between June and September 2024 and followed from birth to 6 months of age. Colostrum samples were collected pre-feeding for bacteriological analysis and BRIX% measurement. Calves' birth weights were recorded, and detailed clinical examinations were performed nine times from birth until weaning. TUS assessments were performed three times, pre- and post-weaning.

RESULTS: Our findings indicate that contaminated colostrum, particularly when contaminated with more than 3 pathogens, significantly impaired passive transfer success ($p<0.005$). All five calves (n=5) that received contaminated colostrum developed clinical BRDC both pre- and post-weaning. Pre-weaning TUS scores showed a significant difference ($p=0.041$) between calves fed contaminated colostrum and those fed pathogen-free colostrum; however, this difference was not significant post-weaning ($p=0.09$). Calves categorized with severe pre-weaning TUS lesions (scores ≥3, n=12) demonstrated a significantly lower average daily gain (0.62 kg/day) until weaning compared to control calves (0.72 kg/day) ($p=0.039$). Post-weaning TUS scores, however, did not significantly differ between these groups.

CONCLUSIONS: Our study confirms a significant impact of poor colostrum quality on the development of pre-weaning BRDC. The lack of a statistically significant post-weaning effect might be attributed to environmental factors, such as suboptimal ventilation in group-housed calves or the limited number of calves receiving pathogen-free colostrum. Overall, these results underscore the critical need for substantial improvements in colostrum feeding hygiene at the investigated farm, since pathogen presence in the colostrum demonstrably compromises calf health and growth performance.

Keywords: calf, TUS, BRDC, colostrum

Prevalence of Respiratory Disease Signs Based on Thoracic Ultrasound and Clinical Respiratory Scores in Unweaned Calves Before- and After Long-distance Transport

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BACKGROUND and OBJECTIVE: Hundreds of thousands of unweaned calves are transported across the European Union each year. Transport exposes calves to multiple stressors, including commingling, pathogen exposure, fasting, and environmental challenges, at an age when their immune system is underdeveloped. However, the prevalence of respiratory disease signs in these calves remains largely unknown. This study assessed respiratory disease prevalence via thoracic ultrasonography (TUS) and clinical respiratory scoring (CRS) in calves transported from Ireland to the Netherlands.

MATERIALS and METHODS: Sixty five unweaned calves were scored (TUS and CRS) before transport in Ireland, shortly after arrival in the Netherlands, and approximately one-, two-, and three- weeks post arrival. The thoracic ultrasound scoring system classified calves as having normal aerated lung tissue in all four lobes (TUS 0), diffuse comet tail artefacts in one to four lobes (TUS 1), lobular or patchy consolidation (TUS 2), lobar consolidation in one lobe (TUS 3), or lobar consolidation in two lobes (TUS 4). Clinical respiratory scores classified calves as having a total score of 0 (healthy), 1 (mild respiratory disease signs), 2 (moderate respiratory disease signs), or 3 (severe respiratory disease signs), based on rectal temperature, coughing, nasal discharge, eye discharge, and ear position. A

weighted Cohen's Kappa test was conducted to compare TUS and CRS.

RESULTS: Pre transport, abnormal TUS (score ≥ 1) affected 53.1% of calves, with 4.7% showing severe signs (TUS ≥ 3). On arrival, abnormalities were observed in 59.4% of calves, while severe cases increased to 12.5%. One week post transport, abnormal and severe scores were 71.9% and 10.9%, decreasing to 67.2% and 15.7% at two weeks, and 57.8% and 6.2% at three weeks. Abnormal CRS occurred in 9.2% of calves pre-transport, 69.2% on arrival, 56.9% at one-week, 52.3% at two weeks, and 40.0% at three weeks post arrival. Severe CRS (score = 3) were only observed post arrival, occurring in 3.1% of calves at one-, two-, and three-weeks. A weighted Cohen's Kappa test yielded a value of 0.038, indicating an agreement close to zero. Severe TUS never correlated to a severe CRS. Across all timepoints, TUS consistently identified more abnormalities than CRS.

CONCLUSIONS: Thoracic ultrasound lesions were common throughout the observation period, while clinical signs were rare pre transport but peaked on arrival, though severe cases remained rare. These findings indicate that relying solely on clinical signs may underestimate true prevalence, and ultrasound provides a more sensitive assessment of transport associated respiratory disease. The poor agreement between TUS and CRS highlights the need for improved screening methods.

Keywords: calf transport, bovine respiratory disease, thoracic ultrasound, clinical respiratory score

Effect of Parenteral Supplementation with Copper, Zinc, and Selenium on Weight Gain and Mineral Status in Suckling Calves

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BACKGROUND and OBJECTIVE: The aim of the present study was to evaluate the effect of parenteral supplementation with copper (Cu), zinc (Zn), and selenium (Se) on weight gain and mineral status in suckling calves.

MATERIALS and METHODS: A completely randomized study was conducted on a farm with a diagnosis of white muscle disease associated with Se and Cu deficiency (Cintra, Córdoba, Argentina). Three-month-old Aberdeen Angus calves (145.5±19.9kg) remained with their dams from the beginning of the study (Day 0, D0) until weaning (Day 90, D90). On D0, calves were stratified by sex and randomly assigned to one of two treatments. The supplemented group (SUP; n=36; 18 males and 18 females) received a subcutaneous dose of 0,3 mg Cu/kg body weight (BW), 1 mg Zn/kg BW, and 0,2 mg Se/kg BW (Suplenut® Se, Biogénesis Bagó SA). The control group (CTRL; n=36; 18 males and 18 females) received no supplementation. Animals were weighed on D0, D29, D62, and D90. At each time point, blood samples were collected to determine plasma Cu and Zn concentrations and whole-blood glutathione peroxidase (GPx) activity as an indicator of Se status. Data were analyzed using linear mixed-effects regression models with repeated measures (lme4 package, R software). Fixed effects included treatment, time, their interaction, and sex, while animal was included as a random effect. Statistical significance was set at $p \leq 0.05$.

RESULTS: Average daily gain (ADG) was not affected by sex ($p=0.35$) but was affected by treatment ($p<0.01$), time ($p<0.01$), and their interaction ($p<0.01$). This interaction was observed because ADG between

D0 and D29 was greater in the SUP group than in the CTRL group (0.844±0.03 vs. 0.627±0.03 kg/day, respectively; p<0.01), while no significant differences were observed between D29 and D62 (SUP: 0.499±0.03; CTRL: 0.431±0.03 kg/day; p=0.11) or between D62 and D90 (SUP: 0.749±0.03; CTRL: 0.692±0.03 kg/day; p=0.18). Treatment also affected overall ADG (D0 to D90; SUP: 0.688±0.02 vs. CTRL: 0.575±0.02 kg/day; p<0,01). GPx activity and plasma Cu concentration were affected by treatment, time, and their interaction (p<0.01); no differences were observed at D0 (GPx: SUP 30.3±1.6, CTRL 26.2±1.6 U/mL hematocrit; Cu: SUP 8.4±1.5, CTRL 8.3±1.5µg/dL), whereas higher values were observed in SUP from D29 onward. Plasma Zn concentration was affected only by time (p<0.01).

CONCLUSIONS: In conclusion, under the conditions of this study, parenteral supplementation with Cu, Zn, and Se increased weight gain in calves deficient in Se and Cu and improved the status of these two minerals.

Keywords: Calves, Copper, Selenium, Parenteral supplementation, Weight gain, Mineral status.

Effect of Herd Size on Pre-Weaning Calf Management, Morbidity and Mortality

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BACKGROUND and OBJECTIVE: Herd size may be a surrogate metric for structural differences in farmer societal categories with best-practice adopters often associated with large herds and traditionalists with small herds. Given the wide, and changing, range in herd sizes in Poland, this study aimed to describe and the effect of herd size on pre-weaning calf management practices and to identify areas for improvement.

MATERIALS and METHODS: Data were collected using a cross-sectional questionnaire survey with five sections (farm characteristics, general calf management, colostrum management, calf feeding and weaning and calf health management and outcomes) from 557 dairy farmers across 9 provinces in Poland. Herd size was categorised as small (<20 cows; 154 herds), medium (21 to 50 cows; 247) and large (≥51 cows; 144). The significance of associations between herd size group and questionnaire responses were analysed using R. Independence was tested with Fisher's exact test and p-values were computed through Monte Carlo simulation with 105 replications.

RESULTS: There was many recommended practices implemented on these farms: harvesting colostrum immediately after calving (68% of farms), feeding colostrum within two hours of birth (90%) and offering more than one colostrum feed (85%). There were also good calf health outcomes: <5% stillbirth rate (84%) and <5% pre-weaning calf mortality (69%). However, there were also many suboptimal practices: not checking calves for FPTI (95%), no calf vaccinations (78%) and no colostrum quality check (83%). There were statistically significant effects of herd size on many variables. Farmers with large herds were more likely to store colostrum for prolonged periods, check colostrum quality, use an oesophageal feeder for colostrum, use a colostrum supplement/replacer, use an autofeeder, feed textured ration, administer electrolytes to diarrhoeic calves and vaccinate calves. In contrast, farmers with small herds were more likely to rear calves individually, store colostrum in a fridge, feed colostrum within one hour of birth and call their vet to cases of calf diarrhoea. They were also more likely to not heat colostrum before feeding, feed only 4-5L milk replacer/day, feed pelleted or ground ration and wean calves early (5-6 weeks).

CONCLUSIONS: Overall, many suboptimal calf management practices were common in this farmer population sample; this is also an issue internationally. Herd size significantly impacted the likelihood of adoption of many practices. Farmers with large herds were, in general, more likely to adopt more contemporary best management practices.

Are Congenital Defects More Common in Calves Derived from *in vitro* Produced Embryos?

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BACKGROUND and OBJECTIVE: The objective of this study was to characterize the main causes of death in calves derived from in vitro produced (IVP) embryos that had been submitted for postmortem examination (PME).

MATERIALS and METHODS: A total of 1,592 embryos were transferred (Dairy Fresh IVP embryos= 328, Dairy Frozen IVP embryos = 328, Beef Fresh = 576, Beef Frozen = 273 and Dairy JIVET (embryos produced from 2-3 month old dairy calves- juvenile in vitro fertilization and embryo transfer, Fresh = 87), resulting in 746 pregnancies that reached fullterm in dairy cows. A total of 50 calves were stillborn or died postpartum, of which 44 calves were submitted for PME (Dairy Fresh = 13, Dairy Frozen = 6, Beef Fresh = 19, Beef Frozen = 6 and Dairy JIVET Fresh = 2). Cause of death (COD) was identified at the PME. The time of death was categorized as 1-5 (1 = died ≤ 30 minutes after birth; 2 = >30 mins and ≤ 24 hours; 3 = > 24 hours to ≤ 7 days; 4 = 8 to 21 days; and 5 = > 21 days).

RESULTS: Mean ± SD gestation length for all calves was 277.7 ± 7.9 days and mean birthweight was 44.5 ± 14.5 kg. Of the 44 necropsied calves, 36.4% weighed > 50 kg. Cause of death was categorized as dystocia (n =15), congenital defect (n = 11), infection (n = 5), multiple causes (n = 4), other (n = 5) and diagnosis not reached (n = 4). Of the 11 diagnoses of congenital defect COD, the organ most affected was the intestines (5), followed by the liver (2) and heart (1). Congenital defect categories identified (but not necessarily as COD) during PME in 28 calves (63.6%) were multiple defects (13), LOS (6), intestinal atresia (4), cardiac defects (2) and other (3). Of the 44 calves that were submitted for PME, congenital defect was found to be a contributor to COD for 47.7% (n = 21 calves). The % of calf deaths for each of the 5-time categories were as follows: 61.4, 13.6, 9.1, 6.8, and 9.1 for categories 1, 2, 3, 4, and 5, respectively.

CONCLUSIONS: Dystocia and congenital defect were the two primary COD identified in calves derived from IVP embryos. This COD profile differs from that of non-IVP calves where dystocia is a primary COD but congenital defect is a much less common COD.

Keywords: Congenital defects, IVP embryos, Calves

Retrospective Investigation of Congenital Malformations in Aborted and Stillborn Bovine Fetuses

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BACKGROUND and OBJECTIVE: In Brazil, epidemiological data on fetal malformations are scarce, particularly in Mato Grosso, the state with the largest cattle herd in the country. The objective of this study was to determine the prevalence, characterize morphological patterns, and assess the involvement of Bovine Viral Diarrhea virus (BVDv) in bovine fetuses with congenital anomalies.

MATERIALS and METHODS: A retrospective descriptive study was conducted by review of 150 necropsies of aborted or stillborn bovine fetuses submitted to the Veterinary Pathology Laboratory of the Federal University of Mato Grosso (LPV-UFMT) between 2004 and 2025. Cases presenting gross findings consistent with congenital malformations were included. Anomalies were classified according to the affected body system and categorized as isolated, multiple, or syndromic. Pooled samples of central nervous system, thymus, and spleen were submitted to immunohistochemistry (IHC) and reverse transcription-polymerase chain reaction (RT-PCR) for detection of BVDv in 15 fetuses. BVDv vaccines are available in the study region.

RESULTS: Of the 150 fetuses evaluated, 26 (17.3%) presented congenital malformations. Most

malformed fetuses originated from farms in Mato Grosso (84.6%), and submissions were more frequent during the dry season (61.5%), especially between August and October, coinciding with the calving period. Dystocic calving was observed in 15.4% of cows. The Nelore breed (beef) was the most affected (50%), reflecting the regional cattle breed profile. Anomalies were predominantly identified in the last third of gestation (69.2%). Multiple malformations were the most frequent presentation (61.5%), followed by isolated musculoskeletal defects (19.2%). The most commonly observed lesions included scoliosis, arthrogryposis, brachygnathism, and hydranencephaly. Among the 15 fetuses tested for BVDv, 5 (33.3%) were positive, with arthrogryposis being the main associated abnormality.

CONCLUSIONS: The prevalence of congenital malformations found is much higher than that expected in the general cattle population. This likely reflects the lethality of some of these malformations. Additionally, some malformations cause dystocia which if severe requires veterinary assistance. Consequently, cases are more likely to be referred to the laboratory - submission bias. The detection of BVDv in a substantial minority of cases indicates the need for more effective on-farm control programmes. The high prevalence of multiple, non-syndromic, defects suggests possible random genetic aetiologies such as de novo mutations (DNMs). It is concluded that congenital anomalies represent a significant proportion of fetal losses diagnosed during the study period, highlighting the need for sanitary surveillance and systematic etiological investigation to reduce reproductive impacts in cattle herds of Mato Grosso.

Keywords: Congenital defects, Aborted, Stillborn

Risk Factors for and Prevalance of Failure of Passive Transfer of Immunity in Calves on Dairy Farms in Türkiye

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BACKGROUND and OBJECTIVE: Failure of passive transfer of immunity (FPTI) remains a major challenge for neonatal calf health, directly affecting morbidity and mortality in dairy herds. Adequate and timely colostrum intake is essential to ensure sufficient IgG absorption. Although information on FPTI in Türkiye is limited, comprehensive data covering different regions remain insufficient. This study aimed to determine the prevalence of FPTI and to evaluate herd- and calf-level factors associated with its occurrence in dairy herds across Türkiye.

MATERIALS and METHODS: Blood serum samples from 1,026 calves in 76 dairy herds across 16 provinces of Türkiye were analyzed using a digital Brix refractometer to assess FPTI, defined as a Brix value <8.5%. Data on potential risk factors—including calf sex, age, breed, parity of the dam, colostrum feeding method, season of birth, region, and herd size—were collected. Herd size was categorized into seven groups based on the number of lactating cows Group I (≤20), Group II (21–100), Group III (101–200), Group IV (201–300), Group V (301–500), Group VI (501–1000) and Group VII (>1000). The effects of dam, calf, and herd management variables on the occurrence of FPTI were examined through the application of logistic regression analysis.

RESULTS: FPTI incidence was 22.4% overall. There was a significant effect of herd size group on

occurrence of FPTI (p=0.002). The lowest prevalence of FPTI was in the largest herd size group (Group VII; 12.3%), and the highest prevalence was in the second smallest herd size group (Group II; 43.4%). There was also a significant effect of herd size group on Brix value. Dam parity (p=0.005), calf breed (p<0.001), birth season (p=0.005) and feeding method (p=0.003) group on occurrence of FPTI. When the calves were compared according to how they received colostrum, the prevalence of FPTI was lower in bottle-fed calves or calves fed with a stomach tube compared to those left with their mothers. Calf age at sampling, calf sex, farm region and colostrum volume fed were not significantly associated with the occurrence of FPTI.

CONCLUSIONS: The prevalence of FPTI observed in this study was consistent with findings reported in other countries. Significant associations were identified between FPTI and both controllable factors, such as colostrum administration practices and season of birth, and inherent factors, including herd size, dam parity, and calf breed.

Keywords: Failure of Passive Transfer of Immunity, herd size, calves, dairy cows

Optimizing Serum Total Protein Cut-offs for Assessing Passive Transfer in Dairy Calves

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BACKGROUND and OBJECTIVE: Calves are born agammaglobulinemic and rely entirely on colostrum to acquire passive immunity. On-farm assessment of colostrum management is typically performed indirectly using serum total protein (STP) measured by refractometry, whereas in laboratory setting immunoglobulin G (IgG) is quantified directly. However, indirect methods may be influenced by calf-related factors, and reported STP cut-offs vary between studies. This study aimed to determine optimal STP cut-offs for assessing passive transfer of immunity in neonatal calves and to evaluate the influence of calf-related factors on these thresholds.

MATERIALS and METHODS: Blood samples were collected from 1–7-day-old calves in 117 commercial Estonian dairy herds with at least 100 cows. STP was measured from centrifuged serum samples by calibrated refractometer and IgG concentration by ELISA. Optimal STP cut-offs were determined for IgG thresholds of < 10 g/L (minimum acceptable level of passive transfer) and ≥ 18 g/L (good passive immunity). Diagnostic performance of STP was assessed using receiver operating characteristic (ROC) analysis. Analyses were performed overall and stratified by calf age, breed, and sex. The study was supported by the Estonian Research Council grant (PSG268).

RESULTS: A total of 1,039 calves were included (3–17 calves per herd). Mean calf age at sampling was 4.1 days. STP ranged from 3.2 to 9.8 g/dL (mean 5.4 g/dL) and serum IgG from 0 to 96.2 g/L (mean 14.6 g/L). Inadequate passive transfer (IgG < 10 g/L) was identified in 34.4% of calves, while 27.8% had IgG ≥ 18 g/L. STP and IgG concentrations were moderately correlated (Spearman's $\rho = 0.67$, $p < 0.001$). The optimal STP cut-off for detecting IgG < 10 g/L was 5.4 g/dL (sensitivity 69%, specificity 86%), and for IgG ≥ 18 g/L was 5.6 g/dL (sensitivity 78%, specificity 73%). ROC analysis demonstrated good discriminatory ability for both thresholds (AUC = 0.86 and 0.81, respectively). For both IgG thresholds, optimal STP cut-offs were higher in Holstein calves and in male calves. Optimal STP cut-offs varied across days of age (range 5.0–5.7 g/L) without a consistent linear pattern. Age-stratified analyses demonstrated highest diagnostic accuracy for IgG < 10 g/L around days 3–5, whereas the accuracy for IgG ≥ 18 g/L was greatest during the first three days of life.

CONCLUSIONS: STP demonstrated good discriminatory ability for assessing passive transfer; however, variation in diagnostic performance and optimal cut-offs across calf subgroups indicates that interpretation should not rely on a single universal threshold.

Keywords: Immunoglobulin G, field diagnostics, age-specific analysis, commercial dairy farms, diagnostic performance

Immune Cell Responses to Bacterial Respiratory Disease Vaccination in Co-housed Calves: Effects of Supplementing Japanese Black (Wagyu) Dams with Probiotic Live Yeast Saccharomyces Cerevisiae Boulardii Cncm I-1079

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BACKGROUND and OBJECTIVE: Japanese Black (Wagyu) calves are often reared for a period alongside their dams after birth. The probiotic live yeast *Saccharomyces cerevisiae* boulardii CNCM I-1079 (SCB) has a positive effect on calves' immune system. However, calves drink milk directly from their dams when kept with them, making it difficult to provide supplementation. In swine, it has been reported that supplementing dams with SCB improves the performance of suckling piglets; nevertheless, there are few reports of this procedure in co-housed cow-calf pairs. This study investigated the effect of maternal SCB supplementation in Japanese Black dams on the immunological response of their co-housed young calves to vaccination against bacterial respiratory diseases.

MATERIALS and METHODS: This study included 20 Japanese Black dams and their calves from a commercial farm. Dams in the SCB group (n = 10) were supplemented with SCB starting one month prior to their expected calving date. Dams in the control group (n = 10) did not receive the supplementation. Calves were separated from their dams at 21 days of life and vaccinated at 21 and 42 days of age. Blood samples were collected at 3, 21 (immediately prior to primary vaccination), 42 (immediately prior to booster vaccination), 45, and 63 days of age.

RESULTS: The number of CD21+ cells in the SCB group calves was higher than that in the control group at 42 days of age ($p < 0.10$). At 45 days of age (3 days after the booster vaccination), the number of CD4+CD45R- cells in the SCB group was significantly higher than that in the control calves ($p < 0.05$), as were the numbers of CD4+CD45R+ and MHC+CD14- cells ($p < 0.10$). By 63 days of age, the SCB group exhibited a significantly higher number of CD4+CD45R+ cells compared with the control group ($p < 0.05$), as well as similarly higher numbers of granulocytes and CD14+ cells ($p < 0.10$).

CONCLUSIONS: Supplementing Japanese Black dams with SCB may have a positive influence on the immune response to vaccination of their co-housed young calves.

Keywords: Japanese Black cattle, Saccharomyces cerevisiae boulardii, Probiotics, Early vaccination

Efficacy of an Intranasal Bovine Coronavirus Vaccine on Lung Health and Growth Performance in Pre-Weaned Calves

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BACKGROUND and OBJECTIVE: Bovine respiratory disease is a significant cause of morbidity and poor growth in milk-fed calves. Bovine coronavirus is a recognised contributor to this disease complex. The objective of this study was to evaluate the effect of an intranasal modified-live bovine coronavirus vaccine on lung health, assessed by thoracic ultrasound, and average daily weight gain (ADG) in pre-weaned calves under field conditions.

MATERIALS and METHODS: A prospective cohort study was conducted on 135 milk fed calves sourced from a single farm. Upon arrival at the calf-rearing facility, calves were housed in group hutches and allocated by row to either receive a commercial intranasal modified-live bovine coronavirus vaccine (Bovilis Nasalgen-C, MSD Animal Health) (n = 70, VACC) or serve as unvaccinated controls (n = 65, CON). Calves were weighed and underwent thoracic ultrasound (TUS) at a mean of 28 days of age (T1), and again 4 weeks later (T2). Lung consolidation was

scored on a 0–3 scale: 0 = no lesions/isolated comet tail, 1 = multiple comet tails and/or lesion/s <1cm, 2= one or more lesions ≥1cm-6cm, 3 = extensive consolidation (≥6cm, abscessation or significant pleural effusion). Calves identified with a TUS of 2 or 3 were treated immediately with an antibiotic and non-steroidal anti-inflammatory.

RESULTS: Time between T1 and T2 varied between 27 and 30 days, with a mean of 29 days. ADG overall was 0.69 kg. An independent-samples t-test showed that average weight gain was significantly higher in VACC calves (0.73 kg) than in CON (0.65 kg) (p = 0.015). No difference was observed in lung scores at T1. At T2, a numeric difference in the amount of calves presenting healthy lungs could be observed (total: 40.74%, CON: 33.85%, VACC: 47.14%), however, sample size was too small to achieve a statistical difference.

CONCLUSIONS: Significantly enhanced growth and ADG was observed in the vaccinated group of pre-weaned calves. These results support using intranasal Coronavirus vaccination as part of a holistic strategy to improve respiratory health and optimize growth in early life.

Keywords: Intranasal bovine coronavirus vaccination, Pre-weaned calves, Bovine respiratory disease, Thoracic ultrasound lung scoring, Average daily weight gain

Quick Thoracic Ultrasonography and the Qtus Score in Outbreaks of Respiratory Disease: Field Experience from Italy

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BACKGROUND and OBJECTIVE: Management of bovine respiratory disease (BRD) remains a major challenge in calf practice. Lung ultrasonography allows earlier and more accurate detection of pneumonia than clinical examination alone. Quick thoracic ultrasonography (qTUS) is a rapid, reliable point-of-care technique for on-farm use, supported by an international training and certification program. The qTUS score classifies calves according to maximal lung consolidation depth as mild (<1 cm), moderate (≥1–<3 cm), or severe (≥3 cm), which are associated with cure and production outcomes. This descriptive study aimed to characterize ultrasonographic findings during respiratory disease outbreaks in Italy.

MATERIALS and METHODS: An Italian practitioner completed the qTUS training program (online modules and a two-day practical session) and was certified as a gold operator (sensitivity and specificity >90%). A descriptive study was conducted in 10 respiratory disease outbreaks in the Lombardy region. An outbreak was defined as >10% of calves showing respiratory signs, assessed at the farmer's discretion. All calves housed in pens with clinical cases were scanned using a 5.6 MHz linear probe and 98% isopropyl alcohol as coupling agent, following the standardized qTUS protocol. Lesions were recorded per cranial and caudal quadrants. Findings included ≤3 B-lines, >3 B-lines, diffuse B-lines, and lung consolidation classified as mild, moderate, or severe. Descriptive statistics were calculated.

RESULTS: Between October 2025 and February 2026, 10 outbreaks in dairy farms were visited. In total, 159 calves were examined: 34% were ultrasonographically healthy, 5% had mild pneumonia, 25% moderate pneumonia, and 36% severe pneumonia. The mean within-herd pneumonia prevalence was 65.9% (±14.1 SD; min 21.0%; max 83.3%). The mean within-herd prevalence of mild, moderate, and severe pneumonia was 20.6±3.9 (0–9.1), 34.7%±13.2 (8.3–53.8), and 65.9%±21.0 (15.4–83.3), respectively. Among calves with mild pneumonia, 75% had one quadrant affected, 12.5% had two, and 12.5% had three quadrants affected. In moderate pneumonia, 43% had one quadrant affected, 23.1% two, 20.5% three, and 12.8% four quadrants affected. In severe pneumonia, 1.8% had one quadrant affected, 15.8% two, 31.6% three, and 50.9% all four quadrants affected.

CONCLUSIONS: Application of qTUS and the qTUS score was feasible and informative under Italian outbreak conditions. As outbreaks were identified based on clinical signs, many calves already had

severe pneumonia with multiple quadrants involved, associated with poorer prognosis and longer antimicrobial therapy. Routine screening to detect subclinical pneumonia may allow earlier intervention and reduce progression to an advanced disease state.

Keywords: qTUS, Pneumonia, qTUS score, Calves, lung consolidation

Why Are the Prevalences of Umbilical Infections in European Dairy Calves So Variable?

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BACKGROUND and OBJECTIVE: The aim was to explain why published prevalences of umbilical infections (UIs) in dairy calves in Europe vary substantially.

MATERIALS and METHODS: A literature search on umbilical health in calves was conducted using PubMed, Google Scholar and Web of Science. Only fully accessible articles in English or German were included. Articles exclusively investigating calves with inflammatory umbilical diseases (100% prevalence) were excluded, whereas studies reporting umbilical diseases in general (e.g., UIs and hernia) were considered. All studies selected included male and female dairy calves (Bos taurus) in Europe.

RESULTS: In 34 articles analysed, the reported prevalences ranged from 0.0% to 73.0%. Nomenclature varied markedly: most studies used "inflammation, infection or omphalitis/omphalophlebitis" (n = 18), followed by "infection" (n = 8), "disorder/disease" (n = 6), and "inflammation" (n = 2). Only six studies mentioned navel care products, while only two described the application method (e.g., dipping). Prevalences were mainly reported at calf-level (n = 27), whereas herd-level prevalence was omitted in 20 studies. Most articles were based on random farm surveys (n = 29), while five studies included only calves affected by umbilical diseases. Calves aged under one month were most frequently investigated (n = 10), followed by calves aged up to six months (n = 7); six studies did not specify age. Clinical examination was the predominant diagnostic method (n = 32), occasionally supplemented by ultrasound (n = 7). Ten studies lacked case definitions for UIs. Diagnoses were mainly performed by veterinary staff (n = 21), sometimes supported by farmers (n = 4), Nine studies relied solely on farmer-reported diagnoses. Across most evaluated categories, important methodological details were frequently missing (e.g., number of calf examinations, number of herds, herd and calf prevalence, observer type). Higher prevalences of UIs were generally reported in large-scale studies (>1,000 calves), studies including only affected calves, those using ultrasound or necropsy, investigations focusing on calves younger than one month, and studies with veterinary diagnosis.

CONCLUSIONS: This review demonstrates the lack of consistent nomenclature for UIs. The wide variation in reported prevalences largely results from heterogenous study designs, limiting comparability. Key influencing factors include study population (size, age, affected or random calves), diagnostic method, and observer type. Detailed reporting of study characteristics is therefore essential. However, many studies lack crucial information for sufficient comparisons (e.g., navel care). Furthermore, UIs need to be assessed at herd level, yet herd prevalences were frequently not reported.

Keywords: dairy calves, umbilical infections, prevalence

Efficacy of Paromomycin Sulfate in Reducing Cryptosporidium Parvum Infection and Diarrhea in Dairy Calves: A Field-Based Trial in Argentinian Field Conditions

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BACKGROUND and OBJECTIVE: Cryptosporidium parvum (C. parvum) is one of the leading causes of neonatal diarrhea in dairy calves, resulting in significant economic losses and animal welfare concerns. The objective of this study was to evaluate the effectiveness of orally administered paromomycin sulfate (PS) in reducing the incidence of C. parvum infection and diarrhea in dairy calves under field conditions.

MATERIALS and METHODS: A randomized controlled preventive trial was conducted between August and December 2025 on two commercial dairy farms with different housing systems: Farm 1 (calves housed individually in raised, roofed hutches) and Farm 2 (calves raised individually, tethered with stakes). A total of 100 calves (50 per farm) were randomly assigned at birth to one of two groups: Control (no treatment) or Treatment (T1). Calves in T1 received oral PS (Parofor®; Huvepharma) at 50 mg/kg body weight (BW) (~2.5 mL/10 kg BW), daily for 7 consecutive days starting on day 3 of life, mixed with the usual liquid diet. Animals were monitored from birth to 70 days of age. Fecal samples were processed using coproparasitological and molecular techniques, and fecal consistency was scored using a Wisconsin-based scoring system. Data were analyzed using R software; survival curves were used to describe C. parvum infection dynamics.

RESULTS: Ninety-eight calves completed the monitoring period; two Control calves died during the trial. C. parvum incidence was highest during the second (54%) and third (16%) weeks of life. All Control calves (100%) became infected with C. parvum, compared to only 26% in the T1 group, representing a 74% reduction in infection incidence. Diarrhea severity differed markedly between groups: Control calves presented 40% severe, 40% moderate, and 20% mild cases, whereas T1 calves presented only moderate (20%) and mild (80%) cases, with no severe cases recorded. Mean duration of diarrhea was significantly shorter in T1 calves (2.5 ± 1.5 days) compared to Control calves (7.4 ± 2.5 days). Additionally, treated calves showed a mean BW advantage of 5.9 kg (95% CI: 4.5–7.3) at week 8 and 7.1 kg (95% CI: 5.7–8.4) at week 10 compared to Control calves.

CONCLUSIONS: Paromomycin sulfate administered orally at 50 mg/kg for 7 consecutive days demonstrated high efficacy in controlling cryptosporidiosis under field conditions. Treatment was associated with a complete elimination of severe diarrhea cases, a significant reduction in diarrhea duration, improved clinical status, and enhanced growth performance in dairy calves, supporting its use as a control strategy in commercial dairy farm under Argentinian field conditions.

Keywords: Dairy Calves, Cryptosporidiosis, Promomycin, Bovine health, Neonatal infection

High-Load Nasopharyngeal Shedding of Bovine Coronavirus Reduces Average Daily Gain in Weaned Calves Independent of Subclinical Pneumonia

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BACKGROUND and OBJECTIVE: Bovine respiratory disease (BRD) is a multifactorial complex often involving co-infections, making diagnosis and management a significant challenge. While bovine coronavirus (BCV) is frequently identified in BRD cases, its primary capability to cause clinical disease and impact performance remains ambiguous. This study aimed to investigate the specific impact of BCV shedding intensity on the average daily gain

(ADG) of weaned calves during a controlled commingling event.

MATERIALS and METHODS: The study evaluated 120 weaned Angus x Holstein calves across three temporal cohorts. Following a 12–14-day quarantine, calves were randomized into four 21-day mixing treatments of varying intensity based on source farm richness and evenness. Deep nasopharyngeal swabs and faecal samples were collected to quantify BCV viral load. Nasal shedding was categorized as none, low (< 10^7 copies/µL) or high (≥ 10^7 copies/µL). Thoracic ultrasonography (TUS) was utilized to identify subclinical lung consolidation. A linear regression model was developed to assess the effect of BCV shedding on ADG, adjusting for cohort, TUS scores (subclinical pneumonia), and mixing pen intensity.

RESULTS: Regression analysis revealed a significant negative association between high-load nasal BCV shedding and calf growth. Calves shedding high viral loads (≥ 10^7 copies/µL). experienced a reduced ADG of approximately 0.35 kg/day ($p = 0.005$) compared to non-shedding baseline animals. Notably, this reduction in weight gain was independent of the presence of concurrent subclinical lung lesions (TUS scores) and the intensity of the commingling environment ($p > 0.10$ for all mixing pen types). Neither low-intensity nasal shedding ($p = 0.95$) nor faecal shedding ($p = 0.50$) were associated with changes in ADG.

CONCLUSIONS: High-load nasopharyngeal shedding of BCV significantly impairs growth performance in weaned calves. Because this reduction in average daily gain (ADG) occurs independently of ultrasonographically detectable subclinical pneumonia and commingling intensity, it highlights the direct metabolic and economic consequences of acute BCV infection. Crucially, the finding that high viral shedding impacts growth regardless of the mixing group structure underscores the risk of transmission across shared airspaces, consistent with previous work demonstrating the airborne spread of BCV between pens. For bovine practitioners, this emphasizes a critical diagnostic limitation: relying solely on thoracic ultrasonography or clinical scoring may miss calves experiencing virus-induced growth setbacks. These findings emphasize the necessity of holistic housing management—specifically accounting for airborne transmission risks between adjacent pens—and targeted viral mitigation strategies to protect calf performance during high-risk transition periods.

Keywords: Bovine coronavirus, average daily gain, viral shedding, subclinical pneumonia, commingling

Comparative Analysis of Different Vaccination Approaches Against the Occurrence of Enzootic Bronchopneumonia (EBP) in a Calf Population of a State Experimental Farm

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BACKGROUND and OBJECTIVE: Enzootic bronchopneumonia (EBP) remains a persistent challenge in calf-rearing operations, even where intranasal vaccination is routinely practiced. On the examined state experimental farm, respiratory disease outbreaks continued to occur despite the established immunization protocol, frequently affecting entire barn sections. Clinical signs ranged from mild coughing to severe respiratory distress, often requiring multiple treatments and indicating substantial co-infections. These disease episodes not only increased the workload for farm personnel and veterinarians but also elevated the risk of premature culling and reduced overall lifetime productivity of affected animals. Against this backdrop, the aim of the present study was to evaluate whether a comprehensive prime–boost vaccination protocol could provide more durable immunity and better health outcomes than intranasal vaccination alone.

MATERIALS and METHODS: The study followed 49 female calves born between November 2023 and February 2024 from birth through the rearing phase. Calves were housed individually during their first two weeks of life and subsequently transferred to group housing, with strict separation between the experimental and control cohorts. The experimental group received a modified-live intranasal vaccine

against bovine respiratory syncytial virus (BRSV) and parainfluenza 3 virus (PI3) starting on day 10 of life, followed by two subcutaneous vaccinations with an inactivated vaccine containing the same viral strains plus Mannheimia haemolytica at the third and sixth week after the initial dose. The control group received only the intranasal vaccination. Standard rearing parameters—including birth data, colostrum intake, feed consumption, live weight, diarrhea incidence, and respiratory clinical scores—were systematically documented by farm staff.

RESULTS: Growth performance outcomes demonstrated notable advantages for the prime–boost group. While birth weights were similar between groups, calves receiving the extended vaccination protocol exhibited an average of 7.9 kg higher body weight at 4–5 months of age compared with controls. Average daily gain was also higher, with an additional 50 g/day observed in the experimental cohort. Clinical scoring of symptoms such as ocular and nasal discharge, coughing, diarrhea, and ear position revealed a 1.5 fold higher cumulative score in the control group, suggesting increased morbidity. Although the differences did not reach statistical significance, they were biologically meaningful within the context of farm-level health management.

CONCLUSIONS: Overall, the findings indicate that a structured prime–boost respiratory vaccination strategy may enhance calf health, reduce the need for therapeutic interventions, contribute to reduced antibiotic usage, and support improved growth performance, thereby increasing both animal welfare and farm profitability.

Keywords: vaccination protocol, calth lung health, prime-boost vaccination

Cow Effects on Brix Levels and Colostrum Quantity

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BACKGROUND and OBJECTIVE: The quality and quantity of colostrum are critical for the passive immunity transfer in dairy calves. This study investigates the associations between various cow and calf factors with Brix levels and colostrum quantity at a commercial farm in the Netherlands.

MATERIALS and METHODS: Data from 2016 to 2023 was analysed, encompassing calving, colostrum, and milk-feeding management in post-natal calves. The dataset included variables such as calf registration number, birth weight, sex, breed, Brix levels, and the dam's colostrum production. Two datasets were created: one with complete Brix measurements and another with colostrum quantity for dams with a minimum of three calvings, resulting in 308 calves from 88 dams and 146 calves from 47 dams, respectively. Univariable analysis was conducted on Brix levels considering season, calf factors (sex, breed, birth weight), and cow factors (parity, previous lactation days, previous 305-day production, previous increased cell count).

RESULTS: Results showed an average Brix level of 24.7% (SD=3.3) and a mean colostrum quantity of 4.8 L (SD=4.3). Brix levels were inversely related to colostrum quantity (β= -0.009, p<.0001) and parity (p=0.002), with higher parity cows having higher Brix levels. Season also impacted Brix levels, with summer showing lower values (23.6%) compared to spring (25.0%, p=0.001). Increased previous lactation days (β= 0.0003, p=0.008) and previous 305-day production (β= 0.00002, p=0.001) were associated with higher Brix levels. Calf factors such as sex, breed, and birth weight showed no significant association with Brix levels.

CONCLUSIONS: Brix levels are influenced by season and cow factors, including parity and previous lactation performance. Further research is recommended to include more management factors so that a farmer will be able to improve Brix levels by management.

Keywords: Colostrum, BRIX, calf performance

Gut and Lung Microbiota in Dairy Calves: Associations with Clinical Respiratory Disease and Respiratory Pathogens

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BACKGROUND and OBJECTIVE: Bovine respiratory disease (BRD) remains one of the main causes of morbidity, mortality, and antimicrobial use in dairy calves. Respiratory health is influenced by bidirectional interactions between gastrointestinal and respiratory mucosa, including the microbiome (the gut-lung axis), for which limited data are available in calves. This study investigated associations between faecal microbiota and clinical BRD and bronchoalveolar lavage fluid (BALF) pathogens, and between BALF microbiota and clinical BRD.

MATERIALS and METHODS: A cross-sectional study was conducted in 20 Estonian dairy herds. Within herds, pre-weaned calves were clinically examined and 10 calves per herd were selected to include animals with and without clinical respiratory disease (n = 200). Calves were retrospectively assigned a California BRD score (range 0–17); exclusion of intermediate scores for clinical BRD analyses resulted in 41 BRD (score ≥5) and 64 non-BRD calves (score 0). Faecal samples and BALF were collected. Bacterial communities were profiled using 16S rRNA gene sequencing, Mycoplasma bovis was detected by PCR, and respiratory viruses by pooled sequencing followed by individual PCR. α-diversity (Shannon index) was analysed using Bayesian mixed-effects regression models, β-diversity (Bray-Curtis dissimilarity) using permutational multivariate analyses of variance, and differential abundance and prevalence using generalized multivariable linear models. This research was funded by the Estonian Research Council (Grant No. PRG2233).

RESULTS: Clinical BRD was associated with 6–14% lower faecal within-sample diversity in younger calves (≤25 days of age), whereas community composition did not differ. At the genus level, BRD calves had increased faecal abundance of Coprococcus and higher prevalence of Prevotella, whereas prevalence of Parasutterella and UBA1417 was decreased. Twenty faecal genera were associated with BALF detection of Mannheimia haemolytica, Mycoplasma bovis, bovine coronavirus, or bovine rhinitis B virus, with distinct genera identified for each respiratory pathogen. In BALF, clinical BRD was associated with higher within-sample diversity in older calves (>28 days of age), differences in community composition, increased prevalence of Aquabacterium, and decreased prevalence of Staphylococcus. BALF community composition differed by BRD status only in calves without diarrhoea.

CONCLUSIONS: These findings support a pathogen-specific gut-lung axis in dairy calves. An age-dependent reduction in faecal bacterial diversity in calves with clinical BRD suggests early life as a critical window for microbiome-targeted BRD prevention, with potential to reduce antimicrobial use.

Keywords: gut-lung axis, bovine respiratory disease, bronchoalveolar lavage, faecal microbiota, 16S rRNA gene sequencing

Associations Between Serum Iron, Vitamin D, Total Iron-Binding Capacity, and Neonatal Diarrhea in Dairy Calves

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BACKGROUND and OBJECTIVE: Despite advances in breeding, neonatal calf diarrhea remains a leading cause of economic loss in cattle farming worldwide. According to the survey of farms included in this

study, it is still the most common health problem in calf rearing, being confirmed at least once in 33.7% of calves in the past year. New early diagnostic approaches are needed to improve control.

MATERIALS and METHODS: The study included 143 calves, aged one to seven days, from 15 selected Slovenian dairy farms. The study was conducted in 2023/2024. The farms were classified by the number of dairy cows and type of farming: nine were small (≤100 dairy cows, 60%) and six were large (>100 dairy cows, 40%). Three small and two large farms were organic (33%), and the remaining ten were conventional (67%). Blood samples were collected from the jugular vein using vacutainers (serum clot activator, 9 mL, Vacuette, Austria). The blood was allowed to coagulate at room temperature, then centrifuged twice at 1,500 g for 10 minutes each. Serum iron was measured colorimetrically with ferrozine (without deproteinization), and total iron-binding capacity (TIBC) with ferric chloride, both on an RX DAYTONA PLUS analyzer (Randox Laboratories, UK). Serum vitamin D was measured by Enzyme Linked Fluorescent Assay (ELFA) on a MiniVIDAS analyzer (BioMerieux, France). All tests followed the manufacturer's instructions. Fecal samples were taken from rectum. The relationships between clinical diarrhea in the studied calves and the values of blood serum iron, TIBC, and vitamin D were examined with Welch t-test (p was set at <0.05).

RESULTS: Among the calves analyzed, the prevalence of clinical diarrhea was 20.3%, while overall diarrhea-related mortality on the surveyed farms was 2.0%. Calves with diarrhea had significantly lower levels of iron ($p<0.05$), TIBC ($p<0.0001$), and vitamin D ($p<0.01$).

CONCLUSIONS: Diarrhea often triggers inflammation and loss of gut barrier function in calves, resulting in blood loss, poor feed intake, and malabsorption. Lower iron levels indicate depleted stores, while reduced TIBC (mainly transferrin) suggests liver dysfunction or an acute-phase response. Transferrin decreases as an inflammatory marker, limiting iron transport and increasing the risk of anemia. Low vitamin D impairs calcium homeostasis, mucosal immunity, and gut repair, potentially prolonging diarrhea by weakening epithelial barriers and altering the microbiota. Results do not prove causation (e.g., did deficiency precede diarrhea?), so confounders must be considered. Future research should include longitudinal studies, intervention trials with vitamin D or iron supplementation, and multi-omics approaches to investigate mechanisms.

Keywords: calf, diagnostics, scours, blood, biochemical parameters, neonatal

Impact of Maternal Serum Selenium Concentration and Dam-Sourced Colostrum Provision on Transfer of Maternal Immunity to Newborn Calves

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BACKGROUND and OBJECTIVE: Transfer of immunoglobulins (Ig) and maternal leukocytes via colostrum to newborn calves are key for their immunity and health. Selenium (Se) serum concentration of dams is suspected to affect the passive transfer of Ig, while the provision of dam-sourced is essential for the transfer of maternal leukocytes. The objective of this study was to investigate associations between maternal Se serum concentration with those of their newborn calves and of dam-sourced colostrum supply on calf mortality rate under field conditions in large commercial dairy herds.

MATERIALS and METHODS: From 113 large dairy herds with a mean herd size of 447 cows, a total of 915 prepartum cows, were examined 3-1 weeks prepartum, including blood sampling. Density of colostrum samples was analyzed using BRIX refractometry. At 1-4 days postpartum, blood samples from their calves were taken. Data on herd-specific management of close-up cows, newborn calves and colostrum supply were collected. Generalized linear mixed effects hierarchically structured regression models were applied to analyze association between maternal Se serum concentration with those of their newborn calves and between calf mortality rate and herd-specific management factors.

RESULTS: In prepartum cows, Se serum concentration was positively associated with the

BRIX value as well as with vitamin D administration and parity (model 1). In model 2, Se serum concentration of prepartum cows as well as colostrum amount administered to calves at first feeding were related to serum Se concentration of their calves. In model 3, Ig serum concentration of calves was related to calves Se serum concentration ($p < 0.01$) and BRIX ($p < 0.01$), but not time and amount of colostrum fed. On farms where calves were immediately provided with dam-sourced colostrum, calf mortality rate was lower. Other management factor associated with higher calf mortality were dust and interval of replacing bucket teats.

CONCLUSIONS: Adequate Se status of prepartum cows is vital for the passive transfer of Ig. According to our data, this effect might be mediated both by higher serum Se concentration of calves and a higher colostral Ig concentration. Presumably, both mechanisms acting synergistically. Se status of prepartum cows was proven to be a key factor of passive transfer of Ig to newborn calves. Managing calves with feeding them with dam-sourced colostrum potentially reduces overall calf mortality within the herd, presumably by ensuring optimal transfer of Ig, maternal leucocytes and selenium.

Keywords: selenium, transfer of immunoglobulins, dam-sourced colostrum provision, calf mortality

Retrospective Pathological Evaluation of Mortality Cases in Cattle from a University Dairy Herd

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BACKGROUND and OBJECTIVE: Understanding mortality patterns in dairy herds is essential for improving herd health management and minimizing economic losses. This study aimed to retrospectively evaluate calf and cow deaths submitted for necropsy and histopathological examination between 2024 and 2026 and to classify causes of death according to age group and pathological system involvement.

MATERIALS and METHODS: A total of 18 cattle cases (calves, young cattle, and adult cows) originating from a university dairy herd and associated facilities were included. Cases underwent systematic necropsy, and selected tissues were examined histopathologically using hematoxylin and eosin staining when applicable. Causes of death were categorized as respiratory diseases, gastrointestinal diseases, septicemic/toxemic conditions, metabolic postpartum disorders, circulatory shock/asphyxia, and miscellaneous causes. Cases with advanced autolysis were recorded separately due to diagnostic limitations.

RESULTS: Respiratory system disorders were the most frequent findings (approximately 33%), primarily bronchopneumonia, fibrinous pneumonia, and pulmonary edema. Gastrointestinal diseases accounted for approximately 28% of cases, mainly hemorrhagic enteritis and typhlitis, some associated with suspected clostridial or endotoxemic etiology. Septicemic or toxemic presentations, including gas gangrene and widespread hemorrhagic diathesis, represented approximately 17% of cases. In adult cows, metabolic and postpartum disorders including hepatic lipidosis, hemorrhagic or septic metritis, and gangrenous mastitis accounted for approximately 17% of deaths. Circulatory shock and asphyxia due to aspiration or mechanical airway obstruction were identified in a smaller proportion of cases. Calf mortality was predominantly associated with infectious respiratory and enteric diseases, whereas adult cow mortality was more frequently related to metabolic imbalance and postpartum complications. Advanced autolysis limited definitive diagnosis in a minority of submissions.

CONCLUSIONS: This retrospective analysis demonstrates that infectious respiratory and gastrointestinal diseases remain the primary causes of mortality in calves, while metabolic and postpartum disorders are significant contributors to mortality in adult cows. Strengthening colostrum management, biosecurity, vaccination protocols, and periparturient metabolic monitoring is crucial to reducing herd losses. Pathological surveillance provides a valuable epidemiological framework for improving herd health strategies in university-based dairy systems

Keywords: Cattle, Necropsy, Mortality, Pathological findings, Retrospective study,

Bacterial Contamination and Antibiotic Susceptibility of Bacteria Isolated from Commercial Milk Replacers for Calves

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BACKGROUND and OBJECTIVE: Milk replacers are widely used in calf rearing as substitutes for whole milk. Some commercial products include probiotic bacteria such as Bacillus spp. or Enterococcus spp. to support intestinal health and improve growth performance. However, the presence of viable bacteria, particularly antimicrobial-resistant strains, may pose a risk for calf production systems. This study evaluated the presence and potential contamination of bacteria and antimicrobial resistance in commercially available calf milk replacers.

MATERIALS and METHODS: Five commercial milk replacers were analyzed using serial dilution and culture on blood agar. For bacterial count, 1 g of milk powder was suspended in 9 mL of sterile saline solution to obtain an initial 10^{-1} dilution, followed by serial dilutions. Total viable counts were calculated and expressed as colony-forming units per gram (CFU g^{-1}). Bacterial isolates were identified using standard microbiological methods, and antimicrobial susceptibility testing was performed. A human infant milk powder was analyzed under the same conditions for comparison.

RESULTS: Total viable counts in the tested milk replacers ranged from 3.1×10^4 to 7.2×10^5 CFU g^{-1}, whereas no bacterial growth was detected in the infant milk powder under aerobic conditions. Three milk replacers (Milk 1–3) that declared the addition of probiotic bacteria, including Bacillus spp. and Enterococcus faecium, showed higher bacterial counts of 5.2×10^5, 7.2×10^5, and 3.9×10^5 CFU g^{-1}, respectively. In contrast, two milk replacers (Milk 4–5) that did not declare the addition of probiotic bacteria showed lower counts of 3.1×10^4 and 1.8×10^5 CFU g^{-1}, respectively. Despite the absence of declared probiotic bacteria, viable bacteria were detected. Enterococcus was isolated from products that did not declare its presence, suggesting possible contamination. Additional isolates included Staphylococcus pasteuri. All isolated bacteria—whether intentionally added or possibly introduced through contamination — demonstrated antimicrobial resistance, including complete resistance to penicillin.

CONCLUSIONS: The results indicate that commercial calf milk replacers may contain viable bacteria, including both probiotic strains and microorganisms introduced through possible contamination. Detection of Enterococcus faecium in products that did not declare its presence suggests contamination during production, processing, or storage. Isolation of environmental organisms such as Staphylococcus pasteuri further supports possible post-processing contamination. Importantly, all isolates showed antimicrobial resistance, including resistance to penicillin, cefuroxime, and colistin. These findings highlight concerns regarding dissemination of antimicrobial-resistant bacteria through calf milk replacers and emphasize the importance of microbiological monitoring and quality control during production.

Keywords: milk replacer, bacterial contamination, antimicrobial resistance, calf nutrition

What Three Areas Should a Dairy Farmer Address to Improve Calf Health? - Results From an Animal Health Ireland Calfcare Prioritization Workshop

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BACKGROUND and OBJECTIVE: Animal Health Ireland (AHI) is a partnership based, non-profit organisation which provides leadership to the Irish

livestock industry, coordinating non-regulatory animal health concerns nationally. The CalfCare Technical Working Group (TWG) of AHI has been actively working on national calf management and health since 2010. In 2025 it was decided to review dairy calf health with a view to prioritising future research and knowledge dissemination activities.

MATERIALS and METHODS: The CalfCare TWG (n=14 members) was convened for an in-person workshop over a half day in September 2025. The TWG membership includes vets (private, government, Teagasc, UCD, pharmaceutical) and agricultural professionals (commercial, Teagasc). The objective of the workshop was to ascertain what were the three (ranked) areas a dairy farmer should address to improve calf health. The workshop format was that of the World Cafe methodology. Post-its were used to elicit rankings from all TWG members of three areas a farmer should address to improve calf health; 41 individual responses were received. These free-text responses were aggregated into six thematic areas within which more specific topics could be identified.

RESULTS: The six broad themes were calf nutrition, colostrum, housing, hygiene, calf stress/diseases and cow nutrition/health. The top three topics within each of the six groups were nutrition (feed volume, water, other), colostrum (volume, feed timing, quality), housing (bedding, ventilation, other), hygiene (feed equipment, colostrum, pens), calf stress/diseases (disbudding, congenital defects, general) and cow nutrition/health (feed, milk fever, body condition). The top five topics ranked across the six groups were colostrum volume, liquid feed volume, timing of colostrum feeding, colostrum quality, bedding, colostrum as first feed and ventilation.

CONCLUSIONS: This workshop prioritised calf management over environmental or cow management. Calf nutrition, colostrum and housing management (in that order) were prioritised over all other themes of calf rearing/health. Colostrum volume, liquid feed volume, and timing of colostrum feeding were prioritised over all other individual management topics to improve dairy calf health. Thus, the three ranked areas that a dairy farmer should address to improve calf health from this prioritisation workshop could be summarised as "feed enough colostrum early enough and feed enough whole milk/milk replacer". The next step is to collaborate with a group of farmers/industry stakeholders to identify relevant, realistic and achievable goals in relation to the areas identified by experts.

Keywords: Calf care, farmer priorities, workshop

Promoting the Health of Individual Calves by Using Specific Colostrum Additives and Phytogenic Substances

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BACKGROUND and OBJECTIVE: The primary causes of calf mortality are diarrhea and respiratory diseases, both of which can be mitigated through improved management on farms. This project aims to reduce disease incidence and improve health, welfare, and performance by using two additives: a colostrum supplement (ImmuneMilk®) and a phytogenic additive derived from Macleaya cordata. While early colostrum intake is essential for survival, phytogenic additives further enhance resilience by improving intestinal health and modulating the immune system.

MATERIALS and METHODS: Comparative feeding trials were conducted on a conventional dairy farm involving several hundred calves during their first four weeks of life. In a series of consecutive trials, groups of 60 calves were divided into trial and control groups (n=30 each). The trials included: Trial 1: 300 g of colostrum supplement within three hours of birth. Trial 2: 300 g of colostrum supplement distributed over 28 days. Trials 3 & 4: Feeding of the phytogenic additive or a combination of colostrum powder and phytogenic additive. On days 1, 14, and 28, researchers collected blood and fecal samples, recorded body weights and animal welfare parameters. Statistical analysis was performed via SAS - Studio, using t-tests for normally distributed data (body weight) and Kruskal–Wallis or Wilcoxon tests for non-normally distributed data, such as total protein (Tp) and IgG levels.

RESULTS: So far, the trial has shown a tendency towards higher body weights in trial groups 1 and 2

on day 28 of life (Control 1: 58.5±7.32 kg vs. Trial 1: 59.4±7.09 kg; Control 2: 64.3±6.35 kg vs. Trial 2: 67.2±6.86 kg). On day 28, Tp and IgG levels were similar between Trial 1 and Trial 2 groups (Trial 1: Tp 55.4±2.81 g/L vs. 54.2±2.78 g/L and IgG 725±181 mg/dL vs. 722±202 mg/dL; Trial 2: Tp 56.9±3.23 g/L vs. 55.1±3.62 g/L and IgG 797±22 mg/dL vs. 806±273 mg/dL for the control and trial groups, respectively).

CONCLUSIONS: First results indicate a clear positive trend in body weight for calves receiving supplementary colostrum powder. Additionally, the first two trials suggest a slight positive trend towards higher total protein levels in the blood. Further evaluations are underway to examine the correlation between these supplements and disease rates, treatment frequency, and general animal welfare. Data for Trials 3 and 4, focusing on the phytogenic additive, are currently being evaluated.

Keywords: Calf-Health, Colostrum, Growth-Performance, Management, Phytogenics, Animal-Welfare

Acute Phase Response in Calves with *Cryptosporidium* spp. Infection and Bovine Respiratory Disease

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BACKGROUND and OBJECTIVE: Bovine respiratory disease (BRD) and cryptosporidiosis cause substantial morbidity and mortality in pre-weaned calves. We hypothesised that *Cryptosporidium* spp. infection and BRD may jointly influence systemic inflammation measurable through acute phase proteins (APPs). Therefore, this study aimed to determine the associations between *Cryptosporidium* spp. infection and inflammatory markers in calves with and without BRD.

MATERIALS and METHODS: This case-control study was conducted on 30 dairy farms across Estonia. From each farm, ten calves under two months of age were enrolled. Five calves with lung consolidations ≥1 cm and five calves without lung consolidations on lung ultrasound (LUS) were selected. All calves (n = 300) underwent clinical examination, and faecal and blood samples were collected from each animal. In the blood plasma and serum, the concentrations of the following immunological markers were quantified: fibrinogen (Fib), haptoglobin (Hp), serum amyloid A (SAA), interleukin-1β (IL-1β), interleukin-6 (IL-6) and interferon γ (IFN γ). *Cryptosporidium* spp. pathogen load (oocysts per gram of faeces, OPG) was determined using an immunofluorescence (IF) method. Associations between *Cryptosporidium* spp. infection status (positive/negative) and inflammatory marker concentrations (outcome variables) were evaluated using linear mixed effects regression models with farm as random intercept and calf age as confounder. An interaction term between calf BRD status and *Cryptosporidium* spp. infection was also included in the model. The study was supported by the Estonian Research Council grant (PRG2233).

RESULTS: *Cryptosporidium* spp. infection was positively associated with Hp (coefficient ± standard error (SE) 0.37 ± 0.14 g/l, p = 0.009) and fibrinogen concentrations (0.13 ± 0.05 g/l, p = 0.007). A statistically significant interaction between BRD category and *Cryptosporidium* spp. status was observed in both models. *Cryptosporidium* spp. infection was associated with higher Hp and Fib responses in calves presenting with clinical pneumonia. In contrast, SAA, IL 6, IFN γ, and IL 1β concentrations showed no relationship with *Cryptosporidium* spp. infection.

CONCLUSIONS: Our findings suggest that *Cryptosporidium* spp. infection and clinical pneumonia may jointly modulate systemic inflammatory responses in young calves, with Hp and Fib reflecting these inflammatory changes most clearly among the markers evaluated.

Keywords: neonatal calf, APP, BRD, Cryptosporidium spp.

The Impact of Extended Versus Short Duration Colostrum Feeding and Milk Replacer Type on Neonatal Calves' Performance, Morbidity, Mortality, and Subsequent Fertility Performance

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BACKGROUND and OBJECTIVE: Colostrum feeding is vital to prevent failure of passive transfer (FPT) in calves. Whether extending colostrum feeding past 24-hours impacts health, growth and reproductive performance is unknown. Milk replacer (MR) powders vary in composition, especially functional protein content. Whether feeding higher levels of functional protein influences growth, health, and reproduction has not been described. Our objective was to conduct a randomised controlled trial that would provide new information regarding these questions.

MATERIALS and METHODS: One-hundred Jersey heifers were enrolled (October 2021-July 2023) for a 2x2 factorial design study. Independent variables included colostrum feeding duration (1 or 4 days) and MR type (isocalorific; 23.8% protein - two different levels of whey protein phospholipid concentrate (WPPC)). Initial colostrum management was the same for all; 2.5 litres fed within 6 hours of birth (Brix value ≥22%), followed by 2.5 litres within 12 hours. Calves fed 1-day colostrum transitioned to MR from their 3rd feed. Calves fed 4-days colostrum received colostrum/transition milk (2.5 litres twice daily) for 3 more days, then moved to MR. MR was fed at 165g/l; 2.5 litres twice daily. Standard husbandry procedures were carried out. FPT was assessed by serum TP (<5.5 mg/dL indicated FPT). Birth, 1-month, 8-week and 10-week weights were obtained. Thoracic ultrasonography identified lung pathology. Treatment records and reproductive data were kept on farm. Only heifers served by AI were included in the first service conception rate (CR) analysis. Two tailed Fisher's exact tests, independent t-tests and multivariable logistic regression modelling were used for data analysis.

RESULTS: 4/50 (8%) 1-day colostrum calves died during the pre-weaning period; all the 4-day group survived (P=0.12). 8/50 (16%) calves from the 1-day group had FPT; 1/50 (2%) calves in the 4-day group had FPT (P=0.04). Multivariable logistic regression showed calves in the 1-day group were 10.98 (95% CI 1.28 – 94.47, P=0.03) times more likely to have FPT. Colostrum feeding duration and MR type had no association with morbidity or growth. MR type had no association with mortality or FPT. First service CR was 58% for calves fed standard MR, and 81% for high WPPC MR (P=0.006). Colostrum duration had no association with first service CR (P=0.217). The combination of 4-day colostrum and standard MR showed a significant reduction in first service CR (P=0.002).

CONCLUSIONS: Extending colostrum feeding positively influenced survival and passive transfer of immunity, and feeding a MR with high WPPC content improved maiden heifer fertility.

Keywords: calf, colostrum, milk replacer, mortality, passive transfer, fertility

Using CalfXpert Optimax Lean Management Principles to Guide On-Farm Youngstock Decision Making - A Case Study

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BACKGROUND and OBJECTIVE: Successful calf rearing reflects management decisions unique to an individual farm. Colostrum management, nutrition and disease prevalence vary between farms, so veterinary advice must be specifically tailored. Lean Management principles, originating from the mid-20th century manufacturing industry to improve efficiency and reduce waste, are increasingly applied in agriculture; neonatal calf management can hugely benefit from these principles. Optimax visits, based on Lean principles, evaluate calf rearing management from start to finish, analysing each step through a whole team farm walk. Breaking down processes and troubleshooting encourages a systematic, holistic approach to generating

achievable targets. This case report demonstrates the impact of applying Lean principles, using the CalfXpert Optimax framework, on calf health and performance in a 300 cow, all year-round calving UK dairy herd. The primary aim was to reduce bovine respiratory disease (BRD) incidence, with an estimated 50% of beef calves and 35% of heifers receiving at least one pneumonia treatment in the preceding year.

MATERIALS and METHODS: The Optimax review prompted discussion around farm specific protocols and concerns, enabling a targeted and realistic action plan. Routine data collection of calf weights, treatment rates, transfer of passive immunity and antibiotic usage by veterinary technicians provided a pre and post Optimax comparison. Immediate actions included quicker feeding of first colostrum, increased milk powder in the first three weeks, amended vaccine protocols and refined BRD treatment. Long term goals included snatch calving, pasteurising colostrum/transition milk and calf housing modifications.

RESULTS: A review was carried out nine months post-Optimax visit and action implementation - youngstock antibiotic usage fell from 15mg/kg to 8mg/kg, and pneumonia recurrence declined from 35% to 2% following vaccine protocol refinement. Improved colostrum management increased the number of calves achieving successful passive transfer (>55g/L) from 50% to 77%. Daily live weight gain (DLWG) rose from 0.78kg/day to 0.91kg/day, with gains in the first 35 days increasing from 0.5kg/day to 0.9kg/day.

CONCLUSIONS: The CalfXpert Optimax strategy provided a structured review of the entire neonatal calf rearing process. Whole team involvement encouraged open farm-specific discussion and identification of practical and realistic improvements. Data monitoring quantified progress, showing real and tangible benefits in enhanced passive transfer, reduced antibiotic use and improved growth rates.

Keywords: Calf Health, Lean management, bovine respiratory disease

Pilot Evaluation of the Feeding Program for Suckling Holstein Calves in Greece

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BACKGROUND and OBJECTIVE: The feeding program of pre-weaned calves is closely associated with their health status and future productivity. The aim of this study was the pilot evaluation of feeding programs implemented on Greek dairy farms.

MATERIALS and METHODS: The following were evaluated: a) the nutritional level between the 3rd and 4th week of age by measuring blood glucose concentration (GLU, target concentration >100 mg/dL), and b) the ability of calves to be successfully weaned by measuring blood β-hydroxybutyrate (BHB, target concentration ≥0.3 mmol/L) 7–10 days before the expected weaning date. A total of 120 and 116 calves from 17 and 18 farms, respectively, were included in the study. Blood sampling and measurements were carried out on farms using a portable device (Abbott Freestyle Precision). In addition, body measurements (heart girth, withers height), estimation of body weight, and clinical evaluation of the respiratory system using the University of Wisconsin calf respiratory scoring system (WISC) were performed. Statistical analysis was conducted using analysis of variance, linear models and Pearson correlations.

RESULTS: Measurement of GLU showed low concentrations (<80 mg/dL) in 31.7%, marginal concentrations (80–100 mg/dL) in 44.1%, and high concentrations (>100 mg/dL) in 24.2% of the calves. In these three groups, the mean age was 25, 26, and 25 days; body weight was 55.1, 59.4, and 63.9 kg; and withers height was 78.2 cm, 79.2 cm, and 81.0 cm, respectively. Eight out of 17 farms (47%) had ≥75% of their calves with GLU >80 mg/dL while the rest (9/17, 53%) had ≤50% of their calves with with GLU >80 mg/dL. The correlation between height and GLU was significant ($P<0.05$) but low ($r = 0.24$). There was no difference between the groups in WISC scores (2.9, 2.7, and 3.0). Of the 116 calves in which BHB was measured, 78 (67.2%) had values <0.3 mmol/L, indicating inadequate adaptation to solid feed and

that they were not ready for weaning. In 14 out of 18 farms (78%) calves with BHBA values <0.3 mmol/L were >50% of the total.

CONCLUSIONS: Measurement of blood glucose and β-hydroxybutyrate concentrations can serve as practical indicators for evaluating and improving feeding programs of pre-weaned calves, supporting optimal growth and development targets, as well as facilitating successful weaning management.

Keywords: Calves, Nutrition, Weaning, Glucose, β-hydroxybutyrate

Calprotectin Concentrations in Faecal Samples of Calves Infected *with Cryptosporidium parvum*

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BACKGROUND and OBJECTIVE: Cryptosporidiosis, caused by the protozoan parasite Cryptosporidium parvum, is a significant contributor to calf morbidity and mortality due to diarrhoea. This study aimed to analyse the concentrations of calprotectin (a protein released by neutrophils during intestinal inflammation) in faecal samples of calves experimentally infected with C. parvum. The hypothesis posited that C. parvum infection increases the faecal concentration of calprotectin, which may serve as a biomarker for intestinal damage.

MATERIALS and METHODS: Faecal samples from eight male calves, experimentally infected with C. parvum and showing typical clinical signs of infection, were analysed. From each calf multiple samples were taken: one before inoculation, at several time points during infection, and one after the trial. Calprotectin was measured using the fCAL immunoassay on the UniCel DxC 600 Synchron (Beckman Coulter). Calprotectin results were combined these results with data on body weight, body temperature, overall health score, faecal volume, faecal consistency, faecal dry matter content, fluid losses, dehydration score, skin tent duration test, and oocyst shedding.

RESULTS: Faecal concentrations of calprotectin were higher in infected calves after inoculation, suggesting intestinal inflammation. Calprotectin levels correlated with several health parameters, including faecal consistency, faecal dry matter content, and oocyst shedding.

CONCLUSIONS: Calprotectin is a promising biomarker for assessing intestinal health in calves infected with C. parvum. The findings suggest calprotectin could enhance disease management and recovery strategies. Further research should validate these findings and establish normal ranges for calprotectin in calves.

Keywords: Cryptosporidium parvum, calprotectine, calf health

Lung Ultrasonography Assessment of Florfenicol and Meloxicam Therapy in Moderate and Severe Bovine Respiratory Disease in Veal Calves

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BACKGROUND and OBJECTIVE: Bovine respiratory disease (BRD) can affect up to two-thirds of veal calves. Antimicrobial treatment is recommended when active bronchopneumonia is identified. Lung ultrasonography (LUS) could be useful to identify lesions as pleurisy and fluid alveolograms/bronchograms that could be more indicative of bacterial infections. The aim of this study was to evaluate the use of a single dose of florfenicol and meloxicam in the treatment of active bronchopneumonia in veal calves.

MATERIALS and METHODS: 145 veal calves were examined three time at week from arrival with LUS

to establish lung lesion score (LLS), ultrasonography score (US), and lesion depth. Wisconsin and California clinical scores were also assessed. Animals that never develop lung lesions were considered as control (CTR, n=50), while calves with LLS≥10.5 and at least 1 clinical sign were considered as BRD-positive, and divided in moderate (1-3cm; MP, n=25) and severe (>3cm; SP, n=43) pneumonias. BRD-positive calves received a bronchoalveolar lavage (BAL) and were treated with a single administration of florfenicol and meloxicam (40mg/kg+0.5mg/kg, Zeleris®, Ceva). Animals were monitored each other day from 1d to 14d, and at 21d and 28d after treatment. Calves were considered fully recovered if LUS indicated healthy lungs or only comet-tail artifacts after treatment. Mixed models were used to evaluated differences among groups.

RESULTS: Treated animals had 91.2% of bacterial infection (*Mannhemia haemolytica*, 28.1%; *Pasteurella multocida*, 32.9%; and *Mycoplasma bovis*, 29.3%) and 87.5% of Coronavirus at BAL microbiology analysis. Clinical scores reduced +1d after treatment in both groups. Overall recovery was 80.9% (96% MP, 72.1% SP). Among them, overall recovery after 1-treatment was 86.5% (91.7% MP, 82.1% SP). Mean time to full recovery was 5.68d in MP, and 9.88d in SP. At treatment, the LLS and US were 11.0 and 2.08 in MP, and 14.3 and 3.33 in SP, respectively. Reduction in MP were significant for LLS at +1, +3, +9 and +21 days (respectively, LLS=6.36; 4.29; 2.33; 0.41); while in US it decreased at +3 and +9 (respectively, US=1.40 and 0.98); both scores were comparable to CTR from +9d. In the SP, the LLS reduced at +1,+5,+9 and +21 days (respectively, LLS=10.9; 6.68; 4.86; 0.49) without differences with CTR from +14d; whereas US decreased at +1, +5, +9, +14,+21 (respectively, US=2.78; 2.22; 1.66; 1.32; 0.87) in SP comparable to CTR from +21d.

CONCLUSIONS: In high risk calves with moderate to severe pneumonia of mixed origin, treatment with florfenicol plus meloxicam rapidly reduced clinical scores and resulted in complete lung recovery.

Keywords: Lung ultrasonography, BRD, Veal calves, Treatment

Effect of UV-C Disinfection in the Housing Environment on Health and Growth Performance in Calves on a Commercial Dairy Farm in Western Germany

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BACKGROUND and OBJECTIVE: The development and health of neonatal calves are critical not only for food safety and animal welfare but also for the economic and genetic success of dairy farms. Structural consolidation of the dairy industry has led to farms with larger herds, resulting in increased pathogen pressure and higher morbidity and mortality in calf rearing (Gulliksen et al. 2009; Torstein et al. 2011). Against the backdrop of the One-Health Concept which emphasizes the interconnectedness of animal, environmental, and human health (WHO 2021) effective control of microbial load in calf housing assumes growing importance. This study evaluates the effect of a standardized UV-C (254 nm) disinfection protocol for calf housing on calf health and development during the first 28 days of life.

MATERIALS and METHODS: A total of 52 calves either housed in boxes or hutches on a commercial dairy farm were enrolled under the farm´s self-monitoring program. All pens were emptied, scraped, and cleaned with cold water and a high-pressure washer prior to calf placement. 25 calves (UV-C group) received additional UV-C disinfection with the EcoProtectorUV-C (Urban GmbH & Co. KG, Hude) of the empty pens immediately before placement and again on day 6 and 14 of life. No further cleaning was performed prior to these in operation UV-C treatments. Health assessments were conducted on days 6, 14, 21, and 28 of life using a predefined scoring sheet. Birth weight, body weights at day 14 and 28, and daily milk intake were recorded, and faecal samples were analysed with a commercial rapid test.

RESULTS: Calves in the UV-C group exhibited a consistently higher average daily weight gain across each rearing interval and overall, with the difference reaching statistical significance up to day 14. The UV-C group also achieved significantly better overall health scores, most notably on day 14, and a significant association was found between UV-C treatment and improved health scoring. Subcomponent analysis revealed that the control group had markedly higher prevalence of diarrhea, cryptosporidiosis, and multimorbidity, and the duration of diarrhea was on average 1.33 days longer than in the UV-C group. Additionally, the UV-C group scored significantly fewer points for respiratory signs and general condition.

CONCLUSIONS: Standardized UV-C disinfection of the calf housing significantly enhanced both, health and growth performance during the first 28 days of life. These findings support the adoption of UV-C treatment as an effective on-farm tool for improving calf rearing outcomes.

Keywords: disinfection, diarrhea, health scoring,

Omphalitis in Neonatal Dairy Calves: Risk Factors and Assessment of Different Navel Care Practices

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BACKGROUND and OBJECTIVE: Omphalitis in neonatal calves presents a relevant animal welfare concern and may predispose affected calves to a reduced well-being and septicemia. Within a large-scale field study, the objectives were (1) to identify animal- and farm-level risk factors for omphalitis and (2) to assess the association between different navel care practices on dairy farms and the omphalitis prevalence.

MATERIALS and METHODS: Between December 2016 and July 2019, dairy farms located in three structurally distinct regions of Germany (North, East, South) were each visited once. During these visits, calves aged 5-21 days underwent a standardized clinical examination, including the assessment of the umbilicus for signs of omphalitis (inflammatory enlargement with or without increased local heat, hyperemia, or pain on palpation). Data on neonatal calf management, including navel care, were gathered through structured interviews with farm or herd managers. Rearing conditions were assessed according to standard operating procedures. Treatment incidences within in the preceding twelve months were extracted from the farms' recordings. The analysis of potential risk factors included 3,445 calves from 567 farms across all regions. The evaluation of specific navel care practices was restricted to Eastern Germany and comprised 1,967 calves from 196 farms. Multivariable (mixed) regression models were applied to evaluate associations between potential influence variables and the omphalitis occurrence at animal and farm level.

RESULTS: At farm level, the median omphalitis prevalence was 25.0%. In contrast, the median reported treatment incidence within in the preceding twelve months was 4.5%. Provision of dry lying areas in the first two weeks of life (IRR = 0.77) and dam-calf separation later than twelve hours after birth (IRR = 0.75) had a positive impact on the omphalitis prevalence in calves. However, a body condition of the dam outside the target range around calving (OR = 1.4) and the omission of navel care (OR = 2.29) increased the odds of omphalitis. Repeated applications (OR = 0.56) and the administration of antiseptics used for navel care into the umbilical cord (OR = 0.38) were associated with lower odds of omphalitis. Wearing gloves during navel care tended to increase the omphalitis odds (OR = 1.34).

CONCLUSIONS: Omphalitis in neonatal calves is a multifactorial disease strongly influenced by hygiene and perinatal management. A clean environment during and after calving is essential to reduce the omphalitis risk and improve calf welfare. Increased awareness and consistent implementation of evidence-based preventive measures by farmers and veterinarians are essential to maintain umbilical health in neonatal calves.

Keywords: dairy calves, omphalitis, risk factors, navel care

Inter-Operator Agreement of a Wound Healing Grading Scale in Dairy Female Calves Disbudded with a Hot Iron

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BACKGROUND and OBJECTIVE: Disbudding is a common procedure in dairy female calves. In recent years, regulations have been implemented in several countries to ensure adequate pain management during the procedure. However, little information has been reported on the prevalence, risk factors, and consequences of wound infections following disbudding. A crucial first step for conducting studies in this field is to have a reliable method to distinguish infected from non-infected wounds. Although at least two scoring systems have been proposed in previous studies, in our opinion they lack sufficient precision—when used individually—to characterize all stages of wound healing. The aim of this study was to develop a new scoring scale, based on two previously published systems, and to evaluate inter-operator agreement among individuals with different levels of experience in wound assessment. We hypothesized that the scale would demonstrate good reliability and could be used in future research or field settings to identify infected wounds.

MATERIALS and METHODS: The scale included five normal wound healing stages and two indicators of infection. Dairy farms clients of the University of Montreal's ambulatory clinic were visited during the summer of 2024 to obtain photographs representing different stages of disbudding wound healing. Photographs were taken randomly on female dairy calves disbudded under two months of age. The target was to collect 10 photographs per normal healing stage (total n = 50) and 25 per infection indicator (n = 50), as classified by one expert. Sampling continued until sufficient photographs were obtained for each category. A questionnaire (Jotform) was created using the collected photographs and completed by five veterinarians and two animal health technicians (AHT). Inter-operator agreement was assessed using non-weighted Fleiss' Kappa (R v4.4.0) (No agreement ≤ 0; slight 0.01–0.20, fair 0.21–0.40, moderate 0.41– 0.60, substantial 0.61–0.80, almost perfect 0.81–1.00).

RESULTS: A total of 111 calves from 18 farms were included, yielding 334 photographs. The final questionnaire contained 98 photographs: 10 for each healing stage and 24 for each infection indicator (two photographs were excluded due to poor quality). For all seven categories combined, Fleiss' Kappa was 0.61 for all operators, 0.66 for veterinarians, and 0.54 for AHT. When classifying wounds as infected versus non-infected, Fleiss' Kappa was 0.78 (all operators), 0.82 (veterinarians), and 0.72 (AHT).

CONCLUSIONS: The scoring scale developed in this study demonstrated substantial agreement for distinguishing infected from non-infected disbudding wounds. It may serve as a useful tool for future research or on-farm assessments.

Keywords: Disbudding, calf health, wounds healing

Early-Life Immune Maturation and Resilience in Dairy Calves: An Ongoing Integrated Longitudinal Study in EUPAHW-SOA20 Subtask 2.1

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BACKGROUND and OBJECTIVE: Early life is a critical window for immune maturation in dairy calves, marked by the transition from passive colostral immunity to autonomous immune competence and

increased susceptibility to infectious diseases. SubTask 2.1 of the EUPAHW-SOA20 project aims to identify early-life biomarkers of immune competence and resilience with an integrated longitudinal approach. The study focuses on Holstein and Simmental (Pezzata Rossa Italiana) calves to highlight any differences in immune development.

MATERIALS and METHODS: Clinically healthy calves are monitored from birth to one year, with six representative time points (day 3, 14, 70, 100, 150, and 12 months). The experimental design closely follows the longitudinal framework developed by INRAE partners within the EUPAHW consortium, enabling harmonized sampling and cross-cohort comparisons of immune and microbiome parameters. The study integrates: (i) immune cell characterization by flow cytometry (monocytes, αβ and γδ T cells, $CD21^+$ B cells); (ii) phagocytic activity, ROS production, apoptosis, and necrosis after ex vivo whole-blood stimulation; (iii) oxidative and metabolic profiling; (iv) expression of immune-related genes; (v) cytokine and chemokine profiling following TLR4 (LPS) and TLR7/8 (R848) stimulation; and (vi) shotgun metagenomics of faecal samples to characterize gut microbiome development.
RESULTS: Preliminary findings reveal dynamic age-dependent immune remodelling. Phagocytic activity declines from the neonatal phase to post-weaning, while oxidative stress markers peak around day 14, indicating increased metabolic demand. Monocyte subsets redistribute, with non-classical monocytes expanding toward weaning, and T-cell maturation increasing in γδ T cells. Functional responses to TLR7/8 stimulation are stronger than TLR4, with a coordinated peak at day 14 involving pro-inflammatory (IL-6, TNF-α), antiviral (IFN-γ, IP-10), and regulatory (IL-10) mediators. Microbial richness and complexity increase over time, supporting a dynamic gut–immune axis.

CONCLUSIONS: Although the study is ongoing and the cohort is expanding, day 14 and the weaning period emerge as critical windows of immune vulnerability and plasticity. Comparative analyses between Holstein and Simmental calves are planned to explore breed-specific differences in immune trajectories, functional responsiveness, and microbiome maturation. In addition, the alignment of the experimental design with the parallel longitudinal study conducted by INRAE will allow direct comparison of key immunological and microbiome-related parameters across cohorts, strengthening the robustness and translational relevance of the findings within the EUPAHW framework. These integrated results aim to inform optimized management, vaccination timing, and breeding strategies to enhance health, welfare, and sustainability in dairy production systems. Research co-funded by the European Union's Horizon Europe-101136346 EUPAHW.

Keywords: Dairy calves, Immune maturation, Early-life immunity, Microbiome, Resilience

Mandibular Osteosynthesis in a Nelore Calf: A Case Report

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BACKGROUND and OBJECTIVE: Mandibular fractures in calves may impair feeding and survival, requiring prompt surgical management. This report describes the treatment and outcome of a 22-day-old Nelore calf with bilateral mandibular fractures.

MATERIALS and METHODS: A 22-day-old Nelore calf weighing 32 kg was treated at the Veterinary Teaching Hospital of the Faculdade de Ciências Sociais e Agrárias de Itapeva, São Paulo, Brazil. The patient presented with dysphagia due to a mandibular injury that occurred during the first days of life, requiring milk administration via oroesophageal tube. Physical examination revealed deformities, abnormal mobility, and pain on palpation in the mandibular region. Radiographs confirmed bilateral fractures of the mandibular body with displacement of bone fragments and periosteal reaction. Anesthesia included xylazine (0,6 mg/kg IM), induction with ketamine (5 mg/kg) and midazolam (0,4 mg/kg IV), and maintenance with isoflurane (1,5–3%) using a 3-L rebreathing bag. Surgery consisted of controlled osteotomy of the bone callus, lesion debridement, smoothing bone edges, anatomical realignment, and internal fixation using a 2.0 locking compression plate. Layered suturing used absorbable polyglactin 2-0 for musculature and nylon 0 for the skin. The animal

was monitored during postoperative follow-up, and a second surgical procedure was later required.

RESULTS: Postoperative treatment included penicillins associated with streptomycin at a dose corresponding to 10.000 IU/kg of penicillin and 4 mg/kg of streptomycin (IM SID for seven days), combined with meloxicam (0,5 mg/kg IM SID for three days). Radiographs confirmed proper mandibular alignment. After two days of hospitalization, the calf was discharged with milk intake from a bottle and later resumed suckling directly from the cow. Skin sutures were removed 14 days after surgery. Thirty days later, the animal returned with an inflammatory reaction associated with the fixation plate, requiring implant removal. Bone consolidation at the fracture site was observed intraoperatively. Postoperative management included a single dose of meloxicam and five days penicillin therapy using the same medications used after the first surgery. Three days later, partial wound dehiscence occurred and was managed by second-intention healing with daily cleaning and topical therapy. Complete healing occurred 10 days after the second surgery. Final evaluation showed maintained mandibular alignment and normal feeding behavior.

CONCLUSIONS: The use of internal fixation with a locking plate provided adequate mandibular stabilization and satisfactory functional recovery in a calf with a bilateral mandibular fracture. Bone consolidation occurred 44 days after surgery, although implant removal was required due to a local reaction.

Keywords: calf, welfare, osteosynthesis, surgery, health

Assessment of the Prevalence and Risk Factors for Disbudding Wound Infections in Calves Disbudded with a Hot Iron

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BACKGROUND and OBJECTIVE: Hot iron disbudding is a common technique in dairy calves but remains a welfare concern due to pain and the risk of wound infection. Data on the prevalence of disbudding wound infections and associated risk factors are limited. This study aims to identify associations between individual factors, such as the failure of passive immunity transfer, the presence of disease, or hygiene conditions and the development of infections at disbudding wound sites.

MATERIALS and METHODS: A pilot prospective cohort study was conducted on 169 female Holstein calves from two dairy farms (around 300 lactating cows each), clients of the bovine ambulatory clinic of the University of Montréal. All calves were disbudded (< 2 months of age) using the same hot iron technique. These calves were followed weekly from birth until the re-epithelialization of the disbudding wounds. In the first week of life, passive immunity transfer was measured on each calf. Then, a clinical follow-up was performed weekly (including the first week) using previously published scoring systems, to identify respiratory and digestive diseases. At each visit, a bilateral lung ultrasound was also performed (defining pneumonia when maximal consolidation depth was ≥ 3cm), and data about calf hygiene were taken. To standardize the hygiene evaluation a previously published score (belly, side, rear) was used and partly modified to include the head (calf considered dirty if at least one area was ≥ 10%). For the post-disbudding period, wound assessment was added at the weekly visits using a previously validated visual scale, which allowed for the categorization of wounds according to their stage of healing and the presence or absence of infection. Basic univariate analyses (Pearson's chi-squared test, the Wilcoxon rank-sum test, and Fisher's exact test) were performed to evaluate the impact of calf-related factors, including health status, failure of passive immunity transfer, and poor

hygiene, on the probability of presenting a disbudding wound infection.

RESULTS: Calves with disbudding wound infections (n=115, 68%) showed the following parameters compared to non-infected ones respectively: failure of passive immunity transfer (19%; 24%, P=0.5), pneumonia (78%; 81%, P=0.6), diarrhea (90%; 89%, P=0.8), unsatisfactory hygiene score (92%; 83%, P=0.08). A significant farm effect was observed (Farm1 and 2, 65% and 90% of calves with infected wounds, P=0.025).

CONCLUSIONS: These preliminary results suggest that farm-related factors can have an important impact on the prevalence of disbudding wound infection. Further studies with more calves and advanced analyses are needed to identify specific risk factors.

Keywords: Disbudding, calf health, wounds healing

Nutritional Evaluation and Optimization of Dry TMR for Calves: Effects on Nutrient Digestibility and Growth Performance

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BACKGROUND and OBJECTIVE: The introduction of dry total mixed ration (TMR) as the first solid feed in calf nutrition has gained increasing interest. In Poland, this practice is particularly popular among farmers; however, the composition, physical structure, and recommended feeding levels of dry TMR have not yet been fully optimized. The aim of this study was to evaluate the nutritional value and apparent nutrient digestibility of a dry TMR used on a commercial dairy farm and to determine the effects of its nutritional optimization on nutrient utilization and growth performance in calves.

MATERIALS and METHODS: The study was conducted in 2024 on a private farm in eastern Poland. Twenty-four newborn calves in the early rearing period were divided into two groups (n = 12). All animals received milk replacer until weaning (up to 2 months of age) and had access to solid feed from the second week of life. The control group was fed the dry TMR (TMR I) originally introduced at the farm, while the experimental group received an optimized ration (TMR II). The reformulation included adjustment of the proportion of fiber and non-fiber carbohydrates, replacement of protein source, addition of yeast, and adjustment of the proportion of microelements. Chemical analyses of individual feed components, TMRs, and faeces were performed in accordance with AOAC [2019]. In addition, in vitro dry matter digestibility was determined using the DaisyII ANKOM system. The observation period lasted 8 weeks. Feed intake, body weight gain, health status and apparent nutrient digestibility were evaluated.

RESULTS: Dry matter intake was similar in both groups. The evaluation of the original dry TMR indicated the need for optimization of its composition in terms of energy supply, protein content, and fiber proportion. Apparent digestibility analysis revealed statistically significant differences ($p \leq 0.05$) between the groups, with higher nutrient digestibility (particularly for protein and fiber) in calves fed TMR II than in those fed TMR I. This was reflected in growth performance, as calves fed TMR II achieved, on average, 15% higher daily weight gains.

CONCLUSIONS: Although the use of dry TMR in calf nutrition is becoming increasingly popular, it requires further investigation. Calves, particularly in the early rearing period, require highly available sources of nutrients and energy. Therefore, more precise recommendations regarding the composition and proportion of individual components in complete dry TMR for calves are needed.

Keywords: calves, nutrition, performance, TMR

The Use of Thermography Method in the Diagnosis of Laminitis and Mastitis in Cows

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BACKGROUND and OBJECTIVE: Laminitis and mastitis are among the most common and economically significant diseases in dairy cattle, affecting animal welfare, milk production, and overall farm profitability. Early detection is crucial for timely treatment and reducing potential complications. Infrared thermography is a non-invasive technique that evaluates surface temperature changes associated with inflammation and altered blood circulation. The aim of this study was to assess the usefulness of thermography in diagnosing laminitis and mastitis in dairy cows.

MATERIALS and METHODS: The study was done on 150 dairy cows aged 2–9 years with 1–6 lactations. Thermographic images of hind hooves and udder quarters were obtained using a TESTO 875-1i infrared camera. During measurements, environmental temperature ranged from 10 to 15 °C, with approximately 50% humidity. A total of 300 hind hoof and 450 udder thermograms were recorded. Temperature measurements were taken at defined anatomical locations of the hoof joints and at the base of each teat. Based on clinical examination and thermographic findings, cows were classified into healthy and affected groups for laminitis and mastitis.

RESULTS: Thermography clearly detected temperature changes associated with laminitis. Cows with visible hind hoof lesions showed higher temperatures compared with healthy animals. The average hoof joint temperature increased by 4.82 °C (23.31%), while the coronary band region temperature was 2.85 °C (12.37%) higher in affected cows. Lactation influenced coronary band temperature by 1.80%, whereas lactation stage affected ankle and coronary band temperatures by 7.50% and 13.40%, respectively. Cow age and somatic cell count did not significantly affect hoof temperature results. In cows with mastitis, elevated udder surface temperatures were observed. The mean temperature of affected front quarters was 2.54 °C (8.77%) higher than that of healthy quarters, while affected hind quarters showed an increase of 1.66 °C (5.64%). Lactation had a minor influence (1.80%), and lactation stage had a minimal effect (0.40%) on front-quarter temperatures. Age and somatic cell count showed no statistically significant influence on mammary gland thermographic findings.

CONCLUSIONS: Infrared thermography appears to be a reliable, non-invasive method for early detection of laminitis and mastitis in dairy cows. The technique allows identification of inflammatory temperature changes in both hooves and mammary glands. Factors such as lactation and lactation stage showed limited influence on the results, while age and somatic cell count had no significant effect. Thermography may therefore serve as a practical screening tool for improving disease detection and herd health monitoring in dairy farms.

Keywords: Cow, Laminitis, Mastitis, Thermography

Infectious Bovine Rhinotracheitis Infection as a Predisposing Factor for Calf Diphtheria

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BACKGROUND and OBJECTIVE: Calf diphtheria is an infection of the oral and laryngeal soft tissues caused by *Fusobacterium necrophorum* following mucosal injury. It typically affects calves 1–4 months of age and is most commonly associated with outbreaks under unhygienic conditions. Known predisposing factors include intercurrent diseases such as persistent bovine viral diarrhea (BVD) virus infection and nutritional deficiencies. Although Infectious Bovine Rhinotracheitis (IBR), caused by bovine herpesvirus-1 (BoHV-1), can occasionally produce shallow erosions on the oral mucosa, it has not previously been linked to calf diphtheria. This study reports cases of calf diphtheria that developed during an IBR outbreak

MATERIALS and METHODS: Over a 40-day period, seven Holstein calves aged 25 - 40 days recovering from acute respiratory disease showed reduced milk consumption, varying degrees of salivation, and malodorous breath. Specifically, two calves had

cheek swelling with diphtheritic plaques in the gingivobuccal sulcus. One calf developed an extensive diphtheritic lesion on the hard palate, while another had a smaller lesion on the hard palate accompanied by fever and inspiratory stridor. A further calf presented with inspiratory stridor, fever, and a swollen, painful laryngeal region on palpation. Another calf showed excessive salivation with diphtheritic plaques on the soft palate and pharynx, and one calf had a lesion on the lateral side of the tongue. Four cases occurred in the same rearing pen (18 calves), while the remaining three were isolated cases in pens of approximately 20 calves each. All animals were fed by automatic milk feeders and had previously received florfenicol and meloxicam for the treatment of respiratory disease. Blood samples were collected for BVDV antigen detection by PCR and for gE antibody detection against BoHV-1 by ELISA. Calves with laryngeal involvement were initially treated with lincomycin-spectinomycin for 5 days plus dexamethasone for 3 days, followed by long-acting amoxicillin until recovery. The remaining calves received long-acting amoxicillin for at least 8 consecutive days and meloxicam twice, 48 hours apart.

RESULTS: The clinical diagnosis in all cases was calf diphtheria. All seven calves were positive for gE antibodies against BoHV-1 and negative for BVD virus antigen. The two calves with laryngeal involvement and the one with the extensive hard-palate lesion recovered after 14 days of treatment; the remaining calves recovered within 8–10 days.

CONCLUSIONS: Under certain conditions, IBR infection may act as a predisposing factor for secondary calf diphtheria during the recovery phase of clinical respiratory disease. Routine vaccination against IBR should therefore not be neglected in prevention programs.

Keywords: calves, IBR, calf diphtheria

Diagnostic Value of Hematological Indices in Neonatal Calves with Systemic Inflammatory Response Syndrome

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BACKGROUND and OBJECTIVE: Systemic inflammatory response syndrome (SIRS) is a critical clinical condition in neonatal calves associated with high morbidity and mortality and can result from both infectious and non-infectious causes. Early diagnosis is essential for improving clinical outcomes. This study aimed to evaluate the diagnostic performance of hematological indices in neonatal calves with SIRS.

MATERIALS and METHODS: A total of 482 neonatal calves diagnosed with SIRS and 39 clinically healthy calves aged between 1 and 30 days were included in the study. SIRS was defined based on the presence of at least two of the following criteria: abnormal body temperature, heart rate, respiratory rate, and leukocyte count. Blood samples were collected from all animals and complete blood count parameters were used to calculate neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (LMR), derived neutrophil-to-lymphocyte ratio (dNLR), neutrophil-to-lymphocyte*platelet ratio (NLPR), systemic immune-inflammation index (SII), systemic inflammatory response index (SIRI), and aggregate index of systemic inflammation (AISI). Diagnostic performance was assessed using receiver operating characteristic (ROC) analysis.

RESULTS: NLR, dNLR, NLPR, SII, SIRI, total leukocyte count, and neutrophil count were significantly higher in calves with SIRS compared to healthy controls ($P<0.05$). The highest diagnostic performance was observed for neutrophil count (AUC=0.82) and total leukocyte count (AUC=0.77). Among the calculated indices, NLPR (AUC=0.78), NLR (AUC=0.76), and dNLR (AUC=0.76) demonstrated the best performance. SIRI (AUC=0.71) showed moderate diagnostic value, whereas SII (AUC=0.64),

AISI (AUC=0.63), PLR (AUC=0.58), and LMR (AUC=0.54) had limited diagnostic utility.

CONCLUSIONS: These findings suggest that simple, inexpensive, and readily available markers such as neutrophil count, leukocyte count, and ratios including NLPR, NLR, and dNLR can be used in clinical practice as supportive tools for the early diagnosis of SIRS in neonatal calves. However, these markers should not be used alone; instead, they should be interpreted together with clinical findings and other laboratory results to improve diagnostic accuracy and support clinical decision-making.

Keywords: SIRS, Sepsis, Calves, Hematological Index

PCR Prevalence of Coronavirus in Dairy Calves with Respiratory Disease in South-Western Poland

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BACKGROUND and OBJECTIVE: Bovine coronavirus (BCoV) is not only an aetiological agent of neonatal diarrhea, but also a viral element of bovine respiratory disease (BRD), a polyetiological disease in the fall and spring months, particularly in young animals, less than a year old. This virus is the first to colonize the mucous membrane of the upper respiratory tract. Transmission of BCoV is mainly through respiratory or fecal-oral routes. When infected with BCoV, within-herd transmission is generally rapid, and infected animals display diverse clinical signs including diarrhea with or without blood, fever, and respiratory signs at variable severity.

MATERIALS and METHODS: The aim of the study was to determine the BCoV prevalence in BRD outbreaks of dairy cattle farms. The study was conducted from January 2022 to January 2025 on 42 dairy herds. The samples came from herds located in following provinces: Greater Poland, Lower Silesian, Opole and Silesian. The farms were divided into 2 groups: smaller farms (under 500) and larger farms (over 500 animals). On each tested farm, 4 to 6 samples (nasal swabs) were collected from calves showing symptoms of BRD. Sampled animals were not previously vaccinated against viral pathogens. From the obtained samples, the genetic material was isolated using a commercial kit "Total RNA Mini Plus". The real-time PCR reaction was carried out using the primers: F: 5'-CTGGAAGTTGGTGGAGTT-3' and R: 5'-ATTATCGGCCTAACATACATC-3' and probe: 5'-CCTTCATATCTATACACATCAAGTTGTT-3'.
Samples with a quantification cycle (Cq) of 37 or less were considered positive.

RESULTS: Genetic material from BCoV was present in samples originating from 88% of the farms (n=37). In smaller farms, BCoV was detected in 79% sites (n=19). All the larger farms(n=18) tested positive.

CONCLUSIONS: BCoV is a ubiquitous infection, and BCoV-specific genetic materials or antibodies have been detected in cattle populations in numerous countries. In our study, we confirmed the widespread nature of this virus and a significant association between presence BCoV and larger herd sizes. This may be due to poor biosecurity measures and a lack of control programs against BCoV, especially among farms with larger herd sizes in Poland. Additionally, close contact between animals in these farms which could potentiate the transmission of BCoV compared to farms with smaller herd sizes.

Keywords: BRD, BCoV, calf

Resistome and Virome Changes Following Fecal Microbiota Transplantation (FMT) in Diarrheic Preweaning Calves

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BACKGROUND and OBJECTIVE: Calf diarrhea is a major cause of morbidity and economic loss in preweaning calves. Fecal microbiota transplantation (FMT) is a promising strategy to restore gut microbial balance; however, its effects on antimicrobial resistance (AMR) and viral communities remain poorly understood. This study investigated resistome and virome dynamics following FMT in diarrheic preweaning calves.

MATERIALS and METHODS: A total of 72 FMT procedures were performed using 24 healthy donors, with 55 resulting in clinical recovery by Day-7. Of these, 60 recipients (49 successful, 11 failed cases), along with their corresponding 24 donors, were randomly selected, and their fecal DNA samples were subjected to shotgun metagenomic sequencing. AMR genes were identified using AMRFinder from MEGAHIT assemblies, and DNA viral communities were profiled using Kraken2.

RESULTS: Healthy donor calves harbored a diverse and abundant resistome gene contigs (177.75 ± 35.44), representing a substantial baseline reservoir. This included predominantly non–high-risk genes (e.g., aadA27, ant (3'')-IIa, blaCARB-2, qnrA), alongside a limited number of clinically relevant determinants (e.g., vanH-D). These findings indicate that even clinically healthy donors carry a complex resistome shaped by environmental and ecological factors. Donors exhibited a consistently rich and stable virome dominated by bacteriophages. Highly abundant and prevalent taxa included Andhravirus andhra (10.38%), Valbvirus ValB1MD2 (8.94%), Eponavirus epona (7.96%), and phages such as Burzaovirus coli (6.54%) and Burzaovirus faecalis (4.95%). These viruses were widely distributed across donor samples, reflecting core virome associated with a stable gut ecosystem. In recipients, successful FMT was associated with coordinated resistome and virome restructuring. The Day-7 Success group showed emergence of donor-associated AMR genes, including blaCTX-M-8, rmt, tet(X) variants, along with shifts in resistance profiles (increase in mph(B), aph(2'')-Ih, vanW, cfxA6; decrease in tet, qnr, dfr, and cat genes). Concurrently, the virome shifted toward a more donor-like composition, with increased prevalence of viruses such as Eponavirus epona, Brigitvirus brigit, and Wotdevirus murinus. Viral community composition differed significantly before and after FMT ($p = 0.02$), indicating successful ecological restructuring. In contrast, failed recipients showed limited microbiome change, retaining distinct AMR profiles (e.g., blaOXA-489, mcr-5.1) and an unstable virome with no significant temporal shift ($p = 0.177$), suggesting limited donor microbiota engraftment.

CONCLUSIONS: Healthy donors harbor a stable, phage-rich virome and diverse resistome that are partially transferred to recipients during successful FMT. These findings emphasize the importance of monitoring donor resistome and virome composition to ensure the efficacy and safety of FMT for calves with diarrhea.

Keywords: Calf, Diarrhea, FMT, AMR, Virome

Longitudinal Study of Lung Ultrasound (LUS) Scores of Untreated Healthy and Untreated Dairy Calves from Birth to 4 Months of Age - An Observational Study

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BACKGROUND and OBJECTIVE: Lung Ultrasound (LUS) has been established as practical diagnostic tool to objectively diagnose early-stage respiratory tract disease under field conditions in young calves. In this study we report the development of LUS

scores over time in clinically healthy and untreated calves from birth to 4 months of age.

MATERIALS and METHODS: Over the course of one year all calves born and raised on a conventional German dairy farm underwent repeated LUS following an established protocol once a month. Calves were first housed in individual hutches until 2 weeks of age and were then moved to well ventilated group pens (5m2/calf) where they were offered up to 12 L of milk replacer per day through an automated milk feeding system. LUS was performed by the same operator on the 2nd to 5th intercostal space (ICS) on both sides of the thorax. Observed lesions were scored into 4 categories (0 to 3) for each ICS. The maximum score (MAX) for each exam, the number of ICS with scores > 0 (FREQ) and the sum of the scores of all ICS (SUM) were determined.

RESULTS: A total of 130 calves were examined between one and 4 times in monthly intervals. Calves had an average daily weight gain of 1.1 kg/d and no antimicrobials or NSAIDs were administered throughout the observation period. A MAX= 0 was recorded in 28%, 33%, 11%, 14% and 14% in calves 0 (hutches), 1, 2, 3 and 4 months old, respectively. MAX=1 was recorded in 63%, 74%, 86%, 79% and 65% in calves 0 (hutches),1, 2, 3 and 4 months old, respectively. MAX=2 was diagnosed in 9%, 4%, 8%,12% and 22% of calves 0, 1, 2, 3 and 4 months old. The highest FREQ and SUM scores were recorded in calves 2 and 3 months old.

CONCLUSIONS: In the present study most cases of mild bronchopneumonia were already diagnosed in the first 2 weeks of life, and before entering group pens. A second bout of mild bronchopneumonia occurred in calves at two months of age, although animals remained clinically healthy. Without treatment several calves tended to overcome disease during the 3rd and 4th month of life, while others appeared to have progressed to more chronic lesions. The results emphasize the importance of including the first weeks of life in any strategy to prevent respiratory disease in calves.

Keywords: Lung ultrasonography, Bovine Respiratory Tract Disease, Diagnosis

Antimicrobial Exposure Disrupts Gut Microbiota Maturation During a Critical Developmental Window in Preweaned Dairy Calves

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BACKGROUND and OBJECTIVE: The establishment of a stable gut microbiota is essential for calf health. Although microbiota maturation is influenced by environmental and management factors, the impact of antimicrobial administration, particularly continuous farm-level use, remains unclear. This study aimed to investigate the relationship between antimicrobial exposure and gut microbiota maturation in preweaned dairy calves across multiple farms.

MATERIALS and METHODS: A longitudinal study was conducted in 165 preweaned dairy calves from 8 farms in Chiba Prefecture, Japan. Fecal samples were collected at 30 and 60 days of age, and from a subset of calves immediately after birth. Antimicrobial usage data were obtained from farm management records and clinical charts. Gut microbiota composition was analyzed using 16S rRNA gene sequencing. Calves were categorized based on antimicrobial exposure into continuous farm-level administration and individual therapeutic administration groups. Microbiota maturation was assessed using alpha and beta diversity metrics, and group differences were evaluated using PERMANOVA.

RESULTS: Gut microbiota composition changed significantly with age (all comparisons: $P < 0.01$). Significant differences in microbiota structure were observed between calves exposed to continuous antimicrobial administration and those without exposure ($P < 0.01$). Between 30 and 60 days of age, significant microbiota development was observed in the non-exposed group ($P < 0.01$), whereas such changes in the continuously exposed group were relatively limited, indicating suppressed microbiota maturation. In contrast, single therapeutic antimicrobial administration had no significant effect on the microbiota development ($P = 0.25$).

CONCLUSIONS: Microbiota development is essential in preweaned calves, and continuous antimicrobial administration may affect the gut microbiota maturation during the critical developmental window between 30 and 60 days of age. Disruption of microbiota development during this period may influence calf health, highlighting the need for optimized antimicrobial use strategies, particularly in early life.

Keywords: Antibiotics, Critical developmental window, Gut microbiota, Microbiota maturation, Preweaned calves

Comparison of Hematological and Blood Biochemical Parameters to Assess the Effect of Iron Substitution in Calves

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BACKGROUND and OBJECTIVE: Assessing the iron status in calves remains a diagnostic challenge. Beside hematologic parameters several serum biochemical markers, including Fe, transferrin and its saturation (TSAT) have been studied in cattle. Parameters such as the reticulocyte hemoglobin (CHr) are established in other species but not in ruminants. We aimed at comparing hematological parameters with the markers mentioned above for their suitability to assess the effect of Fe-substitution in calves.

MATERIALS and METHODS: Thirty dairy calves were assigned to either parenteral (INJ) or oral (ORAL) administration of 1000 mg Fe3+, or a sham treatment (CON) at 3 days of age. Blood was collected before treatment, 3 days after treatment and at 2, 3, and 4 weeks of age. Hematology was conducted and serum was assayed for Fe, transferrin, and TSAT.

RESULTS: Significant treatment x time interaction effects were only determined on the RBC (p=0.03), Hb (p=0.001), PCV (p=0.005), reticulocyte count (p=0.0002) and CHr (p=0.045). INJ showed highest increases in Hb (17,8%) followed by ORAL (6,7%) at 4 weeks. Correlation analysis revealed the strongest associations between Hb and PCV (rho≤0.88, p<0.0001). Hb was found to be correlated with TSAT (rho≥0.50, p≤0,005) and Fe (rho≥0.51, p≤0.004), and to a lesser extent with CHr (rho=0.385, p≤0.0358). CHr showed the strongest positive correlations with Fe and TSAT (rho>0.54, p<0.002).

CONCLUSIONS: Iron supplementation was best reflected in changes of Hb and PCV in these non-anemic calves. Fe and TSAT were associated with Hb without displaying a direct treatment effect. CHr was found to be associated with the availability of Fe, but not with any effect on hematologic the biologic parameters.

Keywords: Iron status, dairy calf, reticulocyte heoglobin, hemoglobin, packed cell volumne

Paromomycin Preserves Gut Microbiota Development While Controlling Neonatal Diarrhea in Preweaned Dairy Calves

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BACKGROUND and OBJECTIVE: Neonatal diarrhea remains a major constraint to calf health and productivity, with early-life gastrointestinal disturbances closely linked to microbiota development. This study evaluated gut microbial dynamics in preweaned Holstein calves receiving paromomycin (Parofor® oral solution) during the neonatal period.

MATERIALS and METHODS: The experiment was conducted at a commercial dairy farm in Brazil. Forty newborn calves met inclusion criteria (eutocic calving, adequate vitality, and confirmed passive transfer). Calves were housed individually and fed whole milk (14% total solids) twice daily, with free access to water and a commercial starter (22% crude protein). Paromomycin was administered orally (Parofor oral solution, Huvepharma, 50 mg/kg body weight) once daily from day 5 to day 11 of life. Fecal samples from a subset of 10 calves were collected at three time points: day 1 (D1; prior to treatment, at 5 days of age), day 7 (D7; end of treatment), and day 21 (D21; 14 days after the end of treatment).

RESULTS: Microbiota composition was analyzed using 16S rRNA gene sequencing, generating 4,801,629 sequences clustered into 6,074 amplicon sequence variants. Alpha diversity indices were primarily influenced by age and individual variation ($P>0.05$), with a progressive increase over time, consistent with microbiota maturation. Reduced diversity observed in some calves at D21 was associated with diarrhea events, indicating transient dysbiosis. These patterns align with previous studies, reinforcing that early-life microbial succession follows a predictable trajectory. At D, the microbiota was dominated by facultative anaerobes such as Escherichia–Shigella and Enterococcus, along with early beneficial genera including Bifidobacterium and Blautia. By D7, a transitional community emerged, with increased abundance of Bacteroides, Collinsella, and Faecalicoccus, reflecting adaptation to anaerobic conditions. At D21, the microbiota exhibited a more mature and stable profile dominated by Blautia, Lactobacillus, and Faecalibacterium, genera associated with short-chain fatty acid production and gut health. Principal coordinates analysis revealed clear clustering by sampling day (PERMANOVA: $F = 4.983$, $R^2 = 0.249$, $P < 0.001$), confirming age as the primary driver of microbial composition. Differential abundance analysis showed reductions in genera associated with gastrointestinal disturbance and enrichment of commensal fermentative bacteria over time. Importantly, no relevant alterations in microbiota development were attributable to paromomycin.

CONCLUSIONS: In conclusion, early-life paromomycin administration does not disrupt gut microbiota maturation and supports the establishment of a stable microbial ecosystem in dairy calves. Therefore, its use is recommended for the treatment and prevention of neonatal diarrhea, as it contributes to reducing diarrhea-associated morbidity and mortality while improving calf performance.

Keywords: dairy cattle, gut microbiota, neonatal diarrhea, microbial maturation.

Paromomycin Reduces Diarrhea Frequency and Oocyst Shedding Compared with Halofuginone in Dairy Calves Naturally Exposed to *Cryptosporidium parvum*

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BACKGROUND and OBJECTIVE: Neonatal diarrhea remains a leading cause of morbidity and economic loss in preweaned dairy calves, with Cryptosporidium parvum recognized as one of the most prevalent enteric pathogens during early life. This study compared the effects of halofuginone and paromomycin on diarrhea occurrence, duration, fecal oocyst shedding, and hydration requirements in Holstein calves naturally exposed to C. parvum under commercial conditions in São Paulo State, Brazil.

MATERIALS and METHODS: Seventy newborn calves were randomly assigned to receive halofuginone (100 µg/kg BW; n = 30) or paromomycin (50 mg/kg BW; n = 40; Parofor oral solution®, Huvepharma) orally once daily for 7 consecutive days (d 5 to 11 of life). Calves were housed

individually, fed whole milk, and monitored throughout the preweaning period. Fecal consistency (score 0–3) was recorded daily, hydration status was clinically assessed, and oral electrolyte therapy was provided when fecal score ≥2. Fecal samples were collected at d 1, 3, 5, 7, and 14 for quantitative oocyst enumeration using sucrose flotation and Neubauer chamber counting.

RESULTS: Colostrum quality and passive transfer of immunity did not differ between groups, confirming adequate early-life management. Median colostrum Brix values were 30.1% and 28.5% for paromomycin and halofuginone groups, respectively (P = 0.226), while serum Brix at 48 h averaged 10.7% and 10.5% (P = 0.447). The temporal diarrhea incidence differed markedly between treatments. Calves receiving halofuginone exhibited an early and pronounced increase in diarrhea beginning at d 8, peaking at approximately 70% around d 19. In contrast, paromomycin-treated calves maintained near-zero incidence during the first 10 days, followed by a moderate peak (~40%). A generalized estimating equation (GEE) model revealed a strong treatment effect (P < 0.0001), with halofuginone-treated calves having significantly higher odds of diarrhea (OR = 4.93; 95% CI: 2.98–8.17). Paromomycin also significantly reduced diarrhea duration, with halofuginone-treated calves experiencing 3.40 times more diarrheic days (95% CI: 2.20–5.24; P < 0.001). The need for oral hydration was greater in the halofuginone group (RR = 1.93; 95% CI: 1.48–2.50; P < 0.001). Oocyst excretion differed over time, with paromomycin-treated calves showing lower and less variable counts, particularly at peak infection (d 5: $4.41 \pm 0.9 \times 10^6$ vs. $6.78 \pm 1.6 \times 10^6$ oocysts/g; P = 0.04).

CONCLUSIONS: In conclusion, paromomycin markedly reduced diarrhea incidence, duration, and clinical intervention requirements, while also mitigating Cryptosporidium shedding. These findings support paromomycin as an effective strategy for controlling neonatal cryptosporidiosis and improving calf health under commercial dairy conditions.

Keywords: dairy cattle, gut health, neonatal diarrhea, enteropathogens.

Transforming Calf Health Under Commercial Conditions: Paromomycin Reduces Diarrhea Morbidity and Improves Growth Performance

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BACKGROUND and OBJECTIVE: Neonatal diarrhea remains one of the most significant constraints to dairy calf health and productivity worldwide, driving antimicrobial use, increasing production costs, and compromising long-term performance. Cryptosporidium spp. is a primary enteric pathogen during early life, and effective control strategies under commercial conditions are still limited. This study evaluated the impact of including paromomycin in a neonatal health protocol on diarrhea incidence, antimicrobial usage, and growth performance in dairy calves.

MATERIALS and METHODS: A longitudinal before–after study was conducted on a commercial dairy farm in Alto Paranaíba, Minas Gerais, Brazil, comparing calves born before (January–September 2025) and after (October 2025–February 2026) the implementation of paromomycin. Passive transfer of immunity was confirmed at 48 h using serum Brix refractometry, with more than 80% of calves achieving values >9.3%. Calves were managed under consistent conditions, receiving whole milk (16% total solids; 6 L/day), live yeast supplementation, and ad libitum starter. After implementation, paromomycin (Parofor Oral Solution®, Huvepharma) was administered orally at 50 mg/kg body weight once daily from day 4 to day 10 of life. Diarrhea incidence between 1 and 15 days of age and average daily gain (ADG) were compared between periods.

RESULTS: The intervention resulted in a marked reduction in diarrhea burden, with incidence decreasing from 165.6% (reflecting multiple episodes per calf) to 9.56% in average. Importantly, the need for parenteral antimicrobial therapy, previously required in all diarrheic calves, was completely eliminated following protocol implementation. Growth performance improved substantially, with ADG from 0 to 30 days increasing from 710 to 854 g/day, and preweaning ADG increasing from 800 to 902 g/day. Weaning weight increased from 90 kg to 97 kg at a similar weaning age (62 days).

CONCLUSIONS: These findings demonstrated that targeted early-life intervention with paromomycin can profoundly improve calf health outcomes under commercial conditions. By simultaneously reducing disease burden, eliminating injectable antimicrobial use, and enhancing growth performance, this strategy represents a scalable and impactful solution for modern dairy systems. The results support a shift toward more sustainable calf-rearing practices aligned with improved productivity and responsible antimicrobial stewardship.

Keywords: Neonatal diarrhea, dairy cattle, Cryptosporidium parvum, Antimicrobial use, growth performance.

Preliminary Study on Salivary Biomarkers of Stress, Redox Status and Passive Transfer in Neonatal Calves: Effects of Colostrum Administration Method and Type

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BACKGROUND and OBJECTIVE: In neonatal calves, passive immunity depends entirely on the timely intake of colostrum, as maternal antibodies are not transferred across the placenta. Although esophageal tube feeding is commonly used in livestock management for practical reasons, its effects on calf welfare are still debated. This preliminary study aimed to compare the effectiveness of passive immunity transfer between administration methods and to evaluate their impact on salivary biomarkers related to stress, inflammation, and redox status.

MATERIALS and METHODS: A total of 35 Holstein calves were included and allocated to two groups based on the method of colostrum administration (17 esophageal tube; 18 bottle-fed). Calves received either natural colostrum (n = 17) or a powdered colostrum replacer (n = 18), depending on availability; this factor was not part of the initial group allocation. Salivary cortisol, adenosine deaminase (ADA), total antioxidant capacity (TAC), total oxidant status (TOS), and oxidative stress index (OSI) were measured at T0, 30 minutes (T30), and 24 hours (T24) after colostrum administration. At 24 hours, ADA, TAC, TOS, and OSI were also measured in serum, together with total proteins (TP) as an indirect indicator of passive immunity transfer.

RESULTS: Delta analysis (ΔT30–T0 and ΔT24–T0) using a linear mixed model revealed significant changes over time for cortisol and ADA, indicating an immediate and transient stress response associated with colostrum administration. No significant differences were observed between administration methods. A slight, non-significant effect of colostrum type was observed for ADA and TOS. Serum total protein levels at 24 hours showed a marked increase compared to baseline, confirming effective passive immunity transfer. Group comparisons indicated that the method of administration did not influence TP levels, while colostrum type showed a trend in favor of natural colostrum, although without reaching statistical significance. Correlation analysis at 24 hours showed a moderate association for TAC, weak correlations for ADA and TOS, and negligible correlation for OSI, indicating that saliva only partially reflects systemic redox status.

CONCLUSIONS: These preliminary findings suggest that passive immunity transfer may be effective regardless of the method of colostrum administration, and that esophageal tube feeding does not appear to increase salivary stress biomarkers compared to bottle feeding. A slight, non-significant trend in favor of natural colostrum was observed. Saliva may represent a promising non-invasive matrix for assessing redox status; however, its reliability appears to vary among

biomarkers. Further studies with larger sample sizes are needed to confirm these results.

Keywords: calf, colostrum, saliva

Effects of Various Dilution Rates and Volumes of Potassium Sorbate on Colostrum Bacterial Contamination Over Time at Ambient and Refrigeration Temperatures

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BACKGROUND and OBJECTIVE: Preservation of colostrum on farm is challenging, since cold temperature storage facilities (refrigerators and freezers) often cannot accommodate the volume of colostrum required to feed calves. Potassium sorbate is a readily available food grade preservative (PSP); shown to be very effective at preserving colostrum at both refrigeration and ambient temperatures. Aims of the current work were to determine the optimal dilution rates (DR) and by volume rate (VR) of PSP to preserve bovine colostrum for feeding to neonatal calves.

MATERIALS and METHODS: Twenty individual cow 'point of feeding' colostrum samples were collected. Three PSP dilution rates (DR) were created (25.4%, 32.2%, and 39.%) by mixing potassium sorbate crystals with 100mL of distilled water. For each dilution, 50 µL (1% by volume) and 25 µL (0.5% by volume) were added to each 5mL aliquot of colostrum to create two different volume rates (VR) for each DR. For each DR and VR, one aliquot was stored at ambient temperature (AT= 23.1°C-24.6°C) and one aliquot was stored at refrigeration temperatures (RF= 3.1°C-4.2°C). Baseline bacteria counts were also measured for each colostrum sample. Aliquots were tested for bacterial contamination at 24 hours, 72 hours and 168 hours. Mixed linear regression models were constructed using TCC and TBC as outcomes of interest, with DR (30 g/100 mL; 40 g/100 mL or 50 g/100 mL), VR (1% by volume and 0.5% by volume) and temperature (RF or AT) as predictors of interest. Survival analysis was also used to account for bacteria counts too numerous to count (TNTC). TNTC was analysed as a 'failure event' and the time frame at which these 'failures' occurred was analysed.

RESULTS: In all cases, 50 g DR was more effective than DR of 30 g and 40 g or no preservative and 1% VR was more effective than 0.5% VR or no preservative at preventing bacterial proliferation at 24 and 72 hours. RF was more effective than AT at preventing bacterial proliferation at 24 hours and 72 hours. Survival models showed a lower hazard of high contamination at VR of 1% and DR of 50 g. A large number of samples in this work were initially contaminated with total bacterial species and this may have biased results, representative of the current situation on dairy farms.

CONCLUSIONS: Potassium sorbate can be used effectively to minimise bacterial proliferation in bovine colostrum at 39% dilution rate and 1% by volume, preferably at refrigeration but also at ambient temperatures.

Keywords: Calves, Colostrum, Preservation

Impact of Passive Immunity and IgG from Transition Milk on Pre-Weaning Dairy and Dairy-Beef Cross Calf Health in a Pasture-Based Block Calving System: A Cohort Study

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BACKGROUND and OBJECTIVE: The objective of this cohort study was to evaluate how passive immunity, measured by serum IgG at 24-36 hours of life, and transition milk IgG intake was associated with mortality, treatment incidence, antibiotic use and growth in dairy calves reared within a block calving system.

MATERIALS and METHODS: A total of 251 calves were included in the study which comprised 139 British Friesian dairy replacements, 92 Wagyu, 19

Hereford and 1 Simmental beef-on-dairy calves. Every calf was blood sampled to measure the level of serum IgG at 24 to 36 hours of age using radial immunodiffusion testing (RID). Excess dam's colostrum and transition milk from milkings 2 - 6 after calving were pooled, pasteurized, and fed to all calves from the second feeding of life to 10 days of age. The transition milk was also tested daily using RID, to determine the total quantity of IgG fed over the 10-d period to each calf.

RESULTS: Calves born in April and May had a higher odds of dying in the preweaning period compared to calves born in February. In addition, for every 1 g/L increase in serum IgG measured at 24 to 36 hours of age, the odds of mortality declined by 12%. Calves with lower serum IgG at 24 to 36 h benefited most from higher IgG in transition milk, as shown by reduced mortality risk. Similar to mortality, every 1 g/L increase in serum IgG at 24 to 36 hours was associated with a 11% decline in the odds of treatment. Those born in March and April also had a higher odds of treatment compared to calves born in February. Calves with serum IgG levels > 22.5 g/L serum IgG gained 0.08 kg/d more compared to those with a serum IgG of < 9.3 g/L. Further, calves fed > 470 g of IgG in transition milk gained 0.07 kg/d more than those that consumed < 260 g of IgG in transition milk.

CONCLUSIONS: This study demonstrated that higher levels of passive immunity were associated with reduced mortality, lower odds of disease treatment and antibiotic use, and improved growth rates, whereas increased IgG intake through transition milk were associated with reduced preweaning mortality, especially in calves with low passive immunity, and improved growth, underscoring the importance of passive immunity and transition milk feeding in block calving systems.

Keywords: pasture-based, block calving, passive immunity, transition milk

Pathogen-Oriented Approach for Intervention and Prevention Of mycoplasma bovis pneumonia in Belgian Dairy Calves Using Qtus and Nbal Diagnostics

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BACKGROUND and OBJECTIVE: Nonendoscopic bronchoalveolar lavage (nBAL) diagnostics and quick thoracic ultrasound (qTUS) enable timely pathogen identification, detection of subclinical pneumonia, and precision treatment. This study describes the effects of a pathogen-oriented approach using nBAL diagnostics and qTUS on ultrasonographic lung health in a dairy farm facing *Mycoplasma bovis* pneumonia, and to assess the influence of lesion severity and housing at first treatment on ultrasonographic cure.

MATERIALS and METHODS: This observational study was conducted on a Flemish dairy farm including 143 calves (aged 2-20 weeks) monitored between May and September 2022 at 10-to-21-day intervals. At the first visit, nBAL samples were collected and analysed by culture and nanopore sequencing. Using the qTUS score, calves were classified as healthy (no consolidation), mild (<1 cm), moderate (≥1 cm to <3 cm), or severe pneumonia (≥3 cm). After a two-day metaphylaxis with florfenicol, treatment duration was adapted to lesion severity and only calves with lung consolidation ≥1 cm at that point were retreated. At each visit, pneumonia prevalence, new cases and ultrasonographic cure (i.e. regression below 1 cm and full reaeration) were assessed. Cure and odds to cure were compared across qTUS categories and housing

type using chi-square tests and binary logistic regression.

RESULTS: At visit 1, lung consolidation ≥1 cm was found in 61.1% (55/90) of calves, with 31.1% (28/90) classified as severe. *M. bovis* and *Pasteurella multocida* were identified in all sampled calves, and management changes were focused on control of *M. bovis*. By visit 2, pneumonia prevalence decreased to 25.5%, resulting in a short-term cure of 58.2% (32/55). Full reaeration after qTUS guided metaphylaxis was seen in 100% (7/7), 59.3% (16/27) and 25.0% (7/28) of the calves with mild, moderate and severe pneumonia respectively (p <.001). After isolation of chronic cases, cure increased progressively over subsequent visits (64-75%). By the final visit, pneumonia prevalence was 13.3% (15/112), resulting in a final cure of 74.5% for the initial cases (41/55). At this final assessment, compared to calves with moderate or mild pneumonia at visit 1, odds for full reaeration were reduced by 77% (OR=0.23; 95%CI: 0.07–0.72) and 73.7% (OR=0.26; 95%CI: 0.08–0.82) in calves initially classified with severe pneumonia. Cure was higher in individually housed calves (100%) (8/8) than in group housed calves (51.1%) (24/47) (p =0.015).

CONCLUSIONS: qTUS guided intervention and nBAL diagnostics enabled pathogen-oriented management, resulting in increased lung health. The associations between lesion severity and housing at start of treatment emphasize the importance of early detection to achieve optimal treatment efficacy.

Keywords: Bovine respiratory disease, calf pneumonia, Mycoplasma bovis, quick thoracic ultrasound, nonendoscopic bronchoalveolar lavage

Heat-killed Lactobacillus Sakei HS-1 Modulates the Number of T Cell Subsets and Monocytes in Preweaned Calves

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BACKGROUND and OBJECTIVE: Preweaned calves remain immunologically immature during the first 30 days of life, despite adequate colostrum intake. Therefore, they are susceptible to infections, which can impair their growth and productivity. Probiotics have been used to support early immune development in calves. Paraprobiotics are non-viable microorganisms with health benefits. Accordingly, they have gained attention as a stable alternative to probiotics. Supplementation with heat-killed *Lactobacillus sakei* HS-1 (HK-LS) reportedly reduces the incidence of diarrhea requiring treatment. However, research on the immunological effects of paraprobiotics in calves remains limited. Therefore, the present study aimed to investigate the immunomodulatory effects of HK-LS supplementation on the temporal dynamics of leukocyte populations in the peripheral blood of preweaned calves.

MATERIALS and METHODS: Twenty clinically healthy Holstein Friesian calves (3 days old; 13 males and 7 females) obtained from local dairy farms were

randomly assigned to either the control (CG, n = 10) or HK-LS administration (AG, n = 10) group. One CG female calf died during the experimental period, resulting in a final sample size of 19 calves. The AG was orally supplemented with HK-LS (1×10^7 non-viable cells/g) twice daily from day 3 (designated as D0). Blood samples from the jugular vein on D0, D7, and D21 were collected. Leukocyte subsets ($CD3^+$, $CD4^+$, $CD8^+$, $WC1^+$, $CD14^+$, $CD45R^+$, and $CD21^+$ cells) were analyzed by flow cytometry. All animal procedures were performed according to institutional guidelines. Data were analyzed using generalized linear mixed models in SPSS.

RESULTS: The lymphocyte and white blood cell counts showed significantly different dynamics between the two groups; however, no significant differences were observed at any time point. The cell counts of the T cell subsets—$CD4^+CD45R^-$, $CD8^+CD45R^-$, and $WC1^+$ lymphocytes—were significantly different between the groups, with the AG showing significantly higher cell counts for $CD4^+CD45R^-$ lymphocytes on D7 and for $CD8^+CD45R^-$ and $WC1^+$ lymphocytes on D21 than the CG. The monocyte subsets ($CD14^+$ PBMC) were significantly different between the groups, with the AG exhibiting significantly higher cell counts on D7 and D21 than the CG.

CONCLUSIONS: HK-LS supplementation may enhance early-life immunity in preweaned calves as evidenced by selective increases in effector/memory-like $CD4^+CD45R^-$ and $CD8^+CD45R^-$ T-cell subsets, γδ T cells, and $CD14^+$ monocytes. However, our study evaluated only the changes in lymphocyte dynamics and did not assess functional immune responses. Further studies elucidating the underlying mechanisms and clarifying the biological significance of these immunological changes are warranted.

Keywords: preweaned calves, paraprobiotics, γδ T cells, monocytes, T lymphocyte subsets

Hazelnut Skin Extract Modulates Markers of Intestinal Barrier Permeability in Neonatal Calves with Diarrhea

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BACKGROUND and OBJECTIVE: Neonatal calf diarrhea (NCD) causes enterocyte injury and disruption of intestinal barrier integrity, particularly in infections such as those caused by *Cryptosporidium* spp., leading to increased epithelial permeability. Polyphenol-rich plant extracts have been proposed as supportive therapeutic strategies due to their antioxidant and anti-inflammatory properties. Among plant-derived sources of polyphenols, hazelnut (*Corylus avellana*) skin is particularly rich in phenolic compounds with documented biological activity. To assess mucosal health, circulating markers such as intestinal fatty-acid-binding protein (IFABP) and tight junction protein 1 (TJP1) are used to evaluate two distinct pathological aspects: enterocyte damage and tight junction disruption, respectively. The aim of this study was to evaluate the effect of hazelnut extract supplementation on serum IFABP and TJP1 concentrations in hospitalized *Cryptosporidium*-positive neonatal calves.

MATERIALS and METHODS: The study was approved by the institutional animal welfare organization of the University of Milan (approval no. 93_2023). Fourteen hospitalized neonatal Holstein heifer calves with severe diarrhea and confirmed *Cryptosporidium* spp. infection were enrolled. All calves received standardized intensive therapy. Animals were randomly assigned to a control group (n = 7), receiving unsupplemented milk, or to a treated group (n = 7), receiving milk supplemented with hazelnut skin extract at 10 g/day (0.5% w/v in the morning feeding) for five consecutive days. Blood samples were collected at admission (T0), day 3 (T3), and day 6 (T6). Serum IFABP and TJP1 concentrations were quantified using commercial ELISA kits (MyBioSource, Inc., San

Diego, CA, USA). Repeated-measures generalized linear models were applied to evaluate group and time effects. Statistical significance was set at $p < 0.05$.

RESULTS: A significant group effect was observed for IFABP concentrations ($p < 0.001$), with consistently higher levels in control calves compared to treated calves across all time points, while no significant time effect was detected. These findings support a potential cytoprotective effect of hazelnut polyphenols, limiting enterocyte damage during the acute phase of infection. For TJP1, a significant time effect was identified ($p = 0.007$), with lower concentrations at T3 compared to T6, whereas no significant group effect emerged. This pattern indicates that although the extract appears to preserve cellular integrity (as reflected by IFABP), tight junction restoration may follow a physiological recovery timeline independent of supplementation.

CONCLUSIONS: Hazelnut skin extract supplementation was associated with reduced markers of enterocyte injury in *Cryptosporidium*-positive neonatal calves, supporting a potential mucosal protective role during severe neonatal diarrhea.

Keywords: Calves, Neonatal calf diarrhea, Corylus avellana, Intestinal barrier permeability, Fatty-acid-binding protein (IFABP), Tight junction protein 1 (TJP1)

Potential Biomarkers for Detection of Intrauterine Infection in Aborted and Stillborn Fetuses

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BACKGROUND and OBJECTIVE: Perinatal mortality (PM) in cattle, occurring from day 260 of pregnancy to 48 hours postpartum causes significant economic losses. Diagnostic challenges, including autolysis or lack of lesions, leave 33-66% of cases without clear cause of death. The objective of this study was to evaluate the reactivity and concentration of selected humoral immune components: complement component C3 and immunoglobulins (IgG1, IgG2, and IgM)—in the (pre-colostral) sera of stillborn and aborted calves. The study aimed to determine if these immunological markers could serve as diagnostic tools to identify intrauterine infections that might otherwise go undetected.

MATERIALS and METHODS: The study was conducted on 52 serum samples collected from stillborn and aborted calves during the 2023/24 calving season in Ireland. The samples were categorized into five groups based on the necropsy-diagnosed cause of death (COD): intrauterine infection (n=5), congenital defects (n=6), birth complications (n=25), DNR (diagnosis not reached, n=9), and other causes (n=7). ELISA tests were employed to measure the concentration of complement C3 and the concentrations of IgM, IgG1, and IgG2. Furthermore, antibody reactivity was tested by ELISA against recombinant proteins (Hsp60 and OMP40 from H. somni), whole bacterial cells (*E. coli*, *S. aureus*), and specific bacterial antigens (LPS from *E. coli*, LTA from *S. aureus*).

RESULTS: Statistical analysis revealed no significant differences between the study groups for most parameters. However, individual variations were observed, with some samples showing markedly higher reactivity. The "infection" group exhibited the highest mean concentration of IgM (121.88 ± 196.56 mg/L) and the lowest concentration of complement C3 (4.45 ± 7.13 ng/mL). In all groups, IgM concentrations were generally higher than IgG1 and IgG2. The DNR group showed numerically highest IgM reactivity towards certain antigens compared to the other groups.

CONCLUSIONS: The presence of specific immunoglobulins in pre-colostral sera across different COD groups confirms that the fetal immune system is stimulated in utero, indicating contact with bacterial antigens during pregnancy, regardless of diagnosed COD. The numerically lowest concertation of C3 in calves with diagnosed infection suggests C3 consumption during the inflammatory response. The strong IgM reactivity observed in the DNR group suggests that some of these calves underwent significant exposure to bacterial antigens in utero which was not detectable at necropsy. The elevated immune markers in individual cases suggest that assessing humoral immunity can provide insights into a fetus's exposure to pathogens. Further research is needed to

establish standardized reference ranges for these components in bovine fetuses with different COD.

Keywords: cattle, pre-colostral serum, intrauterine infection, biomarkers, stillbirth.

Investigation of the Role of Milk Replacer Quality in an Outbreak of Abomasum Disorder in Dairy Calves

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BACKGROUND and OBJECTIVE: Feeding milk replacer is reported as a risk factor for abomasal disorders in calves. However, studies linking milk replacer quality to these disorders are scarce. Hence, the objective of this report was to assess the role of the milk replacer quality during an outbreak of abomasal disease.

MATERIALS and METHODS: A dairy farmer with 120 Holstein-Friesian cows reported a problem with abomasal disorders in his unweaned calves which were housed and fed a milk replacer (powder1). Among 33 heifer calves, 10 were bloated and five died within three weeks. Necropsy confirmed abomasitis and pyloric ulcers. The outbreak resolved after changing milk replacer (powder2). During a farm visit the powder1 was sampled for physical assessment (colour/appearance, stability after reconstitution), total viable bacterial counts, bacterial culture, osmolality, pH and protein, fat, free fat, lactose, moisture and ash content (by Near-Infrared Spectroscopy analysis). Moreover, volatile components profiles of powder1 and powder2 were compared using headspace solid-phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC-MS).

RESULTS: Powder1 was greyish-white with a metallic odour, bitter taste, and persistent aftertaste. A 24 h standing test showed 1% free-fat supernatant and 5% sedimented vegetable protein, indicating poor solubility. Apart from elevated free fat (11.3% of total fat; total fat 15.9%), compositional and bacterial results were within ranges reported for similar products. High free fat suggests a failure of emulsification or spray-drying process intensifying fat oxidation, causing off-flavours, reduced palatability, and peroxide formation that may disturb the digestive environment and favour pathogenic bacteria growth. Seventy-eight volatile components across 11 classes were identified; the main difference was terpene abundance. Powder1 showed 158-fold higher total terpene signal than powder2 (2692 vs 17 million peak area units). Several terpenes were detected only in powder1 (α-phellandrene, o-cymene, α-terpineol, α-thujene, β-pinene, terpineon-4-ol); others were far more abundant (α-pinene, γ-terpinene, camphene, β-myrcene, eucalyptol, p-cymene, d-limonene). The elevated terpene concentrations might be associated with a formulation issue during powder manufacturing. The consequences of terpene exposure are alterations in membrane dynamics and Reactive Oxygen Species levels causing cellular cytotoxicity in the lungs, liver and gastro- intestinal tract.

CONCLUSIONS: The poor milk replacer quality (in this case, high free fat) may have impaired milk replacer digestibility, leading to abomasal disorders. High terpenes concentrations may have also contributed to abomasum disease un this case potentially via cytotoxic effects on digestive cells; however, their role in abomasal disease in calves has not been described previously and requires further investigation.

Keywords: Milk replacer, Free fat, Terpenes, Abomasum disorders, Ulcer, Abomasitis

Health of Veal Calves in the Netherlands: Associations with Transport Age

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BACKGROUND and OBJECTIVE: Long-distance transport of unweaned calves has gained considerable attention due to animal welfare concerns. Under the EU Transport Regulation, a calf may be transported from the age of 14 days, yet this is likely to be increased in the future according to the latest developments in scientific and technical knowledge in this field. Germany already banned transport of calves under 4 weeks, as of January 2023. This provides an opportunity to assess the impact of increased transport age on the health of veal calves in the Netherlands, as ~33% of veal calves in the Netherlands originate from Germany.

MATERIALS and METHODS: Performance of veal calves that arrived at the veal farm at a young age (<28 days at arrival) was compared to those that arrived at a later age (≥28 days at arrival). Data on health parameters (2020-2024): Mortality: census data on country of origin, age at arrival in the veal calf herd, and age at slaughter or death, from 8.8 million veal calves that arrived at a Dutch veal farm during the study period. Records on antimicrobial use (AMU) were available for 5,289 'all-in all-out' production cycles of 800 farms producing white veal. Analysis: multivariable logistic regression models and survival models to quantify association between health parameters and transport age (young vs. old)

RESULTS: Survival rates of veal calves up to slaughter reveal differences between transport age as well as country of origin. Calves born in the Netherlands that arrive young (<d28) in the veal farm had slightly lower survival rates between d28-d60 of their life compared to calves with other (non-German) nationalities that arrived ≥28 days of age. Since the minimal transport age was raised for calves born in Germany (>2023), their survival rates have improved during d28-d75 of their life, but not thereafter. AMU was significantly lower in veal calf herds in which calves arrived ≥28 days of age and were not from German origin (up to 2.85 animal-defined daily doses per 100 days of fattening) AMU was lowest in herds with predominantly Irish calves (≥28 days at arrival). AMU in herds with predominantly German veal calves has not decreased since the minimum transport age was raised in 2023.

CONCLUSIONS: It is assumed that increasing the minimum age at transport will improve health and welfare of unweaned veal calves. Yet this study showed that calves that were transported to veal farms after 28 days of age did not perform unequivocally better.

Keywords: transport age, welfare, veal calves

CATTLE WELFARE

Clinical Efficacy Evaluation of Gamithromycin in Treatment of Diarrhea in Neonatal Calves with Cryptosporidium

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BACKGROUND and OBJECTIVE: Cryptosporidiosis, caused by Cryptosporidium spp is one of the leading causes of neonatal diarrhea in calves, particularly between 5 and 20 days of age. The disease is characterized by weakness, lethargy, and loose to watery diarrhea, and is associated with severe intestinal villous damage due to parasite multiplication at the microvillus border of enterocytes. In Korea, Cryptosporidium has been identified in approximately 38% of diagnosed cases of calf diarrhea, highlighting its clinical and economic importance. Although treatment options remain limited, several macrolide antibiotics have demonstrated activity against cryptosporidiosis. Gamithromycin, a long-acting macrolide, may therefore represent a practical therapeutic option. This study aimed to evaluate the clinical efficacy of gamithromycin in naturally infected neonatal calves.

MATERIALS and METHODS: A total of 115 neonatal diarrheic calves (27 Hanwoo [Korean native cattle] and 88 dairy calves), aged 5–20 days and naturally infected with Cryptosporidium, were enrolled.

Infection status and treatment efficacy were assessed using a rapid calf diarrhea diagnostic kit. Calves in the treatment group received a subcutaneous injection of gamithromycin (6 mg/kg) on day 1, followed by daily oral administration for 7 days of a supportive powder containing glucose, dried citrus, and electrolytes. A second subcutaneous dose of gamithromycin was administered on day 7. Therapeutic outcomes were evaluated on day 14. A control group consisting of 3 Hanwoo and 44 dairy calves infected with Cryptosporidium received no gamithromycin treatment, and mortality rates were compared between groups.

RESULTS: Gamithromycin treatment resulted in clinical and diagnostic improvement in 94 of 115 calves, corresponding to an overall response rate of 81.7%. By day 14, 22 of 27 Hanwoo calves (81.5%) and 72 of 88 dairy calves (81.8%) tested negative for Cryptosporidium. In dairy calves, mortality was markedly reduced in the treatment group (13.6%) compared with the control group (65.9%). Similarly, mortality in Hanwoo calves decreased from 33.3% in the control group to 14.8% following gamithromycin treatment.

CONCLUSIONS: Gamithromycin administration was associated with a high therapeutic response rate and a substantial reduction in mortality in neonatal calves with cryptosporidiosis. These findings suggest that gamithromycin may be a valuable therapeutic option for managing Cryptosporidium-associated neonatal calf diarrhea under field conditions.

Keywords: gamithromycin, diarrhea, calves, cryptosporidium

Developing An Animal Welfare Assessment Protocol for Dairy Farms in Indonesia: On-farm Testing of the Protocol

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BACKGROUND and OBJECTIVE: Since welfare problems vary among systems worldwide, specific welfare assessments are required to capture the particular issues occurring within each system. This study aimed to test and validate a systematic, animal-based welfare assessment protocol tailored to smallholder dairy farms in Indonesia. The goal was also to identify welfare measures that are feasible for routine use and to find strategies to improve dairy cattle welfare on such farms.

MATERIALS and METHODS: Twenty-two welfare measures were tested in 100 smallholder farms belonging to eight dairy cooperatives in four regions of Java, Indonesia. In total, 500 dairy cows were included in the study. Depending on the field condition, the welfare assessment was typically conducted on 4-5 farms/day. The assessments were performed by a single assessor and recorded on printed welfare assessment sheets. All the data from the field notes was managed in Kobo Toolbox and Microsoft Excel before being statistically analysed using IBM SPSS Statistics.

RESULTS: Among 22 measures tested, 15 animal-based measures were considered feasible and practical for use on Indonesian smallholder dairy farms. Based on median prevalence in our study population, hoof and limb-related problems, feed-related problems, cleanliness, and mastitis were the welfare problems most commonly identified by our protocol. There was marked variation between farms in the prevalence of problems identified by our protocol. This finding suggests that, for most measures, there was potential to reduce median prevalence without recommending management practices and approaches that are unsuitable for smallholder farms.

CONCLUSIONS: This study identified 15 key animal-based measures to assess the welfare of dairy cattle in Indonesian smallholder dairy farms. The results of this study thus serve as a useful starting point for further development of a welfare assessment protocol for Indonesian smallholder dairy farms and the creation of evidence-based strategies to improve dairy cattle welfare on these farms.

Keywords: animal welfare assessment, smallholder dairy farms, Indonesia, animal-based, protocol

What Are the Most Physiologically Challenging Phases for Lidia Cows During Traditional Events and Slaughter?

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BACKGROUND and OBJECTIVE: Traditional events involving Lidia cattle expose animals to a sequence of handling procedures that combine intense physical exertion, transport and close human interaction. This pilot study aimed to characterize changes in body temperature (BT), heart rate (HR) and HR variability (HRV) in Lidia cows throughout a complete traditional handling sequence from their rearing farm to slaughter, to identify the phases associated with the greatest physiological challenge.

MATERIALS and METHODS: All procedures were approved by the Animal Experimentation Committee of University of León, in agreement with EU legislation. Four Lidia cows were implanted with biologgers (Star Oddi, Iceland) that recorded BT and HR at 2-minute intervals for four days. HRV indices calculated were SDNN (standard deviation of normal-to-normal intervals), and RMSSD (root mean square of successive differences). Thirteen handling phases (daily activity at rearing farm, penning, transport, horseback cattle drive ("encierro"), two bullring events, lairage, and slaughter) were deemed. Data were analysed using linear mixed-effects models, with handling phase as fixed effect and cow as random effect.

RESULTS: During phase 0 at the farm, cows showed stable baseline values (BT: 37.01°C; HR: 60.1 bpm) and high HRV (SDNN: 20.7; RMSSD: 23.2 ms), indicating low stress and predominant parasympathetic tone. Handling phase had a significant effect on all physiological variables ($P<0.001$). The first long transport phase (2 h 20 min) triggered physiological activation, with increased HR (84.8 bpm) and BT (37.5°C) and reduced RMSSD (14.3 ms), reflecting sympathetic activation and incomplete recovery. The horseback drive induced the strongest response (BT: 39.2°C; HR: 112 bpm) and marked HRV reduction (SDNN: 7.2; RMSSD: 5.8 ms), likely reflecting the cumulative effect of the preceding long-distance transport combined with the absence of a resting period and the high physical intensity of this phase. Subsequent short transport maintained elevated HR and BT with suppressed HRV. Traditional events ("capeas") caused moderate HR increases (82–90 bpm) and HRV reductions. Extended resting periods in the bullring were associated with recovery of autonomic tone, with HRV exceeding baseline values. BT showed delayed normalization after high-intensity phases.

CONCLUSIONS: Lidia cows show phase-specific physiological responses during traditional events and slaughter. The horseback drive is the most demanding phase due to cumulative exertion and limited recovery, while extended rest promotes autonomic stabilization. Parasympathetic indices sensitively reflect stress and recovery. Implantable biologgers effectively identify critical welfare points in traditional handling of fighting cattle.

Keywords: Lidia breed, biologger, temperature, heart rate

From Recognition to Implementation: Stakeholder-Identified Gaps and Requirements for Objective Lameness Detection in Dairy Systems

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BACKGROUND and OBJECTIVE: Problems with the mobility of cows are still one of the most significant welfare and economic challenges in dairy production. Even though mobility scoring is widely promoted, the implementation and consistency are variable. Knowledge of the gap between the perceived importance and practical application is needed for developing objective monitoring systems. This research examined perceptions of prevalence and management of lameness as held by stakeholders, alongside expectations for the next-generation of lameness detection technologies.

MATERIALS and METHODS: A structured online survey was completed by 131 stakeholders within the UK sector, comprising farmers, veterinarians, consultants, and researchers, representing a diverse range of herd sizes. The questions addressed the perceived lameness prevalence, detection methods, economic impact, and expectations regarding the application of technology-based monitoring systems.

RESULTS: Lameness was rated as a very or extremely important for the welfare of herds by 85.5% of respondents. The herd lameness prevalence was estimated within the range of 0% to over 25%. Despite this recognition, detection was predominantly reactive, meaning that it was relying on ad hoc visual observations rather than structured mobility scoring. The main identified economic impacts by respondents included lower milk production, decreased fertility, and the need to cull and replace animals. Other concerns were the time and labour needed for treatment and veterinary costs. Stakeholders showed willingness to consider automated monitoring systems, but under specific conditions. The most important system feature that respondents prioritised was accuracy, followed by user-friendliness and cost-effectiveness. Additional features such as early lameness detection before it becomes obvious, real-time alerts and the ability to integrate with existing farm management software were also mentioned. Respondents preferred simple, easy-to-understand results (e.g. traffic light system) rather than complex data flows. However, they expressed reservations about the level of financial investment they would be willing to make for a new technology. Finally, their views on sharing data with third parties were ambivalent. Many respondents wanted to maintain control over data sharing, raising concerns about trust and governance.

CONCLUSIONS: There is a gap between understanding lameness and its impacts versus implementing effective lameness management. Stakeholders understand that lameness causes welfare and economic problems, however current practices are mainly informal. People agree that we need more objective and automated methods that are accurate, easy to use, and clear in data management. In light of the results and in order to achieve and improve early and reliable detection of lameness, we need to focus on both technology and building trust.

Keywords: Lameness, Mobility, Detection, Welfare, Precision, Technology

Changes of Substance P and Cortisol Concentrations, and of Behavior Following a Repeated Peripheral Electrical Stimulation in German Simmental Calves

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BACKGROUND and OBJECTIVE: Cattle, as prey animals, strongly mask signs of pain, which makes the recognition and the management of pain in cattle a major welfare problem. Various subjective and objective parameters have been described for pain assessment in cattle. Substance P (SP), a neurotransmitter involved in the processing of noxious information, is considered an objective biomarker for nociception. Whereas research about SP in humans and laboratory animals is diverse, basic research work about SP in cattle is rare. The

objectives of this pilot study were to assess plasma substance P (PSPC) and plasma cortisol (PCC) concentrations, vital signs, behavioral parameters, and activity in calves submitted to either an electrical or a sham stimulus.

MATERIALS and METHODS: A total of 24 healthy male German Simmental calves (aged 44 ± 2 days) were included in this study. Calves were randomly assigned to either control (CON) or trial (PAIN) group. Calves in PAIN were submitted to 5 consecutive electrical stimuli of the right hind leg over a duration of 25 minutes, whereas calves in CON received sham stimulation. Blood samples to assess PSPC and PCC were taken before (baseline and at 0 min), during (5 min to 25 min), and after (30 min to 7 hours) the stimulation. Vital signs and behavior were recorded during the stimulation for a duration of 30 minutes, and before each blood sampling. Activity was assessed over 24 hours.

RESULTS: PSPC and PCC did not differ significantly between groups. In PAIN, PSPC decreased during the electrical stimulation, and were significantly lower at 25 min compared with at baseline and at 0 min. In CON, PCC were significantly lower at 4.5 hours compared with at baseline and at 0 min. During stimulation, frequency of "ear movements" was significantly lower, and frequency of "shaking of the legs" significantly higher in PAIN compared with CON calves. In PAIN calves, occurrences of "head held below dorsal line" was significantly higher during, and occurrences of "eye lids half closed" during and after stimulation, compared with CON calves. There were no differences in activity between groups.

CONCLUSIONS: In the absence of tissue damage, electrical stimulation resulted in a decrease of PSPC in PAIN compared with CON calves, even if PAIN calves showed signs indicative of discomfort. The results of this study could affect the use of SP as an objective biomarker if pain is assessed without either concomitant tissue damage or inflammation.

Keywords: analgesia, ethogram, Fleckvieh, nociception, pain, welfare

Influence of Clinical Training on Attitudes of Bavarian Veterinary Students Towards Pain and Pain Assessment in Cattle

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BACKGROUND and OBJECTIVE: The recognition of pain in cattle is difficult, as cattle are prey animals, and thus strongly mask signs for pain and discomfort. Therefore, pain management is a major welfare problem in cattle medicine. The subjective assessment of pain in cattle, as well as the attitudes of the observer towards pain and their use of analgesics are subject to the observers experience and opinions. The objective of this study was to investigate the influence of 12 weeks of clinical training on the attitudes of Bavarian veterinarian students on 1) pain assessment in cattle, 2) the use of analgesics, and 3) questions and statements regarding pain in cattle.

MATERIALS and METHODS: Students were asked to complete two identical questionnaires in their first (week 1) and last (week 12) week of clinical training at a university owned clinic. The questionnaire contained several categories: 1) Demographic data, 2) Recognition of pain in cattle, 3) Pain assessment using a numerical rating scale (NRS), 4) Use of analgesics in cattle, and 5) Questions and statements regarding pain in cattle. Overall, 60 paired questionnaires were included in the statistical analysis.

RESULTS: Most students were aged 20 to 25 years (65%) and female (93%). A total of 72% wanted to work with cattle after university. Out of a selection of 28 behavioral parameters, 6 parameters were recognized as signs for pain in cattle significantly more often following the clinical training. In adult cattle, enucleation of the eye bulb (median NRS 10), claw amputation (median NRS 9), and caesarean section (median NRS 9) were considered the most painful procedures, whereas in calves, both surgical and Burdizzo castration (median NRS 9 each, respectively), as well as laparotomy (median NRS 9)

were scored as most painful. Pain scoring decreased significantly in 9 out of 20 conditions and 3 out of 15 procedures and increased significantly in 1 out 20 conditions in adult cattle. Following clinical training, selected analgesic regimes changed significantly for one out of 8 procedures in calves (tenotomy of contracted tendons). Students' opinions regarding pain recognition in cattle, as well as about farmers' willingness to pay for analgesic treatment differed significantly after 12 weeks at the clinic.

CONCLUSIONS: Practical experience in form of a clinical training influences veterinary students' opinions and attitudes about pain severity and recognition in cattle. The work with farmers had a positive influence on students' opinions about farmers' preferences.

Keywords: analgesics, ruminant, pain assessment, pain management, survey

Implementation of an Animal Welfare Program in Cattle Operations of the Central–Northern Region of Chile

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BACKGROUND and OBJECTIVE: Attention to animal welfare has emerged as a key metric of sustainable livestock farming, inextricably linked to productivity, animal health, and societal expectations. In bovine medicine, welfare indicators are increasingly recognized as key factors in disease prevention, performance, and farm resilience. In this context, an animal welfare program was devised and implemented in the central–north area of Chile, adapted to the climatic, structural, and sociocultural conditions of the region. The program focused on practical, applicable interventions in beef and dairy cattle systems, with limited inclusion of goat operations.

MATERIALS and METHODS: The program was designed by VIRBAC Chile and implemented during 2025. It encompasses on-farm technical visits, group meetings and short theoretical–practical workshops, direct observation of management practices, interviews of producers and farm staff. The evaluation of the potential impact of interventional phase adopted a qualitative approach, focusing on observed behavioral changes in animals, modifications in management routines. producer spontaneous feedback and perception of improvements. The intervention also included alignment of identified sanitary needs with preventive and therapeutic tools available through veterinary industry collaboration, particularly via the portfolio of veterinary medical products.

RESULTS: A cohort of up to 30 producers was directly engaged in the programme, securing the indirect participation of an estimated 80 to 100 supplementary farm staff.. The curriculum encompassed calf management, including care to the newborn and colostrum management, pain management in disbudding and castration, prevention and management of respiratory and enteric diseases. For dairy operations, the focus centred on applied ethology, udder health,low-stress handling and movement alongside the physiological and production sequelae of heat stress. In beef cattle, critical topics included design of restraint facilities, impact of mud and hygiene of the living environment, and formulation of herd health plans. Initial appraisal revealed an increased openness to adopting preventive practices, better structured health SOP, noticeable reduction in rough handling, increased voluntary technical consultations, observable reduction in animal reluctance to handling procedures. Importantly, a shift in producer discourse was identified: animal welfare transitioned from being perceived as an external ethical imposition to a rational productivity-enhancing strategy.

CONCLUSIONS: These findings suggest that the implementation of welfare programmes within traditional production systems is contingent more upon cultural transformation than infrastructural investment. Substantive advancements in bovine production systems can be achieved through interventions that are culturally contextualised, preventative in nature, and technically robust. Animal welfare must be conceptualised not as a peripheral consideration, but as a foundational pillar of modern buiatrics and sustainable livestock agriculture.

Keywords: Cattle welfare, ethology, education, productivity

Pregnant Cows Slaughter: A Systemic Failure or Human Error?

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BACKGROUND and OBJECTIVE: The slaughter of pregnant cows remains an underrecognized issue in livestock production. Although the practice should be avoided, data from various regions worldwide indicate that its prevalence remains considerable, and existing regulatory frameworks are often insufficient. Consequently, the proportion of pregnant cows sent to slaughter has not declined significantly in recent years, continuing to raise ethical concerns and generate economic losses. The aim of this study was to determine the prevalence of pregnant cattle slaughter in central Poland and to identify its underlying causes based on farmer-reported reasons provided at the time of sale.

MATERIALS and METHODS: Data were collected over a one-year period from two cattle slaughterhouses in central Poland. During this time, 1,457 female cattle were slaughtered. Pregnancy status was determined in each case by post-mortem examination of the uterus. The findings were supplemented with farmer-reported reasons for culling, with the possibility of indicating multiple causes per animal. Data from both slaughterhouses were combined and expressed as percentages. Seasonal variation in the slaughter of pregnant females was also assessed. Statistical analyses were performed using the Wilcoxon rank-sum test with continuity correction and the Kruskal–Wallis chi-squared test. Statistical significance was set at $P<0.05$.

RESULTS: A total of 212 pregnant cows were slaughtered, with a mean age of 39 months (range: 18–121 months), accounting for 14.6% of all females included in the study. No significant difference was found between the two slaughterhouses (16.4% vs. 12.6%, $P>0.05$). Seasonal differences were not statistically significant; the proportions of pregnant cows slaughtered in spring, summer, autumn, and winter were 15.6%, 11.8%, 17.4%, and 13.8%, respectively ($P=0.16$). Infertility was the most frequently reported reason for culling (85.5%). Fattening purposes were indicated in 34.4% of cases, advanced age in 9.4%, and herd liquidation in 5.2%. Metabolic disorders (1.4%) and injuries (0.5%) were rarely reported.

CONCLUSIONS: The findings align with reports from other countries, indicating that the slaughter of pregnant cattle remains a persistent issue. The young age of some pregnant females and the predominance of infertility as a reported reason suggest insufficient reproductive monitoring and possible deficiencies in veterinary diagnostics. Reducing the slaughter of pregnant females requires coordinated action, including improved farmer awareness, routine pregnancy screening prior to slaughter, and effective legal regulation.

Keywords: Pregnancy, Cows, Slaughter

Tail Injuries in Dairy Cows: How Much Are Housing Conditions to Blame?

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BACKGROUND and OBJECTIVE: Tail injuries in cattle – particularly fractures resulting in permanent deviation from the tail's main axis – remain an underrecognized welfare issue. Such injuries cause pain, discomfort, and reduced animal welfare. Despite their potential implications, the prevalence of tail injuries has been investigated in relatively few studies. The aim of this study was to assess the prevalence and types of tail injuries in cows, with particular emphasis on the influence of housing conditions.

MATERIALS and METHODS: The study included 2,530 cows. Dairy cows (n=2,384) originated from 47 farms, including 45 herds composed exclusively of Holstein–Friesian cattle. Of the dairy cows, 506 were housed in tie-stall systems (T), whereas 1,878 were kept in free-stall systems (F). Beef cows (n=146; Polish Red, Charolais, and Galloway) came from three extensively managed herds. The farms were located in central Poland and south-eastern Ukraine. Tail injuries were assessed based on the deviation of the tail from its main axis, measured in centimeters, and classified into three categories: ≤3 cm – mild (I); 3–5 cm – moderate (II); >5 cm – severe (III). A separate category included cows with tail avulsion or tail amputation performed either by a veterinarian or by the farmer. Statistical analyses were conducted using the x^2 test or Fisher's exact test, depending on sample size, with statistical significance set at $P<0.05$.

RESULTS: Tail injuries of varying severity were recorded in 558 cows (22.1%), including 83 cows (16.4%) housed in tie-stall systems and 474 dairy cows (25.2%) from free-stall herds. In contrast, only one beef cow (0.7%) was affected ($P<0.001$; mild lesions). Therefore, subsequent analyses were limited to dairy herds. Mild lesions (I) were observed in 270 cows (11.3%; T – 10.9%; F – 11.4%; $P>0.05$); moderate (II) occurred in 161 cows (6.8%; T – 3.8%; F – 7.6%; $P<0.01$), while severe (III) were identified in 34 cows (1.4%; T – 1.0%; F – 1.5%; $P>0.05$). Tail avulsion was recorded in 8 cows (0.3%; T – 0.8%; F – 0.2%; $P>0.05$). Tail amputations were observed exclusively in free-stall herds, affecting 84 cows (4.5%). In 69% of cases, amputation was performed by the farmer (using rubber ligatures), whereas in 31% it was carried out as a veterinary procedure.

CONCLUSIONS: Tail injuries affected about one-fifth of the studied cows, with mild lesions being the most common. Injuries were more frequent in free-stall dairy herds and were nearly absent in extensively managed beef cattle. Further research on this issue is ongoing.

Keywords: Tail injury, Cows, Housing systems

Bovisensor: A New User-friendly Digital Tool to Assess Pain in Cattle

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BACKGROUND and OBJECTIVE: Effective detection and management of pain are fundamental for improving cattle welfare; however, progress is still required to enhance awareness, promote standardized assessment, and ensure the systematic implementation of analgesic strategies. All painful conditions warrant attention, even in the absence of consequences on productivity in adult animals. Consideration must also be given to calves, which experience several painful conditions, yet are often

monitored less frequently and exhibit more subtle or nonspecific pain behaviors. Until recently, pain assessment tools for cattle were limited, with most existing frameworks addressing only a single pathology (e.g., lameness or postsurgical pain).

MATERIALS and METHODS: Under the leadership of Oniris (National Veterinary School of Nantes, France), a panel of veterinary experts employed a structured Delphi method to develop a series of condition-specific, user-friendly pain assessment grids applicable to several common clinical conditions in both adult cattle and young calves. The expert panel comprised twelve specialists from seven different countries, representing both academic institutions and field-based veterinary practices.

RESULTS: This consensus-based process led to the development of a dedicated digital tool: Bovisensor, enabling veterinarians and farmers to generate a standardized pain score for each animal and clinical condition. The tool follows a structured workflow comprising: (i) identification of the animal's age category and sex, (ii) selection of the suspected source of pain, when known, and (iii) recording of the illustrated postural and behavioral indicators observed. Based on these inputs, the tool calculates a score that categorizes the level of pain and subsequently recommends either analgesic intervention or a scheduled reevaluation. The generated report, which includes all recorded clinical signs, can be saved and shared for documentation or follow-up. When analgesic treatment is administered, a reevaluation is encouraged; the expected reduction in the pain score provides an educational feedback for the user, reinforcing the effectiveness of appropriate pain management.

CONCLUSIONS: BoviSensor is a practical decision-support tool designed to assist farmers and veterinarians in improving the detection of pain and implementing appropriate management strategies. Enhancing cattle welfare will require substantial changes in current practices, particularly regarding pain management across a wide range of situations—from calving and dehorning to infectious and traumatic conditions. Effective analgesia represents a worthwhile investment. The routine use of BoviSensor as a standardized pain-scoring system constitutes an essential first step toward increasing awareness at farm level. A trial is currently underway to rigorously evaluate the tool's sensitivity, specificity, and repeatability under field conditions.

Keywords: Pain, Digital, Scoring, Decision, Awareness

Cow-Calf Contact: How Are the Foster Cows Doing?

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BACKGROUND and OBJECTIVE: Among both consumers and dairy producers, there is growing interest in alternative housing systems that better meet the animals' natural behavioral needs. One way to do this is by allowing cows and calves to stay together for a longer period—so called cow–calf contact systems (CCC). In Denmark, most farmers practicing CCC house the calves with foster cows that nurse three to four calves each during the milk feeding period of approximately three months. The purpose of this pilot study was to investigate the development of foster cow health during the first month in the system.

MATERIALS and METHODS: We examined 57 foster cows across seven herds. A clinical examination of all foster cows was conducted within one week after introduction to the system and again after four to five weeks. In addition, farmers were interviewed about the design and management of their foster cow system.

RESULTS: We found that 65% of the foster cows were second to fourth parity, and that cull cows were predominantly used to care for the calves. Most foster cows cared for 3–4 calves, and the farmers selected cows with an average milk yield of 10 liters per calf. There were no significant changes in udder health parameters, including somatic cell count, indicating that overall udder health was not affected—neither positively nor negatively. However, the number of cows with wounds or scratches on the udder increased significantly from 23% at the first examination to 52% at the second, which is presumed to be caused by the suckling calves. The prevalence of cows with swelling of the hocks decreased significantly from 56% on the first examination to 33% on the second. However, this did

not lead to a change in the prevalence of lameness, despite housing in deep bedded pens. Body condition score also did not change from the first to the second examination. The results show that the workload placed on foster cows during the first month does not generally have a negative impact on them. However, it should be noted that caring for calves is demanding, as indicated for example by the wear on the udder.

CONCLUSIONS: The study highlights the importance of focusing on the foster cows and selecting the right animals. Furthermore, knowledge is needed about the impact of being a foster cow for longer than the first month and the use and value of the foster cow after the calves are weaned.

Keywords: cow-calf contact, foster cows, health, welfare

A Study on Behavior and Welfare Characteristics of Holstein Cattle in Manure and Sand Beddings in Free-Stall Barns

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BACKGROUND and OBJECTIVE: Animal behavior is an important indicator of health and welfare in dairy cattle. Bedding material used in free-stall barns plays a critical role in providing comfort and preventing injuries. To our knowledge, this is one of the first studies conducted in Turkey to evaluate the effects of manure and sand bedding on behavioral characteristics and welfare indicators in Holstein cows using real-time monitoring systems. The aim of this study was to evaluate the effects of manure and sand bedding on behavioral characteristics and welfare indicators in Holstein cows.

MATERIALS and METHODS: The study was conducted on 110 Holstein cows aged 2–6 years in a semi-open free-stall dairy farm in Foça, İzmir, Turkey. Animals were randomly divided into two groups (n=55) according to bedding type: manure bedding and sand bedding. Behavioral parameters including standing duration, lying/resting duration, number of steps and standing/lying frequency were monitored using the NEDAP Smarttag Leg – Cow Control System. At the end of the study, carpal and tarsal joint lesions were evaluated using a scoring system. Blood samples were collected from the jugular vein to determine neutrophil–lymphocyte ratio and biochemical parameters including glucose, cholesterol, triglyceride and total protein levels. Statistical analyses were performed using Student's t-test and Mann–Whitney U test with SPSS 22.0.

RESULTS: Carpal and tarsal joint lesion scores did not differ significantly between bedding groups ($P>0.05$). Similarly, serum glucose, total protein and triglyceride levels showed no significant differences. However, several behavioral parameters differed significantly between the groups. Cows housed on sand bedding exhibited significantly longer lying/resting time (689 min) compared with those on manure bedding (638 min) ($P<0.001$). The number of steps was also significantly higher in the sand bedding group (2742 vs. 2380 steps/day; $P<0.001$), while standing/lying frequency was lower (7.97 vs. 9.40 times/day; $P<0.001$), indicating more stable resting behavior. Standing time showed a slight but statistically significant difference between groups (286 vs. 287 min/day; $P<0.05$). In addition, the neutrophil–lymphocyte ratio (1.47 vs. 1.07; $P<0.01$) and serum cholesterol levels (123.62 vs. 108.04 mg/dL; $P<0.05$) were significantly higher in cows housed on sand bedding.

CONCLUSIONS: These results indicate that bedding type influences behavioral indicators of dairy cow welfare in free-stall systems. Sand bedding increased lying/resting time and improved behavioral stability, suggesting greater cow comfort. However, manure bedding was associated with a lower neutrophil–lymphocyte ratio, indicating potentially lower physiological stress.

Keywords: behaviour, cow welfare, holstein, manure, NEDAP smartted leg, sand bedding

Progressing Evidence-Based Pain Management Welfare in Ruminant Livestock in Australia and Beyond

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BACKGROUND and OBJECTIVE: Global livestock industries are under pressure to boost sustainable production while improving ecosystem management and animal welfare. Progress on pain relief is uneven, with differing policies, protocols and attitudes across countries and production systems. A review of 21 years of research and development of safe products for use by farmers to improve pain welfare management in Australia and beyond, was conducted.

MATERIALS and METHODS: Calf and lamb marking involves multiple stressful procedures, making effective pain relief essential. Since 2005, numerous projects across several academic institutions, has facilitated the development and marketing of three separate approaches to pain relief during routine aversive husbandry procedures in livestock, particularly castration and dehorning in cattle and castration and tail-docking in sheep. The adoption of three approaches is reviewed, including: (1) topical wound therapy (Tri-Solfen) to block nociception, (2) oral (Buccalgesic, Butec) or injectable (Metacam) preparations of meloxicam to ameliorate sensitisation, and (3) use of a device for injectable local anaesthesia for ring castration and tail docking in lambs (Numnuts).

RESULTS: Uptake of topical wound therapies including a combination therapeutic topical wound formulation (Tri-Solfen) has been very strong in the sheep industry (~90% for some procedures), with over 170 million animals receiving treatments.However, efforts to encourage the multimodal use of NSAIDs (oral or injectable meloxicam) and the recently developed method of delivering local anaesthesia when rubber rings re used (Numnuts), plus extended action of pain relief, is required. Pain relief for certain procedures has become mandatory in some jurisdictions, increasing uptake across all lamb-marking practices. It is apparent that improving understanding of the mode of action and specific uses of available products by veterinarians and producers helps motivate decisions and overcome resistance to change, enabling realistic expectations that guides the selection of the most appropriate pain-relief products to use on-farm. Surprisingly, extension of this research has led to new therapeutic approaches providing antimicrobial-free pain management of several important viral diseases, for example FMD and Orf infections.

CONCLUSIONS: Despite the complexity of issues involved in improving on-farm animal welfare, evidence-based science confirming efficacy of products, combined with positive producer and veterinarian experience, is driving steady improvement in pain welfare management in Australia and increasingly, beyond.

Keywords: pain, welfare, cattle, sheep, husbandry, change-management

Rethinking the Ideal Dairy Cow Environment: Integrating Stakeholders' Perspectives

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BACKGROUND and OBJECTIVE: Dairy cow housing has been highlighted to be central to animal welfare, health and productivity, as well as key aspect of societal acceptance of dairy farming. Design of housing systems to meet all requirements has remained a complex challenge. This study aimed to explore UK dairy industry stakeholders' perceptions of what the "ideal" dairy cow environment is, including the role of grazing.

MATERIALS and METHODS: A qualitative study design was performed using two focus groups (n = 9) and eleven semi-structured individual interviews with stakeholders across the UK dairy sector. Participants were recruited via convenience and snowball sampling. Discussions explored perceptions of ideal housing and the role of grazing. Data were audio-recorded, transcribed, and analysed using inductive thematic analysis following Braun and Clarke's framework.

RESULTS: Seven key themes emerged regarding the ideal dairy environment. First, the requirements of

the cow, emphasising the need for the environment to allow the cow to express different behaviours and access resources which they desire, with a growing recognition of cow agency and choice, creating positive emotional states. Second, staff requirements highlighted the importance of skilled labour, adequate staffing, and user-friendly infrastructure, linking human wellbeing directly to animal welfare. Third, business requirements reflected the need for profitability, adaptability, and system-specific solutions, reinforcing that no single model suits all farms. Fourth, societal expectations underscored pressures to align farming practices with public perceptions, particularly around naturalness. Fifth, developmental constraints such as planning regulations and existing infrastructure limited the ability to implement ideal systems. Sixth, building design was identified as critical to welfare outcomes, including aspects such as space and cow comfort. Finally, feasibility emerged as an overarching constraint, with participants questioning whether a truly "ideal" system is achievable. Perspectives on grazing were nuanced. While grazing was associated with natural behaviour, enrichment, and public approval, the participants also identified challenges including climatic variability, inconsistent nutrition, and reduced management control. Indoor systems were viewed as offering greater consistency and control, though potentially lacking environmental complexity to provide cows with desired stimulation. Many stakeholders favoured flexible, hybrid systems tailored to farm context, cow type, and external pressures.

CONCLUSIONS: The "ideal" dairy system was conceptualised as dynamic and multi-dimensional, requiring integration of cow welfare, human factors, economic viability, and societal legitimacy. The findings highlight inherent trade-offs between consistency -choice, control - naturalness, and aspiration - practicality. Advancing dairy systems will require context-specific solutions, improved public communication, and innovations that enhance both positive welfare and farm sustainability.

Keywords: Dairy Cow Welfare, Grazing Systems, Sustainable Dairy Farms, Dairy farm environment

Impact of Milking System Type on Human Avoidance Distance Test in Primiparous Cows: Preliminary Results

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BACKGROUND and OBJECTIVE: The objective of this study was to evaluate the impact of automated milking systems (AMS) and conventional milking systems (CMS) on the human-animal relationship in primiparous cows, using Human Avoidance Distance (HAD) test score.

MATERIALS and METHODS: The study was conducted at two commercial dairy farms. These farms were selected based on their milking technologies, with one equipped with CMS and the other with AMS. A total of 70 primiparous Holstein cows were included in the study, comprising 36 animals from an AMS-equipped farm (Farm A) and 34 animals from a CMS-equipped farm (Farm B). The HAD test in the paddock was performed at both farms at 21 d prepartum as well as 5–7 and 25–27 d postpartum. Animals were approached frontally at 1 step/s with a 45° arm extension during HAD testing. For each animal, the flight starting distance (onset of movement) and flight walking distance (total displacement) were recorded. The effects of farm groups and sampling time on the HAD test and flight walking distance were investigated using linear mixed models. Cows were included as a random effect, while farm, sampling time, and their interactions were included as fixed effects. The statistical significance level was set at $P<0.05$.

RESULTS: In Farm A, the average flight starting distance gradually decreased to 1.93 ± 1.15 m, 1.22 ± 0.62 m, and 0.59 ± 0.28 m on day 21 prepartum, days 5–7 postpartum, and days 25–27 postpartum, respectively (P<0.001). Flight starting distance was statistically significantly greater on Farm B than Farm A at days 5–7 postpartum and days 25–27 postpartum, respectively (P<0.001). Flight walking did not differ significantly between on day 21 prepartum and postpartum days (5-7 days and 25-27 days) or across both farms in study (P>0.05).

CONCLUSIONS: In conclusion, human avoidance behavior is not homogeneous across farms with different milking systems. The significant reduction in flight starting distance at Farm A suggests a habituation to human absence over time, whereas the trend at Farm B indicates a more complex behavioral change during the early postpartum period. The flight walking distance patterns were not homogeneous between the two farms. Consequently, farm-specific milking equipment variables must be considered essential for a systematic assessment of human avoidance behavior in livestock. Further studies are needed to investigate the impact of these behavioral changes on milk yield and reproductive performance.

Keywords: Human avoidance distance, flight walking distance, behaviour, milking system

Real-Time Heart Rate Variability Monitoring System for Cattle: A Lot-Based Approach for Automated Welfare Assessment

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BACKGROUND and OBJECTIVE: Heart rate variability (HRV) analysis provides an objective assessment of autonomic nervous system activity and stress levels in livestock. Traditional monitoring methods are invasive, labour-intensive, and limited to single measurements. This study aimed to develop an open-source, low-cost IoT-based system for continuous, real-time HRV monitoring in cattle, enabling automated welfare assessment using adapted physiological parameters specific to the bovine species.

MATERIALS and METHODS: A modular system architecture was implemented using Bluetooth Low Energy (BLE) wearable sensors for RR-interval collection, a Raspberry Pi 5 and ESP32 S3 gateway for edge computing, and a SQLite database for time-series storage. HRV analysis was performed using R 4.3.1 with the RHRV package 4.2.7, processing 5-minute epochs with a minimum of 100 RR-intervals per epoch. Cattle-specific frequency bands were adapted from literature: VLF (0-0.04Hz), LF (0.04-0.1Hz), and HF (0.1-1.0Hz), accounting for bovine respiratory characteristics (8-30 breaths/minute). Seventeen HRV metrics were calculated, including time-domain (SDNN, RMSSD, pNN50), frequency-domain (LF/HF ratio, spectral powers), and nonlinear parameters (Poincaré SD1/SD2, DFA, sample entropy). Artifact filtering removed RR-intervals outside 600-1500ms range (40-150 bpm). A Flask-based web dashboard provided real-time visualization with Chart.js 4.4.4, supporting multi-animal monitoring, interactive graphing, and data export capabilities. The system featured automated quality assessment categorizing epochs as Good (<10% artifacts), Acceptable (<20%), Poor (<30%), or Rejected (≥30%).

RESULTS: The prototype successfully demonstrated continuous HRV monitoring capabilities with an automated analysis pipeline running every 5 minutes via cron scheduling. The web interface displayed real-time metrics across multiple animals simultaneously, with 30-second auto-refresh and touchscreen-optimized controls (7-inch display, 1024×600 resolution). The system architecture supported device-animal pairing management, historical trend analysis with configurable timeframes (7-90 days), and export functionality for scientific documentation. The database schema efficiently managed relationships between readings, pairings, HRV results, and daily aggregated trends. All components operated reliably on Debian GNU/Linux 13 with systemd service management.

CONCLUSIONS: This IoT-based HRV monitoring system provides a functional platform for continuous cattle welfare assessment using adapted physiological parameters. The open-source, modular design enables cost-effective deployment and future expansion. While technical implementation proved successful, clinical validation against reference HRV systems, reproducibility studies, and large-scale field testing (n≥100 animals) remain necessary

before clinical application. Future developments should focus on environmental hardening, battery optimization, and machine learning-based anomaly detection for early disease identification.

Keywords: Heart rate variability (HRV), Cattle welfare, Internet of Things (IoT), Smart farming, Wearable sensors

Immune Performance of Concomitant Administration of Two Inactivated Vaccines Against Multiple Serotypes of Bluetongue Virus in Cattle

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BACKGROUND AND OBJECTIVES: Multiple serotypes of bluetongue virus (BTV) circulate across Europe and pose significant health and management challenges for cattle breeders. Vaccination protocols must ensure strong and protective immunity while avoiding repeated handling of animals that may increase operational constraints and animal stress. Concomitant administration of inactivated vaccines targeting different BTV serotypes could offer a practical solution to facilitate implementation of vaccine programs and streamline field procedures. The objective of this study was to assess whether the simultaneous administration of two inactivated bluetongue vaccines induced an antibody response not lower than that obtained when each vaccine is administered separately. Tolerance of the procedure was also evaluated.

MATERIALS and METHODS: Approximately 70 healthy cattle of various breeds, aged 7-17 months, with confirmed pan-BTV and EHDV anti-VP7 seronegative status and blood qPCR negative at inclusion, were enrolled across five farms in France. Animals were randomized into four groups: BTV-3 inactivated vaccine (BulTaVo®3), BTV 4 and 8 inactivated vaccine (BTVPUR®4-8), both vaccines on the same day, and unvaccinated controls. The injections were performed 21 days apart. Rectal temperature was recorded at each vaccination and for the following two days. Serum samples were collected on Days 0, 21 and 42 upon vaccination. Antibody responses to VP7 were analysed using a commercial competition ELISA (Innovative Diagnostics, Grabels, France). Neutralizing antibody titres targeting VP2 were determined by seroneutralisation tests (SNT) for BTV-3 and BTV-8. Results were compared between groups of concomitant or single-vaccine immunization using Student's and Chi-squared tests.

RESULTS: Clinical assessment showed stable rectal temperatures following vaccination, and no adverse events were reported.

The analyses demonstrated that concomitant administration did not affect either the magnitude or the kinetics of vaccine-induced antibody responses against BTV3 and BTV-8. All vaccinated groups exhibited 100% positive virus-neutralising antibody titres higher than 3.2 $\log_2$ against both viruses following the recommended vaccination regimen, with no statistically significant differences observed between singly vaccinated and with the concomitant vaccination group.

CONCLUSION: This study provides field evidence supporting the compatibility and immunological performance of concomitant administration of two inactivated bluetongue vaccines in cattle. Findings are expected to support practical vaccination strategies that reduce animal handling requirements while maintaining adequate protection against multiple circulating bluetongue virus strains for improvement of vaccination acceptability.

Advancing Dairy Cow Welfare Through Integrated Digital Claw Health Data - Results from the Swiss Claw Health Project

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BACKGROUND and OBJECTIVE: The Swiss Claw Health Project is a nationwide initiative dedicated to improving bovine claw health and welfare. The project demonstrates that providing digital claw health data can support disease control, lameness management, and targeted veterinary visits. Main

objectives were to (i) establish a robust digital infrastructure for data documentation and analysis, (ii) make it accessible and usable to veterinarians, claw trimmers, and farmers, and (iii) identify risk factors, preventive and sanitation measures, decrease lesion prevalences and develop breeding values for claw health traits.

MATERIALS and METHODS: The training of participating claw trimmers ensured high-quality, standardized data collection. All data are stored in a central database as foundation for all analyses and subprojects. Within the database, herd data can be viewed in detail and visualized graphically, for instance, to display time trends or to identify frequently occurring lesions within a herd. Farmers and veterinarians have access to their herd's data, enabling the integration of claw health information into health management. The project team used these data for individual herd consultations, and claw trimmers are increasingly reviewing the data for ongoing monitoring. Adoption by veterinarians and farmers is expected to increase. The digital infrastructure will be handed over to the Swiss breeding organizations to ensure its continued use.

RESULTS: Key project achievements included the establishment of digital documentation among the participating trimmers and the database, by now comprising a dataset of >270,000 trimming records from >1,500 herds. The data enabled effective benchmarking, identification of problem herds for targeted follow-up, and prevalence analyses. Alarm lesions (swelling of the coronet and/or bulb, interdigital phlegmon, white line abscess, ulcer, Digital Dermatitis M2 stage) showed marked prevalence decreases at both herd and individual animal levels from 2020 to 2025, e.g. ulcers 91.25% to 56.13% (herd-level), and 5.92% to 2.99% (animal-level). Risk factors and effective countermeasures were identified, particularly for Digital Dermatitis: protective factors/effective measures included immediate treatment, intensive lameness and lesion monitoring, and rigorous hygiene and biosecurity improvements; risk factors were freestall housing, animal traffic, and high concentrate feeding.

CONCLUSIONS: The integration of digital claw health data can help reduce claw lesion prevalence and enhance dairy cow welfare. The improvement of claw health relies on the collaboration of farmers, veterinarians, and claw trimmers. The results are expected to have international applicability.

Keywords: Claw health, dairy cows, welfare

The Association of Bovine Claw Hoof Lesions on Next Parity Culling in Dairy Cows

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BACKGROUND and OBJECTIVE: Claw horn lesions (CHL) are among the most important problems in high-producing cows and lead to economic losses. This study aimed to evaluate the effect of CHL of primiparous and multiparous cows on the occurrence of lesions in the next parity and to assess their impact on culling risk.

MATERIALS and METHODS: A cohort study was conducted in six dairy farms selected by convenience sampling; cows were divided into 2 groups. Group 1: primiparous cows with CHL (n = 865), Group 2: primiparous cows without CHL (n = 971). The effect of a history of CHL, calving season, stage of lactation (DIM status), stage of pregnancy (DIP), time from the last hoof trimming, affected zone and limb, concurrent infection lesion, Farm and history of reproductive, mastitis, metabolic, and other diseases on the hazard ratio of culling, was assessed using Cox proportional hazards regression (Cox PH).

RESULTS: A total of 1,778 cows were included in the analysis, among which 827 culling events were observed. Fifty-eight observations were excluded due to missing data. The effects of DIP status, occurrence order, and farm were controlled using stratification. DIM status, last calving season, and group were significantly associated with the hazard of culling. Compared with the reference DIM category, cows classified as DIM status 3 and 4 exhibited a significantly increased hazard of culling (HR = 1.49; 95% CI: 1.04–2.13; P = 0.030) and (HR = 1.56; 95% CI: 1.09–2.23; P = 0.016) respectively. DIM status 2 was not significantly associated with culling risk (P = 0.744). Only cows with winter calving showed significantly higher hazard of culling compared with the reference season (HR = 1.30; 95% CI: 1.05–1.60; P = 0.015). A protective effect was seen in group 2, as cows in Group 2 had a significantly lower hazard of

culling compared with the reference group (HR = 0.76; 95% CI: 0.65–0.90; P = 0.001). History of mastitis, reproductive disease, and metabolic disease, concurrent infection, time from hoof trimming to occurrence (both linear and quadratic terms), same limb involvement, and season of birth showed no significant effects on culling risk (P > 0.05).

CONCLUSIONS: History of calving season and occurrence of CHL in first parity recorded as the most important factors in culling in next parities.

Keywords: dairy cow, culling, survival analysis

Can We Help Extensive Farmers Demonstrate Their Cattle Welfare?

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BACKGROUND and OBJECTIVE: Increased societal concerns about farm animals' welfare impact the sustainability of production systems because purchase decisions could compromise the economic viability of those systems, depending on consumers' welfare perceptions. Different AW assessment and certification protocols are used worldwide in intensive dairy and fattening units, but little is done in extensive autochromes systems and breeds in Spain. In this scenario, it is necessary to help farmers to demonstrate that extensive cattle welfare conditions meet consumers´ demands in terms of AW with additional environmental benefits. We aimed to contribute to the sustainability of the extensive suckler beef cattle sector by providing farmers with a tool to evaluate and demonstrate their animals' welfare in Spain.

MATERIALS and METHODS: Twelve of Avileña-Negra Ibérica and sixteen Asturiana de los Valles farms were assessed in spring 2023 and winter 2025 using an adapted Welfare Quality®-based protocol and a complementary animal-based assessment (GO SOSTVAN) designed for extensive farms. Statistical analyses included Student's t-test (Statistica 14.0.0.15) and descriptive analysis.

RESULTS: Due to word count restrictions, we present here only results of good feeding dimension. It was evaluated through the body condition, a direct indicator of their nutritional status. During the first phase of the study, the breeds showed slight differences in the youngest groups (calves and yearlings), and cows of both breeds showed an optimal body condition—3.29 for Avileña and 3.08 for Asturiana, confirming adequate feeding management throughout the year. Rumen fill, an indicator of recent intake, also showed satisfactory values, confirming that the animals do not suffer from nutritional deficiencies even during the harshest winter months, when they are supplemented if pasture is unavailable. Water availability and quality are major challenges in livestock farming, with differences between regions: Asturian farms, located in more humid environments, depend mainly on water supply systems and natural watercourses, whereas Avileña farms rely more frequently on troughs, wells, and ponds. Although the study did not detect any risk situations, because none of the farms had dirty water points, 36% of the drinking troughs on Avileña farms were partially dirty, suggesting the need to reinforce cleaning and water quality control, especially during periods of drought.

CONCLUSIONS: The evaluation protocols are useful to demonstrate that extensive farms of both breeds maintain adequate animal welfare conditions, with satisfactory feeding management throughout the year, and adequate water availability and management without major risks, but improving water monitoring and trough maintenance will be important, particularly in the context of increasing climatic variability.

Keywords: suckler cows, extensive system, autochthonous breed, body condition, rumen fill,

Effects of Quillaja Extracts on *Cryptosporidium* and Productive Performance, *in vitro* and *in vivo* Trials

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BACKGROUND and OBJECTIVE: Infections caused by Cryptosporidium are common in ruminants and current control strategies show variable efficacy. Phytogenic compounds such as extracts from Quillaja saponaria, rich in saponins, may offer antimicrobial and immunomodulatory benefits to mitigate infection and support performance. The objective of this study was evaluated the anticryptosporidial and productive potential of Quillaja extracts (QE) through in vitro assays and in vivo trials in mice and cattle, including growth performance in supplemented steers.

MATERIALS and METHODS: HCT-8 cells were treated with QE or Halofuginone to assess cytotoxicity and infected with Cryptosporidium parvum (AUCP-1) to evaluate anticryptosporidial efficacy. After infection, QE or Halofuginone were added. Parasite growth was quantified by fluorescence. For the in vivo trial, male interferon-gamma knockout mice were divided into four treatments: infected untreated, infected + Halofuginone, infected + QE, and uninfected control. Infected groups received 10^4 oocysts. Treatments were administered orally from 3 days pre-infection to 14 days post-infection. Oocyst shedding (RT-qPCR) and body weight were recorded. Two commercial ruminant trials were conducted. The first evaluated productive performance and serum immune parameters in 40 infected calves during 30 days. The second assessed growth in 1300 rearing steers during 56 days. Both trials included QE supplemented (0.3 g/h/d of saponins) and control

RESULTS: QE and Halofuginone showed similar cytotoxicity (CC50: 90 and 95 µg/mL respectively). QE inhibited ~80% of C. parvum growth at 120 µg/mL, while Halofuginone 90% at 20 µg/mL. No differences in oocyst shedding were observed (p>0.05), although QE showed a numerical reduction. Only the uninfected control gained weight, whereas infected groups lost weight (p<0.05) without differences among them (p>0.05). Calves treated with QE showed a 3.3 kg increase in average body weight compared to the control (p<0.05). Only beta globulin showed significant differences (p<0.05), indicating a more controlled response to the stress. The second trial performed in steers showed a significant increase in body weight of 25 kg for the QE supplemented group (p<0.05). Based on previous literature, saponins may act at the intestinal level in calves, while in steers they may modulate ruminal fermentation.

CONCLUSIONS: Quillaja extracts (QE) showed strong in vitro activity with low cytotoxicity and improved growth performance in cattle, supporting their potential as a phytogenic strategy against cryptosporidiosis. Mouse results differed from cattle trials, suggesting different mechanisms and possible intestinal and ruminal sites of action.

Keywords: Cryptosporidium parvum, Ruminant growth performance, Quillaja Saponaria, Saponins, Phytogenic feed additives

Practical Welfare Enhancements Reduced Mortality on a Commercial Beef Finishing Farm in Slovenia

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BACKGROUND and OBJECTIVE: Animal welfare in intensive beef production systems significantly impacts animal health, productivity, growth performance, and handler safety, while also aligning with ethical standards and regulatory requirements across the European Union. This field report describes practical, welfare-oriented improvements implemented on a commercial beef finishing farm in Slovenia, with a capacity of approximately 1,400 cattle typical of intensive systems.

MATERIALS and METHODS: Cattle are housed at two locations in eight barns, in groups of 7–17 animals in a loose housing system on fully slatted concrete floors. Welfare measures were introduced in phases: artificial lighting adjustment to reduce shaded areas (2019); installation of a duct-based mechanical

ventilation system with automated temperature-dependent airflow control and replacement of narrow glass windows with wider open window sections protected by netting (2022); improvement of drinking water hygiene by oxygenation and disinfection and introduction of an automatic rotating brush as an enrichment measure currently under evaluation (2025). Animal handling was improved by installation of a carousel system and a cattle chute.

RESULTS: Following implementation, cattle move more readily through the facilities, with less hesitation in formerly shaded areas, thereby enhancing handling safety and efficiency. Ventilation and window modifications improved air exchange, light distribution and overall barn climate conditions. Preliminary monitoring of the rotating brush showed a mean recorded operating time of 7.8 h/day, indicating good acceptance by the animals. Average annual mortality decreased from 5.6 % (2019 – 2021) to 3.2 % (2022 – 2025), dropping sharply from 7 % (2021) to 3.5 % (2022) after ventilation and windows modifications, stabilising below 4.0 % thereafter (3.5 % in 2022, 2.5% in 2023, 2.9% in 2024, 3.7% in 2025).

CONCLUSIONS: Practical modifications in housing, ventilation, handling facilities, water hygiene and environmental enrichment are associated with improved management conditions and an approximately 43% reduction in mortality on the farm. Integrating measures to enhance welfare with biosecurity, preventive programs, and nutrition fine-tuning warrants sustainable beef production. However, welfare measures in beef finishing systems remain inconsistently implemented and further progress is needed, particularly in flooring, lying comfort, and environmental enrichment.

Keywords: fattening bovines, loose housing, slatted floors, ventilation, rotating brush, drinking water

DIAGNOSTIC IMAGING AND LABORATORY

Analytical and Clinical Interference of Sample Hemolysis in Evaluating Blood Biochemical and Endocrine Parameters in Cows

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BACKGROUND and OBJECTIVE: Hemolysis is a common cause of errors in laboratory tests as it affects blood parameters and leads to a positive or negative bias. This study aims to examine the relationship between the level of hemolysis (expressed as cell-free hemoglobin concentration, g/L) and the variability of metabolic and endocrine parameters and to determine the threshold level of hemolysis that causes an analytically and clinically significant bias for the twenty most frequently examined blood parameters in cows.

MATERIALS and METHODS: Paired blood samples of 10 mL each were obtained from 30 cows. One was subjected to mechanical trauma and plasma was extracted directly from the other. Hemolyzed and non-hemolyzed samples from the same animal were mixed to obtain final samples with cell-free hemoglobin concentrations of 0, 1, 2, 4, 6, 8, and 10 g/L. Metabolic and endocrine parameters were measured in the blood samples (Rayto Chemray 120 spectrophotometer and automated immunoassay analyzer TOSOH AIA-360) and the linear equation between the level of hemolysis were determined. SPSS statistics software (IBM, USA) was used.

RESULTS: The following threshold values of hemolysis were determined, which correspond to the acceptable analytical (lower value) and clinical (upper value) levels of parameter variability: beta-hydroxybutyrate 0.96 and 4.81; nonesterified fatty acids 0.39 and 3.31; glucose 0.38 and 3.90; albumin 1.12 and 6.11; total protein 1.40 and 6.80; urea 6.62 and 20.1; total bilirubin 0.75 and 5.65; aspartate

aminotransferase, 0.11 and 2.18; gamma-glutamyl transferase 1.71 and 8.90, lactate dehydrogenase 0.01 and 0.11, alkaline phosphatase 0.97 and 2.95; triglycerides 1.56 and 15.5; cholesterol 1.29 and 8.56; calcium 5.68 and 25.7; inorganic phosphates 0.57 and 8.43; magnesium 1.10 and 8.47; insulin 1.15 and 3.89; triiodothyronine 8.19 and 15.6; thyroxine 8.97 and 18.5; and cotrisol 2.78 and 11.22 (g/L cell-free hemoglobin). Three decision levels are available for each metabolic and endocrine parameter: if hemolysis is below the lower (analytical) threshold value, results can be reported without restriction; if hemolysis is between the lower and upper thresholds, the results can be issued with guidance in the form of corrective linear equations; and if hemolysis is above the upper (clinical) threshold, the results and sample must be discarded.

CONCLUSIONS: The results indicate that aspartate aminotransferase, lactate dehydrogenase, nonesterified fatty acids, and insulin are most sensitive to hemolysis, while other parameters can be measured and reported using the correction formulas, even in cases of moderate to severe hemolysis.

Keywords: dairy cows, hemolysis, blood metabolites

Structural and Functional Characterization of Congenital Hydranencephaly in a Calf: Ultrasonography, Mri, Eeg and Histological Correlation

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BACKGROUND and OBJECTIVE: A 13-day-old male *Holstein - Charolais* calf was referred for investigation of severe congenital neurological signs including seizures, impaired suckling reflex, and behavioural abnormalities. Calving was uneventful, passive transfer of immunity was adequate, and no digestive disorders were reported. The herd was officially free of bovine viral diarrhoea virus (BVDV), infectious bovine rhinotracheitis (IBR), and the calf tested negative for BVDV at birth. However, the dam had tested positive for bluetongue virus serotype 8 viremy (BTV-8) approximately 195 days before calving. On admission, the general physical examination was largely unremarkable except for a small uncomplicated umbilical hernia and moderate prognathism. Neurological examination revealed bilateral amaurosis, altered mental status, decerebrate posture, and intermittent horizontal nystagmus, consistent with severe cortical dysfunction. Despite these deficits, the calf was able to stand and ingest limited volumes of milk.

MATERIALS and METHODS:

RESULTS: Trans-temporal ultrasonography revealed a cranial cavity filled with anechoic fluid, crossed sagittally by a hyperechoic linear structure, strongly suggestive of hydranencephaly or severe hydrocephaly. Magnetic resonance imaging under general anaesthesia supported the diagnosis of hydranencephaly, with preservation of the cerebellum and brainstem. Electroencephalography performed without sedation demonstrated a markedly reduced background activity, absence of normal symmetrical rhythms, and frequent generalized spike and spike-and-wave discharges associated with clinical myoclonus, supporting the presence of epileptic activity and severe encephalopathy. Necropsy imaging findings, but revealed a thin residual neural tissue layer surrounding the two hemispheric cavities filled with cerebrospinal fluid. Histological analysis confirmed the presence of neuronal and glial elements within this remnant tissue, explaining the epileptic activity.

CONCLUSIONS: This case highlights the relevance of transtemporal ultrasonography to confirm congenital brain malformations in calves in a field approach. Improved characterization of structural and functional changes in such cases may promote earlier recognition in the field, especially in a context of epizootic emerging diseases. This report further highlights the added value of integrating complementary diagnostic modalities to establish a definitive diagnosis and to refine our understanding of congenital encephalopathies in cattle. Given the known association between *in utero* infection by some BTV strains during mid-gestation and hydranencephaly, this report contributes to the growing body of evidence linking *Orbivirus* exposure to severe neurodevelopmental outcomes. Moreover, the findings support the hypothesis that the pathogenesis of these lesions primarily involves

the destruction of neural precursor cells rather than a vascularitis process.

Keywords: hydranencephaly, calf, MRI, EEG

Development of a Multiplex Real-Time RT-PCR Assay for Simultaneous Detection of Three Major Bovine Enteric Viruses

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BACKGROUND and OBJECTIVE: Bovine viral diarrhea is a major enteric disease complex causing substantial economic losses in the cattle industry worldwide. The principal viral agents include bovine coronavirus (BCoV), bovine viral diarrhea virus (BVDV), and bovine rotavirus (BoRV). In Korea, annual diagnostic surveillance reports approximately 140 BCoV and 190 BVDV cases, with BVDV-associated economic losses estimated to exceed KRW 100 billion. Because clinical signs are nonspecific and mixed infections are frequent, rapid and accurate differential diagnosis is critical. However, most currently available domestic assays rely on conventional PCR platforms, which have limitations in sensitivity, speed, and multiplexing capability.

MATERIALS and METHODS: A multiplex real-time RT-PCR assay was developed for the simultaneous detection of BCoV, BVDV, and BoRV. Primers and probes were designed from conserved genomic regions (BVDV 5'UTR, BCoV N gene, BoRV NSP3 gene) following sequence alignment analysis. Reaction conditions were optimized for both singleplex and multiplex formats. The final amplification protocol consisted of reverse transcription at 50 °C for 15 min, initial denaturation at 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s and 55 °C for 40 s. Analytical performance was evaluated using synthetic RNA transcripts and viral reference materials, including sensitivity, limit of detection (LOD), linearity, cross-reactivity, and fecal inhibition testing.

RESULTS: The multiplex assay demonstrated sensitivity comparable to that of the corresponding singleplex assays, with optimal fluorescence signals observed at an annealing/extension temperature of 55 °C. Using synthetic RNA transcripts, the assay detected down to 1 copy/µL and showed excellent linearity (R^2 > 0.999). Based on repeated low-concentration testing, the limit of detection (LOD) was established at 5 copies/µL for all targets, and the diagnostic cut-off value was set at Ct ≤ 37. No cross-reactivity was observed with other bovine enteric pathogens. Reliable detection was achieved across fecal supernatant concentrations ranging from 0% to 40%. Furthermore, large-scale testing of field clinical bovine fecal samples demonstrated high diagnostic sensitivity and specificity, confirming the assay's applicability under field conditions.

CONCLUSIONS: The developed multiplex real-time RT-PCR assay enables rapid, sensitive, and specific simultaneous detection of three major bovine diarrhea viruses. Compared with conventional PCR methods, the assay improves diagnostic efficiency, reduces turnaround time, and supports early field diagnosis using fecal specimens. This platform is expected to be a valuable molecular diagnostic tool for surveillance and control of bovine enteric viral diseases.

Keywords: Multiplex RT-PCR, BCoV, BVDV, BoRV

Learning Efficacy of a 3-Step Blended Quick Thoracic Ultrasound Training Program for Cattle Practitioners

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BACKGROUND and OBJECTIVE: On-farm thoracic ultrasound techniques have become indispensable for detection and management of respiratory infections in calves. Yet, diagnostic performance is highly variable in novice operators, and structured training programs with objective certification criteria are required to ensure reliable implementation in field conditions. This study

aimed to describe the diagnostic performance and interobserver agreement of practitioners after a structured three-step training and certification program in quick thoracic ultrasound (qTUS) to diagnose calf pneumonia.

MATERIALS and METHODS: In this study, diagnostic performance of 48 practitioners (distributed over 5 sessions in multiple countries between Jan 2024 and December 2025) was assessed after following a 3-step blended training course in qTUS. Step 1: online modules dealt with image recognition, pitfalls, the qTUS point of care ultrasound (POCUS) technique and a series of video exercises. Step 2: a 1.5-day practical session including a theoretical recap and two practical training sessions with a trainer/trainee ratio of 1/5. Step 3: at the end, all participants each scanned 10-18 calves and indicated whether the calf had a lung consolidation ≥1cm. Diagnostic parameters (accuracy [Acc], sensitivity [Se] and specificity [Sp]) were compared to those of an experienced operator. Cohen's kappa was determined. Based on their Se and Sp, participants were certified as bronze (Se and Sp 65-75%), silver (Se and Sp 75-90%) or gold (Se and Sp > 90%).

RESULTS: The 48 practitioners originated from the UK (n=26), Italy (n=8), Belgium (n=6), Poland (n=1), Spain (n=2), Denmark (n=1), Germany (n=1) and Estonia (n=3). The average relative Se and Sp were 0.84 (standard deviation [SD] = 0.16; minimum [Min.]-Maximum [Max.] = 0.5-1) and 0.81 (SD = 0.21; Min.-Max. = 0.29-1), respectively. The average relative Acc was 0.82 (SD = 0.13; Min.-Max. = 0.4-1). Over all sessions, Cohen's kappa averaged 0.60 (SD=0.25; Min.-Max. = 0-1). In total, 22.9% (11/48), 41.7% (20/48) and 14.6% (7/48) achieved bronze, silver or gold operator level, respectively. For 10 participants, Se, Sp or both were below 65%. In total, 52.1% (25/48) practitioners reached substantial to almost perfect agreement with the trainers.

CONCLUSIONS: A structured three-step training and certification program, especially focusing on blended learning and one-on-one contact with experienced trainers, returned a high learning efficacy with good diagnostic accuracy and substantial to almost perfect interobserver agreement in the majority of participants within a defined and feasible training period. The variability in performance highlights the importance of certification in lung ultrasonography, before its implementation in calf health, welfare or sustainability labels.

Keywords: Diagnostic performance, Lung ultrasound, Blended learning, Calf pneumonia, Interobserver agreement

Beyond a Uterus and a Pair of Ovaries. How to Include Ultrasonography with the Linear Transrectal and Convex Probes in the Classic Clinical Examination of the Cow, The bovatus Method, Version 2.0

Camilo Cabra Garcia

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BACKGROUND and OBJECTIVE: The use of ultrasound as a diagnostic method has become standard practice in bovine reproductive management. However, it is also known that few professionals use it as a complement to a routine clinical examination in field conditions. There are many reasons for this: lack of familiarity with the technique, the absence of a step-by-step guide for the examination (protocol), and consequently, a lack of confidence.

MATERIALS and METHODS: The BovATUS (Bovine Abdominal and Thoracic Ultrasound) method scan 7 different regions of the bovine body. It is possible to perform the complete method with the transrectal linear probe, but, the last step should be done with the convex probe and doppler mode. The BovATUS method starts on the left side of the animal. Few organs are seen in this side (due to the rumen size), saving time and effort. In this side, two regions are scanned. Region #1: left thorax: special emphasis must be placed in ventral zones. In this region lung and the heart is seen. Region #2: reticulo-rumen-abomasum. It is caudal to the sternum. Movements of those organs are smooth and constant. Right side; Region #3: left thorax. The same instructions are followed as for the left side. Region #4: pylorus and abomasum. Walls and content must be evaluated carefully. Region #5: small intestine. Constant contractions and the content fill the bowel border to border are normal. Region #6: liver. The parenchyma and vessels should be examined rigorously. Region #7: Right kidney. It is located dorsal and caudal to the last rib. When evaluating the liver, the linear probe will not allow visualization of the caudal vena cava due to its depth. This is where the convex probe

comes in (low frequency). This vein is important in the diagnosis of cardiovascular diseases (shape and size), along with color Doppler (CD) and pulsed wave doppler (PWD) modes examinations of the main hepatic vein (MHV)

RESULTS: With BovATUS V2.0, lesions such as reticuloperitonitis, displacement of abomasum, liver abscesses, ascites, ileus, right renal cysts, lung consolidations, peritonitis, valvular endocarditis, pericardial effusion, among others can be diagnosed or suspected. Adding CD and PWD in the examination of the blood flow of the MHV may suggest tricuspid regurgitation

CONCLUSIONS: Quick, efficient and precise ultrasound examination can be performed by using the BovATUS V2.0 ultrasound method, refining treatment and prognosis (decision-making) at the individual and herd level, and improving general animal health status, welfare, and finances of the farm.

Keywords: ultrasonography, BovATUS, bovine, tricuspid regurgitation, liver, doppler

Preliminary Report on the Determination of the Relationship Between Hematocrit and Number of Infectious Nephrotic Gastrointestinal Nephrotic (INN) Eggs per Gram of Feces in Goats Under Various Production Systems in Mexico

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BACKGROUND and OBJECTIVE: Goat farming in Mexico is of paramount importance, producing 167,000 tons of milk and 48,000 tons of meat annually. Infections caused by gastrointestinal nematodes cause animals to reduce their feed intake by 15 to 20%, resulting in anorexia, tissue damage, toxemia, and anemia. These parasites have a high prevalence because they have adapted to practically all climates where goat farming systems exist in Mexico. They affect both young and adult animals, reducing the efficiency of digestion and nutrient absorption, leading to decreased milk and meat production, and even endangering the lives of growing and maintenance animals. The objective of this study was to determine the relationship between hematocrit and the degree of parasitosis caused by gastrointestinal nematodes (GIN) using the hematocrit-egg count relationship in feces in goats under various production systems in Mexico.

MATERIALS and METHODS: MATERIALS-METHODS: 380 dairy goats (French Alpine, Saanen, Toggerburg) were used in intensive, extensive, and mixed production systems in various regions of Mexico. Blood samples were collected via jugular venipuncture in EDTH tubes, and fecal samples (5 g) were collected for complete blood counts and egg counts using the McMaster test. Results and DISCUSSION: The relationship between hematocrit and the number of eggs per gram of feces in goats was determined using Spearman's rank correlation coefficient, with a significance level of 5%. The data was processed using Microsoft Excel 360© and the analyses were performed using SPSS version 25.

RESULTS: Considering the information from 380 goat records, significant evidence ($p < 0.05$) of an association was found between hematocrit and the number of eggs per gram of feces ($r = -0.195$); however, this total corresponded to three different management systems. When the analyses were performed by production system, in the extensive ($n1 = 9$) and intensive ($n3 = 87$) systems, there was no significant evidence of an association between hematocrit and the number of parasite eggs ($p > 0.05$). However, in the semi-intensive system ($n2 = 284$), this relationship was significant ($p < 0.05$), with a negative association ($r = -0.247$).

CONCLUSIONS: Goats are a species that exhibits a lower degree of anemia when they have a high egg count per gram of feces. More samples will be taken from more animals to standardize reference values and relate hematocrit levels to parasite load. As a preliminary report, this demonstrates the species' resistance to parasitosis, and efforts will be made to standardize the FACH test in this species.

Keywords: goat, parasitosis, hematocrit, Mc Master, anemia

Color Doppler Ultrasonography for Functional Characterization of Abnormal Lung Patterns in Young Cattle at Feedlot Arrival

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BACKGROUND and OBJECTIVE: Thoracic ultrasonography is commonly used for the diagnosis of Bovine Respiratory Disease (BRD) through the identification of abnormal lung patterns such as B-lines and pulmonary consolidations. However, B-mode imaging is essentially morphological and may not reflect lesion biological activity. In other tissues, color Doppler ultrasonography is used to assess tissue vascularization as an indirect marker of inflammatory activity. This proof-of-concept study aimed to assess whether color Doppler ultrasonography provides complementary functional information on abnormal lung findings in young cattle shortly after feedlot arrival.

MATERIALS and METHODS: Twenty-five Limousin young bulls aged 6–8 months were recruited from three French feedlots. Five animals were considered healthy controls based on rectal temperature below 38.7 °C and absence of respiratory signs. Twenty animals presented clinical features compatible with BRD, defined by rectal temperature ≥39.6 °C and/or at least one respiratory sign, together with abnormal thoracic ultrasonographic findings. All animals underwent a single standardized examination during the first 7 days after feedlot arrival under routine field conditions, including clinical respiratory assessment, B-mode thoracic ultrasonography, and color Doppler evaluation of the main abnormal lung area. Doppler settings were optimized for low-velocity blood flow detection. Intralesional vascularization was assessed using a semi-quantitative Doppler vascularization score (DVS): 0 = no detectable signal; 1 = minimal signal; 2 = prominent signal.

RESULTS: Control animals did not show significant changes on B-mode lung examination and no Doppler signal (DVS 0)(n=5). Among 20 clinically affected animals, two main Doppler profiles were identified. Large pulmonary consolidations were associated with minimal or absent vascularization (DVS 0 or 1)(n=6). Limited pleural irregularities with a weak Doppler signal (DVS 1) were observed without a treatment history to clarify whether the lesion is at an early or late evolution stage (n=4). Predominant B-line patterns or small consolidations (<1 cm) were consistently associated with marked vascularization signals (DVS 2)(n=10).

CONCLUSIONS: Color Doppler ultrasonography of abnormal lung findings in young cattle is feasible under field conditions and provides functional information not captured by B-mode imaging alone. The observed dissociation between lesion morphology and vascularization suggests that implementing this imaging technique could improve the interpretation of lung ultrasonographic findings. Further studies are needed to evaluate repeatability and temporal evolution to determine whether this technology may be relevant in BRD management.

Keywords: Bovine Respiratory Disease, Thoracic Ultrasonography, Doppler, young cattle, feedlot, lung lesions

Preliminary Report on the Determination of the Hematocrit-to-gastrointestinal Nematode (gin) Egg Count Relationship Per Gram of Feces in Goats Under Various Production Systems in Mexico

Erika Georgina Hernández Rojas, Ricardo Daniel Pedraza Moreo, Martha Viviana Ávila Ramírez, Ulises Jesús Bautista Pérez, Víctor Hugo del Río Araiza, Jazmin De La Luz-Armendariz, María Guadalupe Sánchez-González, Luis Enrique García-Ortuño, Rodrigo González

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BACKGROUND and OBJECTIVE: Goat farming in Mexico is of utmost importance because it produces 167,000 tons of milk and 48,000 tons of meat annually. Gastrointestinal nematode (GIN) infections cause animals to reduce their feed intake by 15 to 20%, leading to anorexia, tissue damage, toxemia, and anemia. These parasites have a high prevalence because they have adapted to virtually all climates where goat farming systems exist in Mexico. They affect both young and adult animals, reducing the efficiency of digestion and nutrient absorption, leading to decreased milk and meat

production, and even endangering the lives of growing and maintenance animals. The objective of this study was to determine the relationship between hematocrit and the degree of parasitosis caused by GIN by comparing hematocrit to fecal egg count in goats under various production systems in Mexico.

MATERIALS and METHODS: 380 dairy goats (French Alpine, Saanen, Toggernburg) were used in intensive, extensive, and mixed production systems in various regions of Mexico. Blood samples were collected via jugular venipuncture in EDTH tubes, and fecal samples (5 g) were collected for complete blood counts and egg counts using the McMaster test. Results and discussion. The relationship between hematocrit and the number of eggs per gram of feces in goats was determined using Spearman's rank correlation coefficient, with a significance level of 5%. The data were processed using Microsoft Excel 360©, and the analyses were performed using SPSS version 25.

RESULTS: Considering the information from 380 goat records, significant evidence ($p < 0.05$) of an association between hematocrit and the number of eggs per gram of feces ($r = -0.195$) was found; however, this total corresponded to three different management systems. When analyzing production systems, in the extensive (n1=9) and intensive (n3=87) systems, there was no significant evidence of an association between hematocrit and the number of parasite eggs ($p > 0.05$). However, in the semi-intensive system (n2=284), this relationship was significant ($p < 0.05$), with the association being negative ($r=-0.247$).

CONCLUSIONS: Goats are a species that exhibits a lower degree of anemia due to their high egg count per gram of feces. Further sampling from more animals will be conducted to standardize reference values and relate hematocrit levels to parasite load. This preliminary report highlights the species' resistance to parasitosis, and efforts will be made to standardize the FAMACHA test in this species.

Keywords: goat, nematodos gastro intestinal, hematocrit, anemia, McMaster

Clostridium perfringens in Dairy Cattle in Northern Italy: Molecular Characterization and Toxinotype Prevalence (Years 2020-2026)

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BACKGROUND and OBJECTIVE: *Clostridium (C.) perfringens* is a ubiquitous, Gram-positive, anaerobic but aero-tollerant spore-forming bacterium and a major opportunistic pathogen responsible for a wide spectrum of lethal enteric and histotoxic diseases in livestock. *C.perfringens* strains are currently classified into seven toxinotypes (A–G) based on their production of six major toxins. Since virulence is directly linked to these toxins, they serve as the primary targets for developing effective vaccination strategies. This study assessed toxinotype prevalence in northern Italian dairy farms between 2020-2026, within the Parmigiano Reggiano and Grana Padano areas.

MATERIALS and METHODS: The survey was conducted in the provinces of Piacenza, Parma, Reggio-Emilia, Modena, Mantova, and Bologna, on visceral (abomasal/intestinal content) and faecal samples, submitted for diagnostic purposes to the IZSLER laboratories between 2020 and 2026. Samples were diluted 10-fold in buffered peptone water. Then, 100µL were plated on blood agar and incubated anaerobically for 24 hours. Presumptive haemolytic colonies were confirmed via reverse CAMP test and/or MALDI-TOF MS. Strains were genetically characterized using multiplex PCR to detect toxin genes.

RESULTS: Laboratory analyses were conducted on 288 *C. perfringens* strains (132 faecal and 156 visceral), selected from samples with counts exceeding 10^3 in faeces and 10^6 in viscera. Data stratified by year were distributed as follows: 9.4% in 2020; 14.6% in 2021; 12.2% in 2022; 16.0% in 2023; 17.7% in 2024; 22.9% in 2025; 7.2% in 2026 (January-February). The highest number of isolates originated from samples collected in the Parma province

(57.6%), followed by 14.9% in Reggio-Emilia, 9.4% in Bologna, 7.3% in Modena, 5.9% in Piacenza, and 4.9% in Mantova. Toxinotype A was found in 98.6% of the samples, and 20.34% of these (48 out of 284) also carried the beta2 minor toxin genes.

CONCLUSIONS: These findings confirm the widespread presence of toxinotype A, highlighting a significant diffusion of minor toxin genes, specifically the beta2, within the bovine herds in the study area. Since anaerobiosis is difficult to maintain, the tendency of fastidious strains to undergo sporulation could interfere with toxin detection. The molecular characterization of these isolates is essential for the development of targeted control measures: the vaccination is a critical tool to mitigate sudden death losses and safeguard the continued excellence of dairy productions, nevertheless commercial vaccines lack beta2 coverage, supporting the potential importance of autogenous vaccines implementation. Finally, the data has shown a sampling increase in recent years; however, this trend may reflect a reporting bias due to heightened awareness among the stakeholders involved.

Keywords: Clostridium perfringens, Toxinotyping, Molecular characterization, Epidemiology, Dairy cattle

Pilot Study: Using Ct Morphometry to Develop a Prediction Model for Internal Pelvic Dimensions in Holstein Cattle

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BACKGROUND and OBJECTIVE: Fetopelvic disproportion is a major cause of dystocia, and the dam's pelvic size is a significant risk factor. This pilot study aimed to develop models that predict internal pelvic size from externally measured variables.

MATERIALS and METHODS: In eight calves, internal pelvic variables measured by Computed Tomography (CT) were pelvic inlet area (PI_A) and pelvic outlet area (PO_A), which reflect birth canal size. External measurements included six distances (between two tuber coxae [DC], two greater trochanters [DT], two tuber ischiadica [DI], tuber coxae and greater trochanter [DCT], greater trochanter and tuber ischiadicum [DTI], and tuber coxae and tuber ischiadicum [DCI]) and three heights (from tuber coxae [HC], greater trochanter [HT], and tuber ischiadicum [HI]). Multiple linear regression was used to identify significant predictors of internal pelvic size using GraphPad 15 (GraphPad Software, USA).

RESULTS: All internal and external variables showed significant linear correlations ($r > 0.5$, $p < 0.004$). For PI_A, DC, HI, and DCI were retained, yielding the regression equation: PI_A (cm^2) = -55.035+4.689*DC+1.081*HI-2.543*DCI ($r^2 = 0.918$, $p < 0.001$). For PO_A, DT, DCT, and DCI were significant, giving: PO_A (cm^2) = -12.406+1.751*DT-1.603*DCT+1.521*DCI ($r^2 = 0.696$, $p < 0.001$).

CONCLUSIONS: New predictive model for PI_A was thus developed from three selected external pelvic variables, showing higher predictive ability ($r^2 = 0.945$, $p = 0.01$) than existing models. Also, new model was developed for PO_A ($r^2 = 0.696$, $p < 0.001$), which has not been studied in previous studies. This pilot study demonstrates the feasibility of using novel external measurements to estimate pelvic canal size in calves. Further research is required to validate these models and assess their clinical applicability in pregnant or periparturient cows.

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Keywords: Computed Tomography, Fetometry, Pelvic Dimension, Prediction Model

Comparison Of Three Mycoplasma bovis antibody Elisas on Serum From 3-5 Week Old Dairy Calves Using Bayesian Latent Class Analysis

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BACKGROUND and OBJECTIVE: Controlling Mycoplasma bovis is particularly difficult in veal and dairy beef systems that source young calves from multiple origins. Pre-purchase testing may help reduce pathogen introduction, with serology being the most accessible option. Additionally, given current EFSA recommendations to increase the minimum age for calf transport to five weeks, this period represents the relevant time frame for pre-purchase examination of calves. However, antibody test performance in calves aged 3–5 weeks during acute infection is unknown. This study therefore aimed to (1) compare diagnostic accuracy of three commercial antibody ELISAs (ID-Screen, Bio K302 and Bio K432) in 3–5 week old Belgian male dairy calves using Bayesian latent class models at manufacturer and optimized cutoffs, and (2) assess the impact of prevalence on predictive values.

MATERIALS and METHODS: A prospective diagnostic test accuracy study was performed on 194 serum samples from male dairy calves aged from 20-38 days using Bayesian latent class modelling. Samples were categorized using manufacturer and optimized cutoff values. Additionally, predictive values were calculated for each ELISA.

RESULTS: Using the manufacturer's cutoffs, ID-screen, Bio K302 and Bio K432 showed 69.1%, 24.6% and 68.7% sensitivity, and 97.8%, 95.6% and 97.9% specificity, respectively. Optimized cutoffs resulted in 81.4%, 49.7% and 83.8% sensitivity, and 98.9%, 92.5% and 89.2% specificity, respectively. At 7.5% prevalence, PPV was 72.6% for Bio K432, 71.8% for ID-screen, and markedly lower for Bio K302 (31.2%), while NPV was similar for Bio K432 and ID-screen (97.5%) and slightly lower for Bio K302 (94%). At 22.1% prevalence, PPV increased for ID-screen (95.5%) compared with Bio K432 (68.8%) and Bio K302 (65.3%), whereas NPV remained high for Bio K432 and ID-screen (~95%), but decreased for Bio K302 (86.6%).

CONCLUSIONS: In conclusion, at manufacturer's cutoffs, diagnostic accuracy of ID-screen and Bio K432 are similar and outperform Bio K302. However, Se remains low for all three tests. With optimizing cutoffs, there is an important increase in Se, but not enough to serve as screening tests. Overall, the highest diagnostic accuracy was found for ID-screen at an S/P% cutoff value of 30%, which could be applied as a rule-in test to create a feedback mechanism towards the herds of origin. Additionally, optimized cutoffs improve rule-in performance at low prevalence, where small Sp differences strongly influence PPV. Across both manufacturer's and optimized cutoffs, Bio K302 showed poorer rule-out performance than ID-screen and Bio K432, with differences diminishing as prevalence increases.

Keywords: Purchase, Mycoplasma bovis, Diagnostics, Bayesian latent class analysis

Comparison of BA200 and Abbott Analyzers for BHBA Measurement in Pregnant Ewes

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BACKGROUND and OBJECTIVE: The aim of this study was to compare the performance and diagnostic agreement of BA200 and Abbott analyzers in measuring Beta-Hydroxybutyric Acid (BHBA) levels, a critical biomarker for diagnosing subclinical pregnancy toxemia in pregnant ewes. Pregnancy toxemia, particularly in late gestation, is associated with negative energy balance and increased ketone body production, making accurate BHBA measurement essential for timely diagnosis and clinical management.

MATERIALS and METHODS: BHBA levels were measured simultaneously with both devices in serum samples obtained from 147 pregnant ewes, including animals diagnosed with pregnancy toxemia as well as clinically healthy ewes with normal pregnancies. The inclusion of both groups allowed for a comprehensive evaluation of the analyzers across a wide range of BHBA concentrations, reflecting both physiological and

pathological metabolic states. The relationship between devices was evaluated using Pearson correlation, mean differences were assessed with paired-samples t-test, and systematic error was analyzed using Bland-Altman plots. Diagnostic agreement was calculated using Cohen's Kappa coefficient based on the subclinical pregnancy toxemia threshold of 0.8 mmol/L.

RESULTS: A near-perfect correlation was found between the two devices (r= 0.975, P<0.001). However, the Abbott device (M=1.82) yielded statistically significantly higher results compared to the BA200 (M=1.66, P<0.001). Bland-Altman analysis revealed a systematic bias of -0.158 units. While the agreement between devices based on the 0.8 mmol/L threshold was "excellent" (κ = 0.878) clinically significant discrepancies and outliers (e.g., 0.25 vs 1.20 mmol/L) were identified in some samples.

CONCLUSIONS: The BA200 and Abbott analyzers demonstrated high overall agreement and can be reliably used for herd-level screening of pregnancy toxemia. However, the consistent tendency of the Abbott device to yield higher BHBA values, together with the identified systematic bias, may increase the risk of misclassification, particularly in animals with values close to the clinical cut-off of 0.8 mmol/L. In addition, the presence of occasional clinically significant discrepancies and extreme outliers indicates that individual results may differ substantially between devices. Therefore, while both analyzers are suitable for routine use, clinicians should interpret results with caution in borderline cases and take into account device-specific variability to ensure accurate diagnosis and appropriate clinical decision-making.

Keywords: Pregnancy toxemia, BHBA, Sheep, Method comparison, Bland-Altman.

Diagnosis and Ultrasonographic Characterization of Six Cases of Acute Interstitial Pneumonia in Dairy Cattle

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BACKGROUND and OBJECTIVE: Interstitial pneumonias are characterized by inflammation of the pulmonary interstitium, the supportive tissue surrounding the alveoli and blood vessels. Acute interstitial pneumonia (AIP) is an important respiratory condition in adult cattle and may be difficult to differentiate clinically from other types of pneumonia. Thoracic ultrasonography has become a useful tool for evaluating pulmonary lesions in cattle under field conditions. The objective of this study was to describe six cases of acute interstitial pneumonia in adult dairy cattle diagnosed by thoracic ultrasonography and to characterize the ultrasonographic features associated with this condition.

MATERIALS and METHODS: Six adult dairy cattle (≥24 months old) diagnosed with acute interstitial pneumonia were evaluated at the Bovine Clinic of Garanhuns, Federal Rural University of Pernambuco, Brazil, between 2021 and 2024. During this period, AIP represented 7.5% (6/80) of all pneumonia cases diagnosed in adult cattle. All animals presented with acute respiratory signs. Clinical evaluation included respiratory examination, percussion, and pulmonary auscultation. Thoracic ultrasonography was performed to assess pulmonary alterations. Based on clinical suspicion and ultrasonographic findings, animals were treated with antimicrobials (ceftiofur, enrofloxacin, or florfenicol), anti-inflammatory drugs (flunixin meglumine or dexamethasone), a bronchodilator (clenbuterol), and nebulization with bronchodilator agents (camphor oil).

RESULTS: All animals presented acute respiratory signs characterized by tachypnea, polypnea, elbow abduction, expiratory dyspnea, pulmonary hypophonesis, and caudoventral displacement of both lung fields detected by intercostal percussion and auscultation. All animals also exhibited varying

degrees of subcutaneous emphysema along the dorsal region extending from the thorax to the sacral area. Thoracic ultrasonography revealed thickened and irregular pleural surfaces, more than five comet-tail artifacts per scanning field, and increased horizontal reverberation lines. A caudoventral displacement of lung fields was also observed. In animals presenting subcutaneous emphysema, reverberation artifacts originating from the subcutaneous tissue prevented adequate evaluation of deeper structures. Clinical outcome was favorable in 66.7% (4/6) of cases, whereas 33.3% (2/6) resulted in death. Animals that recovered clinically showed a slow and gradual resolution of pulmonary and subcutaneous emphysema. Necropsy findings in the two fatal cases revealed diffuse pulmonary emphysema (interstitial and alveolar), more evident in the diaphragmatic lobes, thickened whitish pleura, and extensive subcutaneous emphysema extending along the dorsal body region.

CONCLUSIONS: Thoracic ultrasonography is a useful adjunct diagnostic tool for acute interstitial pneumonia in adult cattle. Although clinical signs and acute disease progression may raise suspicion for this condition, they are insufficient to differentiate AIP from other forms of pneumonia.

Keywords: bovine respiratory disease, thoracic ultrasonography, pulmonary emphysema, diagnostic imaging, subcutaneous emphysema.

Evaluation of Minimum Inhibitory Concentrations of *Streptococcus uberis* to Benzylpenicillin: A Comparative Field Study Using an Automated Point-of-Care Test and a Standard Laboratory Method

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BACKGROUND and OBJECTIVE: Streptococcus uberis is a major etiologic agent of bovine mastitis. In France, penicillin G remains the drug of choice because stable resistance has not been documented, although ~30% of isolates exhibit increased MIC (≥0.25 µg/mL), and field practitioners report suboptimal cures with standard regimens. Daily intramuscular penethamate achieves high milk penicillin G concentrations for about12 hours; however, troughs before the next dose and wash out of intramammary doses at milking may create subtherapeutic periods. Alternating intramammary benzylpenicillin with intramuscular penethamate 10–12 h later could maintain effective concentrations across the dosing interval. We tested this therapeutic concept in a randomized field trial and benchmarked Mastatest® MIC outputs against Etest® as a reference.

MATERIALS and METHODS: Non GCP randomized field trial conducted across four veterinary practices in France with on site bacteriology and Mastatest® capability. Animals: Lactating cows with non-severe clinical or subclinical mastitis yielding Streptococcus uberis in pure culture. Aseptic quarter milk sampling, cooled (4°C) and processed within 24–48 h. Primary culture at the practice followed NMC derived workflow on COS, CAN, HEKT plates with catalase, coagulase, esculin, and optional Lancefield testing. Milk samples with

presumptive *Streptococcus uberis* isolates were entered into Mastatest®. Reference MICs for benzylpenicillin were obtained by Etest®.

RESULTS: Among 75 Strep. uberis isolates tested by both methods, Etest® classified 92% as penicillin susceptible and 8% as less susceptible using the pre specified method threshold; Mastatest® classified 88% as susceptible and 12% as less susceptible. Taking Etest® as reference, sensitivity was 67%, specificity 93%, PPV 44%, NPV 97% and overall accuracy 91% for Mastatest®. The Mastatest® MIC module showed excellent rule out performance (NPV 97% and specificity 93%), supporting its utility to affirm penicillin susceptibility rapidly at the point of care. Conversely, as expected in a low prevalence "less susceptible" setting, the PPV was modest (44%). In practice, a "susceptible" Mastatest® result can be trusted to proceed with penicillin therapy, while an "intermediate/less susceptible" result should prompt confirmatory testing or clinical caution.

CONCLUSIONS: Parallel laboratory evaluations indicated that Mastatest® provides highly reliable "susceptible" calls for penicillin (NPV 97%; specificity 93%) against Etest®, supporting penicillin first stewardship when Mastatest® indicates susceptibility. These results support using rapid MIC testing to guide penicillin based therapy decisions in practice.

Keywords: Streptococcus uberis, mastitis, benzylpenicillin, MIC

FEEDLOT

Comparison of Tulathromycin Versus Enrofloxacin for the Treatment of Bovine Respiratory Disease Following Metaphylaxis in Us Commercial Feedlots

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BACKGROUND and OBJECTIVE: Macrolide antimicrobial drugs are commonly used for treatment and control of bovine respiratory disease (BRD). The objective of this study was to compare the efficacy of tulathromycin and enrofloxacin for treatment of BRD, following metaphylaxis with tulathromycin.

MATERIALS and METHODS: A total of 483 beef cattle from 35 lots were enrolled in a randomized controlled study. The study took place at a commercial feedlot in Central Nebraska. Eligible lots included cattle entering the feedlot that weighed 550 +/- 200 lbs. and were deemed high risk for developing BRD. All cattle received Tulissin® 100 (tulathromycin injection) injectable solution for metaphylaxis at arrival processing. Enrollment took place at the time of first treatment for BRD. Study cattle did not have pre-existing disease nor comorbidities. Cattle satisfying the study enrollment criteria were randomly assigned to 1 of 2 treatments: Tulissin® 100 (tulathromycin injection) (TUL) or Tenotryl™ (enrofloxacin) injectable solution (TEN). Electronic identification (eID) tags were placed at enrollment and used to match individual carcass data provided by the abattoir. Cattle requiring retreatments followed a uniform treatment regimen with a 4-day post-treatment moratorium. Deep pharyngeal swabs were collected at initial processing and at first, second, and third treatments for BRD from 6 pens. Four pooled samples (5 swabs/pool) per pen were submitted at processing. Samples were submitted to Iowa State Veterinary Diagnostic Laboratory for bacterial culture and

antimicrobial susceptibility testing. The statistical analysis was performed using SAS Version 9.4 (SAS Institute, Cary, NC). Health, performance, and carcass outcomes were compared across treatment groups. Continuous variables were analyzed using linear mixed mode, and binary outcomes were analyzed using generalized mixed models (PROC GLIMMIX). All statistical models included treatment as a fixed effect and lot as the random effect.

RESULTS: No significant differences ($p \leq 0.05$) were observed in health and live performance outcomes. Antimicrobial susceptibility results indicate similar susceptibility profiles among bacterial isolates collected at processing and first treatment. In contrast, bacteria isolated from second and third treatments exhibit resistance to multiple antimicrobial classes. However, the sample size for diagnostics limit drawing significant conclusions of differing susceptibility between the treatment groups, but do lay groundwork for future research.

CONCLUSIONS: This study provides treatment outcome data comparing tulathromycin versus enrofloxacin, following metaphylaxis with tulathromycin. The results of this study indicate that there were no significant differences in health, performance, and carcass outcomes between treatment groups.

Keywords: Antimicrobial resistance, Macrolides, Feedlot

Feeding Behavior, Digestibilty and Metabolic Profile of Wagyu and Wagyu × Holstein Steers in Feedlot

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BACKGROUND and OBJECTIVE: Wagyu–Holstein crossbreeding may improve growth while maintaining carcass traits. We aimed to evaluate feeding behavior, digestibility, and metabolic profile in Wagyu (W; n = 8) and Wagyu × Holstein F1 (WH; n = 8) steers during the finishing period.

MATERIALS and METHODS: A 56-day trial was conducted on a commercial farm using covered feedlot pens (21.6 m²/animal) equipped with smart feeders (Hook SA, Argentina), where animals received a total mixed ration (forage-to-concentrate ratio of 43: 57, based on corn silage, ground corn grain, canola meal, a mineral–vitamin premix, and urea; 45% dry matter, DM; 12.5% crude protein, CP; and 26.7% neutral detergent fiber, NDF) and had access to water. Fecal (two samples per animal per day for two consecutive days) and blood samples were collected every 14 days. Fecal samples were composited by animal to determine apparent digestibility using acid-insoluble ash as an internal marker. Data were analyzed using a mixed model with genetic group as a fixed effect and measurement day and animal as random effects. Means were considered different when $P<0.05$.

RESULTS: Initial and final body weight (BW) were greater for WH than W steers (685 vs. 417±18 kg of average BW for WH vs. W, respectively). Dry matter intake (kg/d) was greater for WH than W steers; however, DM intake relative to BW did not differ between groups (0.91±0.05 %BW/d). Time spent at the feeder (min/d) was almost 30 min lower for WH than W steers, which was associated with shorter average visit duration (−2.7 min/visit), as the number of daily visits did not differ between groups (17.3±1.9 visits/d). In contrast, the DM intake rate at the feeder (g/min) was 64.6% higher for WH than W steers. No differences were detected between groups in DM digestibility, however, CP and NDF digestibility were greater for WH than for W steers (70.8 vs. 63.8±1.8% and 60.3 vs. 49.2±3.1%, respectively). Plasma glucose, urea, total protein, and albumin concentrations did not differ between groups, whereas plasma cholesterol was greater (2.06 vs. 0.89±0.12 mmol/L) and triglycerides tended to be greater (0.19 vs. 0.15±0.02 mmol/L; P=0.10) for WH than W steers. In addition, aspartate aminotransferase concentrations tended to be lower in WH than in W steers (51.9 vs. 63.6±5.8 U/L; P=0.10), whereas gamma-glutamyltransferase concentrations did not differ between groups.

CONCLUSIONS: These results indicate improved digestive and metabolic nutrient utilization efficiency in WH than W steers, achieved through behavioral adjustments at the feeder rather than increased feeding time.

Keywords: beef cattle, nutrition, behavior, metabolism

Fatty Acid Profile of Wagyu Beef: Impact of Cacao By-Product Supplementation

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BACKGROUND and OBJECTIVE: Growing consumer interest in functional foods has positioned the lipid composition of beef as a central quality attribute, particularly due to its content of fats considered beneficial to health, such as monounsaturated fatty acids (MUFA), especially oleic acid, which are associated with improved blood lipid profiles and cardiovascular health. In addition, bioactive compounds such as branched-chain fatty acids (BCFA) have attracted attention because of their potential cardiometabolic benefits. In this context, the Wagyu breed is differentiated by its high degree of marbling and greater proportion of MUFA compared with conventional breeds such as Aberdeen Angus. The use of Cacao by-products from the chocolate industry in finishing diets has been proposed as a nutritional strategy to modulate fatty acid composition. The objective of this study was to evaluate the effect of cocoa by-product supplementation on the fatty acid profile of purebred Wagyu steers and to compare them with an Angus group finished under a conventional feedlot system.

MATERIALS and METHODS: Sixteen Wagyu steers were assigned to two treatments (n = 8): CCH (8% cocoa by-product on a dry matter basis) and SCH (no cocoa inclusion). An additional Angus group (NW) served as a comparative control. All animals were fed total mixed rations. After the finishing period, steers were slaughtered and samples of Longissimus dorsi muscle and subcutaneous fat were collected. Lipids were extracted and analyzed by gas chromatography, and fatty acids were grouped as saturated (SFA), MUFA, polyunsaturated (PUFA), BCFA, and TRANS. Data were analyzed using ANOVA (PROC MIXED, SAS V9.4), considering treatment as a fixed effect ($p < 0.05$).

RESULTS: Results showed that the NW group had a higher proportion of SFA (47.8%) than Wagyu steers (CCH: 45.12%; SCH: 46.73%) ($p < 0.001$), whereas Wagyu exhibited higher MUFA content (CCH: 47.86%; SCH: 46.15%) compared with Angus (45.25%) ($p < 0.001$). Significant differences were detected for BCFA ($p < 0.001$), with SCH (0.46%) > NW (0.44%) > CCH (0.42%), although absolute differences were small. No significant differences were observed for PUFA ($p = 0.081$) or TRANS ($p = 0.482$). In conclusion, Wagyu genetics was the primary determinant of the lipid profile, characterized by higher MUFA and lower SFA proportions compared with Angus.

CONCLUSIONS: The presence and significant variation of BCFA—rumen-derived fatty acids associated in recent studies with potential cardiometabolic benefits—represent an additional component of nutritional value in high-quality beef. Cacao by-product supplementation did not produce consistent improvements in this specific group of fatty acids.

Keywords: branched-chain fatty acids, antioxidant, meat quality

Effect Of Injectable Mineral Supplementation on Feed Intake and Performance of Nellore Cattle in the Finishing Phase

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BACKGROUND and OBJECTIVE: Background and Objective The receiving period in feedlots is a critical moment for cattle health and performance due to the stress of transportation and new environments. This cascade of stressors triggers oxidative stress, systemic inflammation, and immunosuppression, which ultimately reduces feed intake. Injectable mineral supplements bypass the digestive process and antagonistic dietary interactions, making them an efficient strategy to improve antioxidant status and immunity due to their high bioavailability. This study aimed to evaluate the effect of an injectable

multimineral supplement based on phosphorus, selenium, copper, magnesium, and potassium (Fosfosal, Virbac, São Paulo, Brazil) on the intake, performance and morbidity of finishing Nellore cattle.

MATERIALS and METHODS: The study was carried out according to the national ethical law and the principles of laboratory animal care. A total of 1,721 uncastrated Nellore males with an initial body weight (BW) of approximately 371 kilograms and 20 months of age were evaluated. The animals were randomly distributed in 2 groups into pens: control group CON (n=828), receiving only the arrival sanitary protocol (dewormed and vaccine against clostridiums and respiratory diseases) and group FOS (n=893) receiving the arrival protocol plus 10 milliliters intramuscular of injectable multimineral supplement. The 97-day experimental period comprised 15 days of adaptation and 82 days of finishing. Animals received an ad libitum diet twice daily. Dry matter intake (DMI) was measured daily. Cattle were weighed at the beginning and end of the period to calculate average daily gain (ADG). Animals were monitored daily for morbidity and mortality. Data were analyzed using mixed models with significance set at P≤0.05.

RESULTS: There was a significant treatment effect on final ADG (P<0.001) and BW (P≤0.023). The FOS group final ADG (1.965 kilograms/day) and BW (570 kilograms) vs the CON group ADG (1.913 kilograms/day) and BW (568 kilograms). DMI was not significantly affected by the treatments (P≥0.911). The morbidity rate for the CON group was 5.46%, while the FOS group exhibited a comparable rate of 4.93%. No treatment effect was observed on mortality rates (P=0.378).

CONCLUSIONS: The supplementation of injectable multimineral during the feedlot receiving period is efficient at increasing animal performance by providing positive results in final body weight and average daily gain compared to non-supplemented animals.

Keywords: feedlot, feed intake, mineral supplementation, phosphorus

Nutritional Evaluation of Processed Potato Foliage Compared with Alfalfa Hay Using the In Vitro Gas Production Technique

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BACKGROUND and OBJECTIVE: The purpose of this experiment was to compare the metabolizable energy (ME), dry matter digestibility (DMD), production of short-chain fatty acids (SCFA), and in vitro gas production of potato foliage, alfalfa hay, and processed potato foliage, and examine the impact of processing potato foliage with molasses and microbial additives.

MATERIALS and METHODS: Three treatments and three replications were used in the fully randomized design of the experiment. To gather rumen fluid in this case, three fistulated lambs were employed. To gather rumen fluid, three fistulated male Afshar lambs with an average weight of 45.86 ± 3.02 kg, which were kept in a metabolic cage, were used. In the experiment, to evaluate the nutritional value of alfalfa hay, potato foliage, and processed potato foliage, the in vitro gas production was measured at 2, 4, 6, 8, 12, 16, 24, 48, 72, and 96 hours after incubation.

RESULTS: The results showed that at all incubation hours, in vitro gas production for alfalfa hay was significantly higher than potato foliage, and for the processed potato foliage, it was higher than alfalfa hay. In total in vitro gas production volume, there were significant differences among the treatments of potato foliage, alfalfa hay, and processed potato foliage, producing 119.77, 147.98, and 173.76 milliliters of gas per gram of dry matter sample, respectively (p<0.05). Furthermore, in terms of metabolizable energy (ME), digestibility of dry matter (DMD), and production of short-chain fatty acids (SCFA), these values were significantly (p<0.05) higher for alfalfa hay (1.45 Mcal per kg of dry matter, 40.23%, and 4.9 millimoles per liter, respectively) than for potato foliage (1.25, 32.84, and 3.6 respectively) and for the processed potato foliage (1.68, 53.59, and 6.7 respectively) than for alfalfa hay.

CONCLUSIONS: All things considered, processed potato foliage has better nutritional content than alfalfa hay.

Keywords: Potato foliage, In vitro gas production, Microbial processing, Dry matter digestibility, Ruminant nutrition

GENETICS AND BREEDING

Improving Health Traits Through Breeding

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BACKGROUND and OBJECTIVE: Genetic herd management is one of the aspects of management that can contribute to improving cattle health. Monitoring genetic potential and conducting breeding work complements the preventive actions undertaken as part of operational management. This is possible only by including health traits in the selection index, which is the primary tool for breeding work. This study aimed to evaluate the priorities of Polish dairy cattle breeders regarding functional traits and to update the Production and Functionality Index (PF) formula to better align with the requirements of modern dairy farming.

MATERIALS and METHODS: The research methodology involved an online survey of 208 breeders, consisting of 55 questions about the importance of improving health traits for breeders. The survey results were used to decide which traits should be included in the revised PF index and what percentage weighting should be assigned to each group of traits.

RESULTS: The results indicated a strong preference for health-related characteristics. When defining the "ideal cow," breeders rated mastitis resistance highest (3.5 on a 4-point scale), followed by udder conformation and fertility (3.4). In terms of index inclusion, mastitis resistance (4.2 on a 5-point scale), hoof disease resistance (4.1), and feed efficiency (4.0) were identified as the most critical factors. Due to the lack of routine clinical mastitis recording in Poland, somatic cell count (SCC) was utilized as the primary indicator for udder health. Based on these findings, a novel Health sub-index was developed, comprising longevity (44%), SCC (44%), and digital dermatitis resistance (12%). Furthermore, a Functionality sub-index was established to incorporate calving ease (20%) and calf survival (20%). To accommodate these additions while increasing the emphasis on fertility, the relative weights of production and conformation traits were reduced. The revised PF index formula now consists of five sub-indices: 30% Production, 25% Health, 20% Fertility, 15% Udder and Legs Conformation, and 10% Functionality. The proposed formula was subsequently validated through a follow-up survey of 53 active breeders, where 83% of respondents assigned the new index a score of 5 or higher on a 6-point scale.

CONCLUSIONS: By incorporating traits such as digital dermatitis resistance and calf survival, the updated PF index successfully addresses the expectations of Polish Holstein-Friesian breeders. This strategic shift in selection pressure is expected to yield significant improvements in bovine health and fertility while maintaining the population's high production potential.

Keywords: Breeding, Health traits, Selection index, Digital dermatitis resistance

Effects of Genomic Merit, β-Casein Gene Variants (A1 and A2) and κ-Casein Haplotypes on Calving Traits and Calf Growth Traits in Holstein-Friesian Cattle

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BACKGROUND and OBJECTIVE: Bovine A2 milk has gained increasing attention due to its potential human health benefits. Furthermore, milk protein genes, particularly β-casein (CSN2) and κ-casein (CSN3), have attracted considerable attention for their potential influence on milk composition and possible associations with growth-related traits. The

present study aimed to evaluate the effects of β-casein genotypes and κ-casein haplotypes on calf growth traits, including birth weight, weaning weight, and average daily gain (ADG), as well as calving ease. Genomic breeding values for each genotype were also evaluated.

MATERIALS and METHODS: DNA samples extracted from ear tissue samples were genotyped using the Affymetrix Axiom array incorporated into a custom 70K high-density SNP array on a GeneTitan Multi-Channel platform. β-casein genotypes (A1A1, A1A2, and A2A2) and κ-casein haplotypes (AA, AB, AE, BB, BE, and EE) were used in subsequent statistical analyses. The associations between gene variants and growth traits were evaluated using a General Linear Model.

RESULTS: Neither β-casein genotype nor κ-casein haplotype had a statistically significant effect on birth weight. The proportion of explained variance for this trait was relatively low (R^2 = 3.9%), suggesting that birth weight is largely influenced by maternal and environmental factors rather than the investigated milk protein gene variants. In contrast, β-casein genotype had a significant effect on weaning weight (P = 0.032). Calves carrying the A2A2 genotype exhibited the highest estimated weaning weight (97.91 kg), followed by A1A1 (95.23 kg) and A1A2 (93.06 kg). However, κ-casein haplotypes did not show a statistically significant effect on weaning weight. Similarly, β-casein genotype significantly affected average daily gain (P = 0.040). The highest ADG values were observed in calves with the A2A2 genotype (789.9 g/day), whereas the A1A2 genotype showed the lowest growth rate (742.6 g/day). Although κ-casein haplotypes did not demonstrate a significant overall effect, the AE haplotype showed a positive coefficient, suggesting a potential favorable association with daily weight gain. A higher incidence of calving difficulty was observed in cows carrying the A2A2 genotype. Genetic merit exhibited significant effects for both the β-casein and κ-casein genes.

CONCLUSIONS: The findings indicate that β-casein genetic variation may contribute to differences in calf growth performance, particularly for weaning weight and daily weight gain, while κ-casein haplotypes appear to have limited influence on the investigated growth traits. These results highlight the potential relevance of the A2A2 genotype in breeding programs to improve both milk characteristics and growth performance in dairy cattle.

Keywords: Holstein, A2 milk, casein, genomic breeding value, calving, growth rate

Can We Use Genomic Testing to Predict Bovine Clinical Mastitis? A German Field Observation

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BACKGROUND and OBJECTIVE: Genomic testing is a practical tool for predicting future performance and health in dairy cattle. Genomic predictions allow producers to rank and select animals for production traits (e.g., milk yield, fat, and protein), health traits (e.g., mastitis, retained placenta, metritis, ketosis, displaced abomasum, and lameness), and profitability (e.g., net merit index, dairy wellness profit index). The predictive performance of wellness traits has been described. Genomic selection may also help reduce antibiotic use by lowering the incidence of clinical mastitis (CM). The objective of this field observation was to assess whether a genomic mastitis prediction (STA_MAST) was associated with (1) CM incidence and (2) CM recurrence (number of CM events per animal).

MATERIALS and METHODS: The observation was conducted in a large Holstein-Friesian dairy herd in eastern Germany. Heifers (n=222) were genotyped using hair samples analyzed with a genomic test (Clarifide Plus; Zoetis). The mastitis wellness trait prediction (STA_MAST; higher values indicate lower predicted mastitis risk) was used to assign animals to four percentile-based risk groups: bottom 25% (highest predicted risk; n=56), 26–50% (n=55), 51–75% (n=55), and top 25% (lowest predicted risk; n=56). Clinical mastitis events were recorded over two years using herd management software (HERDE, dsp agrosoft, Germany). Associations between the STA_MAST group and CM occurrence were evaluated (chi-square-Test and Fisher-exact-Test, IBM, SPSS) and the number of CM cases per affected animal was summarized by group.

RESULTS: Overall, CM incidence during follow-up was 41.4%. The bottom 25% group (mean STA_MAST 93) had a CM incidence of 58.9%, compared with 16.6% in the top 25% group (mean STA_MAST 109). Animals in the bottom group had increased odds of CM compared with the top group (OR 5.87; 95% CI 2.5–13.7; p<0.0001). Among cows with CM (n=92), recurrence differed between groups. In the bottom group, the distribution of CM cases per affected animal was 54.5% (1 case), 27.3% (2 cases), 3.0% (3

cases), 9.1% (4 cases), and 6.1% (5 cases). In the top group, 63.6% had 1 CM case and 36.4% had 2 CM cases; no cows experienced more than 2 CM cases.

CONCLUSIONS: Clinical mastitis incidence differed between genomic mastitis risk groups, consistent with prior reports validating genomic predictions. Selecting genetically more resistant animals may reduce mastitis treatments and support goals to limit antibiotic use. In this single-herd observation with a limited sample size, STA_MAST also appeared to be associated with CM recurrence. Further studies with larger populations and multiple herds are needed to quantify this association.

Keywords: Dairy cow, Genomic prediction, Clinical mastitis, Antibiotic stewardship

The Relationship of IGF2 g.24507G>T Polymorphism with Fattening Performance and Carcass Traits in Simmental Bulls

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BACKGROUND and OBJECTIVE: Fattening performance and carcass quality are fundamental criteria that directly determine meat production efficiency and economic value in commercial cattle breeding. Investigating the genetic basis of these traits enables early selection of high-yielding animals and the development of effective selection programs. Insulin-like growth factor 2 (IGF2) is a fetal growth and differentiation factor that plays a significant role in muscle growth, myoblast proliferation, and differentiation. IGF2 produces a secreted peptide hormone that plays a crucial role in muscle tissues through both endocrine and local autocrine/paracrine mechanisms. This study aimed to investigate the relationship between the IGF2 g.24507G>T polymorphism and fattening performance and carcass characteristics in Simmental male calves.

MATERIALS and METHODS: A total of 92 Simmental bulls were included in the study. DNA isolation was performed using the "Thermo Scientific GeneJET Genomic DNA Purification Kit." The IGF2 g.24507G>T polymorphism was genotyped using the PCR-RFLP method. The relationship between the polymorphism and the following traits was examined: daily live weight gain, chilling loss, hot carcass weight, chilled carcass weight, final weight, weight gain, hot dressing percentage, and cold dressing percentage. Associations between genotypes and traits were evaluated using a general linear model (GLM).

RESULTS: The G allele was 0.68, and the T allele was 0.32, indicating that the G allele was more frequent. The IGF2 g.24507G>T polymorphism did not conform to the Hardy-Weinberg equilibrium ($P<0.05$). The population genetic parameters estimated for the analyzed locus revealed a moderate level of genetic diversity. The polymorphism information content (PIC) was 0.3407, indicating that the marker was moderately informative. The expected heterozygosity (He) was 0.4352, suggesting moderate genetic variability at this locus. In addition, the effective number of alleles (Ne) was estimated at 1.77, indicating that more than one effective allele contributes to genetic variation within the population. The association analysis revealed that the IGF2 g.24507G>T polymorphism was significantly associated with chilling loss ($P<0.05$).

CONCLUSIONS: In our study, only a potential association with chilling loss was detected. Although IGF2 is an important metabolic marker that plays a central role in many biological pathways, no strong association with carcass traits was identified in the present analysis. Nevertheless, studies with larger sample sizes and the evaluation of interactions among more genetic markers are required to obtain more comprehensive insights. These findings highlight the complexity of genetic regulation of carcass traits and indicate that the role of IGF2 warrants further exploration using multi-locus approaches.

Keywords: Simmental, Carcass, Fattening Performance, IGF2.

Genetics Meets Marination: CAPN1 Variation Modulates the Effects of Marination on Beef Quality

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BACKGROUND and OBJECTIVE: Marination is a common processing technique used to improve meat quality traits such as tenderness, water retention, and color stability. There is no information on how genetic variation in the postmortem proteolytic system may influence beef's response to marination treatments. The objective of this study was to evaluate the combined effects of CAPN1 genotypes and different marination treatments on beef color, pH, Warner–Bratzler shear force (WBSF), and water holding capacity (WHC).

MATERIALS and METHODS: Muscle samples from Simmental bulls (n=96) were subjected to four treatments: non-marinated control, milk marination, olive oil and garlic marination, and lemon juice marination. Meat color parameters (L*, a*, and b*), pH, WHC, and WBSF were evaluated at 0, 24, and 72h of refrigerated storage. Genotyping was carried out using PCR-RFLP. The effects of CAPN1 genotypes, marination treatment, and their interaction were analyzed using a General Linear Model.

RESULTS: At the initial stage (0h), neither genotype nor treatment significantly affected color parameters, indicating that baseline color characteristics were largely independent of the marination treatments. However, after 24h of storage, marination significantly influenced meat color development. The olive oil and garlic treatment resulted in the highest L* values, whereas lemon juice marination produced comparatively lower lightness values. A significant CAPN1 genotype×treatment interaction was detected for L* values at 24h, suggesting that the effect of marination on meat lightness may vary across genetic backgrounds. Differences in WBSF values indicated that marination improved tenderness, likely by enhancing protein degradation and weakening muscle fiber structure. In addition, variations in WHC values among treatments suggested that marination affected the water retention properties of muscle tissue, which may be related to pH changes and alterations in muscle protein functionality. At 72h, both genotype and marination treatments significantly affected certain color parameters. However, genotype×treatment interactions were not significant at this stage, suggesting that marination influence becomes increasingly dominant relative to genotype-related differences over time. A significant genotype×treatment effect on WBSF-0h indicated that the muscle's initial mechanical resistance differed among the experimental groups. This finding suggests that genetic variation in the CAPN1 locus may influence the structural properties of muscle tissue immediately after slaughter. The interaction between the CC genotype and all marination treatments showed more favorable WBSF results than other subgroups.

CONCLUSIONS: Marination treatments together with genotype strongly affect meat quality traits, particularly color and tenderness. These findings highlight the importance of considering both genetic background and processing methods to optimize marination strategies and improve beef quality.

Keywords: Simmental, beef marination, beef color, WBSF, CAPN1, genetic marker

Herd Response of the Use of Genomic Testing to Predict Milk Production, Reproduction, and Udder Heath Performance in a German Large Dairy Operation

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BACKGROUND and OBJECTIVE: Genomic testing is an established tool predicting future performance, rentability, and health parameters. Genomic predictions allow dairy producers to rank and select animals for production traits (e.g., milk yield, fat, and

protein), health traits (e.g., mastitis, retained placenta, metritis, displaced abomasum, lameness), profitability (e.g., net merit) and others. The predictive performance of wellness traits in the USA has been described previously. The objective of this field-based study was to evaluate the predictive value of a commercial test for milk production, mastitis incidence, and reproductive diseases under German conditions.

MATERIALS and METHODS: The study was conducted in a large commercial Holstein-Friesian dairy herd in eastern Germany. The farm had 2,128 total conceptions using genomic-proven bulls. Heifers were genotyped using hair samples analyzed with a commercially available genomic test (CLARIFIDE PLUS, Zoetis Inc., USA). The milk production prediction was assessed using gPTA-MILK (a genomically-enhanced version of PTA (Predicted Transmitting Ability)). The prediction of "days open" was calculated using gPTA-DPR (Daughter Pregnancy Rate). The mastitis wellness trait prediction (STA MAST), metritis wellness trait (STA-Metritis) and lameness (STA-lameness) - higher values indicate lower predicted mastitis risk- were used to assign animals to four percentile-based risk groups: bottom 25% (highest predicted risk), 26–50%, 51–75%, and top 25% (lowest predicted risk). First-lactation phenotypes were recorded in DairyComp 305 (Vas, Madison, USA). Associations between genomic risk groups and realized performance/disease were assessed (Pearson-Chi-Square and Fisher's exact test (SPSS 29. IBM, USA)

RESULTS: Genomic results were available for 153 animals that completed the first lactation. The bottom 25% group (average gPTA-MILK -68) had an average 305DIM milk production of 11,495 kg compared with 14,106 kg in the top 25% group (gPTA-MILK 1,367 kg). The average gPTA-DPR in the bottom 25% group was 134 days compared with 99 days in the top 25% group. Disease incidences were lower in top compared to bottom wellness STA groups: mastitis incidence was 0% in top 25% group vs 38% in the bottom 25% group (p=0.000) and, metritis 0% vs 14% (p=0.014).

CONCLUSIONS: Using gPTA predictions in the present farm was effective at improving milk production while reducing disease in the herd. Disease incidences differed between genomic mastitis risk groups, consistent with prior reports validating genomic US-predictions. Selecting more resistant animals may reduce treatments and support goals to limit antibiotic use. Further studies with larger populations and multiple herds are needed to quantify this association.

Keywords: Genomics, dairy cattle, mastitis, udder health, production, metritis

Phylogenetic Analysis of the Feral Cattle from Isabela Island (Galapagos) and Its Taxonomic Classification Within the Bos *taurus Genus*

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BACKGROUND and OBJECTIVE: The feral cattle of Isabela Island (Galápagos, Ecuador) represent a unique and isolated bovine population that has adapted to extreme environmental conditions, including limited freshwater availability and consumption of saline water. The objective of this study was to perform the first genetic characterization of this population and to explore molecular mechanisms potentially involved in its adaptation to osmotic stress. To establish the phylogenetic placement of this population within the *Bos taurus* lineage, three genes were analyzed. The conserved SLC9A3 gene, which encodes the sodium/hydrogen exchanger NHE3 expressed in renal tissue, served as a taxonomic anchor across *Bos* species. In addition, REN (renin) and AQP3 (aquaporin 3)—both associated with osmotic regulation and renal and hepatic physiology—were examined to identify molecular signatures indicative of environmental adaptation.

MATERIALS and METHODS: Blood samples were collected from the coccygeal vessels of three adult females (CV1, CV2, and CV3). Whole-exome sequencing was performed, followed by a bioinformatic workflow including quality control, alignment to the Bos taurus reference genome using BWA-MEM, and variant calling with GATK/Sentieon. Comparative analyses were conducted with *Bos taurus*, *Bos indicus*, *Bos taurus* × *Bos*

indicus hybrids, *Bos javanicus*, and *Bos mutus*. Percentage identity and phylogenetic trees for SLC9A3, REN, and AQP3 were generated using Clustal Omega and MEGA to evaluate sequence conservation and infer interspecific relationships.

RESULTS: Among the analyzed genes, the highest sequence similarity was observed with *Bos taurus*. The SLC9A3 (NHE3) gene exhibited the greatest homology (CV1: 99.91%, CV2: 99.93%, CV3: 99.91%), confirming strong conservation across *Bos* species. Both REN (CV1: 99.74%, CV2: 99.87%, CV3: 99.76%) and AQP3 (CV1: 99.88%, CV2: 99.88%, CV3: 99.88%) showed close phylogenetic affinity to *Bos taurus*, with AQP3 displaying slightly higher similarity to the *Bos taurus* × *Bos indicus* hybrid (CV1–CV3: 99.82%) compared to REN (CV1 99.53%, CV2 99.65%, CV3 99.61%). This pattern—characterized by a conserved transporter (SLC9A3) and more variable regulatory genes (REN and AQP3)—suggests structural conservation combined with regulatory flexibility, consistent with renal adaptation to saline and water-restricted environments.

CONCLUSIONS: These findings provide molecular evidence supporting the classification of the Isabela Island feral cattle within the *Bos taurus* lineage and suggest genetic adaptations to saline and water-limited environments. Future research integrating physiological assessments—such as urinary osmolality, fractional sodium excretion, and renin–angiotensin–aldosterone system (RAAS) profiling—along with population structure analyses, will be essential to fully characterize this biotype and support its recognition as Ecuador's first native creole cattle breed.

Keywords: Creole breed, Gene, SLC9A3, REN, AQP3, Bovine

GLOBAL WARMING AND ENVIRONMENT

Effect of Birth Season on Inflammatory Response and Oxidative Stress Status in Dairy Calves During the First 90 Days of Life

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BACKGROUND and OBJECTIVE: Maternal heat stress negatively affects health, growth, and future performance of calves. It has been reported that calves born in the summer season are born with a higher inflammatory status and oxidative stress load compared to those born in the winter season. This study aimed to investigate the effect of birth season on the inflammatory response and oxidative stress status in dairy calves during the first 90 days of life.

MATERIALS and METHODS: A total of 41 Holstein calves born in summer (n=20) and winter (n=21) were used in the study. Blood samples were collected from the calves immediately after birth (before colostrum intake; hour 0), at 12 and 24 hours, and subsequently on days 4, 6, 8, 15, 21, 30, 60, and 90. Total protein (TP), Brix percentage, inflammatory parameters (IL-17A, IL-10, IL-6, IL-1β, TNF-α, IFN-γ, haptoglobin, LPS), and oxidative stress markers (NO, PON1, TAS, TOS, OSI) were measured in serum samples. Data was evaluated using Linear Mixed Models

RESULTS: Results showed that TNF-α, NO, and OSI levels were significantly higher in the summer group

compared to the winter group, whereas Haptoglobin, IL-6, IL-10, IL-17A, IFN-γ, and PON1 levels were determined to be higher in the winter group. Although PON1 levels showed a gradual increase from day 15 onwards in both groups, this increase remained at a lower level in the summer group compared to the winter group. LPS and IFN-γ levels showed a decreasing trend from day 30, and IL-1β and IL-10 levels from day 60 until day 90. Although serum TP and Brix values were higher in the winter group, it was observed that the inflammatory response progressed more severely in winter calves.

CONCLUSIONS: In conclusion, although summer calves were born with an oxidative load due to intrauterine heat stress, they developed an optimized and controlled immune response against infections with lower cytokine release. In contrast, the high acute phase and cytokine response in winter calves reflect a more severe and dysregulated inflammatory process. Furthermore, the decrease in LPS and pro-inflammatory cytokines over time in both groups is not an immune deficiency; rather, it is associated with the maturation of the intestinal epithelial barrier and the establishment of immune homeostasis.

Keywords: Calf, Maternal heat stress, Oxidative stress, Cytokines, Fetal programming

Integrated Anti-methanogenic Feed Supplementation to Mitigate the Environmental Impact in Dairy Cattle

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BACKGROUND and OBJECTIVE: The increasing risks associated with global warming and the rising emissions of pollutants and greenhouse gases highlight the need for sustainable mitigation strategies in livestock production. Enteric fermentation contributes substantially to global methane (CH_4) emissions, while also representing a loss of metabolizable energy to the host animal. Marine macroalgae have demonstrated strong anti methanogenic effects in ruminants. The objective of our three year study (2023–2025) was to evaluate the impact of red macroalgae (*Asparagopsis taxiformis, Gracilaria*) supplementation, alone or in combination with yeast based probiotics, on enteric methane emissions, ammonia emissions, nitrogen utilization, rumen fermentation, and milk production parameters in dairy cows.

MATERIALS and METHODS: Holstein cows in early lactation were assigned to dietary treatments across several study periods. Treatments included: Control diet (no supplementation); red macroalgaes (*Asparagopsis taxiformis or Gracilaria*) provided in various formulations; yeast probiotic (*Saccharomyces cerevisiae*); and combined supplementation of macroalgae and yeast probiotic in different ratios and formulations. Dietary treatments were incorporated into the total mixed ration. Methane emissions, rumen fermentation characteristics, nitrogen utilization efficiency, and milk yield and composition were monitored throughout. Rumen microbiota composition, including methanogen abundance, was analyzed to assess microbial shifts associated with supplementation.

RESULTS: Combined supplementation with red macroalgae and a yeast based probiotic led to significant improvements in energy-corrected and fat-corrected milk yields in early lactation cows. Milk fat content increased without reducing overall milk production. Enhanced nitrogen utilization and a more efficient rumen fermentation profile were observed across the combination treatments, with a notable increase in propionate and a simultaneous decrease in ruminal ammonia levels. Supplementation with red seaweeds contributed to a measurable reduction in methanogen abundance and a corresponding decrease in enteric methane emissions, without adverse effects on dry matter intake, feed conversion efficiency, rumen function, or milk quality.

CONCLUSIONS: The incorporation of red macroalgae into ruminant diets, particularly in combination with yeast based probiotics, may represent one of the strategies to improve rumen fermentation, enhance metabolic efficiency, and mitigate enteric methane emissions in the livestock sector, without negatively

affecting milk quality in dairy cows. This study was supported by the Technology Agency of the Czech Republic under the Program Environment for Life. This project was financed within the National Recovery Plan from the European Recovery and Resilience Facility (grant no. SS06020190).

Keywords: rumen, methane emissions, feed additives, seaweeds, probiotics, dairy cows

Impact of Water Stress and Ensiling Duration on the Fermentation Characteristics and Feeding Value of Multi-Species in Arid Conditions

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BACKGROUND and OBJECTIVE: In this study, we evaluated the fermentation characteristics and feeding value of silage produced from a mixture of legume and cereal forage crops grown with or without irrigation (I: irrigated and C: non-irrigated) under arid climatic conditions in Turkey. The plant material used in the study consisted of a mixture of 36% Hungarian vetch (Vicia pannonica cv. Kansur), 34% Field pea (Pisum sativum cv. Kirazlı), 15% Bread wheat (Triticum aestivum cv. Bohemia), and 15% Triticale (Triticosecale cv. Kinerit). The study was conducted using a 2 × 2 factorial arrangement of water stress (Control vs Irrigated) and different ensiling days (30 or 60 days).

MATERIALS and METHODS: The plants were harvested with a forage mower and chopped with a single-row maize chopper. The mean dry matter was 46.55% and 33.80% in the control and irrigated groups, respectively, prior to ensiling. The physical effectiveness factor was determined using the Penn State Particle Separator to calculate physically effective NDF, which was 36.95 and 39.37 for the control and irrigated groups, respectively. Following chopping, the material was ensiled in vacuum bags (approximately 500 g) using a vacuum sealer machine. The forage silage was opened on different days (30 vs. 60 days) to analyze fermentation characteristics and feeding values.

RESULTS: No significant interaction between ensiling days and treatments was seen for pH (P=0.071), Flieg Score (P=0.077), neutral detergent fiber (NDF; P=0.349), acid detergent fiber (SDF; P=0.132), in vitro neutral detergent fiber digestibility (IVNDFD; P=0.296), relative feed value (RFV; P=0.553), and net energy lactation (NEL; P=0.132). However, significant interactions between days and treatments were observed for crude fiber (CF; P=0.028) and in vitro dry matter digestibility (P=0.004). Extending the ensiling duration from 30 to 60 days significantly decreased pH (P<0,001) and improved the Flieg score (P<0.011). Forage silage ensiled for 60 days had greater CF (P=0.015), NDF (P=0494), ADF (P<0.004), IVTDMD (P<0.001) and lower IVNDFD (P=0.616), RFV (P=0.314) or NEL (P<0.001). The irrigated group exhibited a significantly lower pH, Flieg score, IVTDMD, IVNDFD, RFV and NEL compared to the control group

CONCLUSIONS: As a result, non-irrigated forage grown under arid conditions ensures higher fermentation quality and improved digestibility. These findings offer practical strategies for optimizing ruminant nutrition in water-limited environments.

Keywords: Digestibility, feeding value, legume-cereal mixture, water scarcity

Overcoming the Constraints of the “Season”: The Role of the Temperature-Humidity Index in Colostrum Quality

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BACKGROUND and OBJECTIVE: The calendar “season” is often used as a variable in statistical models; however, it is anthropocentric in conception and frequently yields ambiguous results. The aim of this study was to investigate the effect of the Temperature-Humidity Index (THI) during the last month of the dry period on the quality and quantity of colostrum in Holstein cows.

MATERIALS and METHODS: Colostrum samples (from 1,010 cows across 9 farms) were collected within the first 24 hours after calving. Total solids were measured using a digital Brix refractometer, and chemical composition was determined with Milkoscan. Daily temperatures and relative humidities for each farm were obtained from ERA5-Land-Copernicus for the last four weeks of gestation. In total, 30,300 daily records were available, and the mean THI was calculated for each week separately as well as for the entire last month of gestation. Differences between months were assessed using analysis of variance. The effects of parity, colostrum yield, calving to collection interval, dry period length, body condition score, and previous lactation milk yield on colostrum composition and yield; both weekly and for the entire last month, were evaluated using linear models.

RESULTS: The monthly distribution of THI indicated that "season" does not accurately reflect the actual conditions. THI had a significant (negative) effect on colostrum total solids (Brix%) and protein content, in all weeks as well as overall ($P<0.05$). Colostrum yield was significantly (positively) influenced by THI only during the last week before calving ($P<0.05$). In contrast, fat content was not affected at any time. The effects of the other factors were consistent with those reported in previous studies.

CONCLUSIONS: THI provides a more accurate indicator of heat stress compared to "season" and improves the reliability of predictive statistical models. For colostrum quality and yield, recording THI during the last week before calving is sufficient.

Keywords: THI, cattle, season

Effects of an Essential Oil Mixture on Total Gas and Methane Production in Ruminant Diets: An *in vitro* Study

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BACKGROUND and OBJECTIVE: Greenhouse gas emissions from livestock, particularly methane (CH4) produced by ruminants, represent a significant environmental challenge and a loss of dietary energy. This study aimed to investigate the effects of a specific essential oil mixture (EOM) containing eucalyptus oil, peppermint oil, and Mentha arvensis extract on total gas and methane production under in vitro conditions

MATERIALS and METHODS: The study was conducted using the in vitro gas production (HFT) technique. Three different roughage-to-concentrate ratios (30: 70, 50: 50, and 70: 30) were incubated with three different doses of the essential oil mixture (0, 100, and 1000 ppm) in a 3 × 3 factorial design. Total gas production was measured at 48, and methane concentrations were analyzed at 30 and 48 hours of incubation.

RESULTS: Results indicated that the addition of EOM showed a tendency to reduce total gas production ($P=0.064$), with the lowest total gas observed in the 100 ppm treatment groups. Regarding methane emissions, a significant linear decrease was observed at 48 hours of incubation as the EOM dose increased ($P<0.05$). The most effective reduction in methane production was recorded in the concentrate-rich diet (70: 30) supplemented with 100 ppm EOM, which resulted in the lowest methane percentage (13.06%) compared to the control group (19.85%).

CONCLUSIONS: In conclusion, the essential oil mixture containing eucalyptus and mint derivatives demonstrated a potential to mitigate enteric methane production in this in vitro model, particularly at the 100 ppm dosage. These findings

suggest that this specific EOM could be considered a promising feed additive for reducing environmental impact in ruminant nutrition.

Keywords: Essential Oil Mixture, Methane Production, Rumen Fermentation, In Vitro Gas Production.

Milk Fatty Acids Profile and B-hydroxybutyrate as Predictors of Greenhouse Gas Emissions in Dairy Cows Affected by Ketosis

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BACKGROUND and OBJECTIVE: Metabolic disorders such as ketosis are common in high-producing dairy cows. They are associated with alterations in lipid metabolism, milk fatty acid (FA) composition and changes in greenhouse gas (GHG) emissions. Understanding these relationships can provide insight into metabolic status and environmental impact. This study aimed to evaluate the effects of subclinical ketosis (SCK) on milk FA profiles and to explore the predictive capacity of milk FA and blood β-hydroxybutyrate (BHB) for emissions of methane (CH_4) and carbon dioxide (CO_2).

MATERIALS and METHODS: A total 60 multiparous Holstein-Friesian cows were enrolled between 3 and 28 days in milk. Blood BHB concentrations were determined at 7, 14, 21, and 28 days in milk. Animals were classified as affected by SCK (KET; n=17; BHB≥1.0 mmol/L) whereas cows with BHB<1.0 mmol/L at all time points were considered healthy controls (CTR; n=43). The CH_4 and CO_2 emissions were quantified individually through an automated in-barn system during voluntary feed intake. Milk FA were determined by gas chromatography following direct transesterification of milk fat. Statistical analysis was performed using linear mixed-effects models. Furthermore, stepwise forward regression was applied to assess the predictive ability of milk FA and BHB for CH_4 and CO_2 emissions, with variables entering the model at $p<0.10$.

RESULTS: Ketotic cows showed a clear shift in milk FA composition: preformed FA such as stearic acid (C18: 0) and oleic acid (C18: 1ω9) were increased, reflecting enhanced lipomobilization, whereas de novo FA (butyric to myristic acid; C4: 0–C14: 0) were reduced, consistent with impaired mammary synthesis. Odd- and branched-chain FA, including pentadecanoic acid (C15: 0), pentadecenoic acid (C15: 1ω9), heptadecanoic acid (C17: 0), and heptadecenoic acid (C17: 1ω9), were also affected, suggesting changes in rumen microbial activity. Stepwise regression identified key predictors of gas emissions: BHB (+), C18: 1ω9 (+), heptadecenoic acid (C17: 1ω9) (−), and arachidonic acid (C20: 4ω6) (−) (R^2=0.236) for CH_4; C15: 1ω9 (+), C17: 0 (−), C17: 1ω9 (−), C18: 1ω9 (−), linoleic acid (C18: 2ω6) (−), eicosadienoic acid (C20: 2ω6) (−), arachidonic acid (C20: 4ω6) (−), and BHB (−) (R^2=0.340) for CO_2.

CONCLUSIONS: Ketosis significantly alters milk FA composition, reflecting changes in energy metabolism and rumen microbial activity, and these alterations are associated with variation in CH_4, CO_2, and H_2 emissions. The biological mechanisms connecting ketosis and enteric gas production remain largely unexplored. Milk FA profiling, in combination with metabolic indicators such as BHB, provides a promising approach to monitoring cow health and predicting greenhouse gas emissions, offering potential applications for precision nutrition and environmental management in dairy systems.

Keywords: Milk fatty acids, GHG, BHB, Methane

Impact of Cooling Systems on Milk Production and Physiological Responses in Heat-Stressed Holsteins

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BACKGROUND and OBJECTIVE: Heat stress negatively affects the performance, health, and welfare of dairy cows. Selection studies conducted to increase milk yield have lowered the minimum temperature-humidity index (THI) at which temperature begins to affect milk yield negatively; therefore, animals may still experience heat stress even at lower environmental temperatures. Global temperature increases are worsening this situation and threatening productivity and sustainability in the dairy cattle sector.

MATERIALS and METHODS: The study was conducted at the Animal Health and Animal Production Application and Research Center of the Faculty of Veterinary Medicine at Bursa Uludağ University. A total of 40 Holstein dairy cows housed in a semi-closed free-stall barn system were included in the study between July 2025 and September 2025. The animals were divided into two groups: an treatment group (n=22) and a control group (n=18). Both groups were maintained under identical nutritional and management conditions, with the exception of the cooling intervention. The treatment group was managed using a cooling strategy consisting of a sprinkler system coupled with ventilation fans. The sprinkler system utilised nozzles positioned along the feed trough line at 1.5-metre intervals. The control group received no active cooling treatment beyond ventilation fan. Temperature and humidity were recorded every 15 minutes via a HOBO U23 logger to calculate the daily average THI. The respiratory rate per minute and body temperature of animals in the treatment and control groups were measured individually. Performance data, including daily milk yield (DMY), milk conductivity, milk temperature, maximum flow rate, and milking duration, were obtained from herd management software.

RESULTS: Analysis of morning and afternoon milk yields showed no significant difference between groups ($p>0.05$). However, the treatment group exhibited significantly higher evening and total DMY ($p<0.05$). Specifically, treatment vs. control yields were 10.67±0.11 vs. 10.43±0.10 L for evening, and 29.45±0.31 vs. 28.66±0.31 L for DMY. Significant intergroup differences ($p<0.05$) were observed in milk conductivity (9.19±0.27 vs. 12.86±0.66), milk temperature (38.71±0.31 vs. 38.92±0.38), and milking duration (568.78±4.64 vs. 547.08±10.62 s), whereas milk flow rate remained statistically similar. Furthermore, the treatment group showed significantly lower respiratory rates (47.30±0.75 vs. 58.23±2.63 breaths/min) and body temperatures (38.14±0.36 vs. 38.43±0.47°C) compared to the control ($p<0.01$).

CONCLUSIONS: In conclusion, the sprinkler–fan cooling system effectively mitigated heat stress in lactating Holstein cows. The treatment improved daily milk yield and milking parameters while reducing respiratory rate and body temperature, indicating enhanced physiological comfort and productivity under high environmental temperatures.

Keywords: Heat Stress, Holstein Cows, Cooling System, Milk Yield

HERD HEALTH MANAGEMENT AND ECONOMICS

A Comparison of Profitability Between Vaccinated and Unvaccinated Groups on a White Veal Calf Farm in Northern Germany After Implementing Two Different BRD Vaccination Protocols Compared to Non-Vaccinated Animals Under the Same Conditions

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BACKGROUND and OBJECTIVE: This study aimed to assess the economic benefits of vaccinating white veal calves against bovine respiratory disease (BRD)

in a farm with endemic BRD in northern Germany. A total of 1,578 calves were divided into three groups: Group 1 (unvaccinated), Group 2 (vaccinated twice with an inactivated vaccine), and Group 3 (vaccinated using a PRIME-BOOST protocol with a modified live vaccine followed by an inactivated vaccine). The focus was on carcass homogeneity, average carcass weight, and the proportion of slaughter weights exceeding 120 kg.

MATERIALS and METHODS: Calves arrived in September 2024 at an average age of 31–32 days and were housed in an "ALL IN – ALL OUT" system. Vaccination was administered within four days of arrival. Slaughter occurred between March and April 2025, with fattening durations of 188–192 days. Data were collected using VETVET software, including health records, medication, mortality, and individual slaughter data. Nasal swabs from sick calves were analyzed via PCR and bacteriology for BRD pathogens.

RESULTS: Group 1 had 6.75% of carcasses below 120 kg, while Group 2 had 4.8% and Group 3 only 3.98%. Group 2 achieved the highest percentage of carcasses over 170 kg (13.17%), and Group 3 had the highest proportion in the 140–170 kg range (67.17% vs. 60.12% in Group 1). Carcass weight homogeneity was best in Group 3 (94.31% between 110–180 kg), followed by Group 2 (91.82%) and Group 1 (89.97%). Mortality rates were lowest in vaccinated groups: 4.5% in Group 1, 3.56% in Group 2, and 2.47% in Group 3. Average carcass weight gains were +4.47 kg/animal for Group 2 and +4.98 kg/animal for Group 3.

CONCLUSIONS: BRD remains a major challenge in veal fattening due to calf mixing and crowding. Although vaccination was delayed until arrival at the fattening farm, the economic benefits were substantial. Earlier vaccination at the origin farms could potentially yield even better results, though cost responsibility remains unclear. Overall, the study demonstrates that BRD vaccination improves carcass weight, homogeneity, and reduces mortality—leading to higher financial returns. Both vaccination protocols resulted in significantly better outcomes compared to the unvaccinated group, under identical management conditions.

Keywords: Carcass weight, Homogeneity, Mortality, Respiratory disease (BRD), Vaccination protocols, Veal calves

A Clear Decline in Treatment Rate and Mortality Rate on a White Veal Calf Farm in Northern Germany After Implementing Two Different BRD Vaccination Protocols Compared to Non-Vaccinated Animals Under the Same Conditions

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BACKGROUND and OBJECTIVE: This field trial aimed to evaluate the impact of two different vaccination protocols compared to no vaccination on mortality, losses, and treatment rates in a herd of 1,578 white veal calves located on a farm in northern Germany, where bovine respiratory disease (BRD) is endemic. The study enabled a direct comparison between vaccinated and unvaccinated groups under practical farm conditions, focusing on health outcomes and the need for medical interventions.

MATERIALS and METHODS: The calves were randomly assigned to three groups: Group 1 (n=489) remained unvaccinated, Group 2 (n=562) received two doses of an inactivated vaccine, and Group 3 (n=527) was vaccinated using a PRIME-BOOST protocol. This protocol involved an initial intranasal administration of a modified live vaccine containing BRSV and PI3V, followed by two subcutaneous doses of the inactivated vaccine containing BRSV, PI3V, and *Mannheimia haemolytica*. Calves arrived between early and late September 2024 at an average age of 31–32 days and were housed in an "ALL IN – ALL OUT" system. During the first four weeks, calves were housed individually, then grouped in pens of 4–6. Slaughter occurred between March and April 2025. Data were recorded using the VETVET software, including medication, mortality, and disease events. Nasal swabs from sick calves were analyzed via PCR and bacteriology for BRD pathogens.

RESULTS: Mortality rates were highest in the unvaccinated group (4.5%), followed by Group 2 (3.5%) and Group 3 (2.5%). Notably, 59% of deaths in Group 1 occurred after day 113 of the fattening period, compared to 40% in Group 2 and 38% in Group 3.

Individual treatment rates were 14.11% in Group 1, while both vaccinated groups showed reduced rates of 10.30% and 10.24%, representing a 28% decrease. Most treatments occurred before day 57, but Group 1 required more interventions afterward. All groups received equal routine treatments, though Group 1 needed one additional non-routine treatment. PCR results showed no active infection from vaccine strains (CT >30), while other pathogens such as *Mycoplasma bovis, Bovine Coronavirus* and *Pasteurella multocida* were identified.

CONCLUSIONS: BRD remains a significant challenge in veal calf fattening. Although vaccination is not yet standard practice in Germany, this study demonstrates that strategic vaccination protocols can reduce mortality and treatment rates, especially in later fattening stages, leading to lower antibiotic use and improved economic outcomes.

Keywords: Antibiotic reduction, Bovine respiratory disease (BRD), Mortality, prime-boost vaccination, Treatment rates, vaccination protocols

Economic Impact of Udder Asymmetry in a Saanen Dairy Goat Farm in the Netherlands

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BACKGROUND and OBJECTIVE: Udder health plays a crucial role in the economic viability of dairy goat farming. Udder asymmetry negatively affects milk production and can increase culling rates, generating substantial economic losses for farmers. This study quantifies the financial impact of udder asymmetry in dairy goats by analysing the associated reductions in milk yield and resulting opportunity costs. Understanding these losses can help inform improved management strategies and breeding decisions to enhance farm profitability and sustainability.

MATERIALS and METHODS: The study was conducted in December 2023 on a Saanen goat farm in the Netherlands, involving 3,164 lactating goats. An economic study was performed to determine the impact of an imbalanced udder in the herd. Goats were divided into two groups: those with normal udders (n= 2,393) and those with severely imbalanced udders (n= 298). Based on the average daily individual milk production, the individual daily cost of opportunity was calculated in liters, as the average production in goats with severe udder asymmetry was lower. By multiplying the average daily individual production by the number of animals in each group, we obtained the average daily total production by group; and multiplying the individual daily cost of
opportunity of the group with asymmetry by the number of animals, it was obtained the total daily cost of opportunity, which allowed us to know the total annual cost of opportunity. Considering the average price of standardized

RESULTS: Goats with severe udder asymmetry produced 0.215 L less milk per day compared with goats with normal udders. The average daily total production was 9058,53 L in the group with normal udder and 1063,81 L in the group with udder imbalance, which means that the Total Daily and annual Cost of Opportunity was 64,25 L and 23450,81 L, respectively. Imbalanced udders resulted in annual losses of 17,921.11 €, equivalent to 60.14 € per affected goat.

CONCLUSIONS: Severe udder asymmetry in dairy goats leads to measurable reductions in milk yield and, consequently, significant economic losses. These findings highlight the importance of prioritizing udder health through targeted management practices and preventive strategies. Reducing the prevalence of udder imbalance can improve milk production, enhance farm profitability, and contribute to the long-term sustainability of dairy goat operations.

Keywords: udder imbalance, goats, milk quality, economic impact

Effects of Temperature, Mixing Frequency, and Storage Time on Acidified Milk Quality, Physical Characteristics, and Coliform Counts

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BACKGROUND and OBJECTIVE: Milk acidification relies on lowering pH to enable safe storage without refrigeration. Although this practice is commonly used in group housed calf systems, the scientific evidence supporting different preparation and storage strategies remains limited. The objective of this study was to evaluate how environmental temperature, mixing frequency, and time after acidification influence milk quality, physical characteristics, and coliform counts under controlled laboratory conditions.

MATERIALS and METHODS: Three mixing regimens, continuous mixing, mixing every 2 hours, and mixing every 6 hours, were tested at two environmental temperatures (23 °C and 40.5 °C). Milk was acidified with citric acid to a target pH of 4.0–4.2 and inoculated with Escherichia coli, and counts (log CFU/mL) were measured over time using serial dilution. Samples were collected at 0 h (baseline) and at 1, 2, 4, 6, 12, 24, 48, 72, and 96 hours after acidification. The study is ongoing, with completion expected in mid-spring 2026.

RESULTS: Preliminary data show that at 23 °C (73.4 °F), all mixing regimens produced a bacteriostatic effect for up to 96 hours. At 40.5 °C (104.9 °F), all regimens were bacteriostatic for 24 hours, transitioning to a bactericidal effect by 48 hours. Milk pH was most stable under continuous mixing, which also supported more uniform curd formation and steadier bacterial counts, although overall variation across regimens was minimal. Curd formation was negligible across all conditions. However, mixing every 2 or 6 hours resulted in visible separation between milk and citric acid between mixing timepoints, whereas continuous mixing minimized this effect.

CONCLUSIONS: Across the temperature and time combinations evaluated, continuous mixing and mixing every 2 hours produced the most favorable outcomes for milk acidification, including stable pH, minimal physical separation, and desirable bacteriostatic or bactericidal effects. These preliminary findings support their use as practical management strategies for acidified milk preparation and storage

Keywords: Milk Acidification, calf group housing, disease prevention, antimicrobial stewardship, HPAI

Actionable Steps Towards Advancing Antimicrobial Stewardship on the Dairy Farm: Using a Training Program for Improving Early and Accurate Disease Detection in Dairy Cattle by Farm Workers

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BACKGROUND and OBJECTIVE: Antimicrobial stewardship (AMS) promotes the judicious use of antimicrobial drugs to preserve their effectiveness for both humans and animals. On dairy farms, workers play a central role in daily disease detection and treatment decisions within the veterinarian-client-patient relationship (VCPR). Their knowledge and skills directly influence treatment success and the responsible use of antimicrobials. Previous work has identified gaps in farm workers' understanding of animal health and behavior, underscoring the need for accessible, evidence-based training. The

objective of this project was to develop and evaluate a practical, farm worker focused training program designed to strengthen AMS practices, improve recognition of cattle sickness behavior, and enhance early identification of clinical disease signs.

MATERIALS and METHODS: This multi-stage project began in 2018 and included the development of bilingual training materials, incorporation of stakeholder input, field evaluation, and professional editing for online dissemination. The curriculum was piloted on 12 commercial dairy farms, six in Ohio and six in California, with a total of 25 farm workers participating. A longitudinal evaluation assessed changes in knowledge, attitudes, and understanding of AMS principles before and after training. Feedback from participants and farm managers was used to refine the materials. The final training program was made freely accessible through an online platform.

RESULTS: Farm workers demonstrated significant improvements in knowledge related to antimicrobial stewardship, cattle sickness behavior, and early disease recognition. Attitudinal shifts were also observed, including increased confidence in identifying clinical signs and greater alignment with veterinarian-guided treatment strategies. Participants reported that the training was practical, relevant, and easy to apply in daily work. Stakeholder engagement throughout the project strengthened the cultural and operational relevance of the materials and supported successful implementation across diverse farm settings.

CONCLUSIONS: This project produced an accessible, evidence-based training program tailored to dairy farm workers and focused on improving AMS practices and early disease detection. The curriculum led to measurable gains in knowledge and attitudes, reinforcing the value of targeted education for frontline animal care personnel. Ongoing outreach efforts aim to support the adoption and integration of the training into routine farm management to promote sustainable antimicrobial stewardship.

Keywords: antimicrobial stewardship, dairy farm workers, training program, disease identification, veterinary-client-patient relationship

Perceptions of a Cryptosporidium Parvum Vaccine in Dairy Cattle: A Cross Sectional Questionnaire Survey

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BACKGROUND and OBJECTIVE: *Cryptosporidium parvum* is an important cause of neonatal calf diarrhea, associated with increased morbidity, mortality, and antimicrobial use. Vaccination may offer a preventive approach, but field data on farmer experience are limited. The aim of this study was to describe farmer-reported experiences with a *C. parvum* vaccine on Holstein Friesian farms and to assess perceived effects on calf health, treatment use, management practices, and satisfaction.

MATERIALS and METHODS: A cross-sectional questionnaire survey was conducted on five Holstein Friesian farms in Belgium comprising approximately 1,400 lactating cows. All participating farms used a commercially available *C. parvum* vaccine as part of a structured vaccination campaign. The questionnaire explored perceived effects of vaccination on calf health, use of supportive and antimicrobial treatments, management changes (including colostrum/transition milk administration), continuation intentions after the trial period, adverse effects in cows, and overall satisfaction.

RESULTS: All respondents reported noticing a positive effect of dam vaccination on their calves, predominantly describing reduced illness and, when diarrhea occurred, a shorter duration with smoother recovery. Farmers indicated a marked reduction in calf morbidity and mortality and expressed high overall satisfaction with the vaccination program. All participants wished to continue *C. parvum* vaccination beyond the trial, and one reported a sharp increase in diarrhea incidence when vaccination was temporarily discontinued. No negative effects of vaccination were observed in cows. Farmers reported using fewer adjunct treatments (e.g. electrolytes, antibiotics) for diarrhea compared with the pre-vaccination period. All farms provided colostrum or transition milk for 4–5 days,

representing a change from their pre-vaccination protocol. These protocol changes were described as entailing more work but clearly beneficial due to the observed health improvements. Respondents emphasized that fewer calf health problems translated into a more pleasant daily workload.

CONCLUSIONS: On these five Holstein Friesian farms, farmers perceived *C. parvum* vaccination as safe and highly beneficial, with reduced calf morbidity and mortality, decreased need for supportive and antimicrobial treatments, and improved practicability despite additional management effort. While findings are based on self-reported data from a limited number of farms, they support *C. parvum* vaccination as part of integrated neonatal calf diarrhea control programs.

Keywords: Cryptosporidiosis, neonatal calf diarrhea, scours, field experience

Farmer Compliance with Maternal Vaccination Against Neonatal Calf Diarrhea (cryptosporidium Parvum): a Field Evaluation

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BACKGROUND and OBJECTIVE: A novel maternal vaccine [Bovilis® Cryptium®, MSD] based on the Gp40 protein of *Cryptosporidium parvum* was recently developed to deliver passive immunity to calves via colostrum. Pre-clinical work supported safety and efficacy of the vaccine. This study assessed farmer adherence to label directions and evaluated vaccine safety under routine dairy farm conditions.

MATERIALS and METHODS: A longitudinal, multicenter cohort study was conducted from July 2024 to January 2026 on five Holstein-Friesian dairy farms in Belgium with confirmed *C. parvum* pressure and a history of neonatal cryptosporidiosis. Healthy pre-calving cows and heifers were set to receive two doses of Bovilis® Cryptium® 4 weeks apart, with the second dose at least 3 weeks before expected calving, per label. Vaccination and calving records, colostrum management, and calf health data were analyzed. Veterinarians documented any post-vaccination adverse events.

RESULTS: The mean interval between the two primary vaccinations was 4.5 ± 1.0 weeks, with substantial variability (1.5–8.0 weeks) and significant differences between farms (Kruskal–Wallis; $p < 0.001$). The mean interval between the second vaccination and calving was 26 ± 14 days, ranging from 2 to 63 days, again differing significantly between farms (Kruskal–Wallis; $p < 0.001$). No vaccine-related adverse events were reported during the study. Colostrum metrics varied by farm: first milking volume averaged 5.4 ± 3.3 L and Brix 23.9 ± 3.0%, with significant inter-farm differences (Kruskal–Wallis; $p < 0.001$). Calves received, on average, 4.6 ± 1.1 L of colostrum within the first 24 hours (farm range 3.5–6.0 L; Kruskal–Wallis; $p < 0.001$).

CONCLUSIONS: Maternal vaccination with Bovilis® Cryptium® appears practical and safe under field conditions, with no adverse events detected. However, adherence to the recommended dosing schedule is inconsistent: the interval between the two priming doses frequently deviates from label guidance, and the ≥ 21-day window between the final vaccination and calving was missed in approximately 40% of cases. Significant variability in colostrum management among farms underscores the need for ongoing education and support. Strengthening compliance with vaccination timing and optimizing colostrum practices are likely critical to maximize passive transfer and the vaccine's protective impact against neonatal cryptosporidiosis.

Keywords: Cryptosporidium parvum, Vaccination compliance, vaccination schedule

"Cattle Care 24/7: a Holistic Approach Combining Monitoring, Consultation and Preventive Solutions Improving Calf Health and Average Daily Gain on Five Danish Dairy Farms"

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BACKGROUND and OBJECTIVE: "Youngstock rearing in modern dairy herds is increasingly constrained by economic, labor, and administrative pressures that deprioritize calf health. Although awareness of the long-term impact of early-life disease is growing, calf health is often not the first priority. Preventive measures and vaccination are not consistently implemented, on many farms. This gap is critical: calves constitute the genetic and productive future of the herd, and suboptimal early-life management compromises lifetime performance and farm sustainability. Emerging precision technologies, such as youngstock behavior monitoring systems (BMS), provide opportunities for early disease detection and targeted surveillance. However, precision technology can't stand alone, but needs additional targeted and preventive action plans. In this context, we compared calf health and average daily gain (ADG) before and after implementation of the BMS system and a structured vaccination & calf health protocol in five commercial dairy herds."

MATERIALS and METHODS: "Five Danish dairy herds were included in this study. Farms were conveniently selected based on presence of a specific cow monitoring system, the absence of preventive vaccination in the calf population and the willingness of farmer and veterinarian to participate. Selected herds ranged from 550-900 dairy cows. None of them had a neonatal diarrhea (NCDPP) or respiratory (BRDPP) prevention plan before this study. A first batch of 40 calves (CON) were monitored (BMS, ADG, disease diagnosis, treatment monitoring) without intervention. Afterward, a health & economic impact assessment was done, and an action plan was agreed upon. One year after plan implementation, during the same season, a second group of 40 calves (TEST) was equipped with BMS and monitored for the same parameters."

RESULTS: "All herds implemented a prevention action plan, three out of five implemented both a BRDPP and NCDPP. BMS alarms age 1-180 days were reduced by 20 to 67% between CON & TEST. BRD treatments were highly reduced on 3/4 herds implementing a BRDPP. Mortality dropped by > 30% on average (5% CON-3% TEST). ADG was significantly altered in four out of five herds ($p<0.05$): between CON and TEST groups, approximately 150 g/day difference was seen in ADG."

CONCLUSIONS: "On large-scale dairy herds, calf care and behavior monitoring is often difficult and needs to be structured and supported. This study showed that a systematic approach of calf disease prevention can be highly successful: with just a few interventions per farm, an important ADG increase was achieved."

Keywords: Behavior monitoring systems, preventive approach, calf health monitoring, calf health prevention, preventive calf management, holistic calf health management

Breed-Specific Locomotor Behavior for Early Disease Detection in Holstein and Simmental Cows

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BACKGROUND and OBJECTIVE: Automated activity monitoring is increasingly used in precision dairy farming for early disease detection; however, locomotor activity is influenced by multiple biological and management factors, and the relative contribution of parity, breed, and disease status remains unclear under commercial herd conditions. This study aimed to determine the effects of parity, breed, and disease categories on locomotor activity in Holstein and Simmental dairy cows and to evaluate the diagnostic performance of activity-based monitoring for health assessment.

MATERIALS and METHODS: A prospective observational study was conducted in commercial dairy herds between January 2023 and January 2024 using continuous sensor-based locomotor activity recordings. Activity levels were first compared between parity groups in healthy cows, followed by analyses across major disease categories (mastitis, lameness, ketosis, digestive disorders, and reproductive diseases). Linear mixed-effects models were applied to quantify fixed and random effects, and receiver operating characteristic (ROC) analyses were used to evaluate diagnostic accuracy.

RESULTS: Parity did not significantly influence locomotor activity in healthy cows ($p>0.05$), indicating that baseline activity is largely independent of parity under stable physiological conditions. In contrast, disease presence emerged as the strongest determinant of locomotor behavior (−6.57 units; $p<0.001$). Digestive disorders and mastitis produced the largest reductions in activity, while reproductive disorders showed increased but highly variable activity patterns, likely associated with estrus-related behavior. Breed alone had no significant effect; however, a significant disease × breed interaction indicated differential behavioral responses under pathological conditions. ROC analyses demonstrated high specificity but moderate sensitivity, with the highest diagnostic performance observed for mastitis and digestive disorders.

CONCLUSIONS: Locomotor activity primarily reflects health status rather than parity or breed in dairy cows. Sensor-based activity monitoring provides biologically meaningful and clinically applicable information for disease detection, particularly in inflammatory and metabolic disorders. These findings support the integration of locomotor activity into precision herd-health algorithms and highlight the need for multi-parameter sensor approaches to improve early detection sensitivity.

Keywords: locomotor activity, disease detection, Holstein, Simmental

Advancing Veterinary Paraprofessional Practice in Kenya: Outcomes of a National Continuous Professional Development Training Initiative (2024–2025)

Peter Canisius Lugonzo

Zoetis Africa

BACKGROUND and OBJECTIVE: Continuous professional development (CPD) is critical for strengthening the technical capacity of veterinary paraprofessionals (paravets) to address emerging challenges in animal health and production systems. In Kenya, increasing demands for quality assurance, regulatory compliance, antimicrobial stewardship, and inclusive service delivery highlight the need for structured, scalable, and competency-based training models. Despite their critical frontline role, paravets often face limited access to standardized refresher training. This study reports the design, implementation, and outcomes of a national CPD program delivered in collaboration with the Kenya Veterinary Paraprofessional Association to enhance clinical competencies, promote gender equity, and expand nationwide access through a hybrid learning platform.

MATERIALS and METHODS: Between March 2024 and November 2025, six regional two-day CPD trainings were conducted annually, integrating in-person and virtual participation. The competency-based curriculum addressed priority technical areas, including biosecurity, udder health management, poultry production systems, and diagnostic approaches. Sessions combined expert-led lectures, case-based discussions, and practical demonstrations to enhance applied learning. Attendance and gender-disaggregated data were systematically recorded by the national veterinary regulatory board-Kenya Veterinary Board, with participants earning 16 CPD points per training. Descriptive statistical analyses were performed to assess participation trends, gender representation, repeat attendance, and modality preference, while structured feedback surveys evaluated participant satisfaction and perceived training impact.

RESULTS: The program achieved sustained national engagement. In 2024, total participation comprised 1,445 virtual and 805 in-person attendees; in 2025, participation increased to 1,508 virtual and 707 in-person attendees. Female participation remained

substantial, accounting for 39.9% of attendees in 2024 and 36.4% in 2025, demonstrating progress toward inclusive capacity development. Participant feedback indicated high satisfaction with content relevance, technical depth, and applicability to practice. Beyond skills enhancement, the initiative strengthened industry linkages, supported market entry for a new animal health distributor, increased institutional visibility of the training provider, and promoted adoption of evidence-based veterinary practices. Most respondents reported increased confidence in clinical decision-making and intention to adopt improved biosecurity and diagnostic practices.

CONCLUSIONS: The hybrid CPD model demonstrates an effective, scalable framework for professional capacity building in Kenya. By integrating regulatory accreditation, gender-responsive participation strategies, and flexible delivery modalities, the initiative provides a replicable model for sustainable workforce development in comparable low- and middle-income settings.

Keywords: Animal Health, Clinical Competency Development, Capacity Building, Evidence-Based Veterinary Practice, Gender Inclusion

Does Sending Dairy Heifers Out to Be Contract/custom Reared Impact Their Lactational Performance?

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BACKGROUND and OBJECTIVE: As dairy herds expand, farmers prioritise the 'milking platform' (land adjoining the milk parlour) for milking cows and some farmers move their replacement dairy heifers off the farm altogether to be reared by a contract/custom rearer. Heifers leave preweaning and return on the point of calving two years later. This may have implications for heifer health, growth and subsequent milk production. The impact of this management practice on primiparous milk production has not been examined. Hence, the objective of this study was to evaluate the impact of rearing strategy (contract-rearing vs. home-rearing) and associated co-factors, growth rate, body condition score (BCS), and age at first calving (AFC) on first lactation milk yield in dairy heifers.

MATERIALS and METHODS: A single cohort of 3,905 heifers (2,445 contract/custom-reared, 1,460 home-reared) from 97 pasture-based, seasonally calving, dairy farms was individually monitored in a longitudinal study from calfhood to the end of their first lactation. Body weight and BCS were recorded at four farm visits. Average daily gain (ADG) was calculated in three developmental phases; pre-breeding (calfhood to second grazing period), post-breeding (second grazing season to second winter housing period) and overall growth (calfhood to second winter housing period). First lactation performance data (305-day milk production), AFC, and breeding indices were retrieved from the national cattle breeding organisation (ICBF). Three multivariable mixed-effects models (one for each of the three rearing periods) were constructed to assess the impact of several independent variables on first lactation milk yield.

RESULTS: There was no significant difference in median first lactation milk production between heifers reared at home (4,951 kg) and those contract/custom-reared (4,877 kg) consistent with a negligible difference in ADG (0.73 vs 0.70 kg/d, respectively). In the pre-breeding period model, higher pre-breeding ADG was significantly positively associated with increased milk yield. Results from the post-breeding period model, demonstrated a significant positive association between post-breeding ADG and milk yield. This model also demonstrated a positive effect of higher pre-breeding BCS on milk yield. The overall growth model again demonstrated a significant positive association with milk yield. Across all models, lactation length positively impacted milk yield, although it interacted negatively with AFC, where older calving heifers had lower milk yields.

CONCLUSIONS: The practice of contract-rearing of heifers did not adversely affect first lactation milk yield compared to home-rearing, indicating that both approaches to heifer rearing can achieve similar outcomes when growth is managed effectively.

Keywords: Contract/custom rearing, dairy heifers, milk production

Integrated Control of *Mycoplasmopsis (mycoplasma) bovis* in Dairy Calves: Efficacy of a Modified Live Vaccine in a Multi-modal Management Program

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BACKGROUND and OBJECTIVE: An integral approach is essential for effective control of *Mycoplasmopsis (Mycoplasma) bovis* in dairy operations, given the pathogen's ability to persist in herds and evade conventional interventions. Fundamental management practices, including rigorous colostrum and maternity protocols, optimal nutrition, sanitation, well-designed housing, ventilation, and the judicious use of antibiotics and vaccination, form the backbone of disease control. The objective of this analysis was to evaluate mortality of calves before and after inclusion of a multifaceted management approach to controlling endemic *M. bovis*.

MATERIALS and METHODS: Data were sourced from the Valley-Ag VAS dairy management software, which documented 62,856 calf births and a total of 133,931 health and management events from 2020 to 2025. Events recorded included birth, sale, death, pneumonia cases in hutches for calves younger than 73 days, pneumonia in hutches for calves older than 73 days, and instances of diarrhea. As part of a comprehensive management strategy including diagnostics, colostrum management, improvement of facility, and vaccination protocols designed by the herd's veterinarian, the facility began vaccination of animals in July 2022 with a modified-live *M. bovis* vaccine (Protivity®, Zoetis). The vaccination consisted of two doses: the first administered within the first week of life and a booster given 21 days after the initial dose. Calves were also vaccinated against respiratory viruses (IBR, PI3, and BRSV), given at 3 days of age and boosted at 45 days of age. Daily data entry was conducted by the dairy's dedicated health team and management staff. All records underwent cleaning and refinement in Microsoft Excel, with subsequent graphical analysis performed using Microsoft Power BI software to ensure clarity and rigor in data interpretation. Records obtained from 2020-2022 are identified as PRE, with data obtained between 2023-2025 as POST.

RESULTS: Following application of this multifaceted strategy, the facility observed a marked decrease in overall calf mortality. In PRE, the average mortality was 34.5% as a proportion of all calves born, and in POST, the average mortality was 13.3% as a proportion of all calves born. Pneumonia-specific deaths averaged 19% when calves were in hutches (<72d) in PRE, and 10.7% of deaths in POST years. For calves >73 days of age, 15.5% of deaths were pneumonia-specific in PRE years, and 9.3% in POST years.

CONCLUSIONS: The results suggest that integrating diagnostics, enhanced management, and a cellular immunity-targeting vaccine is effective in controlling *Mycoplasmopsis bovis* in commercial calf operations, though good management and husbandry remain essential components alongside vaccination.

Keywords: mycoplasmopsis bovis, vaccination, management, dairy calf

Heat maps: A Consulting Tool for Visualizing Lameness Using the Example of a Herd with Digital Dermatitis

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BACKGROUND and OBJECTIVE: A heat map is a two-dimensional data visualisation technique that uses colour to represent the magnitude of individual values within a data set. Heat maps provide an intuitive and quick overview of large amounts of data. We have therefore created heat maps of the locomotion score according to Sprecher et al. (1997), to evaluate the lameness of a dairy herd over time and facilitate the identification of cows with recurring lameness.

MATERIALS and METHODS: As part of the monitoring of a serious claw health problem

involving digital dermatitis, the locomotion scoring was carried out every three months over a period of four years (January 2022 to December 2025) on a dairy farm in northern Germany with 80 cows and an annual milk yield of 11,000 kg. The locomotion score consists of five grades. To create the heat maps, each score was assigned a colour. To illustrate the lameness situation of the entire herd over time, the relative frequencies of the locomotion scores were first presented as stacked 100% column diagram and heatmaps of all animals were prepared, as well as heatmaps clustered according to lactation number.

RESULTS: The herd's locomotion score improved very gradually, with several setbacks along the way (December 2025: score 1: 41.8%, score 2: 40.5%, score 3: 16.5%, score 4: 1.3%, score 5: 0.0%; target values according to Cook et al. (2016): scores 1 + 2: > 93%, score 3: < 7%, score 4: < 1%, score 5: 0%). The heat maps show that virtually all animals in the herd had a score of ≥ 3 over time and that, despite intensive therapeutic measures, these animals very rarely returned to a score of 1. This shows that preventive measures to improve claw health were clearly insufficient in the past. From November 2024 onwards, the herd's mobility score tends to improve, as preventive measures such as spraying the claws with an approved claw care product were carried out more consistently than before.

CONCLUSIONS: Heat maps of the locomotion score are useful for visualising claw health trends at herd level. They also provide valuable insights into locomotion score dynamics at cow level. Therefore, they offer information that would otherwise be lost when only the prevalence of lameness is shown. Heat maps can be used by veterinarians and farmers in particular to evaluate the effectiveness of preventive and therapeutic measures at herd level.

Keywords: herd health, lameness, heat map

Understanding Barriers to Accessing Veterinary Services Among Cattle Producers in Rural Alberta

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BACKGROUND and OBJECTIVE: Veterinary workforce shortages in rural regions pose challenges for cattle producers seeking timely and appropriate veterinary services. While access to veterinary care is frequently discussed in relation to herd health and cattle practice sustainability, limited empirical research has examined cattle producers' perspectives on accessing veterinary services. The objective of this study was to explore beef cattle producers' experiences accessing veterinary services in Alberta and to identify barriers and facilitators relevant to herd health management and cattle practice delivery.

MATERIALS and METHODS: A qualitative study design was used. Semi-structured interviews were conducted with cattle producers from cow-calf and feedlot sectors. Participants were recruited using purposive sampling through intermediaries, followed by snowball sampling. Interview guides and analysis were informed by the Levesque Access to Care Framework to structure discussion of service accessibility from both a supply-side and demand-side. Interviews were audio-recorded, transcribed verbatim, and analyzed using a hybrid inductive and deductive thematic analysis approach.

RESULTS: Data collection and analysis were ongoing at the time of abstract submission. Preliminary analysis indicates that cattle producers experience multiple barriers to accessing veterinary services, including geographic distance, affordability, and suitability of services, with variation across production systems and regional contexts. The Access to Health Care framework provided a practical structure for identifying and organizing barriers and facilitators relevant to cattle production and veterinary practice.

CONCLUSIONS: Findings from this study will contribute to understanding how access to veterinary services influences herd health management and cattle practice sustainability in rural regions. Results may inform workforce planning, service delivery models, and policy initiatives aimed at improving access to bovine veterinary care. Final results will be available at the congress.

Keywords: access to care, rural, beef production, qualitative

Uganda Crane Creameries Cooperative Union (UCCCU) Pooled Clostridial Vaccination Campaign: Improving Herd Health and Farmer Resilience

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BACKGROUND and OBJECTIVE: Clostridial diseases have long caused rapid and fatal infections among cattle in South-Western Uganda, leading to significant economic losses among smallholder dairy farmers. These infections progress swiftly, rendering antibiotic treatment ineffective, and thus require proactive prevention through vaccination. Both UCCCU and partner cooperatives have reported increasing cases of sudden death syndrome, especially among society farmers who have not previously vaccinated against clostridial. Objectives Main OBJECTIVE: The project sought to reduce livestock mortality and enhance dairy productivity by implementing a large scale Clostridial vaccination program across nine districts. It aimed to strengthen herd immunity, build cooperative based animal health capacity, and promote long term sustainability in disease prevention among smallholder farmers. Specific OBJECTIVES: 1. Vaccinate 200,000 cattle against Clostridial diseases within one year. 2. Enhance farmer awareness and knowledge on Clostridial disease prevention. 3. Strengthen veterinary and extension capacities through training and provision of vaccination equipment. 4. Reduce cattle mortality by at least 20% across targeted cooperatives.

MATERIALS and METHODS: The intervention employed a cooperative-led vaccination model leveraging pooled mobilisation and service delivery. UCCCU coordinated vaccine procurement, distribution, supervision, and field logistics, while Zoetis supported capacity building, provision of cold-chain equipment, automatic vaccination guns, and training of extension workers and para-veterinarians. Vaccines were accessed through a cooperative check off system, enabling farmers to pay gradually through milk proceeds.

RESULTS: UCCCU vaccinated 185000 cattle, trained and equipped 50 para veterinarians, and successfully reduced mortality among cooperatives that adhered to regular vaccination schedules. These experiences inform and strengthen the scaling of the current initiative.

CONCLUSIONS: The campaign led to a measurable reduction in cattle mortality, improved herd immunity, and increased dairy productivity. It strengthened cooperative resilience to future disease outbreaks and promoted long term sustainability through retained vaccination equipment, improved veterinary systems, and continuous farmer sensitization.

Keywords: Uganda Crane Creameries Cooperative Union, Clostridia, Vaccination, Campaign, Uganda

From Disease Prevention to Climate Resilience: Herd Health Management in Portuguese Small Ruminant Systems

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BACKGROUND and OBJECTIVE: Improving animal health is a key strategy for sustainable livestock production and climate change mitigation. In Mediterranean regions such as Portugal, small ruminants play an important role in sustainable farming systems due to their adaptability to marginal environments. However, diseases such as neonatal infections, mastitis, parasitic infections, and poor body condition can compromise productivity and animal welfare. This study aimed to

evaluate herd health management practices in two small ruminant farms in northern Alentejo (Portugal) and identify preventive veterinary strategies to improve herd health, productivity, and sustainability.

MATERIALS and METHODS: Data collection focused on herd health status and preventive management practices in small ruminant farms. Information was obtained through interviews with farmers, farm workers, and the attending veterinarian, as well as through field observations and farm records. Two case studies were analyzed. Farm 1 consisted of approximately 500 meat sheep (White Merino crossbreed) managed in a semi-extensive system with continuous natural mating. Farm 2 included 400 dairy goats (Murciana breed) managed in an intensive system with controlled breeding using natural mating and artificial insemination. Farm facilities were evaluated according to housing structure, feeding and watering systems, flooring, animal density, humidity, ventilation, and environmental conditions.

RESULTS: In Farm 1, inadequate housing conditions—including lack of lateral protection, poor drainage, and high humidity—were associated with a high prevalence of neonatal diseases and compromised lamb survival. Omphalitis and arthritis affected 31% of confined lambs, while neonatal diarrhea occurred in 52% of animals. Post-partum ewes also presented poor body condition (BCS 2/5), indicating nutritional and management challenges during the periparturient period. Corrective measures included improvements in flooring and drainage, reorganization of animal groups by production stage, implementation of preventive health plans, and adjustments to reproductive and nutritional management. In Farm 2, excessive bedding humidity and deficiencies in milking management were associated with mastitis problems (9% clinical and 12% subclinical), elevated somatic cell counts, and omphalitis in 11% of kids. Corrective actions included improved hygiene and bedding management, revision of milking procedures, newborn management protocols, nutritional adjustments, and the implementation of structured herd health planning.

CONCLUSIONS: Structured herd health management programs improve animal health, productivity, and welfare in small ruminant systems. Preventive veterinary strategies—including housing, hygiene, nutrition, reproduction, and biosecurity—support disease control and herd resilience. In Mediterranean regions such as Portugal, these approaches are essential for sustainable livestock production, climate resilience, and improved farm efficiency, and represent important opportunities for young veterinarians and veterinary nurses working in small ruminant health management.

Keywords: Climate resilience, Herd health management, Preventive veterinary medicine, Small ruminants, Sustainable livestock production

Looking Beyond Efficacy to Evaluate Intervention Implementation on Ruminant Farms

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BACKGROUND and OBJECTIVE: Traditionally, intervention evaluation focuses on efficacy, however implementation evaluation goes beyond efficacy to explore why an intervention did or did not have the expected effect. It is particularly valuable in real world settings, where contextual factors may influence outcomes. Poor outcomes under real world conditions may often reflect failures in implementation rather than inherent shortcomings of the intervention. This study represents part of a larger pilot trial exploring the use of a framework to guide the process of uptake of preventive medicine practices on five ruminant farms in the North of England. Interventions and strategies relevant to each individual farm were identified and implemented. Proctor's Implementation Outcomes (PIOs)—a taxonomy describing key outcomes related to the implementation process— was used with the objective of evaluating how the interventions were implemented in practice.

MATERIALS and METHODS: Farmer–veterinarian pairs participated in one or two facilitated planning

meetings, followed by implementation of agreed intervention(s) over a 6–9 month period. Mid point monitoring and end point review meetings were also conducted. Planning meetings focused on identifying relevant farm specific interventions. Review meetings were guided by questions aligned with PIOs. Inductive qualitative content analysis of meeting transcripts identified the interventions chosen. Deductive qualitative analysis, coded to PIOs, was used to analyse the review meeting transcripts to evaluate the implementation of these interventions.

RESULTS: Implementation outcomes varied across interventions, with exceptions to most general patterns. Adoption and fidelity were generally high, and where fidelity was lower, adaptations were typically well reasoned. Acceptability and cost were discussed infrequently but were usually viewed positively, with cost framed mainly in terms of realised benefits. Appropriateness was also positive, though alternative interventions were rarely considered. Penetration was generally low, with interventions remaining add-ons rather than becoming embedded in existing practices. Sustainability was rarely considered beyond a year. Limited structured follow up restricted assessment of several outcomes, and some factors—such as perceived value—were often implied rather than explicitly stated. Overall, farms implemented interventions largely as intended, but with exceptions. The limited attention to several implementation outcomes highlights the opportunity for targeted post implementation follow up to compare expectations with real world performance and inform future implementation.

CONCLUSIONS: Implementation evaluation provides valuable insights into on farm change processes, revealing both aspects of successful implementation as well as opportunity to further understand and improve on it. PIOs offer a practical and usable framework for improving understanding of implementation on ruminant farms and strengthening future uptake of preventive practices.

Keywords: Implementation science, Human behaviour change, framework, veterinary, preventive medicine

Interim Safety Evaluation of a Phytogenic Dry-Off Tablet in a Large-Scale Field Trial in Dairy Cows

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BACKGROUND and OBJECTIVE: The dry-off period represents a critical transition in the lactation cycle of dairy cows, characterized by major metabolic, hormonal and immunological adaptations. Phytogenic interventions have recently gained interest as potential tools to support physiological adaptation during this period. Following a preliminary pilot trial suggesting beneficial effects of a phytogenic dry-off tablet containing an Allium-derived compound on metabolic parameters, a large-scale randomized blinded field study was initiated to evaluate its safety and potential benefits under commercial conditions. As the trial is still ongoing and blinding remains maintained, this abstract focuses exclusively on safety observations.

MATERIALS and METHODS: A randomized, double-blinded field trial is being conducted in 11 commercial Holstein dairy herds across three countries (Belgium, Luxembourg and Germany). To date, 251 cows have been enrolled. Dry-off management consisted of abrupt cessation of milking without selective dry-off. Cows were randomly assigned to two blinded groups: one receiving the StopLac® tablet and one receiving placebo tablet. A biological monitoring subgroup of 80 animals was followed for metabolic and biochemical parameters (including AST, CPK, GLDH and GGT) measured at predefined time points. Clinical events (mastitis, abortions and other health disorders), milk production parameters and somatic cell count (SCC) dynamics were monitored from dry-off to 100 days in milk. Descriptive statistics were used to assess group homogeneity and evaluate safety based on clinical outcomes and biological reference ranges.

RESULTS: Baseline demographic and management data indicate good homogeneity between the two blinded groups, supporting the robustness of the study design. Interim clinical observations do not raise any safety concerns: the incidence of mastitis, abortions and other clinical conditions remains low

and comparable between groups, with overlapping survival curves and no emerging safety signal. Somatic cell count dynamics from dry-off to early lactation are similar in both groups, with no indication of increased subclinical mastitis. Milk yield immediately after calving is also comparable. In the biological monitoring subgroup, metabolic and biochemical parameters remain within expected physiological ranges, with no consistent pattern suggesting treatment-related risk. No parallel changes were observed in hepatocellular markers (GLDH, GGT) or clinical indicators of metabolic disease. Some enzymes (AST, CPK) show moderate differences between groups.

CONCLUSIONS: This interim analysis from an ongoing large-scale blinded field trial indicates that the phytogenic dry-off tablet is well tolerated under commercial farming conditions. No safety concerns have emerged regarding clinical health, udder health indicators or metabolic biomarkers during the transition period.

Keywords: Dry-off, transition, high producing dairy, phytogenic

Longissimus Dorsi Muscle Thickness at Drying off Is Associated with Colostrum Quality in Holstein Cows

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BACKGROUND and OBJECTIVE: Colostrum immunoglobulin concentration is critical for passive immunity transfer. Brix refractometry is widely used as an indirect estimate of immunoglobulin concentration. This study evaluated associations between ultrasonographic measurements of longissimus dorsi muscle thickness (LDT) and backfat thickness (BFT) of Holstein cows during the dry period and colostrum Brix values.

MATERIALS and METHODS: A total of 192 multiparous Holstein cows from five dairy farms were included. LDT and BFT were measured at four timepoints relative to calving: -45d (drying-off window, corresponding to 60 to 45 days before calving), -21d, -7d and 0d (calving). Within each timepoint, cows were classified separately into LDT and BFT terciles corresponding to low, moderate and high values for that timepoint. Colostrum samples were evaluated using a Brix digital refractometer (PAL-1, ATAGO, Fukaya-shi, Saitama, Japan). The same general linear modelling approach was applied separately at each timepoint. The initial models included herd, parity (2, 3, 4+), LDT tercile, BFT tercile, dry-period length (covariate) and relevant interactions as potential predictors of Brix values. Results are reported as estimated marginal means (EMM) with 95% confidence intervals (CI).

RESULTS: Mean (± standard deviation) Brix value was 25.4 % (± 4.1). Statistically significant associations were observed between LDT terciles and colostrum Brix only at -45d. Parity × LDT tercile interaction was associated with Brix (P = 0.020), whereas LDT tercile as a main effect (P = 0.090), BFT tercile (P = 0.173), and parity as a main effect (P = 0.169) were not. Within parity 4+, cows in the high LDT tercile had higher (P = 0.009) Brix (28.8 %, 95% CI: 26.9-30.6) than the low tercile cows (23.5 %, 95% CI: 20.8-26.2). Within the high LDT tercile, parity 4+ cows had higher Brix than parity 2 cows (adjusted mean difference 3.5 %, P = 0.009) and parity 3 cows (adjusted mean difference 3.7 %, P = 0.007). No significant associations were observed at −21d, −7d or 0d.

CONCLUSIONS: Greater skeletal muscle reserves at drying-off, reflected by greater LDT measured 60 to 45 days before calving, were associated with higher colostrum Brix measurements in older cows. In contrast, subcutaneous fat reserves, assessed as BFT, were not associated with colostrum Brix measurements. These findings suggest that the level of muscle reserves at drying-off may be linked to subsequent colostrum immunoglobulin synthesis and could inform targeted late lactation and dry

period management strategies aimed at optimising colostrum quality and passive immunity transfer.

Keywords: Colostrum, Dairy cattle, Skeletal muscle, Transition

Predicting Carcass Traits from Heart Girth and Body Condition in Holstein Dairy Cows under Commercial Farm Conditions

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BACKGROUND and OBJECTIVE: Accurate, on-farm prediction of carcass outcomes for cull dairy cows supports timely and economically sound culling decisions. This study evaluated whether heart girth (HG) and body condition score (BCS), supplemented by lactation stage (quarters in milk; QIM) and lactation number (LA), can predict corrected carcass weight and carcass classification outcomes (conformation and fat cover) in Holstein dairy cows under commercial farm conditions.

MATERIALS and METHODS: Data were collected from 12 commercial Holstein herds located in Brandeburg (Germany). Herd managers measured HG (cm) and assessed BCS (1–5; Edmonson et al., 1989) in cows destined for slaughter. Carcass traits (hot carcass weight corrected for trimming losses, conformation class, fat cover class) were obtained from slaughter documentation and standardized to numeric scores. Multiple linear regression models were developed for corrected carcass weight, conformation, and fat cover using HG, BCS, LA, and QIM. The dataset was split within farms into training and validation subsets.

RESULTS: The final dataset included 3,934 cows (mean lactation 3.22; mean days in milk 245; mean HG 214 cm; mean BCS 2.96; mean carcass weight 290 kg). Model performance was moderate (coefficient of determination: training 0.649; validation 0.584). Interpreted coefficients were biologically coherent: each 1-point increase in BCS was associated with +27.69 kg corrected carcass weight; each 1 cm increase in HG with +2.34 kg; each additional QIM with +5.93 kg; each additional lactation with −0.26 kg. Conformation and fat cover classes were positively associated with HG and BCS, with BCS contributing more strongly to fat cover.

CONCLUSIONS: HG and BCS, augmented by QIM and LA, predicted corrected carcass weight and EU classification outcomes with useful accuracy under commercial conditions. The approach is low-cost, implementable without scales, and suitable for integration into herd management systems to estimate slaughter value and support culling decisions. Emphasis on standardized measurement and data quality is important. The model was integrated into a digital application that can be downloaded for Android in the Google Play Store: "HF Schlachtprognose Tool" - available in German and English The study was conducted in the scope of a European Innovation Partnership (EIP)-agri project ("Die Entwicklung des KUH-mehr-WERT Navigators").

Keywords: culling, economics, key figure, slaughter

Effects of Calving Complications on Survival, Performance, Health, and Fertility in German Holstein Dairy Cows

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BACKGROUND and OBJECTIVE: Calving complications—twin birth, stillbirth, and dystocia—are key determinants of reduced performance, longevity, and welfare in dairy herds. This study quantified their short- and long-term effects across lactations under commercial conditions, focusing on survival, milk yield, fertility, health disorders, and subsequent calving traits.

MATERIALS and METHODS: We analyzed 36,187 calvings (2018–2022) from 12 commercial dairy herds in Brandenburg, Germany (average herd size: 574 German Holstein cows). Analyses were conducted separately for lactations 1 to 5+, using linear models with herd and the calving-related factor as fixed effects. Risk factors were: twin birth (single vs. twin); stillbirth (live vs. stillborn/death within 48 h; singletons only); calving difficulty (unobserved, slight, moderate, severe/surgery; singletons only); and a combined calving outcome for observed singletons (live/easy, live/difficult, dead/easy, dead/difficult; difficult = moderate to severe difficulty).

RESULTS: Overall, 3.6% of calvings were twin births and 6.1% were stillbirths. Moderate calving difficulty occurred in 9.4% of single births, and severe difficulty in 1.3%. The proportion of twin births increased with parity. Calving complications were consistently associated with higher culling risk and reduced survival across lactations. Milk production was significantly reduced, particularly in multiparous cows. Reproductive performance declined, including lower first-service conception rates and overall conception rates, as well as poorer subsequent calving outcomes. Prior complications strongly increased the risk of retained placenta, metritis, metabolic disorders, and subsequent stillbirth. The combined calving outcome showed coherent and often highly significant associations with disease incidence and survival.

CONCLUSIONS: Twin births, stillbirth, and difficult calvings substantially impair survival, production, fertility, and health across lactations in German Holsteins. Preventive strategies—structured calving management, optimized facilities, staff training, systematic birth monitoring, and early twin diagnosis—promise meaningful economic gains and improved animal welfare. The study was conducted in the scope of a European Innovation Partnership (EIP)-agri project ("Die Entwicklung des KUH-mehr-WERT Navigators").

Keywords: herd health management, culling, stillbirth, dystocia, twinning

Management Factors Influencing Colostrum Quality and Contamination and Bovine Respiratory Disease in Pre-weaned Calves on Mid-atlantic Dairy Farms in the United States

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BACKGROUND and OBJECTIVE: This cross-sectional study aimed to evaluate farm-level management factors influencing colostrum quality, colostrum hygiene, and the risk of bovine respiratory disease (BRD) in pre-weaned dairy calves. From February to June 2025, 34 dairy farms in Pennsylvania, Virginia, and North Carolina were visited once.

MATERIALS and METHODS: Farms collected ≥14 colostrum samples during the two months preceding the visit, and a questionnaire covering colostrum, housing, and calf management was administered at the visit. A total of 660 frozen colostrum samples were analyzed for Brix % and bacterial contamination (Aerobes, Coliforms, E. coli). Additionally, 12 calves aged 22–60 days were randomly selected per farm for respiratory health scoring and thoracic ultrasonography.

RESULTS: Across farms, good-quality colostrum (Brix ≥22%) averaged 76.82% (n = 507; SE = 1.60%). Mean contamination prevalence was 21.97% (n = 145; SE = 1.60%) for Aerobes, 11.36% (n = 75; SE = 1.20%) for Coliforms, and 17.88% (n = 118; SE = 1.50%) for E. coli. Across all models evaluated, none of the farm-level management practices showed statistically significant effects on Brix quality. A beta-binomial model identified several management practices associated with reduced contamination ($P < 0.05$). Not changing bedding in the calving pen significantly reduced the risk of aerobic and coliform contamination. Adding bedding more than six times per month in the calving pen also decreased aerobic and coliform contamination. Checking the calving pen more than six times/d decreased aerobic and coliform risk, as well as a 3-step feeding equipment cleaning. Harvesting colostrum within three hours reduced aerobic contamination. The risk of aerobic, coliform, and E. coli contamination increased when colostrum was not heat-treated. Alternative colostrum sources or mixing fresh with alternatives reduced E. coli, while mixing pregnant heifers with cows increased it. Across 357 calves evaluated, herd-level BRD prevalence ranged from 0.00% to 91.70% (mean = 21.10%, SE = 2.20%). A multivariable Poisson model identified several management practices that significantly affected the risk of BRD ($P < 0.05$). Using plastic hutches and combining mechanical with natural ventilation reduces BRD risk, whereas not providing a second colostrum feeding, relying solely on mechanical ventilation, and not assessing transfer of passive immunity increases BRD risk.

CONCLUSIONS: These findings show that specific farm-level management practices meaningfully influence both colostrum bacterial contamination and the risk of BRD in pre-weaned calves, underscoring the importance of coordinated hygiene, feeding, and housing strategies to improve early life calf health on dairy farms.

Keywords: Bovine respiratory disease, Colostrum quality and contamination, Management practices

Effects of Rumen-Protected Choline Supplementation on Colostrum Quantity and Quality and Lactation Performance in Dairy Cow

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BACKGROUND and OBJECTIVE: In dairy cattle breeding, colostrum quality and quantity are important parameters affecting calf survival and disease incidence. It is known that heifers in their

first lactation period generally produce colostrum in low quantities and of poor quality. Studies have shown that adding choline chloride to rations increases colostrum quantity and quality. Choline supplementation is also thought to have a positive effect on milk yield. This study was conducted to evaluate the effects of rumen-protected choline supplementation on colostrum quality and quantity, as well as lactation performance, in first-calf and multiparous dairy cows of the Simmental breed.

MATERIALS and METHODS: During the study period (March 2025 - June 2025), all animals were kept under the same housing and environmental conditions. The study was conducted on 90 animals, 44 in the experimental group and 46 in the control group. Animals in the experimental group were fed a ration supplemented with rumen-protected choline (Kemin-Choligem (choline chloride (600 g/kg), hydrogenated vegetable oil)) for 21 days prior to calving (Close Up), while no choline was added to the rations of animals in the control group. The colostrum quantity and quality, as well as milk yields, of the cows in the groups were measured. Intra-group comparisons were performed using the Paired Samples T Test or Wilcoxon Test, while inter-group comparisons were performed using the Independent Samples T Test or Mann Whitney U Test, based on the normality of the data distribution. SPSS (version 28.0) software was used for statistical analysis, and a significance level of $P<0.05$ was adopted.

RESULTS: Colostrum quality (Brix) and quantity (kg) were significantly higher ($p = 0.001$) in primiparous animals whose rations were supplemented with choline compared to animals in the control group. (Experimental 6.97 kg/27.59 Brix, Control 5.2 kg/24.95 Brix). In multiparous animals, no significant difference was detected between groups in terms of colostrum quantity, while colostrum quality was significantly higher in the experimental group compared to the control group ($p = 0.013$) (Experimental 5.58 kg/26.18 Brix, Control 6.33 kg/Brix 24.09). No difference was detected between groups in milk yield at 305 days of lactation in both primiparous and multiparous animals ($p = 0.58$; $p = 0.570$ Trial 7176 kg / Control 6724 kg).

CONCLUSIONS: The results of the study indicate that supplementing the ration of primiparous and multiparous cows with choline chloride increases colostrum quantity and quality as well as milk yield.

Keywords: Colostum Quantity and Quality, Lactation Performance, Choline Chloride, Dairy Cattle

IMMUNOLOGY AND VACCINOLOGY

Impact of Mastitis Vaccination on Clinical Mastitis Rates in 27 Belgian Commercial Dairy Farms

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BACKGROUND and OBJECTIVE: Mastitis remains the most prevalent disease in dairy cattle and represents a major challenge for animal welfare, milk quality, and farm profitability, accounting for a substantial proportion of antibiotic use on dairy farms and raising concerns among stakeholders. Preventive strategies, such as vaccination against major mastitis pathogens, are increasingly adopted. Given the limited availability of multi-farm data under commercial Belgian conditions, this study assesses the association between mastitis vaccination and changes in clinical mastitis rates.

MATERIALS and METHODS: A retrospective, multi-farm observational before–after study was conducted in 27 Belgian dairy farms from farm registers. Each farm served as its own control by comparing a 12-month prevaccination period (Y0) with the subsequent 12-month period under vaccination (Y1). Vaccination was implemented under veterinary supervision and guidance, with a commercially available multivalent vaccine against Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli), and/or a monovalent vaccine against Streptococcus uberis (S. uberis). The vaccine against S. aureus and E. coli was administered as two injections at a one-month interval, followed by booster injections every 2.5 to 3 months in lactating cows, dry cows, and heifers. The vaccine against S. uberis (3 farms) was administered as three injections at one month intervals, followed by booster injections every six months. In farms using both vaccines, booster injections were performed according to the respective schedules of each vaccine. Clinical mastitis was analysed as an annual herd-level rate, calculated as the number of recorded clinical mastitis cases relative to the number of lactating cows. Data were analysed using a linear regression model with group as a factor and “farm” as a random factor.

RESULTS: The average clinical mastitis rate decreased significantly from 61.07% (± 35.4) in Y0 to 34.74% (± 23.0) in Y1 ($p < 0.001$), corresponding to an average relative reduction of 43.11%. At the farm level, 23 out of 27 farms (85.18%) showed a reduction in clinical mastitis rate, with 40.7% of farms achieving relative reductions greater than 50%. Four farms showed an increase in clinical mastitis rate during Y1.

CONCLUSIONS: Mastitis vaccination was associated with a significant reduction in clinical mastitis rates in Belgian dairy farms, despite heterogeneity in farm systems, herd sizes, management practices, pathogen profiles, and vaccination strategies. Certain farms experienced outbreaks caused by pathogens not targeted by the implemented vaccine. Given the multifactorial nature of mastitis, vaccination should be considered as part of a broader mastitis control program.

Keywords: mastitis, dairy, vaccination, udder, e.coli., aureus

The Comparison of a Vaccination Protocol Containing Live BRSV, Mannheimia haemolytica Leukotoxoid and Inactivated Histophilus somni to an Inactivated Multivalent Vaccine on the Antibiotic Usage and Mortality on a Belgian Rose Veal Farm

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BACKGROUND and OBJECTIVE: Respiratory diseases remain a major cause of morbidity and mortality in veal calves, leading to substantial economic losses. Vaccination is an important preventive measure, but the practical effectiveness of different respiratory vaccination strategies is not always well established. The aim of this study was to compare two respiratory vaccination protocols in veal calves on antibiotic consumption and mortality.

MATERIALS and METHODS: This study was conducted on a Belgian veal farm that finishes approximately 2,400 calves in batches of about 600. Most calves were male Holstein Friesian, arriving at 2 to 4 weeks of age and slaughtered at 8 months. Two batches that entered in February and September 2023 received an inactivated vaccine against bovine respiratory syncytial virus (BRSV), parainfluenza type 3 (PI3), and Mannheimia haemolytica, with a booster 4 weeks later (control group; n=567 and n=598). Two subsequent batches that entered in January and March 2024 received a live BRSV vaccine plus an inactivated vaccine against M. haemolytica leukotoxin and Histophilus somni, with both products given shortly after arrival and again 5 weeks later, under veterinary supervision (test group; n=587 and n=595). Outcomes were mortality during the rearing/fattening period and antibiotic consumption, expressed as Defined Daily Dose (DDD). Both total antibiotic consumption as the antibiotics used for respiratory disease were analysed, and they were split into injectables (individual treatments) and oral antibiotics (group treatments).

RESULTS: The number of calves that died during the rearing/fattening period decreased from 3.4% (33 calves) in the control group to 1.1% (10 calves) in the test group, showing a significant reduction of 67% ($p<0.01$). The total antibiotic consumption decreased from 58.42 DDD in the control group to 41.25 DDD in the test group, showing a reduction of 29%. Oral antibiotics decreased from 55.2 to 39.16 DDD, and the injectables decreased from 3.22 to 2.09 DDD. The antibiotics used for respiratory disease decreased from 43.57 DDD in the control group to 25.42 DDD in the test group, representing a 42% reduction. Oral antibiotics decreased from 40.79 to 23.71 DDD, and the injectables decreased from 2.78 to 1.71 DDD.

CONCLUSIONS: Adapting the vaccination protocol from an inactivated vaccine against BRSV, PI3, and Mannheimia haemolytica to the combination of a live BRSV vaccine with an inactivated vaccine against Mannheimia haemolytica leukotoxin and Histophilus somni resulted in a significant reduction of mortality (-67%) and a reduction of 42% the antibiotics used for respiratory disease.

Keywords: veal, bacterial pneumonia, vaccination, histophilus, BRSV

Immunological Response to a New Multivalent Recombinant BVD Vaccine Used as a Booster of Live Attenuated Vaccines Against BVDV-1 and BVDV-2

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BACKGROUND and OBJECTIVE: A novel subunit vaccine comprises five bovine respiratory and reproductive viral antigens, including recombinant glycoproteins of BVDV-1 and BVDV-2. It is intended to protect cattle from transplacental infection caused by BVDV. This study evaluated the immunological response induced by this vaccine when used as a booster to live attenuated BVD vaccination.

MATERIALS and METHODS: Two farms were enrolled: Farm 1 included 53 heifers previously vaccinated with a live attenuated BVDV-1/2 vaccine; Farm 2 included 45 heifers previously vaccinated with a live attenuated BVDV-1 vaccine. All vaccinations were administered in accordance with the Summary of Product Characteristics (SPC). One year after the last vaccination, animals were randomly assigned to three groups (A, B, and C). In Farm 1, groups comprised 18, 18, and 17 animals; in Farm 2, each group included 15 animals. Groups A and B received the new vaccine: Group A received one dose, and Group B received two doses three weeks apart. Group C was revaccinated with the same vaccine used previously on each farm. Blood samples were collected in farm 1 at D0, D50, D90, and D180, in farm 2 at D0, D41, and D56, to assess neutralizing antibodies (SN) against BVDV-1. Serum samples were analyzed using a neutralization test in MDBK cells in 96-well plates to quantify SN antibodies against BVDV-1. Data were processed using RStudio and Microsoft® Excel, and statistical significance was set at $p<0.05$.

RESULTS: Before vaccination (D0), SN titers against BVDV-1 were similar across groups in both farms (Farm 1: 6.64±0.28, 7.34±0.30, 7.08±0.45; Farm 2: 4.09±0.40, 3.76±0.31, 3.46±0.43). In farm 1, group B had significantly greater ($p<0.05$) seroneutralizing antibody titers against BVDV-1 compared to groups A and C at D50 and D180. On D50, the average antibody titers for BVDV-1 were 7.16±0.45, 9.16±0.20, and 7.29±0.29, respectively, and on D180, the average titers were 7.08±0.57, 8.62±0.31, and 7.25±0.33. In both cases, the differences between group B and group A and C were statistically significant. In farm 2, after vaccination, antibody titers increased across all groups, with no significant differences between them. At D56, the average BVDV1 titers were 6.75±0.47, 6.18±0.49 and 6.34±0.39 respectively.

CONCLUSIONS: The new recombinant E2 glycoprotein vaccine induces a high immunogenic response against BVDV-1 and can be used as a booster after a live attenuated vaccine containing BVDV-2 and/or BVD-1. One dose maintains the seroneutralizing antibody levels achieved with previous vaccines, whereas two doses yield higher levels after vaccination with the vaccine containing BVDV-1 and BVDV-2.

Keywords: marker, BVD, booster, vaccination, E2, fetal protection

Economic Evaluation of the Impact of Vaccination Against Staphylococcal Mastitis in a French Sheep Flock

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BACKGROUND and OBJECTIVE: Staphylococci are the main pathogens responsible for mastitis in dairy sheep flocks. Implementation of a mastitis control program is an essential step in improving milk quality and disease prevention. One of the measures included in these control programs is vaccination. The most obvious losses are caused by clinical mastitis. Losses from clinical mastitis are obvious, with reduced yield and high culling risk However, due to the high prevalence of subclinical mastitis compared to clinical mastitis, it is important not to underestimate the costs it will entail. The objective of this study was to evaluate the economic impact of vaccinating against Staphylococcal mastitis compared to a period before vaccination.

MATERIALS and METHODS: The study was performed in a conventional managed dairy sheep Lacaune flock in the Roquefort Area (Aveyron,

France). All animals were vaccinated against Staphylococcal mastitis according to the recommended administration program. The control group is a season without vaccination (N=471 animals, November 2021-April 2022). The first year of vaccination (N=483 between November 2022 and April 2023. Second Year of vaccination (N=486 between November 2023 and April 2024), Third year of Vaccination (N=529 between November 2024 and April 2025) With the base price of milk, and adding the bonuses or penalties due to Bulk Tank Somatic Cell Count (bSCC) and Staphylococci excretion, we calculated each year's milk earnings. We also assessed economic losses from clinical mastitis for each year. Fixed (€/sheep) and variable (€/l) costs are extracted from French Livestock Institute data.

RESULTS: Clinical mastitis culls fell about fifteenfold from the control season to year 3. Fewer staphylococcal cases, together with improved reproduction, increased the number of lactating ewes in the final season. Milk quality improved across all three vaccinated years, resulting in higher bonuses. Profitability was consistently positive, with gains of €41 per ewe in year 1 versus control (ROI 7.8), €33 in year 2 (ROI 6.2), and €31 in year 3 (ROI 5.8).

CONCLUSIONS: Vaccination against Staphylococcal mastitis is an effective tool in controlling subclinical and clinical mastitis caused by Staphylococci on the farm. Vaccination against Staphylococcal mastitis is profitable, with a positive ROI. Each euro invested in the vaccine yielded a return between 7,8€ and 5,8€.

Keywords: sheep, mastitis, vaccination, staphylococcus aureus, milk quality

Field Performance of a Novel Multivalent Viral Marker Vaccine in a French Beef Cattle Herd

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BACKGROUND and OBJECTIVE: Bovine Respiratory Syncytial Virus (BRSV), Parainfluenza 3 virus (Pi3), and Bovine Viral Diarrhea Virus (BVDV) are major causes of Bovine Respiratory Disease (BRD). BRD is one of the most prevalent causes of morbidity and mortality among beef cattle in France, thus prevention is key to reducing its impact. In this country, one of the strategies to prevent BRD is vaccinating calves, but adults are no longer vaccinated against BRD. The objective of this field experience was to evaluate the vaccination of the adults against BRD and its impact on the mortality and morbidity of the calves born from vaccinated adults versus the previous year without vaccination against BRD

MATERIALS and METHODS: The study was conducted in a conventionally managed beef Charolais herd in the Auvergne Region (France). All adult animals were vaccinated with a multivalent vaccine containing a live BRSV, inactivated Pi3, and BVDV1 E2 and BVD2 E2 proteins, according to the recommended administration program (N=362 calves born from those adults between August 2024 and February 2025). The control group is a period with only BVDV1 live and BVDV2 live vaccination (N=373 calves born between August 2023 and February 2024), according to the recommended administration program. The calf vaccination protocol remained unchanged across these two seasons: BRSV live intranasal vaccination in the first week of age; Mannheimia haemolytica, serotype A1, leukotoxoid; and Histophilus somni inactivated vaccine, two doses, three weeks apart from the second week of age. For instance, the calves' BRD morbidity in this farm was 31,2% in 2020-2021, 31,8% in 2021-2022, and 23,9% in 2022-23. BRD Mortality was 20-21 1,3%, 21-22 1,9% and 22-23 2,2% Housing ventilation was audited in December 2021, and no severe air-circulation problems were identified.

RESULTS: The calves' morbidity due to BRD observed during the study period was 6,6% (N=24) compared to 12.6% (N=47) during the control period, representing a 52.3% reduction in morbidity. Significant difference between vaccination protocols (p-value: 0.001*) The calves' mortality due to BRD observed during the study period was 0% (N=0) versus 2,1% (N=1), during the control period. No significant difference between vaccination protocols (p-value: 0.15)

CONCLUSIONS: Vaccinating adult Charolais cattle with a multivalent viral marker vaccine containing BRSV, PI3, BVDV1, and BVDV2 was associated with lower calf BRD morbidity and a reduction in BRD case fatality compared with the previous season when adults received only BVDV1 and BVDV2 vaccination. Additional studies across herds and seasons will help confirm these findings under routine field conditions.

Keywords: BRD, cattle, vaccination, BVD, BRSV, beef

Reduction of Bovine Respiratory Disease Treatments in Adult Cows on a Commercial Dairy Farm in the Netherlands Following Vaccination with a Histophilus Somni and Mannheimia Haemolytica Leukotoxoid Vaccine

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BACKGROUND and OBJECTIVE: In recent years, an increase in the incidence of Bovine Respiratory Disease (BRD) in adult dairy cows has been observed in the Netherlands. The objective of this poster is to evaluate the effect of a vaccine containing Histophilus somni and Mannheimia haemolytica leukotoxoid on BRD treatments in adult cows in a commercial dairy farm in the Netherlands.

MATERIALS and METHODS: A closed commercial dairy farm in the Netherlands (144 cows) experienced severe problems with BRD and pericarditis in cows aged two years or older. In March 2024, a bronchoalveolar lavage (BAL) was performed on a cow with BRD. The sample was tested with a quantitative multiplex Polymerase Chain Reaction (PCR) assay. Mannheimia haemolytica (Ct 29) and Pasteurella multocida (Ct 26) were detected. Additionally, a pericardial aspirate was collected from a heifer with pericarditis and analyzed using the same PCR, detecting Histophilus somni (Ct 36). Vaccination protocol: vaccination from two months of age with a vaccine containing Histophilus somni and Mannheimia haemolytica leukotoxoid was performed. According to the assisting veterinarian's recommendation, the first primovaccination was administered in May 2024, followed by a second dose four weeks later and a booster ten months after. Treatment data were collected from May 2023 through April 2025. The incidence of BRD treatments during the twelve-month pre-vaccination period (May 2023–April 2024) was compared with that of the twelve-month post-vaccination period (May 2024–April 2025). Treatment records were derived from medication administration logs maintained by the farmer and the attending veterinarian. A treatment episode was defined as a new case when an interval of at least 14 days occurred between consecutive treatments. Herd size remained stable, averaging 146 cows (older than two years of age) before primovaccination and 141 cows (same age) thereafter.

RESULTS: Statistical analysis (Chi-square test) shows a significant 59% reduction in BRD treatments among cows older than two years of age in the twelve months following the first primovaccination (p-value: 0.03648). A total of 20 BRD treatments (13.7% of the herd) were administered in twelve months before the first primovaccination, compared to 8 treatments (5.7% of the herd) in twelve months after the first primovaccination.

CONCLUSIONS: Vaccination with a vaccine containing Histophilus somni and Mannheimia haemolytica leukotoxoid on a dairy farm with major BRD problems in adult cows shows a significant 59% decrease in BRD treatments, reinforcing its potential role in preventive measures against BRD in adult cows.

Keywords: BRD, vaccination, bacterial pneumonia, leukotoxoid,

Storage and Handling of Calf Vaccines in Danish Herds: A Survey-Based Assessment

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BACKGROUND and OBJECTIVE: Proper storage and handling of vaccines are essential to ensure their efficacy in livestock health management. This study, conducted in November 2025, evaluated practices related to vaccine use in Danish cattle herds, aiming to identify areas for improvement. In Denmark, vaccines are under veterinary prescription, but farmers are allowed to administer vaccines after completing a course in medicine handling.

MATERIALS and METHODS: At a calf conference, a questionnaire was distributed to people working with cattle in Denmark, with 87 responding to the survey. Of these, 66 had administered vaccines within the past two years and were included in this survey. The survey addressed vaccine storage,

administration practices, handling of leftover doses, and attitudes toward vaccination.

RESULTS: 63.6% of the respondents reported they had received instructions on vaccine handling. Most (93.9%) reported that vaccines were stored in refrigerators, yet only 4.8% of these checked the temperature at least once a week. Administration practices varied: Of 41 respondents who had been vaccinating calves within the past two years, 41.5% administered vaccines directly from the refrigerator, while 39.0% allowed them to reach room temperature. Handling of leftover doses revealed that 87.8% either discarded the remaining vaccine or vaccinated additional animals, whereas 12.2% stored it for later use. Confidence in administration was generally high, though needle change for every 1–5 calves was reported by 42.5%. Intranasal vaccines were used by 41.5%, but 35.3% of those who used this route had not received training for intranasal administration. Attitudes toward vaccination were positive; most respondents felt they understood how vaccines work, but expressed an interest in gaining more knowledge.

CONCLUSIONS: While Danish cattle producers generally adhere to recommended vaccine practices, targeted interventions in storage monitoring, administration hygiene, and education could further optimize vaccination outcomes and improve herd health.

Keywords: storage, handling, vaccines, training, survey

Immunological Response of a New Multivalent Marker Vaccine Used as a Booster of a Monovalent, Inactivated Vaccine Against Infectious Bovine Rhinotracheitis

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BACKGROUND and OBJECTIVE: A novel subunit vaccine comprises five bovine respiratory and reproductive antigens, including IBR (gE-/tk-double-gene-deleted). This study focused on the immunological response induced by this new vaccine used as a booster after an inactivated IBR marker vaccine.

MATERIALS and METHODS: A total of 48 heifers previously vaccinated with an inactivated vaccine against infectious bovine rhinotracheitis virus were enrolled in the study. Nine months after the first vaccination, these animals were randomly distributed into three groups of 16 animals each. Two of these groups were vaccinated with the new multivalent marker vaccine; Group A received one dose, and Group B received two doses three weeks apart. Group C was vaccinated again with the same vaccine used nine months before. Blood samples from all animals were collected on the day of vaccination (D0), 8 weeks (D56), 3 and 6 months after (D92 and D183) to assess neutralizing antibodies (SN) against IBR and ELISA for gB antibodies and gE antibodies, to assess that no external infection occurred during the trial. Serum samples were analysed using a neutralization test in GBK cells in 96-well plates to quantify SN antibodies against IBR, and an IDEXX ELISA was used to analyse antibodies against IBRgE and IBRgB. RStudio and Microsoft Excel 365 (Microsoft Corp.) were used to calculate primary results and perform statistical analyses. A p-value <0.05 was considered a significant result.

RESULTS: Regarding ELISA IBRgE results, all animals remained negative throughout the trial (D0 to D183), indicating no viral circulation. Before vaccination (D0), the average SN titres of groups A, B, and C were similar for IBR (5.95±0.23, 5.54±0.36, and 6.11±0.18, respectively) and for ELISA IBRgB (94.13±0.20, 94.19±0.19, and 94.13±0.24, respectively). No statistical differences were observed at D0 and in any of the other time points. At the end of the study (D183), the average titres for SN and for ELISA maintained the serological response for all groups. Regarding SN the average results were (6.51±0.05, 6.49±0.05 and 6.15±0.15, respectively), whereas in ELISA IBRgB, the average results from this period were (94.79±0.17, 95.07±0.15 and 94.87±0.21, respectively). No statistical differences were observed at any of the analyzed time points

CONCLUSIONS: The new, multivalent marker vaccine induces a strong immunological response against IBR and, when used as a booster for a previous vaccination with monovalent inactivated IBR vaccine, maintains high antibody levels comparable to those obtained with an initial, monovalent IBR vaccine.

Keywords: IBR, DIVA, vaccination, switch, respiratory, reproductive

Immunological Response to a New Multiviral Marker BVDV-1/2 Vaccine (E2 Recombinant Glycoprotein), Used as a Booster After Inactivated BVDV-1 Vaccination

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BACKGROUND and OBJECTIVE: A novel subunit vaccine comprising five different bovine respiratory and reproductive antigens, including BVDV-1 and BVDV-2 (E2 recombinant glycoprotein). The vaccine is intended to protect cattle from transplacental infection caused by BVDV. This study focused on the immunological response induced by this new vaccine, used as a booster after an inactivated BVD-1 vaccine.

MATERIALS and METHODS: A total 48 female heifers previously vaccinated with an inactivated vaccine against bovine viral diarrhoea virus were enrolled in the study. Nine months after the first vaccination, these animals were randomly distributed into three groups of 16 animals. Two of these groups were vaccinated with the new vaccine, Group A received one dose and Group B received two doses three weeks apart. Group C was vaccinated again with the same vaccine used nine months before. Blood samples from all animals were collected at the day of vaccination (D0), 8 weeks (D56), 3 and 6 months after (D92 and D183) to assess neutralizing antibodies (SN) against BVDV-1 and BVDV-2. Serum samples were analysed using a neutralisation test in MDBK cells in 96-well plates to quantify SN antibodies against BVDV-1 and BVDV-2. The RStudio program and Microsoft ® Excel 365 were used to calculate primary results and perform statistical analysis. A p-value <0.05 was considered a significant result.

RESULTS: Before vaccination (D0), the average SN titers of group A, B, and C were similar for BVDV-1 (3.00±0.38, 3.26±0.35, and 2.58±0.26, respectively) and BVDV-2 (2.78±0.18, 2.65±0.13, and 2.47±0.07, respectively). No statistically significant differences were observed. After that, group A and B had significantly greater ($p<0.05$) seroneutralizing antibody titers against BVDV-1 and BVDV-2 compared to group C from 8 weeks to 6 months after vaccination. On D56, the antibody titers increased in all groups for BVDV-1 (8.47±0.39, 9.53±0.07 and 5.07±0.47, respectively) and BVDV-2 (7.27±0.29, 8.49±0.10 and 3.63±0.31, respectively). On D92, the average titers remained high for groups A and B, whereas they were lower in group C for BVDV-1 (8.02±0.41, 9.36±0.14, and 4.16±0.39, respectively) and BVDV-2 (7.29±0.33, 8.51±0.07, and 3.41±0.28, respectively). Six months after vaccination, these differences were more evident in groups A and B compared to group C against BVDV-1 (7.44±0.29, 8.26±0.23, and 3.43±0.33, respectively) and BVDV-2 (6.15±0.32, 7.14±0.26, and 2.60±0.08, respectively).

CONCLUSIONS: When used as a booster after an inactivated BVDV-1 vaccine, the new multiviral marker vaccine elicits strong immune responses against both BVDV-1 and BVDV-2, yielding higher seroneutralizing antibody titers against BVDV-1 and BVDV-2 than the initial inactivated BVDV-1 vaccine.

Keywords: BVDV-1, BVDV-2, vaccination, reproduction, fetal protection,

Comparison of the Immunological Response of a New Multivalent Marker Vaccine Used as a Primary Vaccination Against BVDV-1, BVDV-2, and IBR

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BACKGROUND and OBJECTIVE: A novel subunit marker vaccine comprising five different respiratory and reproductive antigens, including double-deleted gE-/tk- IBR, BVDV-1, BVDV-2 recombinant E2 glycoproteins, BRSV and PI3, has been developed to protect cattle against these key viral pathogens. This study focused on the immunological response induced by this new vaccine, used as a primary vaccination compared with a live attenuated vaccine and an inactivated vaccine against BVDV-1, BVDV-2, and IBR.

MATERIALS and METHODS: A total of 51 female calves were enrolled in the study. These animals

were distributed into 4 groups: A, B, C, and D of 15, 15, 13, and 8 animals, respectively. Group A received the new multivalent marker vaccine, group B received an inactivated IBR and BVDV vaccine, group C received a live attenuated BVDV-1/-2 vaccine, and group D remained unvaccinated and, were used as a control group. The vaccinations were administered according to the manufacturer's instructions for each product. Day 0 was the day of the first vaccine administration in group A. All animals were sampled at D0, D90, D210, D270, D330, D390, D450, D570 and D600 to assess seroneutralizing antibodies (SN) against IBR, BVDV-1 and BVDV-2. Serum samples were analysed using a neutralization test in MBDK cells in 96-well plates to quantify SN antibodies for BVDV-1 and BVDV-2 and in GBK cells to quantify SN antibodies against IBR. The RStudio program and MICROSOFT® EXCEL 365 (Microsoft Corp.) were used to calculate primary results and to perform statistical analysis. A p-value <0.05 was considered a significant result.

RESULTS: Regarding results for BVDV-1, group A had significantly greater ($p<0.05$) seroneutralizing antibody titres compared to groups B, C, and D on study days: D90, D210, D270, D330, D450, and D600. For BVDV-2, group A had significantly higher ($p < 0.05$) seroneutralizing antibody titres than groups B, C, and D on study days D90 and D210. Nevertheless, on study days: D270, D330, D390, D450, D570, and D600, there were no significant differences between groups A and C, but both groups had significant differences ($p<0.05$) compared with groups B and D. Regarding IBR results, groups A and B had significantly higher ($p < 0.05$) seroneutralizing antibody titres than groups C and D on study days D270, D330, D390, D450, D570, and D600.

CONCLUSIONS: The new multivalent marker vaccine elicits strong immune responses against BVDV-1, BVDV-2, and IBR, and is not inferior to the other vaccination protocols evaluated.

Keywords: DIVA marker vaccine, BVDV E2 recombinant glycoprotein, IBR gE-deleted tk-deleted, respiratory, reproductive

Assessment Of F41 Valence in *E. Coli* Vaccine Strain Ec/17. Practical Consequences

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BACKGROUND and OBJECTIVE: Neonatal Calf Diarrhea (NCD) is recognized worldwide as one of the biggest challenges for both the beef and dairy industries. Enterotoxinogenic *Escherichia coli* (ETEC) strains are commonplace causes of NCD. Vaccination of pregnant dams upgrades the protective value of colostrum, and commercial vaccines are available, all of them offering protection against the F5 (K99) fimbria of *E. coli*. However, other adhesins contribute to the pathogenicity of ETEC, such as F41. Although F5 and F41 are frequently produced simultaneously by ETEC strains, objectives of this work were to characterize the presence of F41 in vaccine strain EC/17, and to check whether immunization with a commercial vaccine formulated with EC/17 can elicit an immune response to F41 adhesin.

MATERIALS and METHODS: Identification of genes encoding for F41 was carried out through methods of molecular biology with primers designed by others. The investigation was extended to evaluate sequences encoding for F4, F5 and F6 fimbriae. Finally, a set of 42 European strains were tested, including EC/17 and EC/42, a challenge strain. Serological response to F41 was explored in rabbits (routine batch control test, ELISA), from 12 distinct commercial batches of a vaccine formulated with EC/17. Finally, 13 cattle were enrolled in a vaccination test. Animals (n=10) were injected with a single dose (3 ml) of the same commercial vaccine, the remaining 3 animals were used as negative control. Blood samples were taken twice, before vaccination and 21 days later. Blood serum samples were examined by indirect ELISA for detection of F41 antibodies.

RESULTS: This research first assessed the specificity of the F41 primers and confirmed the sequence identity of the targeted *E. coli* F41+ gene. Presence of genes coding for F41 adhesin was confirmed in EC/17, and in EC/42 as well. Across the

tested collection of *E. coli* strains examined, F5 and F41 fimbriae occur together in 23.8% of strains, including EC/17 and EC/42. A commercial vaccine formulated with strain EC/17 as antigen, was able to evoke a significant antibody response against both F5 and F41 adhesins in laboratory animals (rabbits, $p<0.025$) and in target animals (cattle, $p<0.001$).

CONCLUSIONS: When a commercial EC/17-*based* vaccine is administered, an immune response against F5 and F41 is observed.. Since both of *E. coli* strains (vaccination strain EC/17 and challenge strain EC/42) have been confirmed for F41 valence, it is therefore possible to state that commercial EC/17-based vaccines are able to protect against *E. coli* F5 an F41-*related* NCD.

Keywords: Neonatal calf diarrhea, F41, vaccine

Impact of the Introduction of a New *Cryptosporidium parvum* Vaccine into Vaccination Protocols of Pregnant Cows on the Immune Response Elicited by a Vaccine Against Bovine Rotavirus, Coronavirus and *E. Coli K99*

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BACKGROUND and OBJECTIVE: Neonatal calf diarrhea (NCD), caused primarily by *Escherichia coli* K99 (ECK99), *Cryptosporidium parvum*, bovine rotavirus group A (RVA), and bovine coronavirus (BCoV), continues to pose a major global problem for both the beef and dairy industries. Although vaccines targeting RVA, BCoV, and *E. coli* K99 have been available for prophylactic use in pregnant dams for many years, an anti-*C. parvum* vaccine is a relatively new commercial introduction. While these established modern vaccines have demonstrated safety and efficacy, their current marketing authorizations contain limited data concerning the safety of simultaneous administration, particularly when the products are sourced from different manufacturers. Consequently, this study was designed to assess the immune response of cows to a multivalent vaccine (RVA, BoCV, ECK99) given concurrently with an anti-*C. parvum* vaccine, and to monitor for any unexpected local or systemic adverse reactions.

MATERIALS and METHODS: The compatibility of the new anti-*C. parvum* vaccine and the multivalent vaccine was assessed in a study approved by the Ethical Committee for Animal Use of Virbac (EU-ERC/202410-06). Sixty-one dairy cows and heifers, in their last trimester were vaccinated with the new anti-C. parvum vaccine on D0. On D30, all animals got a booster shot. On D36±1 all were injected on the opposite side of the neck with the multivalent vaccine. Blood samples were collected on D0, D30, and D58 to evaluate the immune response to Rotavirus A (RVA), Bovine Coronavirus (BoCV), and E. coli K99 (ECK99) using commercial ELISAs. Immune responses to the three antigens were presented semi-quantitatively across five classes (negative to ++++). Seroconversion was defined as an increase of at least one class. Animals were closely monitored for any potential adverse effects during the week following the injections.

RESULTS: No adverse local or systemic reactions were reported beyond those detailed in the product's summary of characteristics. Given the European commercial setting, a high percentage of the animals had prior exposure to NCD agents, with frequent seropositivity to Rotavirus A (RVA) and Bovine Coronavirus (BoCV). Consequently, seroconversion rates observed were 80.3% for ECK99, 62.3% for BoCV, and 44.3% for RVA. A statistically significant shift in the distribution of patients across ELISA classes was noted between Day 0 and Day 58 (Chi-squared test, $p<0.05$).

CONCLUSIONS: The concurrent administration of the two vaccines looks safe and does not impair the immune response to the multivalent vaccine.

Keywords: neonatal calf diarrhea, C. parvum, vaccine, safety

Safety and Serological Response of a Multivalent Marker Vaccine Against Bovine Respiratory Disease Administered in Two to Four Weeks Old Veal Calves

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BACKGROUND and OBJECTIVE: Bovine respiratory disease (BRD) affects up to 61% of veal herds, with particularly high incidence in pre-weaned calves. This highlights the need for safe early-life vaccination. Recently, a new multivalent marker vaccine designed to protect cattle against BRD, has proven to be safe and efficacious in older calves. Evidence in younger animals remains limited. This study evaluated safety and serological responses when the vaccine was administered to veal calves at 2 to 4 weeks of age.

MATERIALS and METHODS: Seventy-one veal calves (male and female) from a commercial Spanish feedlot, representing different origins and breeds, were enrolled in the study. Each calf received two intramuscular doses (2 mL) at 2–4 weeks of age, with a booster administered 21 days later. Animals were monitored from th e first vaccination day(D0) until 12 weeks post-vaccination (W12). Safety assessments were performed in a subgroup of fifteen calves and included rectal temperature, systemic and local reactions, and general health observations, at D0, D0+4h, D1, D2, D20, D21, D21+4h, D22, and D23. Serum samples were collected at D0, D21, W6, and W12. TheW12. The immune responses were evaluated using commercial ELISA kits: BoHV-1 gB and gE (IDEXX), BVDV p80 (CIVTEST), and Total BVDV antibodies (IDEXX).

RESULTS: No anaphylactic reactions were reported in any of the 71 vaccinated animals during the monitoring period. Within the safety subgroup, 33% of calves developed transient hyperthermia after the first dose and 30% after the second dose. None of the calves required specific treatment, and all recovered within three days, consistent with the expected range described in the Summary of Product Characteristics (SPC). No systemic or local adverse reactions beyond the anticipated transient fever were observed. Serology demonstrated 100% seroconversion to BoHV-1 gB by D21. At D0, 66.7% of the animals were gE-negative, and this status was reduced until W12, with 76.9% of animals remaining gE-negative. These findings are consistent with the vaccine profile and highlight the vaccine's efficacy. In addition, robust BVDV-specific antibody responses were detected, with 100% seroconversion achieved by W6, confirming the vaccine's immunogenicity in this age group.

CONCLUSIONS: The administration of the multivalent marker vaccine in calves at 2–4 weeks of age demonstrated a similar safety profile to the standard (SPC) profile, reinforcing its potential role in early-life immunization strategies against BRD.

Keywords: BRD, Veal, Caves, Respiratory, vaccination, protection

Clinical Mastitis Outcomes Following S. uberis Vaccination in a Pasture-Based South African Dairy Herd

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BACKGROUND and OBJECTIVE: Mastitis is a common disease in dairy cattle and a major driver of antimicrobial use. Streptococcus uberis is an environmental mastitis pathogen that can be associated with pasture-based or mixed management systems, where cows may have greater exposure to soil, mud, manure, and wet grazing areas. Infections can cause clinical mastitis, reduce milk yield and quality, increase treatment costs, and increase the risk of culling. This study evaluated the impact of a S. uberis mastitis vaccine on the incidence and risk of clinical mastitis and on milk production in a large South African dairy herd.

MATERIALS and METHODS: A total of 1,188 dairy cows were enrolled and assigned to a vaccinated group (n=587) and a non-vaccinated control group (n=596). Vaccination was implemented under veterinary supervision using a commercially available monovalent vaccine against S. uberis. The

protocol included three 2 mL intramuscular doses administered in the neck at 5-week intervals (November 2021 to January 2022), followed by a booster dose at 6 months (July 2022). Clinical mastitis cases and milk yield were recorded and monitored for 13 months, from February 2022 to February 2023.

RESULTS: Over the 13-month study period, the incidence of clinical mastitis was significantly lower in cows vaccinated with the S. uberis vaccine (31.7%, 186 cases) than in the non-vaccinated control group (44.1%, 263 cases), corresponding to a 28.1% reduction in total cases. Risk analysis showed that vaccination was associated with a significant 30% lower overall risk of clinical mastitis (risk ratio = 0.703; P<0.001). The strongest effect was observed for S. uberis mastitis, with a significant 42% reduction in risk among vaccinated cows (risk ratio = 0.584; P=0.002). In addition, cows that developed S. uberis mastitis produced numerically more milk in the vaccinated group (23.8 kg/day) than in the control group (22.9 kg/day).

CONCLUSIONS: Vaccination was associated with a significant reduction in clinical mastitis cases. It also appeared to lessen the economic impact of S. uberis infections, as vaccinated cows that became infected maintained numerically higher milk yields. Overall, these findings indicate that vaccination can be a useful component of an integrated mastitis control program, alongside established preventive measures and herd management practices.

Keywords: mastitis, vaccination, RSA, streptococcus, uberis

Simultaneous Intranasal Vaccination Against Bovine Respiratory Syncytial Virus, Parainfluenza-3 Virus, and Bovine Coronavirus Reduces Antibiotic Use in Dutch Veal Calf Settings

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BACKGROUND and OBJECTIVE: Preventing respiratory tract infections -especially after transport and commingling- remains a major challenge in the veal calf and dairy beef sectors. In these systems, vaccinating animals upon arrival may be one of the most practical approaches; however, the effectiveness of this strategy under such extreme conditions is still largely unproven. The aim of this study was to determine whether simultaneous intranasal vaccination on arrival against bovine respiratory syncytial virus(BRSV), parainfluenza-3 virus(PI3), and bovine coronavirus(BCoV) significantly reduces antibiotic use in Dutch veal calf fattening operations.

MATERIALS and METHODS: A multicenter historical control trial was conducted on eleven veal calf fattening farms in the Netherlands between December 2024 and March 2026, including approximately 15,000 calves. Upon arrival at the fattening units, calves received simultaneous intranasal vaccination with two separate live attenuated vaccines (Bovilis® Intranasal RSP® Live (MSD) for BRSV/PI3, and Bovilis® Nasalgen®-C (MSD) for BCoV). Both vaccines were not mixed and each one was given in a different nostril to prevent vaccine interference. Throughout the study, antimicrobial use(number of oral metaphylactic group treatments and Defined Daily Doses [DDD] administered), performance indicators (average daily gain, mortality, and lower end percentage), and overall farmer satisfaction were recorded and compared with data from the two preceding fattening cycles. Comparisons were made using generalized linear mixed regression models with fattening unit included as a random effect. Statistical analyses were conducted in R (R Core Team 2025). Significance was set at p<0.05.

RESULTS: No significant differences were found in any of the measured performance indicators between vaccinated and non-vaccinated cohorts. However, the number of oral metaphylactic antibiotic group treatments was significantly lower in vaccinated calves compared to non-vaccinated calves (beta = -0.77; 95%CI -1.5 to -0.07; p=0.011). In addition, there was a clear tendency toward reduced DDD in the vaccinated cohort (beta = -8.0; 95%CI -21.0 to 4.9; p=0.10). Overall, veal farmers reported higher satisfaction when the vaccination protocol was implemented.

CONCLUSIONS: Simultaneous non-mixed intranasal vaccination on arrival against BRSV, PI3, and BCoV in Dutch veal calf fattening units was linked to a marked reduction in oral metaphylactic antibiotic

group treatments and a tendency toward lower DDD, without negative effects on performance indicators. The observed increase in farmer-reported satisfaction further underscores the relevance of this vaccination approach. Overall, these results suggest that intranasal respiratory vaccination on arrival at the veal farm is a valuable strategy to support antimicrobial stewardship in veal calf production under field conditions.

Keywords: intranasal vaccination, Bovine respiratory disease, BRD, Veal calves

Comparative Beta Toxin-Specific Antibody Responses in Camels Vaccinated with Two Commercial Clostridial Vaccines: A Controlled Field Study

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BACKGROUND and OBJECTIVE: Clostridial infections represent a significant health risk for camels and effective vaccination is essential to prevent outbreaks in Saudi Arabia. This study compared the immunogenicity of two commercial vaccines (by quantifying antibody responses to Clostridium perfringens beta and epsilon toxins in camels.

MATERIALS and METHODS: Forty camels were randomly allocated to two groups balanced by baseline antibody titers. Group 1 received One Shot Ultra® 8 (2 mL, SC; Zoetis) and Group 2 received Bar-Vac 10 (2 mL, SC). A booster dose was administered 4 weeks after the primary vaccination. Blood samples were collected at baseline (pre-vaccination), 3 weeks post-primary vaccination, and 5 weeks post-booster. Antibody titers were measured and compared within and between groups over time, Statistical analyses were performed using SPSS version 20. Antibody titers across the three points within groups were compared using the Friedman Test, while the Mann-Whitney U Test was employed for between groups comparisons of toxins antibody levels.

RESULTS: For beta toxin, One Shot Ultra® 8 elicited a highly significant increase in mean titers from 30.2 ± 5.1 (baseline) to 76.3 ± 8.1 (post-primary) and 80.6 ± 6.4 (post-booster; $P < 0.001$). Bar-Vac 10 induced a modest rise from 25.3 ± 4.9 to 34.2 ± 7.9 and 36.9 ± 8.3 ($P = 0.002$). Post-booster beta toxin titers were higher in the One Shot Ultra® 8 group than in the Bar-Vac 10 group ($P = 0.013$). Variability in beta toxin responses was lower with One Shot Ultra® 8 (IQR = 6.2) than with Bar-Vac 10 (IQR = 9.8). For epsilon toxin, titers increased in both groups to similar levels (One Shot Ultra® 8: 10.4 to 68.9; Bar-Vac 10: 10.6 to 59.3), with no differences between groups ($P = 0.12$).

CONCLUSIONS: Both vaccines increased antibody titers, but One Shot Ultra® 8 elicited higher and more consistent beta toxin antibody responses than Bar-Vac 10, reflecting a more robust immunological response. Although animals were not directly challenged with the toxins to assess clinical efficacy, these immunogenicity results provide strong surrogate markers indicative of protective potential. Further studies are warranted to confirm the vaccines' protective efficacy and the durability of the immune response. Our data suggest that One Shot Ultra® 8 elicits a robust and potentially superior immune response against Clostridium perfringens beta toxin, positioning it as a leading choice for effective vaccination of camels.

Keywords: Antibody Response, adaptive immunity, beta toxin

Effect of Dam Vaccination Against c. Parvum on Gp40-antibody Levels Under Field Conditions

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BACKGROUND and OBJECTIVE: Neonatal calf diarrhea (NCD) remains one of the most important causes of morbidity and mortality in young calves. *Cryptosporidium parvum* (Cp) is a key pathogen in this syndrome. A new commercial vaccine allows dam vaccination against Cp, targeting its Gp40 glycoprotein. Under field conditions, Gp40 antibodies (Gp40Ab) provide a

measurable indicator of pathogen-specific passive immunity transferred from dam to offspring. This study evaluated how maternal Cp vaccination and colostrum provision influence calf serum Gp40Ab levels, with a focus on quantifying the postvaccination increase.

MATERIALS and METHODS: A cross-sectional field study was conducted on four dairy farms in Germany. Calves aged 2–7 days (n=137) were sampled. Of these, 92 calves received colostrum from nonvaccinated dams, while 45 calves received colostrum from dams vaccinated with a commercially vaccine inducing Gp40Ab production. All calves received at least 2 L of colostrum within the first 12 hours of life. Serum Gp40Ab levels were determined using an in-house ELISA. Passive immune transfer (PIT) was also evaluated using two techniques: ELISA-IgG (<10 g/L cutoff) and digital Brix Refractometry (<8.3% cut off). Associations among vaccination status, PIT indicators, and calf serum Gp40Ab levels were analyzed using linear and mixed regression models ($p<0.05$).

RESULTS: Across all farms, the strongest signal was the clear and consistent rise in calf serum Gp40 levels following maternal Cp vaccination. Calves receiving colostrum from vaccinated dams demonstrated substantially higher Gp40Ab than calves from nonvaccinated dams, indicating effective transfer of Cp-specific antibodies. The magnitude of this increase was biologically robust and highly statistically significant (Δ =7.1 Titer (2Log); $p<0.001$). Higher Gp40 titers in vaccinated groups were independent of general PIT status, demonstrating that the effect was vaccination specific rather than merely the result of better overall IgG transfer.

CONCLUSIONS: Under real farm conditions, Cp dam vaccination significantly elevates Gp40 antibody concentrations in neonatal calves, demonstrating strong transfer of pathogen specific immunity. These findings reinforce the value of prepartum vaccination as a targeted strategy to enhance early-life protection against *C. parvum*.

Keywords: neonatal calf diarrhea, Cryptosporidium parvum, serology, gp40 antibodies, dam vaccination

Preliminary Evaluation of Needle-Free Injector for Subcutaneous Vaccination in Cattle

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BACKGROUND and OBJECTIVE: Lumpy skin disease (LSD) remains a significant concern for cattle production and is primarily controlled by subcutaneous vaccination in South Korea. Under field conditions, variability in injection depth may lead to inconsistent vaccine deposition within target tissue layers. Needle-free injector system have been applied in swine production and are considered to reduce variability related to needle-based injection. This study aimed to conduct a preliminary ex vivo evaluation of needle-free injector system for subcutaneous vaccine delivery in cattle.

MATERIALS and METHODS: An ex vivo study was performed using the original Anijet system, and modified configurations adjusting spring force (7% and 12%) combined with 0.3 mm and 0.4 mm nozzle diameters. Trypan blue (1 ml per injection) was used for tracer. Fresh bovine hide was collected from local abattoir on the day of experimentation, rinsed with phosphate-buffered saline (PBS), and maintained at 4°C prior to ex vivo evaluation. Injection sites were approximately 3 cm apart. The original Anijet was tested in eight injections (four with hair and four without hair). Modified configurations (7% and 12% spring force; 0.3 mm and 0.4 mm nozzles) were evaluated in four replicate injections each. Injection performance was qualitatively assessed based on macroscopic dye deposition patterns. Cross-sectional examination was used to classify tracer localization within the dermis, subcutaneous tissue, or deeper muscle layer.

RESULTS: The original Anijet configuration resulted in dermal deposition in all injection (8/8), without

reaching the subcutaneous layer. For the 7% spring configuration, the 0.3 mm nozzle achieved subcutaneous deposition in 3/4 injection without muscle extension, whereas the 0.4 mm nozzle showed primarily dermal localization. The 12% spring configuration produces less. Consistent outcomes; subcutaneous deposition was observed in 1/4 injection (0.3 mm nozzle), while other injections remained dermal or extended into muscle tissue. Overall, the 7% spring combined with the 0.3 mm nozzle demonstrated the most consistent subcutaneous localization without visible muscle penetration.

CONCLUSIONS: Spring force adjustment influenced penetration depth in this ex vivo bovine skin model. The upgraded needle-free injector demonstrated potential for subcutaneous delivery under the tested conditions. Further in vivo evaluation will be conducted to confirm its reproducibility under physiological conditions.

Keywords: needle-free injector, subcutaneous, vaccination, cow.

Consecutive Intranasal–Parenteral Vaccination Against BRSV, PI3 And mannheimia Haemolytica in Young Calves with Maternal Immunity

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BACKGROUND and OBJECTIVE: Heterologous prime–boost vaccination uses two different vaccine types to stimulate multiple immune pathways. An intranasal live vaccine can trigger early mucosal and innate immunity even when maternal antibodies are present, while a later injectable inactivated vaccine can enhance systemic and humoral responses. This strategy may offer broader and longer-lasting protection compared to homologous vaccination. Two studies evaluated such a regimen in young calves with maternal immunity: an intranasal live attenuated BRSV–PI3 vaccine (Bovilis INtranasal RSP Live) in the first week of life, followed by a single inactivated BRSV–PI3–*Mannheimia haemolytica* (Mh) vaccine (Bovilis Bovipast RSP) at six weeks.

MATERIALS and METHODS: Two studies were conducted: Study A with 20 calves, Study B with 30 calves, all possessing antibodies against BRSV, PI3, and Mh. Calves were divided into vaccinated (intranasal live vaccine at Week 1, inactivated vaccine at Week 6) and unvaccinated groups. In Study A, eight calves per group were challenged with PI3 at Week 22 and BRSV at Week 24; clinical signs, rectal temperatures, and nasal shedding were monitored, and lung lesions assessed post-mortem. In Study B, all calves were challenged with Mh at Week 9, observed for clinical signs, and lung tissues sampled after euthanasia for lesion scoring and bacterial recovery.

RESULTS: After PI3 challenge, all calves shed virus, but vaccinated calves had significantly lower peak viral loads and shorter shedding (P = 0.0002). They also developed higher PI3-specific antibodies post-challenge. Following BRSV challenge, both groups showed respiratory signs, but vaccinated calves had lower disease scores and fewer lung lesions (3/8 vs. 6/8). BRSV shedding lasted one day less in vaccinated calves, and antibody titers were significantly higher (P = 0.0115). In Study B, vaccinated calves had significantly reduced coughing (P = 0.0462), lower rectal temperatures, fewer early euthanasia, reduced lung consolidation (P = 0.0367), and less Mh recovered from lungs (P = 0.0286).

CONCLUSIONS: In the studies reported here, the heterologous intranasal–injectable prime–boost strategy induced protection against BRSV, PI3, and Mh in calves with maternal antibodies, lowering pathogen replication, disease severity, and lung pathology.

Keywords: heterologous prime-boost, maternal antibody interference, bovine respiratory disease, mucosal immunity

Field Experience of Morbidity, Mortality, and Performance in Spanish Beef Calves Before and After Introduction of a Commercially Available Modified-live Vaccine Against *Mycoplasmopsis (mycoplasma) bovis*

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BACKGROUND and OBJECTIVE: Mycoplasmopsis bovis is a major threat to the cattle industry. It can evade host immunity and contributes to the bovine respiratory disease (BRD) complex. Targeted management practices, including vaccination, are therefore important for herd health. This study aimed to evaluate morbidity, mortality, and performance in a Spanish feedlot before and after introduction of a commercially available modified-live M. bovis vaccine (Protivity®, Zoetis).

MATERIALS and METHODS: Data were obtained from a commercial feedlot in Huesca and used to compare morbidity, mortality, and performance rates before (2023; PRE) and after (2024; POST) introduction of a M. bovis vaccination program (Protivity®: 2 mL s.c. at arrival + 2 mL s.c. 21 days later). Commercial conditions, management practices, and BRD treatment protocol were consistent across years. The PRE cohort included 968 animals and the POST cohort 710 animals. The arrival health protocol was unchanged in 2024 except that a beta lactam antibiotic was used for metaphylaxis instead of a macrolide.

RESULTS: The percentages of calves receiving one, two, and three BRD treatments were 32.2%, 14.8%, and 3.8% in PRE, versus 19.9%, 3.7%, and 0.8% in POST, respectively. Mortality following the first BRD treatment (case fatality) was 10.8% in PRE and 3.4% in POST. Overall BRD attributable mortality was 2.1% in PRE versus 1.0% in POST. Treatment cost per slaughtered animal and death loss per slaughtered animal were €4.2 and €32.6 in PRE, versus €2.8 and €17.6 in POST, respectively. The total value per slaughtered animal was €97 higher in POST compared to PRE, yielding a vaccine return on investment of 9.7: 1.

CONCLUSIONS: In this before–after field comparison, the introduction of a commercial modified-live M. bovis vaccine was associated with lower BRD treatment rates, reduced case fatality and overall BRD attributable mortality, and improved slaughter value, yielding a high ROI. While causal inference is limited by the observational design, these results support integrating M. bovis vaccination into feedlot health programs under real world conditions.

Keywords: Mycoplasmopsis bovis, modified-live, vaccine, feedlot, BRD

Field Experience of Morbidity, Mortality, and Performance in Crossbreed Suckling Calves Before and After Introduction of a Modified Live *Mycoplasmopsis (mycoplasma) bovis* Vaccine in Spain

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BACKGROUND and OBJECTIVE: Mycoplasmopsis bovis can evade host immune defenses and be a major contributor to the bovine respiratory disease (BRD), increasing morbidity and production losses. This study aimed to evaluate morbidity, mortality, and performance in suckling crossbreed calves, before and after incorporation of a commercially available modified live M. bovis vaccine (Protivity®, Zoetis) into the farm health protocol.

MATERIALS and METHODS: A retrospective, farm level assessment was conducted using records from a farm operating six weaning facilities in Spain (Ternera Gallega, Region Certified Meat). Outcomes were compared before vaccination (PRE) and after introduction of the vaccine (POST). In POST, calves received 2 mL s.c. of a modified live M. bovis vaccine (Protivity®) at arrival and a second 2 mL s.c. dose 21

days later. The remaining health program and BRD treatment protocol were unchanged across years, including vaccination against BRD associated viruses and a standardized treatment protocol. Data included feed intake (milk and/or starter), BRD treatment rate, mortality, and average daily gain (ADG). Primary analyses matched the same seasonal period (June–September) in 2024 (PRE; n=1,281) versus 2025 (POST; n=797). Additional analyses compared the full year 2024 without vaccination (PRE 2; n=3,770) versus 2025 after vaccine introduction (POST 2).

RESULTS: In data recorded from June–September, morbidity, total BRD treatments, and mortality were respectively 45.3%, 72.4%, and 3.0% in PRE versus 23.21%, 33.5%, and 1.8% in POST. ADG was 0.76 kg/day in PRE and 0.81 kg/day in POST. Using vaccine cost as the denominator, the return on investment (ROI) through weaning was 2.27: 1. In the full-year comparison, total BRD treatments and mortality were respectively 76.2% and 4.4% in PRE-2 versus 33.5% and 1.8% in POST-2. ADG increased was 0.78 kg/day in PRE-2 and 0.81 kg/day in POST-2. In this comparison, the vaccine ROI was 2.48: 1.

CONCLUSIONS: In this before–after field comparison, the introduction of a commercial modified-live M. bovis vaccine into calves' health protocol was associated with lower was associated with reduced BRD morbidity and mortality, improvements in growth performance, and favorable ROI. While causal inference is limited by the retrospective, observational design, these findings support integrating M. bovis vaccination into calves' health programs under real world conditions.

Keywords: Mycoplasmopsis bovis, modified-live vaccine, BRD, calves

Field Experience of Mortality and Growth Performance of Holstein Male Suckling Calves Before and After Introduction of a Commercially Available Modified-Live Vaccine Against *Mycoplasmopsis (mycoplasma) bovis* in Spain

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BACKGROUND and OBJECTIVE: Mycoplasmopsis bovis is a major threat to the cattle industry. It can evade host immunity and contributes to the bovine respiratory disease (BRD) complex. Targeted management practices, including vaccination, are therefore important for herd health. This study evaluated mortality and performance in suckling Holstein male calves – sourced from France and the Czech Republic and raised in Spain through weaning – before and after introduction of a commercially available modified-live M. bovis vaccine (Protivity®, Zoetis).

MATERIALS and METHODS: A retrospective analysis was conducted on a farm with two weaning facilities to compare BRD-related mortality and average daily gain (ADG) from suckling to weaning before (PRE, data from 2021–2024) and after the introduction of M. bovis vaccination (POST, data from 2025). Calves in POST received a commercially available modified-live M. bovis vaccine (Protivity®: 2 mL s.c. at arrival + 2 mL s.c. 21 days later). Other vaccinations and the BRD treatment protocol were unchanged across years. Farm records included milk/feed intake, mortality, and management costs. A total of 5,275 calves were weaned in PRE and 1,036 in POST; initial body weight averaged 60 kg in PRE and 65 kg in POST, with calf sourcing unchanged.

RESULTS: The ADG through weaning was 0.89 kg/day in PRE versus 0.92 kg/day in POST. Management costs and total operational costs (management + feed) per kg gained were respectively €0.58/kg and €2.29/kg in PRE versus €0.46/kg and €1.87/kg in POST. Of critical importance, calf mortality was 5.5% in PRE versus 3.7% in POST. Using vaccine cost as the denominator, the return on investment through weaning was 4.05: 1.

CONCLUSIONS: In this before–after field comparison, the introduction of a commercial modified-live M. bovis vaccine into Holstein male calves' health protocol was associated with improvements in growth performance, lower mortality, reduced costs, and favorable ROI. While causal inference is limited by the retrospective, observational design, these findings support integrating M. bovis vaccination into Holstein male calves' health programs under real world conditions.

Keywords: Mycoplasmopsis bovis, modified-live vaccine, BRD, Holstein male calves

Field Experience of Mortality and Performance in Crossbreed Suckling Calves Through Weaning and Transition Periods After Introduction of a Commercially Available Modified-live Vaccine Against *Mycoplasmopsis (mycoplasma) bovis*

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BACKGROUND and OBJECTIVE: Mycoplasmopsis bovis is a major threat to the cattle industry. It can evade host immunity and contributes to the bovine respiratory disease (BRD) complex. Targeted management practices, including vaccination, are therefore important for herd health, but field data across the suckling, weaning, and transition periods are limited. This study evaluated calf mortality and growth performance from the start of suckling through the end of the transition period (90 days on feed) before and after introduction of a commercially available modified live M. bovis vaccine (Protivity®, Zoetis) in a commercial calf raising operation in Spain.

MATERIALS and METHODS: A retrospective analysis was conducted on a commercial calf-raising farm to compare mortality and average daily gain (ADG) from suckling to the end of transition period before (PRE, data from 2021–2024; n = 5,017) and after the introduction of M. bovis vaccination (POST, data from 2025; n = 927). Crossbred suckling calves in POST received a commercially available modified-live M. bovis vaccine (Protivity®: 2 mL s.c. at arrival + 2 mL s.c. 21 days later). Other vaccinations, feeding and BRD treatment protocols, and calf sourcing were unchanged across years; mean initial body weight was approximately 70 kg across both PRE and POST periods.

RESULTS: The ADG from suckling to the end of transition period was 1.048 kg/day in PRE versus 1.180 kg/day in POST. Calf mortality was 3.8% in PRE versus 1.4% in POST. Using vaccine cost as the denominator, the return on investment through the transition period was 5.96: 1.

CONCLUSIONS: In this before–after field comparison, the introduction of a commercial modified-live M. bovis vaccine into crossbred suckling calves' health protocol was associated with improvements in growth performance, lower mortality, and favorable ROI between the start of suckling through the end of the transition period. While causal inference is limited by the retrospective, observational design, these findings support integrating M. bovis vaccination into calves' health programs under real world conditions.

Keywords: Mycoplasmopsis bovis, modified-live vaccine, BRD, suckling calves, transition

Development and Evaluation of a Novel BRV–BCV–ETEC Combination Vaccine for the Prevention of Neonatal Calf Diarrhea in Korea

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BACKGROUND and OBJECTIVE: Neonatal calf diarrhea (NCD) is a leading cause of morbidity and mortality in pre-weaned calves worldwide and is primarily associated with infections by bovine rotavirus (BRV), bovine coronavirus (BCV), and enterotoxigenic Escherichia coli (ETEC). In the Republic of Korea, prevention of NCD relies largely

on imported vaccines, which may not fully reflect the antigenic and epidemiological characteristics of locally circulating pathogens. Therefore, the development of vaccines based on domestic field isolates is essential for improving disease control in regional cattle populations. In the present study, we developed an experimental trivalent vaccine containing field-derived BRV (G6P[5] and G8P[5]), BCV, and ETEC F17 strains isolated from diarrheic calves in Korea.

MATERIALS and METHODS: To evaluate vaccine safety, immunogenicity, and the transfer of maternal antibodies, pregnant cows were immunized twice during late gestation prior to parturition. Neutralizing antibody responses against each antigen were measured in maternal serum, colostrum, and sera from newborn calves. Protective efficacy was assessed by orally challenging calves with virulent BRV, BCV, and ETEC strains, followed by monitoring of clinical signs, incidence and severity of diarrhea, pathogen shedding, and intestinal pathological changes.

RESULTS: No local or systemic adverse reactions were observed in vaccinated cows, indicating an acceptable safety profile. Vaccination elicited marked increases in virus-neutralizing antibody titers against BRV and BCV as well as antibody responses against ETEC, and these antibodies were efficiently transferred to calves via colostrum. Following challenge exposure, calves born to vaccinated cows remained clinically normal, with no evidence of diarrhea or mortality. In contrast, control calves developed persistent diarrhea accompanied by increased pathogen shedding and characteristic intestinal lesions. Histopathological examination further demonstrated preservation of intestinal villous architecture and epithelial integrity in calves from vaccinated dams, whereas control animals showed villous atrophy, epithelial damage, and inflammatory changes consistent with enteric infection. Notably, no statistically significant difference in protective efficacy was observed between the medium-dose and high-dose vaccine formulations.

CONCLUSIONS: Collectively, these findings demonstrate that maternal immunization with a trivalent vaccine based on locally derived BRV, BCV, and ETEC strains can effectively confer passive protection against NCD in neonatal calves. The comparable protective efficacy observed with the medium-dose formulation suggests that this dose may provide a practical and cost-effective basis for the development of domestically produced vaccines for the prevention of neonatal calf diarrhea in Korea.

Keywords: Bovine neonatal calf diarrhea (NCD), Bovine Coronavirus (BCV), Bovine Rotavirus (BRV), enterotoxigenic Escherichia coli (ETEC), Inactivated Vaccine

Field Evaluation of Safety and Immune Response of An Inactivated Vaccine Against EHD-8 in Cattle

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BACKGROUND and OBJECTIVE: Epizootic Haemorrhagic Disease serotype 8 (EHD-8) emerged in Southern Europe in 2023, prompting rapid development of an inactivated vaccine. This field study aimed to assess the humoral immune response induced by vaccination with an inactivated vaccine (Hepizovac®) in naïve and previously infected heifers and to evaluate the antibody response following a booster dose administered six months after primary vaccination.

MATERIALS and METHODS: The study was conducted in two dairy farms in south-west France previously affected by EHD-8. Eighty Prim'Holstein heifers (6–24 months) were enrolled and allocated into three groups: unvaccinated sentinels (n=20), a group (n=40) receiving a two-dose primary vaccination with Hepizovac® (21 days apart), and a group (n=20) receiving the same primary schedule plus a booster six months later. Blood samples were collected every 7 to 8 weeks from D−21 to D316 and analysed using a commercial anti-VP7 competitive ELISA. Serological dynamics were evaluated separately for animals initially seronegative and those naturally infected before vaccination. Clinical monitoring recorded major side effects, hyperthermia (rectal temperature above 39.5°C) and local reactions.

RESULTS: At D−21, 49 animals were seronegative and 31 non-negative. Sentinel animals remained serologically stable, supporting the absence of viral

circulation throughout the study. The vaccine demonstrated good safety, with no major adverse reactions in naïve or previously infected animals. Six transient hyperthermia events occurred after the first dose; none followed the second. Local reactions occurred in 22 heifers after the first injection and 20 after the second. In seronegative heifers (n=37), the primary vaccination triggered a strong humoral response, with mean %S/N decreasing from 78% at D−21 to 21% at D42. No significant change was observed in previously infected animals (n=23). The six-month booster elicited a strong amnesic response in all revaccinated animals regardless their status at the start of the study (%S/N = 9% for both naïve (n=12) and previously infected cattle (n=8)). For seronegative animals at D-21, the level of antibodies was higher after booster than after primary vaccination.

CONCLUSIONS: This field study confirms that Hepizovac® is safe in both naïve and previously infected animals. Vaccination induces a strong primary humoral response in naïve cattle. Previously infected animals showed limited responses to primary vaccination but reacted favourably to booster administration. Booster vaccination effectively enhanced humoral immunity, supporting its relevance in managing EHD-8 immunity in cattle populations.

Keywords: Epizootic Haemorrhagic Disease, Vaccine, Safety, EHDV, Cattle

Adenosine Metabolism and Its Impact on Monocyte and Neutrophil Phagocytic Activity in Neonatal Calves

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BACKGROUND and OBJECTIVE: Neonatal calves are highly susceptible to infectious diseases due to limitations in early innate immune responses despite passive immunity from colostrum. Purinergic signaling, particularly extracellular adenosine, regulates innate immune cell activity, but its role in neonatal calf immunity remains unclear. This study investigated age-related changes in adenosine metabolism and their effects on neutrophil and monocyte function.

MATERIALS and METHODS: Nine neonatal calves and five adult cattle were included in the study. Calves were sampled at birth (baseline), 48 h, and at 7, 14, and 28 days of age, whereas adult cattle were sampled once. Blood samples were collected for cell isolation, plasma adenosine measurement, and determination of serum adenosine deaminase (ADA) and 5'-nucleotidase (CD73) activity. Adenosine receptor expression (A1, A2A, and A3) on monocytes and neutrophils was evaluated by Western blot. Isolated cells were resuspended at 1×10^5 cells/mL and incubated with adenosine (100 µM), caffeine (50–200 µM), or their combinations, followed by exposure to FITC-labelled Escherichia coli (MOI = 30) to assess phagocytic activity.

RESULTS: Plasma adenosine concentrations (mg/L) in calves were 0.0456 (0.0130–0.0795) at birth, 0.0097 (0.0040–0.0180) at 48 h, 0.0120 (0.0054–0.0355) at day 7, 0.0091 (0.0044–0.0370) at day 14, and 0.0112 (0.0041–0.0151) at day 28, whereas adult cattle showed 0.0542 (0.0267–0.0677). Serum CD73 activity (U/L) was 3.2 (2.0–4.8) at birth, 4.0 (2.8–4.8) at day 2, 3.2 (2.0–3.6) at day 7, 4.8 (3.2–7.2) at day 14, and 6.4 (5.2–9.6) at day 28, while adult cattle showed 8.0 (4.8–13.25). Serum ADA activity (U/L) was 201.6 (158.4–230.4) at birth, 259.2 (259.2–288.0) at day 2, 259.2 (230.4–288.0) at day 7, 230.0 (186.5–259.0) at day 14, and 230.4 (201.6–230.4) at day 28, compared with 172.8 (158.4–230.4) in adult cattle. Adenosine treatment significantly increased neutrophil phagocytic activity compared with the control group ($p < 0.0001$). In contrast, all tested doses of caffeine showed an antagonistic effect against adenosine, attenuating the adenosine-induced increase in phagocytic activity ($p < 0.0001$). Western blot analysis confirmed the expression of A1, A2A, and A3 adenosine receptors in neutrophils at all sampling times. Among these receptors, A2A exhibited the strongest signal intensity, followed by A1, whereas A3 showed the lowest expression.

CONCLUSIONS: These findings suggest that adenosine signaling may play an important role in regulating neutrophil function in neonatal calves.

Keywords: adenosine metabolism, innate immunity, purinergic signaling

One Year Duration of Immunity in Sheep for a Vaccine Against Bluetongue Virus Serotype 3 (BTV 3)

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BACKGROUND and OBJECTIVE: The Blue Tongue Virus (BTV) is the causative agent of a severe seasonal transboundary disease affecting ruminants. Since its emergence in the Netherlands in 2023, Bluetongue virus serotype 3 (BTV3) has continued to spread widely across Europe, with significant impacts on animal health and causing severe losses for farmers. Mass vaccination of herds is the most efficient tool against the disease and establishing the duration of immunity (DoI) provided by BTV3 vaccine (Bultavo 3) in sheep is therefore essential to enable long-term control strategies. This study aimed to evaluate the efficacy of the vaccine one year after administration.

MATERIALS and METHODS: Eight animals (32 - 36 days old) were vaccinated with one-shot of BTV3 inactivated vaccine at a minimum dose and challenged one year later. One group of 8 non-vaccinated animals served as control. One year after primary vaccination, all animals were challenged with a virulent BTV3 heterologous strain, isolated from the 2023 outbreak in The Netherlands. Animals were observed for clinical signs and rectal temperatures for 21 days post-challenge and presence of viral RNA in serum were measured using a BTV3 specific qRT-PCR at regulator intervals.

RESULTS: The vaccine demonstrated a good safety profile, with no local or systemic reactions following administration. Following challenge, clinical signs like oedema, locomotion troubles, anorexia, apathy, conjunctivitis and/or nasal discharge, and hyperthermia were observed in all control sheep. In contrast, none of the vaccinated animals developed clinical signs. Presence of BTV3 RNA was detected in blood samples from all control animals from day-3 post challenge and persisted until study termination. No viral RNA was detected in any of the vaccinated animals at any sampling time following challenge one year after vaccination, corresponding to a 100 % reduction in viral load compared with controls.

CONCLUSIONS: In conclusion, in this study, a single dose regimen of the inactivated Bultavo 3 vaccine conferred robust one year protection in sheep, preventing clinical disease and viraemia after heterologous challenge. These results demonstrate that vaccination not only protects individuals but also effectively blocks onward transmission, supporting its use as a key component of BTV 3 control strategies in an increasingly enzootic European context. Bultavo® 3 is a trademark of Boehringer Ingelheim Vetmedica GmbH, used under licence

Keywords: BTV-3, Bultavo 3, vaccine, sheep, duration of immunity

Up to One Year Duration of Immunity in Cattle for a Vaccine Against Bluetongue Virus Serotype 3 (BTV 3)

Jiri Nezval[1], Milan Huňady[1], Lucie Střelcová[1], Martin Tkáč[1], Juraj Kučerák[1], Lore Van Hecke[2], Mathieu Chevalier[2], Hélène Gaude[2]

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BACKGROUND and OBJECTIVE: The Blue Tongue Virus (BTV) is the causative agent of a severe seasonal transboundary disease affecting ruminants. Since its emergence in the Netherlands in 2023, Bluetongue virus serotype 3 (BTV3) has continued to spread widely across Europe, with significant impacts on animal health and causing severe losses for farmers. Mass vaccination of herds is the most efficient tool against the disease and establishing the duration of immunity (DoI) provided by BTV3 vaccine (Bultavo 3) in cattle is therefore essential to enable long-term control strategies. This study aimed to evaluate the efficacy of the vaccine six months and one year after administration.

MATERIALS and METHODS: BTV3 inactivated vaccine at a minimum dose was administered to 16

calves (21 - 36 days old) with 2-shots, 21 days apart, and 16 calves were left unvaccinated. Half the animals in each group were challenged at 6 and 12 months after vaccination respectively, with a virulent BTV3 heterologous strain, isolated from the 2023 outbreak in The Netherlands. Clinical signs, rectal temperatures and general condition were recorded daily for 21 days in all animals and viral RNA was measured by a BTV3 specific qRT-PCR.

RESULTS: The vaccine showed a good safety profile, with no local or systemic reactions following either administration. Following challenge, clinical signs like conjunctivitis and/or nasal discharge and hyperthermia were observed in all control cattle. All vaccinated animals were protected from development of clinical signs at both 6 months and 1 year after vaccination. Presence of BTV3 RNA was detected in blood from all control animals. Following challenge 6 months after vaccination, viraemia was absent in all vaccinated calves at all sampling times. Following challenge 1 year after vaccination, viral RNA was undetectable in 7 out of 8 vaccinated animals, and only transient, low level viraemia occurred in one calf. Vaccination reduced viral load 100% and 96.3% after 6 months and 1 year, respectively.

CONCLUSIONS: In conclusion, a two dose regimen of the inactivated Bultavo 3 vaccine conferred robust one year protection in cattle, fully preventing clinical disease. The vaccine provided complete prevention of viraemia after 6 months and significantly reduced viraemia after one year. These results demonstrate that vaccination not only protects individuals but also effectively blocks onward transmission, supporting its use in BTV 3 control strategies. Bultavo® 3 is a trademark of Boehringer Ingelheim Vetmedica GmbH, used under licence

Keywords: BTV-3, Bultavo 3, vaccine, cattle, duration of immunity

A Multi-Omics Approach for Extracellular Vesicles Characterization: Deciphering the Immunomodulatory Potential of Buffalo EVs from Colostrum to Mature Milk

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BACKGROUND and OBJECTIVE: Extracellular vesicles (EVs) derived from milk are promising bioactive components due to their ability to mediate intercellular communication and modulate immune responses. Naturally secreted by mammary epithelial and immune cells, they carry a complex cargo of proteins, lipids, and nucleic acids, including miRNAs, which contribute to their immunomodulatory potential. Milk EVs influence immune function, gut barrier integrity, and inflammatory processes, with potential applications in the treatment of inflammatory and autoimmune diseases. Exploring species-specific differences is crucial to identify optimal sources for clinical and therapeutic applications. This study aimed to characterize the small RNA cargo and metabolites of colostrum EVs (ColosEVs) and milk EVs (mEVs) at two lactation stages (T1 – 15 days, T2 – 60 days post-parturition) in Italian Mediterranean buffalo, using two omics techniques: next-generation sequencing (NGS) and high-performance liquid chromatography–mass spectrometry (HPLC-MS).

MATERIALS and METHODS: EV preparations were characterized by nanoparticle tracking analysis (NTA) and scanning electron microscopy (SEM), confirming vesicles in the expected nanometric range and typical morphology. Representative NTA profiles showed mean particle sizes of ~175.6 ± 3.2 nm in ColosEVs and ~174.0 ± 1.5 nm in mEVs, with concentrations in the order of 10^12 particles/mL (approximately 4.1 × 10^12 particles/mL in ColosEVs and 2.3 × 10^12 particles/mL in mEVs). Conventional flow cytometry was applied through both single-EV and bead-based approaches, monitoring the expression of tetraspanins including CD9, CD81, and CD63.

RESULTS: Colostrum contained more EVs than mature milk, whereas mEV_T1 and mEV_T2 displayed higher molecular diversity and shared the highest number of features. We identified 51 miRNAs enriched in ColosEVs, 81 in mEV_T1, and 79 in mEV_T2. Metabolite analysis revealed 379 molecules particularly contained in ColosEVs compared to mEVs, while 619 and 764 were enriched in mEV_T1 and mEV_T2, respectively. Preliminary integrative multi-omics analyses (mixOmics, R) showed clear class-specific clustering of ColosEVs, mEV_T1, and mEV_T2 in integrated heatmaps and PCA, together with strong miRNA–metabolite associations (correlation cut-off r = 0.85), supporting stage-dependent remodeling of EV molecular cargo. Functional analysis of class-specific miRNA targets highlighted biological categories related to immune/stress response, DNA/RNA metabolism, protein modification/signaling, and transport/localization, while metabolite pathway enrichment suggested dynamic changes in amino acid, lipid, carbohydrate, and signaling pathways across lactation.

CONCLUSIONS: This systems biology approach is essential to decipher the molecular messages of buffalo EVs across lactation stages and to better understand their roles in immune regulation, neonatal development, and potential clinical applications.

Keywords: miRNA, meatbolomics, transcriptomics; NGS; HPLC-MS; immunomodulation, inflammation

Immunogenicity of a Trivalent Inactivated Bovine Respiratory Disease Vaccine Containing Local Pl-3 and BoHV-1 Isolates

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BACKGROUND and OBJECTIVE: Bovine respiratory disease complex (BRDC) is one of the most economically significant infectious disease syndromes affecting cattle worldwide. Major viral agents associated with BRDC include bovine herpesvirus type 1 (BoHV-1), bovine parainfluenza virus type 3 (PI-3), and bovine viral diarrhea virus (BVDV). The antigenic compatibility between vaccine strains and circulating field strains is considered an important determinant of vaccine efficacy. This study aimed to evaluate the immunogenicity of trivalent inactivated vaccine formulations containing local isolates of BoHV-1 and PI-3 together with the reference BVDV NADL strain, and to compare humoral immune responses induced by different adjuvant systems in experimental animal models.

MATERIALS and METHODS: Three candidate trivalent inactivated vaccine formulations containing BoHV-1 strain 8640, PI-3 strain ET-81, and BVDV NADL were prepared using different adjuvants: aluminum hydroxide ($Al(OH)_3$), ISA206, and ISA51. Following vaccination, immunogenicity was evaluated in BALB/c mice. Animals were immunized with four doses at two-week intervals. Neutralizing antibody responses against each viral component were assessed using a serum neutralization assay. Blood samples were collected on days 30 and 90 in mice.

RESULTS: Neutralizing antibody responses varied according to both viral antigen and adjuvant formulation. Among the three viral components, the PI-3 strain ET-81 and BHV-1 strain 8640 consistently induced strong humoral responses in the murine model in the ISA51 and ISA206 formulations. BoHV-1 induced antibody responses which increased over time. In contrast, antibody responses against the BVDV NADL strain remained comparatively low, especially in the aluminum hydroxide formulation. Statistical analysis showed that both PI-3 and BoHV-

1 induced significantly higher antibody titers than BVDV ($p < 0.01$), whereas the difference between PI-3 and BoHV-1 was not statistically significant.

CONCLUSIONS: The results demonstrate that the local PI-3 strain ET-81 and BHV-1 strain 8640 elicited the strongest humoral immune responses among the viral components included in the trivalent vaccine formulation. Oil-based adjuvants, ISA51 and ISA206, enhanced the magnitude and persistence of antibody responses compared with aluminum hydroxide. These findings suggest that locally circulating PI-3 and BoHV-1 strains may represent promising antigenic components for the development of improved BRDC vaccines.

Keywords: Adjuvant comparison, Bovıne Respıratory Dısease, Inactivated vaccine, Local strain

Effects of Colostrum Supplementation with Capsaicin on Passive Immunity Transfer, Health and Growth of Dairy Heifers

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BACKGROUND and OBJECTIVE: Due to the epitheliochorial structure of the bovine placenta, calves do not receive passive immunity in utero. Therefore, colostrum quality and quantity in the first hours of life are critical for transfer of passive immunity (TPI). This study investigates the effect of capsaicin on the absorption of immunoglobulins, metabolites, and enzymes to optimize TPI in neonatal calves, and evaluates its impact on heifer health and growth.

MATERIALS and METHODS: In an Ecuadorian herd of crossbred Holstein cattle at 2500 m altitude, the calves were randomly assigned to one of two treatments groups: a control group (CON, n=8) receiving colostrum equivalent to 10% of their body weight from a colostrum bank via orogastric tube at 1, 12, and 20 hours post-birth, and a capsaicin group (CAP, n=8) receiving 40 mg capsaicin/kg body weight with each colostrum feeding. Adverse effects, immunoglobulins and metabolites were evaluated at 48 hours, while morbidity and mortality, height at the withers, weight, and average daily gain were recorded at 21 months.

RESULTS: The animals exhibited no adverse effects. At 48 hours post-colostrum administration, the CAP group showed significantly elevated serum IgG(21.6 ± 0.43 g/L) compared to the CON group (15.5 ± 0.78 g/L; $p < 0.05$) this represents an increase of 39.35% compared to CON. Total protein, glucose, cholesterol, triglyceride, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase levels in the CAP group were also elevated compared to the CON group ($p < 0.05$).Regarding morbidity and mortality, in the CON group, two animals died from pneumonia and one from diarrhea; and in the CAP group, one animal was euthanized due to a fracture. At 21 months, the CAP group demonstrated significantly greater mean height at the withers (1.39 ± 0.01 m vs. 1.33 ± 0.01 m, p=0.010), weight (385.6 ± 12.18 kg vs. 332.7 ± 12.74 kg, p=0.019), and daily gain (0.610 ± 0.01 kg vs. 0.516 ± 0.02 kg, p=0.019) compared to the CON group.

CONCLUSIONS: The results indicate that administering 40 mg of capsaicin per kilogram of body weight along with colostrum is safe, improves passive transfer of immunity, as well as health and growth in Holstein crossbred calves raised at altitude in Ecuador.

Keywords: Capsaicin, colostrum, immunoglobulins, calves.

Neonatal Calves Exhibit Early Immunocompetence with Distinct Lymphocyte and Cytokine Responses to Bacterial Stimulation

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BACKGROUND and OBJECTIVE: Neonatal calves are born agammaglobulinemic and rely on colostrum-derived passive immunity. Although traditionally considered immunologically immature, evidence shows they can mount early adaptive immune responses with functional biases. This study characterized temporal dynamics of lymphocyte subsets and cytokine production following bacterial stimulation during the first month of life in Holstein calves with adequate passive transfer.

MATERIALS and METHODS: Twenty-eight clinically healthy Holstein heifer calves were evaluated at 3, 7, 14, and 28 days of age. Peripheral blood mononuclear cells (PBMC) were isolated and stimulated with concanavalin A (ConA), Escherichia coli, Staphylococcus aureus, and Staphylococcus hyicus, plus unstimulated controls, and cultured for 3 days at 37°C. Cells were analyzed by flow cytometry (FlowJo) to quantify $CD4^+$, $CD8^+$, $WC1^+$, and $CD25^+$ lymphocytes. Cytokines (IL-10, IL-4, IL-17, IFN-γ) were measured by ELISA. Temporal effects were assessed using one-way ANOVA with Tukey's test.

RESULTS: Unstimulated $CD4^+$ lymphocytes increased from 25.28 ± 18.21 at D3 to 32.23 ± 19.30 at D28 ($p = 0.0051$). $CD8^+$ cells rose markedly from 9.11 ± 11.57 to 35.80 ± 22.18 ($p < 0.0001$). $WC1^+$ lymphocytes peaked at D14 (50.54 ± 22.56) and returned to baseline at D28 (25.61 ± 22.24) ($p < 0.0001$). $CD25^+$ expression remained low and stable (1.38–2.00; $p = 0.83$). $CD4^+$ cells showed temporal changes only under Staphylococcus aureus ($p = 0.015$). $CD8^+$ and $WC1^+$ lymphocytes were significantly modulated under all stimuli ($p < 0.05$), with $CD8^+$ highest at D28 and $WC1^+$ peaking at D14. $CD25^+$ cells varied under S. aureus ($p < 0.0001$) and Staphylococcus hyicus ($p = 0.025$). IL-10 was minimal except under S. aureus, which increased at D7 and D14 and declined at D28 ($p < 0.001$). IL-4 remained low under most conditions but increased under ConA ($p = 1.9 \times 10^{-6}$) at D14 and D28, and under S. aureus ($p = 0.006$), with a modest rise under Escherichia coli at D28 ($p = 0.004$). IL-17 was absent basally but induced by ConA ($p = 0.0027$), S. aureus ($p = 0.02$), and S. hyicus ($p < 0.05$), increasing at D14 and D28; E. coli induced a modest increase at D28 ($p = 0.03$). IFN-γ was detected but showed no temporal differences ($p > 0.05$).

CONCLUSIONS: Neonatal calves show early immunocompetence, with coordinated lymphocyte and cytokine responses. Activation of $WC1^+$ γδ T cells and increased IL-17 between days 14 and 28 indicate a pro-inflammatory bias.

Keywords: Calf immunity, Passive immune transfer, Neonatal immune development, Lymphocyte subsets, Cytokine production.

INFECTIOUS DISEASES

Prevalence of Histophilus Somni in a Dutch Commercial Dairy Herd Affected by Bovine Respiratory Disease

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BACKGROUND and OBJECTIVE: Histophilus somni is frequently associated with bovine respiratory disease (BRD). Little is known about the prevalence of this pathogen on dairy farms in the Netherlands. The objective of this poster is to evaluate the prevalence of Histophilus somni on a commercial dairy farm in the Netherlands, which experienced severe problems with BRD. From January through October 2025, 26 adult cows (18% of a herd of 145 cows) were treated for BRD. During the same period, 45 individual treatments were administered within the group of calves aged two weeks to four months (n=25). Calves within this group received metaphylactic oral antibiotic treatment three times.

MATERIALS and METHODS: In February 2025, bronchoalveolar lavage (BAL) samples were collected from two cows with BRD and analyzed using a quantitative multiplex polymerase chain reaction (qPCR) assay. In April 2025, three pooled BAL samples from untreated calves aged two weeks to four months with BRD were analyzed by qPCR. Additionally, three individual BAL samples from different calves within the same group were cultured for Histophilus somni. In June 2025, 47 samples were collected for qPCR analysis. Both nasal and vulva or preputial swabs were collected from healthy animals, including calves aged younger than two weeks (n=5), calves aged two weeks to four months (n=5), dry cows and heifers (n=5), and a bull (n=1). Additional samples were collected from the bulk

tank (n=1), air (n=2), soil (n=3), teat buckets (n=4), drinking troughs (n=3), and calving ropes (n=1).

RESULTS: Histophilus somni was detected by qPCR in two out of two BAL samples from cows with BRD, two out of three BAL pooled samples from calves with BRD. Culturing on Histophilus somni was positive in one out of three individual BAL samples from calves with BRD. This is 63% (n=8) of samples from animals with BRD. Furthermore, Histophilus somni was detected by qPCR in three out of five nasal swabs from dried off heifers and cows, the nasal and preputial swabs from the bull, the drinking trough of calves aged younger than two weeks and the calving ropes. This is 15% (n=47) of samples from healthy animals and other materials.

CONCLUSIONS: The prevalence of Histophilus somni in a Dutch commercial dairy herd affected by BRD was 63% (n=8) of samples from animals with BRD and 15% (n=47) of samples from healthy animals and other materials.

Keywords: Histophilus Somni, BRD, prevalence, reproductive, respiratory

New Kid on the Block? Bovine Coronavirus Detection in Different Types of Respiratory Samples and Interactions with Other Pathogens and Season

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BACKGROUND and OBJECTIVE: Bovine coronavirus (BCoV) is mainly known as a cause of neonatal calf diarrhea and winter dysentery. However, recently BCoV was shown to also be a (previously disregarded) player in the bovine respiratory disease complex (BRD). BCoV mainly infects the upper respiratory airways, influencing innate immunity barriers such as the mucociliary elevator to be less effective and thus allowing other pathogens easier access to the lungs to cause further damage. This implies that diagnostics could be influenced by the type of sample taken (sampling upper or lower airways). BCoV caused diarrhea has a seasonal pattern, while little is known of BCoV linked BRD. This study aimed to evaluate the incidence of BCoV in different types of BRD samples, the possibility of seasonality (which could allow targeted vaccination programs) and the possible influence on other pathogens.

MATERIALS and METHODS: 2.186 bovine respiratory samples (867 nasal swab,203 bronchoalveolar lavage (BAL),1116 lung), sent in during the years 2024-2025, were analyzed at the ARSIA lab by use of a multiplex PCR (Vetmax Ruminant Respiratory, Thermofisher). All samples were evaluated for the presence of three viral (BRSV, PI-3 and BCoV) and four bacterial pathogens (*P. multocida, M. haemolytica, H. somni* and *M. bovis*). A multivarious logistic regression analysis was performed on the PCR results, including season, sample type, recent purchase and geography as parameters. Unilateral factors having a $p<0.2$ were included in the multivarious analysis with a backwards stepwise approach. Significance was set at $p<0.05$.

RESULTS: Fourteen percent (95%CI: 12.63-15.59) of all samples tested positive on BCoV, making it the most identified virus (BRSV: 12.67%, PI3: 5.35%). Bacterial prevalences were the following: *P. multocida* 54.39%, *M. haemolytica* 30.19%, *M. bovis* 24.02%, *H. somni* 21.73%. Swab samples were significantly more BCoV positive (OR BAL 0.38, OR lung 0.29, $p<0.001$). Seasonality was observed: significantly more samples were positive in autumn (OR 1.63) and winter (OR 2.85) compared to spring and summer. Both *M. haemolytica* & *M. bovis* presence increased the odds of BCoV identification (OR 1.36 [95%CI 1.04-1.77] and OR 1.95 [95%CI 1.47-2.55] respectively). BCoV presence was a significant risk factor for the detection of *M. bovis* (OR 2.12 [95%CI 1.46-3.08], $p<0.001$), but not *M. haemolytica*.

CONCLUSIONS: A higher proportion of BCoV-positive samples was found when using nasal swabs compared to BAL or lung samples. BCoV appears more frequently in BRD samples collected in autumn and winter, when stables are typically more crowded. The link between *M. bovis* and BCoV warrants further research.

Keywords: Bovine respiratory disease, Coronavirus, Mycoplasma bovis

Bovine Coronavirus Circulation in Romanian Feedlot Calves: Respiratory, Enteric and Environmental Detection

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BACKGROUND and OBJECTIVE: Bovine coronavirus (BCoV) is increasingly recognized as a contributor to the bovine respiratory disease (BRD) complex. Data describing (single or simultaneous) respiratory, enteric and environmental circulation in Romanian feedlots are limited. As dams in many Romanian herds are routinely vaccinated with Bovilis® Rotavec® Corona, maternal antibodies may be present in younger calves and can hinder serological surveillance. Therefore, other tests need to be used to determine the prevalence of this disease. OBJECTIVES: To assess the prevalence of BCoV in Romanian feedlots using a multimatrix sampling approach (nasal swabs, rectal swabs, serum and slurry).

MATERIALS and METHODS: Five Romanian feedlots were sampled in 2023. A total of 198 calves were tested at arrival. Nasal and rectal swabs underwent BCoV PCR testing. Serum was tested for anti-BCoV antibodies using a BIO-X BCoV ELISA, reflecting natural exposure and/or maternal immunity in younger animals. Slurry samples were collected to evaluate environmental contamination and also underwent BCoV PCR testing.

RESULTS: BCoV was frequently detected across all matrices. Respiratory presence of BCoV was confirmed in 134/198 nasal swabs, enteric shedding in 131/198 rectal swabs, and 4/5 slurry samples were PCR positive. Serology showed high antibody detection rates (188/198), consistent with widespread exposure and/or maternal immunity, depending on age. PCR-based findings confirm active BCoV circulation at feedlot level.

CONCLUSIONS: Romanian feedlot calves show extensive respiratory, enteric and environmental circulation of BCoV. While serology may reflect both natural exposure and maternal immunity, PCR results clearly demonstrate active viral presence. These findings highlight the importance of preventive measures at feedlot entry, given the role of BCoV in the BRD disease complex.

Keywords: bovine coronavirus, feedlot calves, BRD complex, PCR detection, environmental contamination

A Decade of BRD in Czech Cattle: Insights into Pathogen Burden and the Emerging Role of Bovine Coronavirus in Young Calves (2015–2025)

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BACKGROUND and OBJECTIVE: Bovine respiratory disease (BRD) remains one of the most important health challenges in young calves, causing substantial losses. BRD outbreaks are typically associated with multi-pathogen infections, which may exacerbate disease severity. Bovine coronavirus (BCoV) is increasingly recognized as a relevant respiratory pathogen in calves, potentially acting as a primary agent and predisposing animals to secondary bacterial infections. The aim of this study was to estimate the prevalence of major BRD-associated pathogens in BRD-affected Czech cattle herds and to evaluate temporal trends in BCoV prevalence and characterize BCoV co-infections within the BRD complex.

MATERIALS and METHODS: A total of 828 individual Bronchoalveolar Lavage (BAL) samples were collected from calves up to five months of age showing clinical signs of BRD on 86 Czech farms on 99 outbreaks between 2015-2025. Sampling was repeated on several farms following new BRD outbreaks. At each outbreak, three or six BAL samples per farm were collected at a single time point and pooled in the laboratory (three individual samples per pooled sample). Pathogens were detected using commercial real-time RT-PCR/PCR assays (Biosellal, France). Samples from 44 BRD outbreaks were tested for 7 pathogens: Pasteurella multocida, Mannheimia haemolytica, Mycoplasma bovis, Histophilus somni, BCoV, bovine respiratory syncytial virus (BRSV), and bovine parainfluenza virus type 3 (BPIV-3). From 2019 onwards, influenza D virus (IDV) was included in the diagnostic panel and tested on 55 further BRD outbreaks.

RESULTS: Ninety-two outbreaks (92.9%) were positive for at least one BRD-associated pathogen. Pasteurella multocida was the most prevalent pathogen (88.8%), followed by bovine coronavirus (57.5%). The prevalence of BCoV increased from 12.5% in 2015 to 85.7% in 2022. Among BCoV-positive samples, co-infection with Pasteurella multocida was detected in 96.5% of cases and with Mannheimia haemolytica in 47.3%. Overall, Mannheimia haemolytica was detected in 39.3% of outbreaks, Histophilus somni in 38.3%, BRSV in 16.1%, Mycoplasma bovis in 11.1%, and BPIV-3 in 9.0%. Influenza D virus was not detected.

CONCLUSIONS: This long-term BAL-based study demonstrates a high and increasing prevalence of bovine coronavirus in BRD-affected Czech cattle herds and its frequent co-infection with major respiratory pathogens. These findings support the important role of BCoV within the BRD complex and emphasize the need for comprehensive preventive strategies, including vaccination, to reduce respiratory disease in young calves.

Keywords: Animal Health, Calf health, Infectious disease, BRD, Bovine coronavirus (BCoV), Laboratory results

Evaluation of Minimally Invasive Group Sampling for Diagnosis of Viral Agents with the Greatest Impact in Ruminant Production in México

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BACKGROUND and OBJECTIVE: Background. Viruses infecting ruminants cause systemic or multisystemic clinical signs, leading to decreased production. Diagnosis currently requires invasive individual sampling, which causes stress and reduces the immune response. To minimize stress during sampling, a minimally invasive group sampling method using cotton strings was developed. Objective. To determine the capacity of a minimally invasive group sampling method for the molecular identification of viral agents affecting ruminant production.

MATERIALS and METHODS: A total of 36 corral in different production units were studied: 12 goat, 12 sheep, and 12 cattle. The selection criterion was the observation of clinical signs characteristic of the viral infections to be identified. Oral fluid (OF) samples were collected by placing a cotton string in each pen for 5 to 15 minutes, depending on the species. Conventional oral swab (OS) sampling was performed on three animals in each corral.

RESULTS: The results obtained demonstrate that oral fluid collection using a minimally invasive method with cotton strings allows for molecular diagnosis of Leukemia virus (Lev), Langerhans virus (LvPR), Herpes simplex virus type 1 (HvBo1), Herpes simplex virus type 1 (HvCa1), Pov, BVDV, RSV, and Rv in intensive goat, sheep, and cattle production units.

CONCLUSIONS: Oral fluid sampling using a minimally invasive group sampling method with cotton strings is the preferred method for molecular diagnosis of viruses belonging to the families Retroviridae (bovine leukosis and small ruminant lentivirus), Herspesviridae (capri and bovine herpesvirus), Flaviviridae (bovine viral diarrhea virus), Poxviridae (contagious ecthyma), and Paramyxoviridae (respiratory syncytial virus and respirovirus) that are associated with clinical signs that cause decreased welfare and production in goats, sheep, and cattle in Mexico.

Keywords: sampling, diagnosis, ruminant,

Exploring Alternative Cytokines as Potential Biomarkers for Mycobacterium Bovis Infection in Cattle

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BACKGROUND and OBJECTIVE: Mycobacterium bovis (M. bovis) is the primary agent of Bovine tuberculosis (bTB) in cattle. It represents both a threat to human health and the cattle industry worldwide. Improving bTB diagnostic performance in cattle represents a key step in eradicating the disease. The interferon-gamma (IFN-γ) release (IGRA) blood assay is routinely used in the diagnosis of M. bovis infection, but additional cytokines might be useful as biomarkers of this infection in cattle. In our study, we evaluated the utility of sixteen immune cytokines as diagnostic biomarkers of M. bovis infection.

MATERIALS and METHODS: Fifty-five cattle were used in this study: healthy animals (N = 19), infected (IFN-γ test positive, no post-mortem lesions; N = 17), and affected (IFN-γ test positive, visible post-mortem lesions; N = 19). Heparin blood samples were stimulated in vitro with bovine purified protein derivative (PPD-B), alongside controls. After 18–24 h of incubation, plasma were collected and levels of 16 key cytokines were measured: IL-1α, IL-4, IL-6, IL-10, IL-17, IL-36Ra, MIP-1α, IP-10, MCP-1, TNF, VEGF-A, IFN-γ, IL-23, IL-27, IL-35, and THBS-1.

RESULTS: We observed that both M. bovis exposed cattle (both infected and affected) released higher levels of PPD-B specific IFN-γ and IP-10. On the contrary, only cattle belonging to the affected group released higher levels of PPD-B specific IL-4, IL-17, and TNF compared to healthy subjects. Canonical discriminant analyses (CDA) indicated that IFN-γ, IP-10, IL-4, IL-17, and TNF could be useful biomarkers for infection status. Only a modest separation between infected and affected cattle was observed in the CDA analysis, nevertheless our data suggested that IL-4, IL-10, and TNF might improve, at least in part, the differentiation of cattle in diverse stages of TB infection.

CONCLUSIONS: Our data suggest that the parallel measurement of IFN-γ and IP-10 might improve the diagnosis of M. bovis infection in cattle in terms of sensitivity and specificity, whereas IL-4, IL-10, and TNF might allow the differentiation of cattle in diverse stages of TB infection. Overall, the data generated in our study provide a foundation to improve the diagnosis and staging of M. bovis in cattle. The study was funded by the Italian Ministry of Heath (grants numbers IZS ME 06/24 RC and IZS LER 02/23 RC).

Keywords: Mycobacterium bovis, IFN-γ, biomarkers, cytokines, chemokines.

Mycobacterium Caprae: A Challenge for Disease Control in Tuberculosis-free Regions

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BACKGROUND and OBJECTIVE: Infection caused by Mycobacterium caprae (M. caprae), a member of the Mycobacterium tuberculosis complex (TB), represents a growing epidemiological threat to cattle; despite being usually associated with goats and wild animals, this is particularly concerning in regions already officially recognized TB-free. Lombardy is one of the areas with intensive livestock farming in Europe, recognized TB-free since 2010. Despite this, seven TB outbreaks were identified at the beginning of 2025 and for some it was not possible to establish the origin of the infection. The aim of this study was to highlight the vulnerability of TB-free areas to the introduction of M.caprae.

MATERIALS and METHODS: In Italy, the official control program for bovine TB follows the provisions reported in 2020/689 EU Regulations, which is based on TB testing, post-mortem inspection at slaughterhouse and animal movement control. Surveillance is conducted annually. In case of suspicion, it is necessary to perform an investigation on the farm where the animal comes from and to carry out in-depth analyses of the suspect animal (PCR, bacteriological culture and histopathology). In case of positive results molecular characterization is performed by spoligotyping and mycobacterial interspersed repeat unit-variable number of tandem (MIRU-VNTR).

RESULTS: Based on the results of the surveillance activities conducted in 2025, seven outbreaks of M.caprae were confirmed and the investigations identified different sources. Three cases were related to the introduction of infected animals from other EU countries, identified either through investigations or lesions detected at slaughter. Molecular analysis of these cases revealed different spoligotypes, suggesting different origins. For the other outbreaks, it was possible to identify the farm that triggered the transmission cycle of the infection and reconstruct the links between the involved farms (movements of adult bovine or calves), but it was not possible to trace the origin of the infection. However, all the isolates from these outbreaks shared the same spoligotype and MIRU-VNTR, confirming a common source of infection.

CONCLUSIONS: The introduction and spread of M. caprae in Lombardy, although rapidly controlled, highlights the vulnerability of TB-free regions, especially if located in territories at risk of introduction of M. caprae. It is worth mentioning that due to its epidemiological cycle, the control of TB due to M.caprae is particularly challenging, since it can infect multiple species including wild animals, limiting the effectiveness of control measures which are applied to domestic animals. Therefore, continuous surveillance, strict animal movements control and rapid investigations remain essential to mitigate this risk.

Keywords: Mycobacterium caprae, TB-free areas, TB surveillance

Assessment of Bohv-1 Abortion and Viremia Protection in Seronegative First-Calf Heifers After Intranasal Vaccination in Pregnant Heifers

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BACKGROUND and OBJECTIVE: Herpes virus (BoHV-1) may manifest in multiple clinical forms in cattle including respiratory disease and mid-term pregnancy loss. The objectives of this study were: 1) To demonstrate safety of a commercially available, modified live, intranasal BoHV-1/BRSV/PI3V/Pasteurella multocida/Mannheimia haemolytica vaccine when administered to BoHV-1 seronegative pregnant heifers; 2) to illustrate the ability of this vaccine to protect against respiratory signs in face of BoHV-1 challenge and; 3) demonstrate the pregnancy-sparing attributes of this vaccine.

MATERIALS and METHODS: Seventy-nine seronegative (to BoHV-1), unvaccinated breeding-age heifers received prostaglandin and were bred via estrous detection for artificial insemination or natural service. Pregnancy and seronegative status were confirmed 90 days post-breeding with 47 heifers confirmed pregnant. 45 days later, 22 heifers were enrolled as controls and 25 heifers were vaccinated at 5 months gestation intranasally with 2cc of a commercially available, modified live vaccine (Bovilis Nasalgen 3-PMH) and housed in bio-secure pastures. At 6 months gestation, 20 head from each group were randomly selected and intranasally challenged with (5ml; 2.5 ml/nostril) of BoHV-1 Colorado strain isolated from an IBR fetal abortion at 1x108/ml. Prior to challenge, pregnancy was confirmed and a serum sample collected. Post-challenge they were observed 3x/daily until calving, for clinical scoring as well as abortion assessment. If abortion was observed, samples were collected to evaluate viral infection.

RESULTS: No negative clinical signs or abortions occurred after vaccination, and all 40 animals were confirmed pregnant at the time of challenge. Post-challenge, several controls showed nasal discharge over a 5-day period, starting at day 6, whereas only one vaccinate had nasal discharge on day 7 ($p< 0.05$). Four abortions were observed from the control group whereas all 20 of the vaccinates carried their calves to term ($p< 0.05$). The 4 abortions all occurred within 2 months of challenge, were autolyzed, and virus was detected by PCR, virus isolation, and IHC in kidney of only the 3rd abortion. Serum testing showed that all aborting heifers were seronegative for several other abortive pathogens, except one with a low Leptospirosis pomona titer not indicative of recent infection. Pre-colostral blood samples were taken from calves born to 3 vaccinates and 3 controls and were all BoHV-1 seronegative. 24 hours after colostrum ingestion, all calves were BoHV-1 seropositive.

CONCLUSIONS: This vaccine is safe in BoHV-1 seronegative, pregnant heifers. Most clinical signs and all abortions happened in the control group; among the vaccinates, only one showed nasal discharge for a day, with no abortions observed.

Keywords: BOHV-1, abortion, Prevention, Intranasal, Vaccination,

A Retrospective Analysis of Bovine Respiratory Disease Pathogens Identified from Nasal and Nasopharyngeal Swab Samples from Uk Herds

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BACKGROUND and OBJECTIVE: Bovine respiratory disease (BRD) is a multifactorial disease process which causes significant morbidity, mortality and economic loss in the UK cattle population. Multiple viral and bacterial agents are implicated in the BRD complex, with co-infections being reported to increase disease severity. Developing a greater understanding of the aetiology of BRD and the interactions between the pathogens in UK herds may help veterinarians and producers develop evidence-based treatment and vaccination protocols, leading to reduced antimicrobial usage and improved preventative herd health management. This study aimed to describe the frequency of and interaction between bacterial and viral pathogens detected by RT-PCR from clinical BRD samples submitted over a two-year period to a UK laboratory.

MATERIALS and METHODS: Data were analysed from 395 clinical BRD tests submitted to a Devon-based laboratory by UK veterinary practices via a Boehringer Ingelheim subsidised sampling scheme between January 2024 and December 2025. All submissions consisted of up to three nasal or nasopharyngeal swab samples which were pooled at the laboratory prior to analysis. Samples were tested using RT-PCR to detect nucleic acids from the following bacterial and viral species commonly associated with BRD: M.haemolytica; P.multocida; H.somni; M.bovis; RSV; BoHV-1; PI3 and BCoV.

RESULTS: The majority (n=385; 97.5%) of tests were positive for at least one pathogen, with bacterial species (n=383; 97.0%) more commonly detected than viral species (n=227; 57.5%). Two or more pathogens were found in 89.4% (n=353) of tests, with 83.3% (n=329) of tests detecting multiple bacterial species and 15.7% (n=62) of tests detecting multiple viral species. Most tests were submitted in Winter (n=152; 38.5%), followed by Autumn (n=129; 32.7%), Spring (n=66; 16.7%) and Summer (n=48; 12.1%). Among the bacterial pathogens, P.multocida was most commonly detected (n=368; 93.2%) while BCoV was the most commonly detected virus (n=131; 33.2%).

CONCLUSIONS: The bacterial species implicated in BRD are found commensally in the upper respiratory tract, meaning their significance in single isolation from nasal or nasopharyngeal swab samples can be difficult to interpret. However, as shown in this dataset, the majority of clinical BRD cases demonstrate co-infection with multiple bacterial and viral species which is significant given that multiagent BRD infections are known to be associated with more severe clinical disease. Despite some limitations due to the study's retrospective nature and single laboratory data source, the results presented demonstrate the aetiological factors which must be considered when treating and developing prevention strategies for BRD on UK farms.

Keywords: Bovine respiratory disease, bronchopneumonia, bacteria, virus, molecular diagnostics

Emerging Bovine Respiratory Pathogens in Romania: Molecular and Pathological Findings

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BACKGROUND and OBJECTIVE: The bovine respiratory disease (BRD) complex constitutes one of the leading causes of economic losses in both the beef and dairy cattle industries. This study aimed to assess the prevalence of respiratory pathogens and their associated lesions in Romanian cattle farms.

MATERIALS and METHODS: A total of 174 samples (46 lung, 67 nasal swabs, and 61 bronchoalveolar lavage samples) were analysed by real-time PCR between 2020-2025, targeting the most commonly detected pathogens associated with BRD: Bovine respiratory syncytial virus (BRSV), Bovine Parainfluenza virus type 3 (PI3), Bovine Coronavirus (BCoV), Influenza D virus (IDV), Bovine Viral Diarrhoea virus (BVDV), Pasteurella multocida (PM), Mannheimia haemolytica (MH), Mycoplasma bovis (MB), and Histophilus somni (HS). Pathogen prevalences during the period 2020–2022(P1) were compared to findings of 2023-2025(P2) to assess temporal changes and associated lesions. Additional testing for Bovine Rhinitis Virus type A(BRVA), Bovine Rhinitis Virus type B(BRVB), and Bovine Adenovirus type 3(BA3) was performed for only P2. Lung tissue samples were also analysed by histopathology. All analyses were conducted at the Synevovet Laboratory (Romania).

RESULTS: The most frequently detected bacterial pathogen was PM, with a P1 prevalence of 29%, decreasing to 21% in P2. This was followed by MB (16% to 14%),MH (13% to 15%) and HS (9% to 6%). Among viral pathogens, BCoV showed an increase from 15% to 20%. Respiratory viruses such as PI3 (5% P1&P2), BRSV (5% to 3%) and BVDV (3% P1&P2) maintained relatively constant values, suggesting a persistent endemic circulation. BRVA, BRVB, and BA3 were included exclusively in P2, with prevalences of 7%, 8%, and 2%, respectively. Histopathology showed pulmonary lesions matching detected pathogens. Key findings were bronchointerstitial pneumonia with thickened alveolar septa, inflammatory cells, and airway epithelial degeneration. PM bacterial infections led to suppurative bronchopneumonia; viral pathogens caused interstitial pneumonia and epithelial changes.

CONCLUSIONS: The results highlight a constant predominance of the bacterial agents PM, MB and MH, along with BCoV, which remain the main pathogens involved in BRD. PM recorded the highest prevalence, with a decreasing trend, while BCoV showed an increase in recent years. Viruses as BRVA&B and BAV3 were shown to be present in a minority of samples. The data indicate epidemiological changes in the structure of respiratory pathogens, highlighting the need to update prevention, diagnosis and control strategies in cattle herds.

Keywords: Bovine health, Laboratory diagnostic, Infectious disease, BRD Patogens,

Prevalence of Salmonella Antibodies in Bulk Tank Milk from Dairy Herds in Flanders, Belgium

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BACKGROUND and OBJECTIVE: The prevalence of salmonellosis in Belgian cattle has been studied only sporadically, partly because Belgium lacks a national control program comparable to neighboring countries. Field observations suggest increasing Salmonella occurrence in cattle herds, potentially contributing to rising human Salmonellosis cases. Bulk tank milk (BTM) antibody testing can provide a herd-level indicator of Salmonella exposure. This study aimed to estimate the prevalence of

Salmonella in Flemish dairy herds using a province-weighted design to obtain a representative regional estimate and allow provincial comparisons.

MATERIALS and METHODS: A cross-sectional study was performed on 135 dairy farms in Flanders using province-proportionate stratified random sampling from the official registry of active dairy farms. The target sample size (130 herds) was calculated assuming an expected prevalence of 50%, a 10% margin of error at 95% confidence, a design effect of 1.1, finite population correction (N = 4,210 herds), and 25% inflation for nonresponse; minor deviations led to 135 sampled herds. Farms were allocated by province (West Flanders, East Flanders, Antwerp, Limburg, Flemish Brabant). One BTM sample per farm was collected during routine milk collection and tested with a commercial ELISA for Salmonella antibodies. Herds were classified as positive or negative using the manufacturer's cut-off; no borderline results occurred. Apparent prevalence and 95% confidence intervals (CIs) were calculated overall and by province.

RESULTS: Of 135 herds, 18 were ELISA-positive, giving an overall apparent herd-level prevalence of 13.3% (95% CI: 8.1–20.3%). Provincial prevalences were 17.9% (5/28; 95% CI: 6.1–36.9%) in Antwerp, 15.4% (6/39; 95% CI: 5.9–30.5%) in East Flanders, 13.3% (6/45; 95% CI: 5.1–26.8%) in West Flanders, 7.7% (1/13; 95% CI: 0.2–36.0%) in Limburg, and 0% (0/10) in Flemish Brabant. Provincial estimates did not differ significantly, but prevalence tended to be higher in herd-dense areas. No province appeared clearly free from exposure, except Flemish Brabant, however cautious interpretation as a result of the limited sample size is necessary.

CONCLUSIONS: This province-weighted survey suggests that roughly one in seven to ten dairy herds in Flanders has detectable Salmonella antibodies in BTM, indicating ongoing or past herd-level exposure. These estimates provide baseline information for spatial analyses, risk factor investigations, and the design of targeted Salmonella control or vaccination strategies in the Flemish dairy sector.

Keywords: Salmonella, Antibodies, Salmonella dublin, tank milk

Whole-Genome Sequence of Mycobacterium bovis Strain CJ-30 Isolated from a Farmed Elk (Curvus elaphus nelson) in Korea

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BACKGROUND and OBJECTIVE: Mycobacterium bovis (M. bovis), a member of the Mycobacterium tuberculosis complex, is the causative agent of bovine tuberculosis in a wide range of domestic and wild animals. In this study, we report the draft genome sequence of M. bovis strain CJ-30, isolated from the lung of a farmed elk (Cervus canadensis) in Korea.

MATERIALS and METHODS: This strain CJ-30 was a predominant genotype, accounting for 33.6% of the MIRU-VNTR genotypes identified in cattle and deer in Korea. Genomic DNA was extracted from a culture-confirmed isolate and sequenced using the Illumina platform.

RESULTS: The draft genome is approximately 4.33 Mb in length, with a GC content of 65.6%, and contains 3,940 predicted protein-coding sequences, along with tRNA and rRNA genes. Comparative genomic analysis revealed high sequence similarity to the reference strain AF2122/97 and identified genomic regions characteristic of M. bovis.

CONCLUSIONS: This genome sequence provides valuable insights into the molecular epidemiology, transmission dynamics, and potential host adaptation of M. bovis circulating in domestic deer populations in Korea.

Keywords: Mycobacterium bovis, Whole-Genome Sequence, Elk

Surveys for Antibodies Against *Coxiella burnetii* in Dairy Cattle Herds in Finland Indicates Continuous Low-Level Exposure

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BACKGROUND and OBJECTIVE: Q fever is a zoonotic disease caused by *Coxiella burnetii*, an obligate intracellular bacterium. The disease is endemic worldwide apart from New Zealand. Domestic ruminants such as cattle, sheep and goat are considered as a main reservoir of *C. burnetii*. The seroprevalence rates reported in cattle population by different countries vary greatly, ranging up to 84%. Of the Scandinavian countries in Denmark increasing prevalence, up to 79 %, have been reported. In Sweden, prevalence of antibodies shows large regional differences, whereas in Norway the disease appears to be absent or at least the prevalence is very low. In Finland, antibodies against *C. burnetii* were first detected in 2008 in a bovine animal tested due to export. In the following investigations on the farm of origin other seropositive animals were found and *C. burnetii* bacteria was detected in bulk tank milk (BTM) by PCR. The aim of this study was to monitor the occurrence of *C. burnetii* antibodies in BTM of Finnish dairy herds to illustrate possible changes in prevalence.

MATERIALS and METHODS: The samples were collected as a part of Finnish national monitoring for bovine diseases in 2009, 2018 and 2025. The BTM samples from randomly selected dairy herds were tested for *C. burnetii* antibodies (ELISA). The proportion of herds tested ranged from 7-10% in different years. Apparent prevalence was calculated with Epitools.

RESULTS: The number of tested herds in 2009, 2018 and 2025, were 1238, 485 and 329, respectively. The apparent prevalence of *C. burnetii* antibodies was 0.2% (0.08-0.6%, 95% CI) in 2009, 1.2% (0.6-2.7%) in 2018, and 1.2% (0.5-3.1%) in 2025.

CONCLUSIONS: The prevalence of *C. burnetii* antibodies in Finnish dairy herds can be considered low, and there are no indications of increasing trend. Although the current prevalence is low, continued monitoring is necessary to determine whether an increase similar to that observed in some other European countries might occur.

Keywords: seroprevalence, apparent prevalence, Coxiella burnetii, Q fever

Seroprevalence of Bovine Viral Diarrhea Virus in Omani Cattle from Different Governorates

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BACKGROUND and OBJECTIVE: Bovine viral diarrhea virus (BVDV) is a major cause of production losses and reproductive disorders in cattle worldwide, but data from the Arabian Peninsula remain scarce. This study aimed to estimate the seroprevalence and spatial distribution of BVDV infection in cattle across Oman and to identify major risk factors to inform national control strategies.

MATERIALS and METHODS: A cross-sectional survey was implemented between August 2017 and July 2018 in all 11 governorates of Oman using a two-stage sampling design. In total, 1,758 cattle from 442 herds were randomly selected from all wilayats and stratified by age, breed and sex. Sera were tested for antibodies against BVDV using a commercial competitive ELISA targeting anti-p80-125 (NSP2-3) antibodies. Herd management, movement and health data were collected via structured questionnaires, and herds were geo-referenced for spatial analysis.

RESULTS: Overall, 402 of 1,758 cattle were seropositive, yielding an individual seroprevalence of 22.9% (95% CI 20.9–24.9). At herd level, 203 of 442 herds were positive, corresponding to a seroprevalence of 45.9% (95% CI 41.2–50.7). Herd-level seroprevalence was highest in Dhofar (89.6%) and moderate to high in several northern governorates, indicating widespread circulation with a distinct hotspot in the south. Individual seroprevalence ranged from 7.8% in Muscat to 64.0% in Dhofar. Older cattle (>5 years, 30.6%), crossbred (27.6%) and imported animals (23.7%), and females (23.9%) had higher odds of seropositivity. At herd level, higher seroprevalence was associated with location in coastal and highland areas of Dhofar (87.2%), transhumant herds (92.8%), herd size >50 head (72.2%), use of common pastures (57.1%), absence of quarantine for new introductions (47.1%) and reported history of diarrhea (68.4%).

CONCLUSIONS: This first national-scale investigation demonstrates high and spatially clustered BVDV seroprevalence in Omani cattle herds, with Dhofar emerging as a key hotspot. A comprehensive control strategy integrating strengthened biosecurity, enforced quarantine, targeted testing and strategic vaccination is urgently warranted, particularly in high-density and transhumant systems.

Keywords: Bovine Viral Diarrhea, Seroprevalence, Cattle, Oman

Detection of Circulating Cell-Free DNA in Farmed Elk (Curvus elaphus nelsoni) Naturally Infected with Mycobacterium bovis in Korea

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BACKGROUND and OBJECTIVE: Bovine tuberculosis (BTB) is a chronic infectious disease primarily caused by Mycobacterium bovis (M. bovis) In Korea, deer including elk are raised for antler production, however, routine tuberculosis surveillance has not been consistently implemented, even though the tuberculin skin test (TST) can be applied to deer.

MATERIALS and METHODS: In this study, we evaluated the detection of circulating cell-free M. bovis DNA in blood using a one-tube nested real-time PCR assay targeting the rpoB and IS6110 sequences of M. tuberculosis.

RESULTS: Among the 51 deer tested, 27 (52.9%) were positive by the tuberculin skin test, and 17 (33.3%) showed granulomatous lesions in the lungs. Furthermore, single-tube nested real-time PCR performed on plasma samples from deer with gross lesions yielded a positivity of 64.7% (11/17). Among the 27 deer positive on the tuberculin skin test, 40.7% (11/27) were PCR-positive.

CONCLUSIONS: These results demonstrate the potential utility of circulating cell-free DNA as a direct molecular diagnostic method for M. bovis, offering a less invasive alternative to conventional immunological testing and post-mortem tissue-based culture or PCR methods.

Keywords: Mycobacterium bovis, circulating cell-free DNA, Elk, one-tube nested real-time PCR

Detection Of Neospora Caninum in Bovine Abortion Cases from a Major Cattle-Producing Region of Brazil

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BACKGROUND and OBJECTIVE: The protozoan Neospora caninum is a major cause of abortion in cattle and is widely distributed in Brazil. Confirming its role in abortion requires correlating pathogen detection with compatible histological lesions. This study aimed to determine the prevalence of PCR-positive aborted bovine fetuses for N. caninum and to compare PCR results with histopathological and immunohistochemical (IHC) findings, including geographic distribution across different biomes.

MATERIALS and METHODS: A retrospective observational study was conducted between 2012 and 2024, involving 192 bovine abortion cases submitted to the Veterinary Pathology Laboratory in Mato Grosso from 176 farms. Gestational age ranged from 3 to 8 months. All fetal brains were tested by real-time PCR for N. caninum. All fetuses were examined histologically, in a blinded evaluation, across multiple organs, and were also subjected to IHC in brain, skeletal muscle, and heart. Epidemiological data, degree of autolysis, gestational age, and geographic origin were recorded, including classification of farms within the Cerrado, Amazon, and Pantanal biomes.

RESULTS: In total 43 out of 192 cases (22.4%) tested positive by PCR for N. caninum. The apparent prevalence was 24% at the farm-level. Most positive cases were in the state of Mato Grosso (74.4%), mainly distributed across the Cerrado (43.8%), Amazon (40.6%), and Pantanal (15.6%) biomes. The majority herds were dairy (54.8%) and 40.5% were beef. N. caninum abortions occurring mainly during the second trimester. No significant gross lesions were observed, and 76.7% of fetuses were autolyzed. Among PCR-positive cases, 69.8% had histological lesions consistent with protozoal infection; non-suppurative encephalitis (70%), non-suppurative myositis (53.3%) and myocarditis (36.7%). In fetuses with cerebral liquefaction, skeletal muscle lesions were essential for histological diagnosis. IHC was positive in only 33.3% of cases with, indicating lower sensitivity compared to PCR.

CONCLUSIONS: N. caninum is an important cause of bovine abortion across different biomes of Central-Western Brazil. Although Mato Grosso has the largest beef cattle population in Brazil, most cases involved dairy cattle, probably due to closer pregnancy monitoring and smaller herd sizes on dairy farms. PCR proved to be an effective screening tool with good agreement with histopathology, whereas IHC showed limited sensitivity. Skeletal muscle emerged as an important ancillary tissue, particularly in cases with brain autolysis. These findings reinforce the importance of an integrated diagnostic and epidemiological approach in the investigation of reproductive losses in cattle production systems.

Keywords: Neospora caninum, abortion, Brazil

Unravelling the Mechanism of GP40-Mediated Passive Immunity Against Cryptosporidiosis

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BACKGROUND and OBJECTIVE: *Cryptosporidium spp.* are among the leading causes of diarrhoeal disease worldwide, second only to rotavirus. In humans, cryptosporidiosis accounts for approximately 200,000 deaths annually, disproportionately affecting children under five years of age and immunocompromised individuals. In livestock, the disease imposes substantial economic losses estimated at billions of USD each year. Despite its major impact on global public and animal health, highly effective therapeutics and vaccines remained unavailable until recently. Recently a licensed livestock vaccine was developed, designed to confer passive immunity through bovine colostrum targeting the *Cryptosporidium parvum* GP40 antigen. Although this vaccine significantly reduces disease severity, it does not prevent parasite transmission or oocyst shedding. This study investigated the mechanisms underlying passive immunity mediated by bovine colostrum using an in vitro infection model alongside microbiome profiling of vaccinated and unvaccinated calves.

MATERIALS and METHODS: A blocking assay was performed using Colo-680N cells infected with excysted *C. parvum* oocysts. The binding and inhibitory activity of hyperimmune bovine colostrum (HBC), non-immune colostrum (NC), and a

monoclonal anti-GP40 antibody (4E9) were evaluated using fixed-cell immunofluorescence microscopy.

RESULTS: At a single stock concentration, both HBC and NC demonstrated significant blocking activity, indicating inherent anti-cryptosporidial properties in both preparations. However, serial dilution experiments revealed distinct concentration-dependent differences, with enhanced inhibitory activity observed in HBC at specific thresholds. These findings support antigen-specific vaccine-induced effects, while the activity observed in NC likely reflects pre-existing, non-specific anti-*Cryptosporidium* antibodies resulting from prior environmental exposure of dams.

CONCLUSIONS: Having identified concentration ranges at which HBC exhibits superior anti-cryptosporidial activity relative to NC, ongoing work will further characterise these effects using functional blocking assays. These studies aim to elucidate the immunological mechanisms driving passive protection, including host innate immune responses, parasite replication dynamics, and host cell apoptosis. In parallel, faecal microbiome profiling of vaccinated and unvaccinated calves will assess vaccine-associated shifts in gut microbial composition and potential pro-inflammatory dynamics.

Keywords: Cryptosporidium, gp40 antigen, immuno-flourescence microscopy, bovine colostrum, passive immunity

First Isolation of *Moraxella bovoculi* from Infectious Bovine Keratoconjunctivitis (IBK) Cases in Chile

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BACKGROUND and OBJECTIVE: Infectious bovine keratoconjunctivitis (IBK) is the most common ocular disease of cattle worldwide, characterized by photophobia, blepharospasm, lacrimation, corneal edema, and corneal ulcers with several degrees of scarring that could culminate in corneal rupture and permanent blindness, resulting in decreased weaning weight of animals and economic losses. The causative agent of IBK has historically been cited as *Moraxella bovis*, the only pathogen to reproduce IBK-like ocular lesions in experimental models. Otherwise, in recent years, the frequency of isolating *M. bovoculi* from IBK cases has increased, leading to its postulation as a relevant causative agent of IBK and thus arousing interest in studies of this pathogen. However, the experimental reproduction of the disease has not yet been achieved, and its role in the disease remains unclear. To the best of the author's knowledge, there are no published studies on IBK and its etiological agents in Chile. The present study reports for the first time the presence of *M. bovoculi* associated with IBK cases in Chile.

MATERIALS and METHODS: Thirteen isolates suggestive of *Moraxella* spp. (small colonies with complete hemolysis on blood agar plates and Gram-negative diplococci) were obtained through microbiological culture from IBK cases from four different farms in southern Chile. For species identification, DNA from colonies from pure culture was extracted and subjected to PCR-RFLP and PCR targeting the partial 16S rRNA gene, followed by sequencing.

RESULTS: Electrophoresis of PCR-RFLP products from all isolates showed two bands (150 and 450 bp), indicative of *M. bovoculi*, and BLASTn analysis of the 16S rRNA sequences showed 100% identity with *M. bovoculi* sequences.

CONCLUSIONS: All isolates in the present study were identified as *M. bovoculi*, indicating the absence of *M. bovis*, considered the principal causal agent of IBK. Recent studies support a progressive shift in etiological prominence toward *M. bovoculi*, both as the predominant agent and as the only microorganism detectable in clinically affected animals, a situation that could explain the ineffectiveness of some prevention programs based on animal immunization that include vaccines usually based on *M. bovis* antigen, which may not be effective against other *Moraxella* species. This highlights the importance of recognizing the *Moraxella* species involved in IBK. Future studies on IBK prevalence and the etiological agents

involved are necessary to enhance vaccination strategies and optimize existing vaccines based on locally generated evidence.

Keywords: Infectious bovine keratoconjunctivitis (IBK), Moraxella bovoculi, Moraxella bovis, Moraxella spp., PCR-RFLP., 16S rRNA gene

Is the Interferon-gamma Release Assay Able to Predict the Confirmation of Tuberculosis at Slaughter in the Mediterranean Water Buffalo (bubalus Bubalis)?

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BACKGROUND and OBJECTIVE: Official ante-mortem tests for tuberculosis (TB) in Mediterranean buffaloes (Bubalus bubalis) include single and comparative intradermal tests and the interferon-gamma (IFN-γ) release assay (IGRA). In IGRA, the IFN-γ production by blood lymphocytes stimulated with bovine or avian purified protein derivatives (PPDs) is quantified by ELISA and expressed as optical density (OD); results are positive when the bovine PPD response exceeds baseline and avian PPD response by ≥0.1 (ΔOD). Animals that test positive to any ante-mortem test are classified as TB suspects. According to EU regulations, TB is confirmed by detecting visible lesions (VLs) at post-mortem examination (PME) and/or identifying Mycobacterium tuberculosis complex (MTBC) by culture. Given the limitations of both ante- and post-mortem tests, this study aimed to evaluate the positive predictive value (PPV) of IGRA in predicting TB infection and to compare median ΔOD levels between confirmed (M+) and non-confirmed (M−) TB cases.

MATERIALS and METHODS: This work combines a longitudinal study (Study1) and a retrospective study (Study2), including 36 and 818 IGRA+ buffaloes, respectively. All animals were classified as TB suspects and slaughtered. A case was confirmed when VLs were detected at PME and/or MTBC was identified. The PPV was calculated as the proportion of M+ among IGRA+ cases and median ΔOD values were compared using the Wilcoxon rank-sum test.

RESULTS: The disease was confirmed in 13/36 (Study1) and 488/818 (Study2) suspected cases, corresponding to PPVs of 36.1% and 54.8%, respectively. Specifically, VLs at PME were found in 11/13 (84.6%) cases in Study1 and 452/488 (92.6%) cases in Study2, whereas MTBC was identified in 9/13 (69.2%) and 80/488 (16.4%) cases, respectively. In both studies, M+ cases showed higher median ΔOD values (Study1: ΔOD= 0.52, IQR= 0.86; Study2: ΔOD= 0.97, IQR= 1.04) compared with M- cases (Study1: ΔOD= 0.17, IQR= 0.3; Study2: ΔOD= 0.68, IQR= 0.86), with a p-value <0.05.

CONCLUSIONS: The findings showed low PPVs in IGRA, suggesting a low sensitivity of the confirmatory tests, given the limitation of both microbiology and VLs detection at PME. Moreover, IGRA interpretation must be individually tailored to the specific needs and context in which it is applied as the prevalence of the disease and performance fluctuation during low-prevalence eradication phases may also have an impact on PPVs. Higher median ΔOD levels were identified in M+ TB cases, suggesting a robust species-specific immune response to PPDs stimulation in buffaloes. Further research is warranted to explore this phenomenon.
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Keywords: Tuberculosis, Buffalo, Diagnostics

Ways to Implement New Cattle Vaccines Against Mycoplasma Bovis: Drivers for Adopting Calf Vaccination from the Farmers' Perspective

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BACKGROUND and OBJECTIVE: Calf vaccination can reduce disease burden and antibiotic use in cattle, but uptake varies across regions and production systems. Farmers' needs and decision drivers for adopting vaccination as a first-line option are not well understood. *Mycoplasma bovis* causes respiratory disease and immunosuppression, resulting in substantial economic losses. This study aimed to identify drivers for adopting vaccination, using *M. bovis* calf vaccination as a use-case.

MATERIALS and METHODS: A survey based on an established questionnaire adapted from the RESET Mindset Model was conducted among German farmers between November 2024 and February 2026. Items were rated for importance (0 = not important, 10 = essential) and complemented by open responses. The model assessed key behavioral cues: guidelines/rules/regulations, education/information/awareness, social pressure/changing society, economic incentives (ROI), and tools/feasibility.

RESULTS: Data were collected from 36 farmers (23 male, 13 female) from Northwest (72%) and Southeast Germany (28%). Participants were aged 18–35 years (70%), 36–50 years (14%), 50–60 years (14%), and >60 years (2%). Farm types included dairy (48%), mixed dairy and beef (26%), and beef (26%). Overall, 59% of operations reported vaccinating animals; vaccination was reported by 66% of dairy farms and 84% of beef farms, most commonly targeting BRD. Awareness of *M. bovis* was high (86% overall; dairy 80%, beef 100%). Farmers primarily associated *M. bovis* with BRD (40% of responses) and less frequently with arthritis (10%). The strongest drivers for adoption were expected animal health benefits (median 10) and farm-level economics/ROI (median 9). While only 33% expressed specific interest in product price, respondents emphasized the importance of farm-specific profitability. Education and information (median 7), tools and feasibility factors such as practical protocols and perceived effectiveness (median 7), and veterinarian recommendation (median 7) were also influential. In contrast, "use by other farmers" was of low relevance (median 4). Social pressure, image considerations, and regulatory guidance were weaker overall (median 6), though more relevant among female farmers and those aged 18–35 years.

CONCLUSIONS: Implementation strategies should prioritize farm-calculable ROI, clear and practical information, and easy-to-use protocols and tools, supported by veterinary guidance. Because rules, norms, and social pressure were not strong drivers overall (despite being more relevant in younger and female subgroups), messaging focused solely on regulation or peer uptake is unlikely to substantially increase adoption at population level.

Keywords: calf vaccination, M. bovis, farmer decision-making;

Prevalence Of Brd Cattle Pathogens in Germany Using Non-Endoscopic Bronchoalveolar Lavage

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BACKGROUND and OBJECTIVE: For diagnosis of bovine respiratory disease complex (BRDC), deep nasopharyngeal swabs, bronchoalveolar lavage (BAL), and transtracheal wash are commonly used (Van Driessche et al., 2017). This report summarizes results obtained under field conditions using a non-endoscopic bronchoalveolar lavage (neBAL) method. Previous work showed good on-farm practicality and safety for this technique (Löschner et al., 2024).

MATERIALS and METHODS: During routine veterinary support, five veterinarians from Zoetis Germany collected BAL fluid (BALf) from calf groups showing clinical signs compatible with BRDC using a neBAL examination set (Easy Lavage, IVET, Germany). Sampling occurred from January 2024 to December 2025. On each farm, BALf from 3–5 calves was pooled and tested by multiplex real-time PCR (VetMAX™ Ruminant Respiratory Screening PCR Kit,

Thermo Fisher Scientific Inc., USA). Associations between pathogen detection (presence/absence) and year (2024 vs. 2025) and region (Northwest, East, South, Southwest) were analyzed in IBM SPSS Statistics v29.0 using chi-square and median tests; p≤0.05 was considered significant.

RESULTS: 129 pooled samples (53 in 2024, 76 in 2025), mostly from dairy operations were collected. The vaccination status was not documented. The neBAL procedure was easily and rapidly performed on farm; no preparation or sedation of the animals was required. The results are consistent with those of Löschner et al. (2024). Only 5.4% of the samples were negative for all targets. The most frequently detected pathogen was *Pasteurella multocida* (87.6% of the samples), no differences between year (p=0.18) and region (p=0.18) observed. *Mannheimia haemolytica*: 43.4% positive; year (p=0.33); region (p=0.35). *Histophilus somni*: 43.4%; year (p=0.19), region (p=0.18). *Mycoplasmopsis bovis*: 40.3%; year (p=0.33), region (p= <0.001, Max 72% Northwest, Min 23% South). *Bovine coronavirus*: 26.4%; year (p=0.7), region (p=0.38). BRSV: 12.4%; year (p=0.75), region (p=0.83). Parainfluenza-3: 6.2%; year (p=0.83), region (p=0.86).

CONCLUSIONS: Under field conditions, neBAL provided a minimally invasive and practical method to collect BALf from calves in a short time without sedation, enabling pathogen-specific BRDC diagnostics. *P. multocida* was the most frequently detected pathogen overall, while *M. bovis* showed marked regional predominance in Northwest Germany. The neBAL method appears suitable for routine veterinary practice to support targeted therapy and prophylactic decision-making in BRDC cases.

Keywords: Diagnosis, BAL, BRD

Antimicrobial Susceptibility Patterns of *Salmonella* Dublin Strains Isolated from Dairy Cattle in Italy (2022–2025)

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BACKGROUND and OBJECTIVE: Salmonella enterica subsp. enterica serovar Dublin (S. Dublin) is a bovine-adapted zoonotic pathogen responsible for invasive disease in cattle and humans. In dairy herds, it frequently causes severe systemic infections in calves, while adults may remain asymptomatic carriers, contributing to persistence and production losses. The increasing occurrence of antimicrobial and multidrug resistance further complicates control and therapeutic strategies, often limiting treatment to supportive care, highlighting the growing impact of this pathogen on animal health and dairy productivity. This study aimed to assess the antimicrobial susceptibility of S. Dublin strains isolated from clinically diseased dairy cattle in Italy during 2022-2025.

MATERIALS and METHODS: A total of 261 S. Dublin strains were included in this study, obtained from symptomatic dairy cattle submitted to the diagnostic laboratories of the Istituto Zooprofilattico Sperimentale of Lombardia and Emilia-Romagna (Northern Italy) between 2022 and 2025. Samples, including carcasses, fetuses, and viscera, were collected from animals on dairy farms where outbreaks occurred. Isolation followed standard bacteriological methods throughout the study. Biochemical identification used the EnteroPluri-Test (Liofilchem, Teramo, Italy), and serotyping followed ISO/TR 6579-3: 2014. Antimicrobial susceptibility was tested on 226 isolates via broth microdilution to determine minimum inhibitory concentrations (MIC), following CLSI guidelines. Susceptibility to ampicillin, amoxicillin-clavulanic acid, colistin, enrofloxacin, florfenicol, flumequine, sulfisoxazole, tetracycline, and trimethoprim-sulfonamides was assessed using a commercial panel (Thermo Fisher Scientific, Waltham, MA, USA). MIC was defined as the lowest concentration preventing visible growth. Results were interpreted according to CLSI breakpoints (VET08, 2018; M100,

2019), except for colistin and enrofloxacin (EUCAST v11, 2021) and flumequine (CASFM, 2020). Intermediate and resistant phenotypes were classified as non-wild type (NWT).

RESULTS: In the examined population, ampicillin (222/226, 98.2%) and colistin (78/226, 34.5%) showed higher percentages of NWT strains followed by florfenicol (76/226, 33.6%), sulfisoxazole (42/226, 18.6%), enrofloxacin (38/226, 16.8%), tetracycline (24/226, 10.6%), trimethoprim-sulfamethoxazole (11/226, 4.9%) Amoxicillin-clavulanic acid (4/226, 1.8%) and cefotaxime (2/226, 0.9%) exhibited the lowest proportion of NWT strains. Out of the 226 strains analysed, 89 (39.4%) isolates exhibited multidrug-resistant (MDR) profiles.

CONCLUSIONS: This study reports antimicrobial resistance in S. Dublin from dairy cattle, highlighting MDR strains. Ampicillin resistance is of concern, as it is used as first- and second-line therapy for enteric and septicemic infections, respectively, while colistin use should be minimised under national and international guidelines. Treatment options are further limited because cephalosporins and aminoglycosides are often ineffective in vivo against Salmonella. Overall, these findings emphasize the challenges in controlling infections and promoting prudent antimicrobial use in dairy herds.

Keywords: Salmonella Dublin, antimicrobial resistance, dairy cattle

Initial Experiences in the Detection of *Mycoplasmopsis bovis* in Calves Using a Diagnostic Rope

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BACKGROUND and OBJECTIVE: Detecting Mycoplasmopsis bovis (M. bovis) is important for management decisions on prevention, hygiene, and targeted therapy of bovine respiratory disease (BRD) in calves. Chewing ropes are established as a diagnostic tool, and M. bovis has been described in calf saliva (Bernheim et al., 2025). Therefore, saliva sampling via chewing ropes may be an alternative or a useful complement to existing sampling methods for respiratory pathogens. This study evaluated a new non-invasive, cost-effective, and easy-to-use sampling approach using salivette-coated cotton ropes ("diagnostic rope", DR) in calves. The objectives were to assess practicality and to estimate diagnostic value based on the detection rate of M. bovis.

MATERIALS and METHODS: Seventeen calf groups with clinical signs of BRD from 16 farms were examined (15 dairy farms; 2 groups from a calf-rearing unit). Groups comprised 5–10 calves aged 4–12 weeks. DRs (BioCheck GmbH, Leipzig, Germany) were placed within easy reach of all animals for 30–60 minutes. Collected saliva was analyzed by PCR for M. bovis (VetMAX™ Ruminant Respiratory Screening Kit, Thermo Fisher Scientific, USA). For indirect comparison, detection rates from pooled non-endoscopic bronchoalveolar lavage (neBAL) samples—obtained from 3–5 clinically affected calves per farm across 80 farms and PCR tested—were used (Löschner et al., 2024).

RESULTS: DR sampling was non-invasive, less costly, and easier to perform in terms of time and labor, requiring no direct animal handling. The M. bovis detection rate using DR saliva samples was 24%. In indirect comparison, Löschner et al. (2024) reported a 43% detection rate using neBAL. Because the comparison was not conducted in parallel within identical animal groups, results should be interpreted as a practical, field-based assessment. DR acceptance varied between individuals and appeared reduced in BRD-affected calves. Differences in sample matrix, sampling technique, and potential method sensitivity may also have influenced detection.

CONCLUSIONS: Diagnostic ropes for saliva collection appear suitable for detecting respiratory pathogens in calves, as shown here for M. bovis. The method is animal-friendly, practical, and cost-effective. Further studies are needed to fully evaluate diagnostic performance, ideally including parallel comparisons with neBAL, serology, and nasal swabs.

Keywords: detection, M. bovis, diagnostic rope, BRD

Seroprevalence and Antibody Dynamics Following Lumpy Skin Disease Vaccination in Korean Native Cattle

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BACKGROUND and OBJECTIVE: Lumpy skin disease (LSD) is a major infectious disease of cattle and was first detected in the Republic of Korea in October 2023. Vaccination is the primary strategy for LSD prevention, and the Republic of Korea has maintained an ongoing vaccination program. However, field data comparing antibody responses by the number of vaccine doses received remain limited. This study evaluated the association between the number of vaccine doses received and humoral immune responses to lumpy skin disease virus (LSDV) under commercial farm conditions.

MATERIALS and METHODS: A total of 730 Hanwoo (Korean native cattle) aged 1–12 years were included at baseline. Samples were collected at two time points (baseline, n=730; and 6 months later), with paired results available for 661 cattle. LSDV-specific antibodies were measured using the ID Screen® Capripox double antigen Multi-species ELISA kit (IDvet®, Montpellier, France), following the manufacturer's instructions. No clinical LSD outbreaks were reported during follow-up. Seropositivity was compared across dose groups defined by the number of vaccine doses received.

RESULTS: Baseline seroprevalence of LSDV-specific antibodies was 64.1% (468/730). Seropositivity increased with the number of vaccine doses received (21.0% after one dose, 47.5% after two doses, and 76.5% after three doses). In the one-dose group with paired results (n=113; first vaccinated at 6 months of age), seropositivity increased from 20.4% (23/113) to 40.7% (46/113) at 6 months; 22.1% (25/113) seroconverted (negative to positive), whereas 1.8% (2/113) seroreverted (positive to negative). In the three-dose group with paired results (n=450), seroconversion occurred in 2.7% (12/450), whereas seroreversion occurred in 16.2% (73/450)

CONCLUSIONS: Under field conditions, LSDV antibody seropositivity increased with the number of vaccine doses received, supporting booster-based vaccination strategies to achieve higher post-vaccination seropositivity. The 6-month follow-up demonstrated both seroconversion and seroreversion, indicating that antibody status is dynamic over time and highlighting the need for time-point–appropriate serological monitoring to guide evidence-based vaccination policy.

Keywords: Lumpy skin disease, Vaccination, Seroprevalence, Korean Native Cattle

Antibiotic Treatment in Calves Impacts Respiratory Microbiome Diversity and Reduces the Severity of Respiratory Disease Induced by Influenza D Virus, Bovine Coronavirus and Resistant Mycoplasma Bovis

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BACKGROUND and OBJECTIVE: Bovine Respiratory Disease Complex is one of the most pressing health, welfare and economics challenges in cattle production worldwide, and a major antimicrobial consumption source. The disease is multifactorial, driven by a combination of environmental stressors, holobiont factors, and respiratory pathogens (bacteria and viruses). When appropriately used, antibiotics have shown to be effective against respiratory bacteria in young cattle. Recent studies in human showed that the use of antibiotics for respiratory conditions also alters the microbiome of the respiratory tracts and potentially contributes to modify the infectivity and pathogenicity of respiratory bacteria and viruses. This experimental study aimed to determine the effect of an antibiotic treatment on the respiratory microbiome of calves and its consequence on induced respiratory infections with Influenza D virus (IDV), bovine coronavirus (BCoV) and a strain of Mycoplasma bovis that is highly resistant to antibiotics.

MATERIALS and METHODS: 14 pre-weaned calves were randomly assigned in 2 groups based on antibiotic treatment: (i) infected without antibiotics (Inf_NonATB), (ii) infected 3 days after antibiotic treatment with tilmicosin (12.5 mg/kg, twice daily by oral route) from day -5 to -3 (Inf_ATB). On day 0 (D0) all calves were co-inoculated (French official ethical approval) by nebulization using a mask and compressor system, delivering 10^7 $TCID_{50}$ of IDV and BCoV and 10^{10} CFU of highly tilmicosin resistant Mycoplasma bovis. From D-5 to D+21, clinical signs were monitored by daily examination and regular thoracic ultrasound. Pathogens loads were quantified by qPCR on bronchoalveolar lavages (BAL) and deep nasal swabs, with additional isolation for M.bovis. Inflammatory status was assessed by cytokines dosage in BAL and total blood counts. At D21, calves were euthanized and respiratory lesions were recorded.

RESULTS: The usage of tilmicosin greatly impacts nasal microbiome alpha-diversity, suggesting a reduction in the abundance of some dominant taxa, leading to a more evenly distributed microbial diversity. Clinical examination indicated that Inf_ATB calves show significantly less severe respiratory clinical signs and lung lesions than Inf_NonATB calves. These differences could not be explained by differences in pathogens replication in the upper and lower respiratory tracts, since IDV, BCoV and M. bovis loads were similar between the two groups. However, cytokines and blood leukocytes count showed significant differences in host inflammatory response, probably explaining the differences in clinical signs.

CONCLUSIONS: These results suggest that antibiotic treatment with tilmicosin could limit the severity of BRDC induced by respiratory viruses and resistant bacteria, probably by modulating the respiratory microbiota and the host inflammatory response.

Keywords: Respiratory disease, calf, antibiotic, microbiome, virus, mycoplasma

Safety of Simultaneous Btv-3 and Btv-8 Vaccination in Dairy Cows: A Field Study

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BACKGROUND and OBJECTIVE: Bluetongue virus (BTV) is a vector-borne disease of ruminants that causes considerable economic losses in cattle and sheep. Following the emergence of BTV-3 in north-western Europe in 2023 and the continued risk of BTV-8 reintroduction, vaccination has become increasingly important. In Belgium, a national vaccination programme against BTV-3 and BTV-8 was implemented in 2025. Because cattle had to be vaccinated against both serotypes within a limited timeframe, simultaneous administration of both vaccines could facilitate herd management. The aim of this observational field study was therefore to evaluate the safety and tolerability of simultaneous administration of two inactivated bluetongue vaccines (BLUEVAC-3 and BLUEVAC BTV8) in dairy cows under commercial farm conditions.

MATERIALS and METHODS: The study was conducted on a Belgian commercial dairy farm with 141 lactating cows. Twenty-nine clinically healthy cows were selected based on parity and days in milk. Animals received both vaccines subcutaneously on two occasions (18 April and 9 May 2025), administered simultaneously at separate injection sites on opposite sides of the neck. Milk yield and milk temperature were automatically recorded using an automated milking system. Data were collected from three days before to five days after each vaccination. Injection sites were inspected for local reactions on days 1, 4 and 7 after vaccination, and health events and treatments were recorded in the herd management system.

RESULTS: Twenty-nine dairy cows were monitored. After the first vaccination, milk temperature remained stable (39.1–39.3 °C). Milk production also remained stable, ranging between 42.0–42.5 kg before vaccination and 42.2–43.1 kg after vaccination. Following the second vaccination, a mild increase in milk temperature was observed (39.4–39.6 °C), returning to baseline from day 3 onwards. Milk production remained stable, ranging between 41.8–42.3 kg before vaccination and 41.3–42.5 kg after vaccination. No systemic adverse

reactions were observed. Mild local swellings occurred in a small number of animals after the second vaccination and resolved within seven days.

CONCLUSIONS: Simultaneous administration of BLUEVAC-3 and BLUEVAC BTV8 was well tolerated in dairy cows under field conditions. Vaccination did not negatively affect milk production and resulted only in a mild and transient increase in milk temperature after the second vaccination. Mild local swellings observed in a small number of animals resolved spontaneously. These findings indicate that simultaneous vaccination with both inactivated bluetongue vaccines can be safely applied in dairy herds and may facilitate vaccination programmes targeting multiple BTV serotypes.

Keywords: Bluetongue virus, BTV-3, BTV-8, dairy cows, vaccination, field study

Serological Survey of Major Bovine Viral Infections in Dromedary and Cattle Herds in Tunisia

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BACKGROUND and OBJECTIVE: Cattle and dromedary farming represent essential livestock sectors, contributing to meat and milk production as well as rural socio-economic stability in Tunisia. However, limited epidemiological data are available regarding the circulation of major bovine viral pathogens in mixed farming systems. In particular, information on Bovine alphaherpesvirus 1 (BoAHV-1), Bovine alphaherpesvirus 2 (BoAHV-2), Bovine gammaherpesvirus 4 (BoGHV-4), Bovine Viral Diarrhea Virus (BVDV), and Enzootic Bovine Leukosis (EBL) remains limited. In this study, we hypothesized that cattle and dromedary herds might be exposed to these viral infections. Therefore, we evaluated the serological positivity of BoAHV-1, BoAHV-2, BoGHV-4, BVDV, and EBL in selected herds.

MATERIALS and METHODS: The survey was conducted in March 2024 in the Tataouine Governorate. Four farms were selected: two extensive dromedary farms (No. 1 and No. 2) and two intensive dairy cattle farms (No. 3 and No. 4). Each farm included 50 animals, for a total of 200 sampled tested. Serum samples were tested for specific antibodies using commercial ELISA kits according to manufacturers' instructions. Antibodies against glycoproteins gB and gE of BoAHV-1, total antibodies against BoAHV-2, BoGHV-4, BVDV, and antibodies against glycoprotein 51 (gp51) of EBL were investigated. In addition, all sera were subjected to a virus neutralization test for BoAHV-1, following international reference standards, to confirm serological findings.

RESULTS: The dromedary sera showed no significant serological evidence of viral circulation, with the except in farm No. 1, where a low rate of EBL seropositivity rate (2%) was detected. In contrast, cattle herds demonstrated clear evidence of viral exposure to the viruses investigated. Farm No. 3 showed BoAHV-1 seroprevalence ranging from 4% to 10%, a seropositivity of 48% for BoGHV-4, 40% for BVDV, and 14% for EBL. Farm No. 4 showed a seroprevalence of BoAHV-1 ranging from 6% to 10%, 56% for BoGHV-4, 35% for BVDV, and 4% for EBL. No antibodies against BoAHV-2 were detected in any of the four farms tested.

CONCLUSIONS: This preliminary survey demonstrates moderate seroprevalence of BVDV and BoGHV-4 and lower prevalence of BoAHV-1 and EBL in cattle, while dromedaries showed only minimal exposure to EBL. These findings highlight the need for structured surveillance programs and targeted control strategies to limit viral spread in cattle herds in mixed livestock systems. Further studies at the national level are needed to better define the dynamics of infection and support viral diseases control measures in Tunisia.

Keywords: BoAHV-1, BoAHV-2, BoGHV-4, BVDV, EBL

Field Evaluation of Safety of An Inactivated Vaccine Against EHD-8 in Pregnant and Lactating Dairy Cows

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BACKGROUND and OBJECTIVE: Epizootic Haemorrhagic Disease serotype 8 (EHD-8) emerged in Southern Europe in 2023, leading to the rapid development of Hepizovac®, an inactivated vaccine against the virus responsible for the disease (EHDV-8). Market authorization studies reported good tolerance and no major adverse events; however, no data was available on its administration in pregnant and lactating dairy cows. This study was therefore conducted to assess the safety of the vaccine in this specific animal category.

MATERIALS and METHODS: A total of 36 pregnant and lactating Prim'Holstein cows, aged over 30 months and 5–6 months into gestation, were enrolled from two dairy farms in northern France which is an EHD-free area. Animals were randomized into two groups: Group 1 received two 4-mL subcutaneous injections of Hepizovac® administered three weeks apart, and Group 2 received 4 mL of NaCl 0.9% following the same route of administration and the same schedule. Clinical examinations, rectal temperatures and local reactions were recorded before each vaccine injection (D0 and D21) and for three days after each injection (D1, D2, D3, D22, D23 and D24).

RESULTS: No major adverse effects were observed in both groups. One premature calving was recorded after the second injection, but no link to vaccination was established. No hyperthermia above 39.5 °C occurred in either group after injections. Local reactions consisted mainly of small nodules at the injection site. After first injection, 4 animals were concerned in the vaccinated group and one in the control group. After the second injection, 4 and 3 cows had small nodules in the vaccinated and the control groups, respectively.

CONCLUSIONS: Under field conditions, Hepizovac® was well tolerated in pregnant and lactating dairy cows, with no major safety concerns identified. These findings confirm previous authorization data and are consistent with results from parallel studies conducted in dairy heifers in endemic areas.

Keywords: Epizootic Haemorrhagic Disease, Vaccine, safety, EHDV

Current Data on Serological Survey for Contagious Bovine Pleuropneumonia in Poland

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BACKGROUND and OBJECTIVE: Contagious bovine pleuropneumonia (CBPP) is highly contagious disease of cattle and water buffalo, listed by the World Organisation for Animal Health. Despite the eradication of CBPP in most countries worldwide, the disease persists in sub-Saharan Africa. The risk of introducing the disease into countries free of CBPP is constantly present. To assess the occurrence of CBPP in Poland, serological monitoring of cattle is carried out annually in selected regions of the country. The study aimed to evaluate the presence of specific antibodies to Mycoplasma mycoides subsp. mycoides, an etiological agent of CBPP, in cattle from selected regions of Poland in 2024-2025.

MATERIALS and METHODS: A total of 1,600 bovine serum samples were collected between 2024 and 2025 (800 samples in both years). Samples were obtained from 526 cattle herds in total, across all six regions of Poland. The number of samples tested in individual regions was: 320 in the eastern region, 240 in the central region, 316 in the northern region, 400 in the north-western region, 160 in the southern region and 164 in the south-western region. The samples were analysed using a commercially available complement fixation test according to the manufacturer's requirements. If a positive or doubtful result was obtained, the samples were retested using the same method.

RESULTS: The results of the two-year survey showed a total of 109 doubtful results, which constituted less than 7 per cent of the samples. The remaining 1,491 samples were interpreted as negative. In 2024, the doubtful results in the amount of 92 came from cattle in all six regions of Poland as follows: 7 (the eastern region), 10 (the central region), 17 (the northern region), 41 (the north-western region), 15 (the

southern region) and 2 (the south-western region). In 2025, the number of doubtful results was significantly lower at 17. These results came from cattle in the eastern (9), central (5), northern (1) and north-western (2) regions. Retesting of the doubtful samples using the same method ultimately gave a negative result.

CONCLUSIONS: The survey indicates the need to continue determining the occurrence of CBPP in the Polish cattle population. All results were ultimately found to be negative; however, a certain percentage of doubtful results was observed, which favourably decreased in the second year of the survey.

Keywords: contagious bovine pleuropneumonia, cattle, complement fixation test

First Detection and Antimicrobial Susceptibility of Mycoplasma bovis isolated From Calves with Respiratory Disease in Chile

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BACKGROUND and OBJECTIVE: Bovine respiratory disease (BRD) is a multifactorial syndrome and one of the leading causes of morbidity, mortality and economic losses in calves worldwide. Among the bacterial agents involved, Mycoplasma bovis (M. bovis) is recognized as an important pathogen associated with pneumonia, chronic respiratory disease and treatment failure in calves. In Chile, M. bovis has mainly been reported in cases of bovine mastitis, and its involvement in respiratory disease remains poorly documented. The objective of this study was to detect and isolate M. bovis from lung tissue of calves with BRD in Chile and to describe the antimicrobial susceptibility profile of field isolates.

MATERIALS and METHODS: Between May and December 2022, lung tissue samples were collected from 13 calves with clinical findings compatible with BRD from two dairy farms in southern Chile. Calves were euthanized and necropsied at the Animal Pathology Institute, Universidad Austral de Chile. Lung samples were cultured on modified Hayflick agar for Mycoplasma spp., and colonies showing the characteristic “fried egg” morphology were considered compatible with Mycoplasma. These colonies were analyzed by conventional PCR to confirm M. bovis. In addition, DNA extracted directly from lung tissue was analyzed using a species-specific real-time PCR assay.

RESULTS: Colonies compatible with Mycoplasma spp. were obtained from 7 of 13 (53.8%) lung samples, and all isolates were confirmed as M. bovis by conventional PCR. Real-time PCR detected M. bovis DNA in 11 of 13 (84.6%) lung samples, including four samples negative by culture, highlighting the higher sensitivity of molecular detection compared with culture. Other bacterial pathogens were also isolated from lung tissue, including Trueperella pyogenes (n=4) and Pasteurella multocida (n=1). Viral pathogens were not investigated. Antimicrobial susceptibility was evaluated using the Epsilometer test (E-test), determining minimum inhibitory concentrations (MICs) for erythromycin, azithromycin, tetracycline and spectinomycin. All seven isolates showed MIC values >256 µg/mL for erythromycin and azithromycin. MIC values for tetracycline ranged from 0.75 to 2 µg/mL, while MIC values for spectinomycin ranged from 2 to 12 µg/mL. Although veterinary-specific clinical breakpoints for Mycoplasma spp. are not well established, tetracycline MIC values were close to proposed resistance thresholds, whereas spectinomycin MIC values remained relatively low.

CONCLUSIONS: This study reports the first isolation of M. bovis from lung tissue of calves with BRD in Chile and suggests that this pathogen may contribute to respiratory disease in Chile. The isolates obtained provide a basis for future genomic and epidemiological studies of M. bovis associated with BRD in Chile.

Keywords: Bovine respiratory disease, Mycoplasma Bovis, PCR, Antimicrobial susceptibility

Systemic Use of Levamisole Associated with Two Topical Treatments for Ulcerative Lesions in the Udder of the Udder of Lactating Cows

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BACKGROUND and OBJECTIVE: The aim of this study was to evaluate and compare the efficacy of two topical treatments associated with the systemic use of levamisole in ulcerative lesions suggestive of stephanofilariasis. A lactating herd of 2,200 Holstein cows housed in a free stall was inspected for the presence of ulcerative udder lesions.

MATERIALS and METHODS: A total of 112 cows with lesions were identified, and a sample of 30 cows was underwent diagnosis by direct examination, all of which tested positive. The 112 cows were then randomly allocated into two treatment groups: HC - HoofCare® (n=59) and PC – positive control (n=53), treated with a formulated paste (Triclorsil® with Unguento®). Both topical treatments were applied every 48 hours until complete healing or until 60 days had elapsed. All animals received two subcutaneous applications of levamisole (7.5 mg/kg), administered 30 days apart. Lesions were evaluated by infrared thermography every 15 days (five time points) and photographed for quantitative analysis of lesion area using ImageJ software every 30 days (three time points). Due to variation in lesion size, they were classified into three subgroups: small (≤ 10 cm²), medium (>10 cm² to 25 cm²), and large (> 25 cm²). Data were analysed and compared across time points and treatment groups using Mixed Linear Models.

RESULTS: Thermographic evaluation did not show significant differences at any time point in within or between the groups, where the HC group presented an initial median temperature (D0) of 38.2°C and PC with 37.5°C. Planimetric analysis revealed no a significant difference between treatments for small lesions, although a significant reduction (P<0.001) in the percentage regression index was observed in the HC group between D0-D30 and D0-D60. Medium lesions showed significant differences only at D30 and D60, as well as in the regression index between D0-D60 in the CP group. Large lesions demonstrated significant area reduction between D30 and D60 in both groups, with decreases of 7.5 cm² and 6.4 cm² in the HC group, and 9.89 cm² and 15 cm² in the CP group. Complete healing occurred in three cows in the HC group and seven cows in the CP group.

CONCLUSIONS: It is concluded that both topical treatments combined, when combined with levamisole administration, are effective in treating ulcerative udder lesions consistent with stephanofilariasis. However, 60-day treatment period was insufficient for complete healing, suggesting the need to extend topical therapy and levamisole administration until full lesion healing.

Keywords: Stephanofilaria spp., trichlorfon, ivermectin, ulcerative dermatitis

Epidemiological Investigation of Ibr Outbreaks with Probable Indirect Transmission in a High-Density Dairy Cattle Area

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BACKGROUND and OBJECTIVE: Infectious Bovine Rhinotracheitis (IBR), caused by Bovine Herpesvirus 1 (BoHV-1), remains widely prevalent in cattle worldwide. Direct nasal contact is the primary infection route. Thus, herd incursion occurs mainly through animal introduction, although indirect transmission via fomites or personnel is increasingly recognized. In Galicia (Spain), a voluntary IBR control program has been implemented through Cattle Health Associations (ADSG), combining serological monitoring, where gB-ELISAs detect antibodies induced by infection or

vaccination, while gE-ELISAs specifically identify field virus infection, enabling differentiation between infected and vaccinated animals (DIVA gE-deleted marker vaccines). Herd status is classified annually based on bulk milk testing and young animals' serology (9–36 months). Despite this program, new infections still emerge in herds previously classified as IBR-free. This study aimed to analyze the temporal evolution of IBR seroprevalence in a high-density dairy area between 2023 and 2025 and to investigate outbreaks, identifying associated epidemiological factors.

MATERIALS and METHODS: A retrospective sero-epidemiological study was conducted in 101 herds (n=13,910 animals) included in the same ADSG. A total of 12,876 serological determinations were performed (gB-ELISA=4,337; gE-ELISA=8,539). Twenty-two IBR outbreaks, detected between May 2024 and July 2025 were further investigated through historical serology (2021–2022), nasal swabs for BoHV-1 PCR in herds with respiratory disease, and a structured epidemiological questionnaire completed by ADSG veterinarians.

RESULTS: At herd level, seropositivity increased from 14.85% in 2023 to 42.57% in 2025, while intra-herd seroprevalence rose from 5.5% to 16.8%. Newly infected herds occurred in high herd density areas. In the twenty-two investigated herds, marked intra-herd seroconversion was observed. Clinical presentations ranged from severe respiratory outbreaks, to late-term abortions or subclinical infections. In eight herds, gB-positive animals detected in 2021–2022 suggested reactivation of latent infections. More than 50% of affected herds (55–59% in 2024-2025) had not introduced new animals, infection appeared to spread from nearby affected farms (located<2km), and all incoming cattle were gE-negative. Identified biosecurity gaps included lack of fencing, shared mixer wagons (14 herds), absence of visitor control, and overlapping veterinary teams. Notably, BoHV-1 was detected by PCR on a veterinarian's jacket, supporting possible mechanical transmission. None of the affected herds were previously vaccinated against IBR.

CONCLUSIONS: In high-density dairy regions, insufficient biosecurity and lack of vaccination allow BoHV-1 circulation despite voluntary control programs, through both reactivation of latent infections and indirect transmission. Marker vaccination combined with strict biosecurity is essential to maintain IBR-free status.

Keywords: IBR, BoHV-1, seroprevalence, biosecurity, dairy cattle

Presence of *Mycoplasmopsis bovis* (*M. bovis*) Antibodies in Bulk Tank Milk in German Dairies

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BACKGROUND and OBJECTIVE: Mycoplasmopsis bovis (M. bovis) is a common cattle pathogen associated with respiratory disease, otitis, arthritis (especially in calves), and mastitis and arthritis in adult cattle. Detecting antibodies in bulk tank milk (BTM) by ELISA is a practical approach to screen dairies as M. bovis antibody–positive or –negative. This study aimed to provide an overview of BTM antibody status in German dairy herds using samples collected from June 2023 to January 2026.

MATERIALS and METHODS: BTM samples were submitted by veterinarians and herd managers from Holstein Friesian and Simmental herds across Germany. Herds were categorized by cow numbers: <50 (9.9%); 51–100 (42.4%); 101–250 (23.7%); and >251 (24.1%). Samples were tested for M. bovis antibodies using the ID Screen® Mycoplasma bovis Indirect ELISA (IDvet, France) at the Bavarian Animal Health Services laboratory (Poing, Germany) or the private milk quality laboratory bovicare GmbH (Schönow/Bernau, Germany). Cut-off (CO) categories followed the manufacturer's instructions: 0 (<30%), 1+ (≥30%), 2+ (≥50%), 3+ (≥100%), 4+ (≥150%).

RESULTS: A total of 783 dairy herds were tested. Overall, 377 herds (48.1%) were positive for M. bovis antibodies, indicating prior pathogen contact. Among positive samples, CO categories were: 2+ (52.2%), 1+ (22.5%), 3+ (21.2%), and 4+ (4.3%). Herd size was associated with antibody presence (p<0.001; Kruskal–Wallis test; IBM SPSS 29.0): 73% of herds with <50 cows were antibody-negative, compared with 26.1% of herds with >251 cows. No differences were detected between herds with 51–100 vs. 101–

250 cows (p=0.3). Antibody status did not differ across years (p=0.16).

CONCLUSIONS: Across multiple years, a substantial proportion of German dairy herds showed evidence of M. bovis exposure based on BTM antibody detection. Larger herd size was associated with higher likelihood of antibody positivity. BTM ELISA testing is a practical screening tool to identify herds with evidence of pathogen contact.

Keywords: M. bovis, bulk tank milk, screening, ELISA

Comparative RNA-seq Analysis of Gene Expression in Mycobacterial Infection in *Bubalus bubalis* and *Bos taurus* Blood

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BACKGROUND and OBJECTIVE: Bovine tuberculosis is a major infectious disease affecting ruminants. Transcriptomic profiling represents a promising strategy to identify host biomarkers for early diagnosis. The aim of this study was to compare blood RNA expression profiles in Mediterranean buffalo (Bubalus bubalis) and cattle (Bos taurus) during mycobacterial infection to identify potential diagnostic markers and investigate species-specific immune responses.

MATERIALS and METHODS: Blood samples were collected from tuberculosis-free herds and outbreak herds. Whole blood was processed both as untreated and after 24-hour in vitro stimulation with phosphate-buffered saline (control) or bovine purified protein derivative (PPD-B). Total RNA was extracted and assessed for quantity and quality using microfluidic electrophoresis. RNA-Seq libraries were generated and sequenced, producing ~375.8 Gb of data from 5 infected and 5 uninfected animals per species, in all examined conditions. Raw reads were quality-trimmed and mapped to the reference genomes (Bos taurus ARS-UCD2.0; Bubalus bubalis NDDB_SH_1). Gene counts were obtained and differential expression was analysed using DESeq2 in R.

RESULTS: High-quality reads were retained (>98% paired end) for both species, with mean mapping rates above 82%. In both species, expressed genes represented approximately 74% of the total annotated genes. Using a p-value threshold of 0.05, the number of differentially expressed genes (DEGs) differed markedly between the two species, with a predominance of upregulated genes in Bos taurus and downregulated genes in Bubalus bubalis. This indicates a species-specific transcriptional response under the investigated conditions, suggesting the involvement of distinct regulatory mechanisms between the two species.

CONCLUSIONS: RNA-Seq analysis revealed clear differences in the blood transcriptomic response to mycobacterial infection between cattle and buffalo. Cattle showed a broader transcriptional activation, while buffalo displayed fewer DEGs and mainly downregulated transcripts. These results support the presence of species-specific host–pathogen interactions and highlight the value of comparative transcriptomics for identifying candidate biomarkers useful for infectious disease surveillance in ruminants. Research supported by project IZS ME 14/22 RC "Evaluation of biomarkers for mycobacterial infection in Mediterranean buffalo (Bubalus bubalis) and cattle (Bos taurus)".

Keywords: Gene expression profiling, Mycobacterial infection, Bubalus bubalis, Bos taurus,

The Role of Veterinary Practitioners in Participatory Epidemiology – Early Detection of the Re-Emergence of Schmallenberg Virus Using a Mobile Phone App

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BACKGROUND and OBJECTIVE: XLVets Ireland, an independent group of veterinary practices (220 vets, 53 practices), has been monitoring bovine congenital defect since 2021 using a mobile phone app (Defect Tracker). Given that Schmallenberg virus (SBV) causes the arthrogryposis-hydranencephaly syndrome (AHS) it may be possible to monitor this disease using the same application. The first cases of SBV were detected in Ireland in 2012/13. This outbreak conferred a variable degree of immunity on the national herd. No subsequent cases were detected until the second outbreak in 2017/2018. Then in 2024/25 a third outbreak occurred. This pattern suggested that SBV outbreaks may be cyclical ('Schmallenberg-years' every 4-6 years). Hence, the objective of this study was to determine if the Defect Tracker could be used to detect the reemergence of SBV in the national cattle population.

MATERIALS and METHODS: Passive/scanning surveillance for animal diseases is operated in Ireland via the national Regional Veterinary Laboratory (RVL) network. The LIMS of these six labs was interrogated to retrieve SBV PCR-positive diagnoses in necropsied bovines for the most recent outbreak in 2024 (1 Sept. – 31 Dec.)/2025 (1 Jan. – 21 Mar.). In parallel with this virus testing, the Defect Tracker azure cloud database was interrogated to detect the reporting of ankylosis and torticollis (characteristic externally observable lesions in cases of clinical SBV) in bovines between 2023 and 2025.

RESULTS: The first signs of a new SBV outbreak were detected in the autumn of 2024 by the RVLs when they detected the virus in two deformed calves at necropsy, both in the same laboratory. The number of positive cases increased substantially in spring 2025 when 17 additional SBV PCR-positive diagnoses were made, in five laboratories. Data from the Defect Tracker recorded ankylosis in 6, 13 and 59 cattle in 2023, 2024 and 2025, respectively while torticollis was recorded in 1, 5 and 33 cattle in the same periods. Thus, in total ankylosis/torticollis was recorded in 7, 18 and 92 cases in 2023, 2024 and 2025, respectively.

CONCLUSIONS: Taken together, these findings confirm that there was a new outbreak of SBV in Ireland in 2024/2025 due to infection in summer/autumn, 2024. From this, it can be extrapolated that the next outbreak of SBV may occur in 2028/31. There was a high degree of temporal concordance between the laboratory SBV PCR diagnoses and the veterinary practitioner clinical SBV presumptive diagnoses. This outcome highlights the potential role for veterinary practitioners in participatory epidemiology, both nationally and internationally.

Keywords: Schmallenberg virus, congenital defects, veterinary practitioners

Current Data on Serological Survey for Contagious Agalactia in Poland

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BACKGROUND and OBJECTIVE: Contagious agalactia (CA) is a serious and economically important disease syndrome of sheep and goats, listed by the World Organisation for Animal Health. Mycoplasma agalactiae (M. agalactiae) is considered the most important etiological agent of CA. In Poland, CA is subject to mandatory registration. Regular monitoring of sheep and goats for CA has been applied in Poland for years. The study aimed to assess the presence of anti-M. agalactiae antibodies in the serum of sheep and goats from 2024 to 2025.

MATERIALS and METHODS: The study was conducted between 2024 and 2025 using a total of

400 serum samples (200 samples per year). Samples were collected from 258 sheep and 142 goats, originating from a total of 144 herds across all 16 voivodeships of Poland. The presence of specific antibodies to M. agalactiae was determined in the serum samples using a commercial ELISA kit according to the manufacturer's requirements.

RESULTS: The results showed no presence of specific antibodies to M. agalactiae in any of the tested samples.

CONCLUSIONS: In previous years of monitoring sheep and goats for CA in Poland, some false results were indicated, which fortunately were not confirmed in the current survey. Despite the negative results, there is a constant need to screen sheep and goats for CA to prevent its introduction into the country.

Keywords: Contagious agalactia, Mycoplasma agalactiae, sheep, goat, ELISA

Characterization Of Cytopathogenic Bovine Viral Diarrhea Virus Strain During a Respiratory Outbreak in Calves

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BACKGROUND and OBJECTIVE: Bovine Viral Diarrhea Virus (BVDV) remains a globally significant cattle pathogen. While non-cytopathogenic (ncp) biotypes are typically responsible for persistent infections and most field cases, cytopathogenic (cp) strains are classically associated with sporadic Mucosal Disease (MD) in persistently infected animals. However, the involvement of cp strains in acute high-mortality outbreaks affecting susceptible calf cohorts is less frequently documented. The outbreak involved severe respiratory distress, erosive–ulcerative mucosal lesions, and bronchopneumonia. This study aimed to characterize a cp BVDV-1a strain (isolate 11227) recovered from a severe outbreak in an officially "Disease-Free" certified enterprise, where 45 suckling calves died within four days.

MATERIALS and METHODS: Necropsies were performed on two affected calves, and tissue and mucosal swabs were collected for histopathological and virological analyses. Initial screening was performed using commercial ELISA kits for major bovine pathogens. Virus isolation was conducted in pestivirus-free MDBK cells, and cultures were monitored for cytopathic effects (CPE) over serial passages. The isolate was biotyped by immunoplaque assay and confirmed by indirect immunoperoxidase monolayer assay (IIPMA). Molecular characterization was carried out by RT-PCR targeting the 5'UTR region of the viral genome, followed by Sanger sequencing and phylogenetic analysis using the Maximum Likelihood method with 1000 bootstrap replicates.

RESULTS: Histopathological examination revealed pseudomembranous glossitis and enteritis with crypt necrosis, diffuse interstitial pneumonia, and fibrinoid-necrotic vasculitis. CPE characterized by cell rounding and lysis were observed in MDBK cells beginning at the fourth passage. At the fifth passage, virus titers reached 1.5 $\log_{10}$ $TCID_{50}$/mL in lung-derived samples and 4.5 $\log_{10}$ $TCID_{50}$/mL in spleen-derived samples. Immunoplaque and IIPMA confirmed the cytopathogenic phenotype. RT-PCR produced a 288-bp amplicon, and the high-titer spleen isolate was sequenced. PCR assays for other major respiratory pathogens were negative. Phylogenetic analysis classified isolate 11227 within the BVDV-1a subgenotype, clustering closely with reference strains NADL and Singer.

CONCLUSIONS: The characterization of a cp BVDV-1a strain from this outbreak indicates that cp biotypes may be associated with acute and severe disease in susceptible calf cohorts, extending beyond their classical association with sporadic MD. The outbreak involved severe respiratory distress, erosive-ulcerative mucosal lesions, bronchopneumonia, and systemic vasculitis. The rapid progression and clustering of cases may reflect efficient horizontal transmission under high-density management conditions. These findings emphasize that cp BVDV should be considered in the differential diagnosis of high-mortality events and highlight the need for continuous surveillance, even in herds with biosecurity management.

Keywords: Bovine viral diarrhea virus, Cytopathogenic, Suckling calves, Virus isolation, BVDV-1a

Validation of the Syringeability of a Fixed Combination of Florfenicol and Meloxicam Through a New More Accurate Method and Comparison with Florfenicol-Based Products Commonly Used in Bovine Respiratory Disease (BRD)

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BACKGROUND and OBJECTIVE: Syringeability is a key parameter for any parenteral formulation intended for use in cattle and is particularly critical in young calves. Solutions for injections must be easily administered to ensure the welfare of both the animal and the operator-whether a farmer or a veterinarian, especially under challenging winter conditions where increased viscosity can hinder proper delivery.

MATERIALS and METHODS: Historically, syringeability of injectable formulations was assessed by applying a constant manual force to the syringe plunger and recording the time required for the product to pass through a needle of predefined gauge. This approach was limited by operator dependent variability and inconsistent application of force. A new syringeability evaluation method was implemented to improve standardization and measurement accuracy. A calibrated texturometer was used to apply a constant and predefined plunger speed of 100 mm/min over a 10 mL displacement. During the test, the instrument continuously measured the force required to extrude the formulation, expressed in Newtons (N).The lower the force is, the easier the injection is. Each formulation was tested six times under identical conditions to assess repeatability. For each set of six measurements, the coefficient of variation (CV) was calculated, expressed as a percentage. A low CV was interpreted as high repeatability of the syringeability profile. Two different temperatures (5°C and Room Temperature) and two needle gauges (1,2 mm and 0,8 mm) were tested. Four products were compared: Zeleris® (florfenicol 400 mg/mL and meloxicam 5 mg/mL, Ceva), Resflor® (florfenicol 300 mg/mL and flunixin meglumine 16.5 mg/mL, MSD), Nuflor®300 (florfenicol 300 mg/mL, MSD), Florkem® (florfenicol 300 mg/mL, Ceva).

RESULTS: The results of this study clearly demonstrated the high syringeability of Zeleris®: At 5°C, the force needed to inject Zeleris through a 1,2 mm needle was 23N. Zeleris® syringeability was better than the other florfenicol-based products both at 5°C (23N for Zeleris, 33N for Florkem, 68N for Nuflor 300 and 83N for Resflor) and at room temperature. The superior performances of Zeleris® may be explained by the various excipients used in its formulation.

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Keywords: Syringeability, texturometer, compliance, well-being

Herd Level Seroprevalence and Detection of *Mycoplasmopsis bovis (Mycoplasma bovis)* from 2021-2026 in Portuguese Cattle Herds

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BACKGROUND and OBJECTIVE: Mycoplasmopsis (Mycoplasma) bovis is a critical pathogen in beef and dairy cattle worldwide. Control of M. bovis is challenging due to its role in immunosuppression due to its ability to evade the host immune system and contributes to the bovine respiratory disease complex. Outbreaks of M. bovis cause substantial risks to animal health and economic losses from reduced milk yields, treatment failures, mortality, and premature culling across the beef and dairy cattle industries. The objective of this study was to estimate herd-level seroprevalence of M. bovis across cattle production systems and regions in Portugal over time.

MATERIALS and METHODS: Seroprevalence convenience sampling occurred at a herd level between 2021-2026 from farms in Northern and Southern Portugal, and the Azores. During the sampling period, 94 dairies, 9 dairy beef, and 27 feedlot operations were sampled. Samples per farm ranged from 5-12, with an average of 10 samples collected per farm. Dairy herds were sampled from groups aged 2–6 months. Dairy beef operations were sampled 60–90 days after arrival, and feedlots 30–40 days after arrival. Serum antibodies to M. bovis were assessed using two indirect ELISAs at a commercial laboratory (ID Screen Mycoplasma bovis Indirect IDVET and BIO K302 Monoscreen AbELISA Mycoplasma bovis Indirect BIOX). In addition, pneumonic lung tissue was collected from necropsies at each production type for detection of M. bovis (dairy farms: n = 21; dairy beef farms: n = 7; feedlots: n = 47) and tested using the VetMAX Ruminant Respiratory Screening Kit (Applied Biosystems, Thermo Fisher Scientific) with real-time PCR. Data for both seroprevalence and tissue detection of M. bovis are presented as presence/absence of M. bovis in samples, with percent positivity as a percentage of total samples collected.

RESULTS: Herd-level seropositivity was detected in 45/94 dairy operations (47.9%), 9/9 dairy beef operations (100.0%), and 24/27 feedlots (88.9%). M. bovis was detected in pneumonic lung tissue in 14/21 dairy samples (66.7%), 7/7 dairy beef samples (100.0%), and 43/47 feedlot samples (91.5%).

CONCLUSIONS: Over a multi-year sampling period, Mycoplasma bovis was widely detected across Portuguese cattle operations with herd-level seropositivity in dairies (47.9%) and higher prevalence in dairy beef (100.0%) and feedlots (88.9%). Frequent detection in pneumonic lung tissue supports extensive circulation of M. bovis and its involvement in respiratory disease, underscoring the need for continued surveillance and targeted control in high-risk production systems.

Keywords: Mycoplasma bovis, Portugal, Herd-level seroprevalence, Detection in lung lesions

Tricks of the Trade: Understanding Cattle Purchasing in the Context of An Endemic Statutory Disease and Associated Control Efforts

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BACKGROUND and OBJECTIVE: Bovine Tuberculosis (bTB) is an endemic infectious disease of cattle in Ireland. Statutory eradication efforts began in the 1950s, but despite considerable progress, eradication has not been achieved. The movement of undetected infected cattle through trade into previously uninfected herds has been identified as an important transmission route between herds. Herds recently released from movement restrictions following the removal of test-positive animals have been shown to be at increased risk of subsequent breakdowns (i.e., detection of bTB within a herd). The limited sensitivity of the intradermal (tuberculin) diagnostic test results in some infected cattle remaining undetected. These animals may continue to spread bTB among herd mates. If such an animal is unintentionally sold, infection may be introduced into the buyer's herd. Thus, purchasing cattle is a known risk factor for disease incursion. Risk-informed and risk-based trading (RBT) has been suggested as a potential mitigation measure, facilitating the informed and responsible trade of cattle. However, as this approach hinges on farmer compliance, farmer acceptability is crucial to its success. Therefore, understanding how bTB is perceived in the context of the broader stressors experienced by farmers may provide policymakers with insights into how/why farmer buying decisions occur, and whether future policies would be accepted or resisted.

MATERIALS and METHODS: Irish cattle farmers were recruited using purposive quota and snowball sampling within a qualitative social science framework. Ten dairy and eleven beef suckler farmers were interviewed. Interviews were audio-recorded, anonymised and transcribed. Reflective thematic analysis, following Braun and Clark's six-phase framework, was used to analyse the transcripts. Themes were discussed and refined with a second coder for improved validity.

RESULTS: Interviews lasted an average of 19 minutes (range: 10-62). Four themes emerged: (1) the multifaceted nature of cattle purchasing, balancing desirable animal traits, affordability, and the risk of various infectious diseases; (2) the influence of the presence of bTB on purchasing decisions; (3) how farmers' lived experiences shape farmer welfare and influence perceptions of bTB control; and (4) hopes, fears, and adjustments of risk-based trading interventions necessary for improved acceptance by farmers.

CONCLUSIONS: Cattle purchasing decisions are shaped by farmers' preferences, practical needs, finances, social influences, experience, and the broader political context. Understanding how bTB risk fits among these factors can help explain compliance with bTB risk–informed recommendations. Many farmers want more disease-risk information when purchasing, but support for RBT policies varied widely.

Keywords: Bovine Tuberculosis, Cattle Trade, Farmer Decision-Making, Risk-Based Trading, Livestock Disease Management

Bacterial Suppurative Meningoencephalitis in a Newborn Calf Caused by *Klebsiella pneumoniae*: A Case Report

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BACKGROUND and OBJECTIVE: Bacterial meningitis and meningoencephalitis are uncommon but severe neurological diseases in neonatal calves and are often associated with high mortality. The pathogens most frequently involved in bovine central nervous system infections include Escherichia coli, Histophilus somni, and Listeria monocytogenes, although other opportunistic bacteria may occasionally be implicated. Klebsiella pneumoniae is a Gram-negative bacterium commonly related to bovine mastitis and systemic infections in humans, but neurological disease in calves is rarely reported. The objective of this study was to describe the clinical presentation, diagnostic findings and pathological characteristics of suppurative meningoencephalitis.

MATERIALS and METHODS: A 16-day-old male Guzerat calf produced through in vitro fertilization was admitted to the Department of Medicine and Husbandry of Ruminants at the Faculty of Veterinary Medicine and Animal Science, National Autonomous University of Mexico. The animal had a six-day history of respiratory distress, fever, dehydration, depression, and recumbency followed by neurological signs. Clinical examination revealed tachypnea, tachycardia, positive jugular pulse, dilation of the nostrils, ataxia, opisthotonos, nystagmus, and seizures. Diagnostic procedures included complete blood count and cerebrospinal fluid analysis. Blood samples were obtained through jugular venipuncture using EDTA tubes.

Cerebrospinal fluid was collected by cisternal puncture after sedation with xylazine and evaluated by cytology and bacteriological culture for diagnosis

RESULTS: The complete blood count revealed leukocytosis with neutrophilia, monocytosis, hyperfibrinogenemia, and reactive thrombocytosis consistent with inflammation. Erythrocyte morphology showed acanthocytes and keratocytes suggesting mechanical erythrocyte damage. Cerebrospinal fluid appeared turbid and cytological examination demonstrated marked mixed septic pleocytosis composed mainly of neutrophils and macrophages with increased protein concentration. Gram staining revealed numerous Gram-negative bacilli. Bacteriological culture of the cerebrospinal fluid produced abundant growth of Klebsiella pneumoniae confirming infection of the central nervous system. Necropsy findings included severe suppurative meningoencephalitis with inflammatory exudate on the ventral brain surface, suppurative meningomyelitis of the spinal cord, subdural hematoma, extensive epidural empyema, and moderate multifocal interstitial pneumonia. No other significant lesions were observed elsewhere.
CONCLUSIONS: This case documents suppurative meningoencephalitis caused by Klebsiella pneumoniae in a neonatal calf, an uncommon etiology in bovine medicine. Diagnosis was established through integration of clinical findings, cerebrospinal fluid analysis, bacteriological culture, and post-mortem examination. The report highlights the diagnostic importance of cerebrospinal fluid evaluation in calves presenting neurological signs. Although infectious meningoencephalitis is rare in bovines, opportunistic pathogens should be considered in differential diagnoses of neonatal neurological disease. Early recognition and comprehensive laboratory investigation are essential to identify unusual etiological agents and to improve understanding of central nervous.

Keywords: Klebsiella pneumoniae, Suppurative meningoencephalitis, Neonatal calf, Central nervous system infection, Cerebrospinal fluid analysis, Bovine infectious disease

Regional Prevalence of *Mycoplasma bovis* on 58 Closely Located Dairy Farms: Associations Between Bulk Milk Tank Antibody Test Results and Potential Risk Factors

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BACKGROUND and OBJECTIVE: *Mycoplasma bovis* (*M. bovis*) is a bacterial pathogen which causes persistent mastitis, pneumonia and arthritis in cattle, resulting in considerable economic losses worldwide. Its prevalence is increasing globally. A cross sectional study showed antibodies in the majority of dairy herds in the Netherlands, with higher risk linked to larger herd size and cattle introduction, but not to geographical region. As demand for support in managing outbreaks grows, this study aimed to improve understanding of *M. bovis* epidemiology on dairy farms located close together within a small geographical area.

MATERIALS and METHODS: Bulk milk tank (BMT) samples from 58 dairy farms serviced by the same veterinary practice were tested using the IDscreen® *M. bovis* antibody ELISA (Innovative Diagnostics), with an S/P% ≥30 considered positive. A brief questionnaire on potential risk factors (RF) was completed by the attending veterinarian and livestock farmer at sampling. Cluster analysis and multivariate logistic regression were carried out using SaTScan™ v10 and STATA/SE 17, based on test results, geographical coordinates and survey metadata from the farms.

RESULTS: Many farms (97%, n=56) were located within a maximum distance of 15 kilometers from each other. The herd level prevalence based on BMT antibodies was 25.9%. Positive farms showed no significant spatial clustering. At 1.7% of farms, M. bovis had ever been detected before. Farms reporting typical clinical signs—such as chronic pneumonia in youngstock, persistent mastitis, arthritis and/or otitis media—had a significantly higher likelihood of being antibody positive (Odds Ratio 6.46; p = 0.03).

Some RF showed tendencies to be associated with antibody-positivity ($p < 0.15$).

CONCLUSIONS: Despite the absence of prior diagnoses of *M. bovis* on nearly all farms, regional herd prevalence was comparable to national levels. For herds showing suggestive clinical signs, BMT antibody testing may provide a useful initial screening tool for detecting exposure. The distance of farms from positive farms did not seem to be an important risk factor, even though the density of dairy farms in the area was high. The relatively small sample size limited the power to identify significant associations with other surveyed RF; however, some interesting trends among surveyed factors warrant further investigation in larger or additional regions. A subset of farms has agreed to ongoing monthly BMT testing, of which results will be available at the time of the WBC.

Keywords: {Mycoplasma bovis}, regional herd prevalence, Risk Factors, Bulk Milk Tank, Antibody ELISA

Findings from BTV-Related Field Pilot Studies in the Netherlands

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BACKGROUND and OBJECTIVE: In September 2023, bluetongue virus serotype 3 (BTV-3) emerged in the Netherlands for the first time. Within a few months thousands of cattle herds became infected and farmers reported severe clinical signs, including reduced milk production and increased mortality. In 2024 the Netherlands encountered the second year of the epidemic with again many infections in cattle herds. In September and October of this year, the Royal GD Veekijker observed an increase in reports concerning calves with blue eyes and keratoconus. Then, January 2025, the Veekijker received numerous reports of newborn calves with central nervous symptoms, ranging from "dumb" calves to calves collapsing and dying. Additionally, hydrocephalus or hydranencephaly was diagnosed significantly more frequently at Royal GD in the first quarter of 2025.

MATERIALS and METHODS: Two pilot studies were performed. The first study concerning the 'blue eyes' aimed at answering questions regarding the onset, clinical picture, progression, recovery, and association with BTV-3. The pilot study included a dairy farm, a dairy cattle rearing farm, and a veal calf farm. Overall, ten calves were included for clinical examination and additional diagnostics. A second pilot study, aiming to gain more insight into the development of congenital brain abnormalities (CBA) was initiated. Pathology data was analyzed to see if there was an increase in CBA. Farmers of the twenty herds which send in a calf with CBA in the months January and February 2025, were interviewed. The background of the 20 included calves, farm management practices and a possible link with the BTV-3 outbreak in 2024 was investigated

RESULTS: The "blue eyes" were not present at birth. From the ten examined calves nine had varying degrees of corneal edema, four presented keratoconus and tested fluorescein positive, three showed corneal scarring. Nine calves tested bluetongue virus and antibody positive as well as the mothers that were tested. There was a clear rise of CBA at the end of 2024 and the start of 2025. Approximately 10% of the farms were vaccinated for BTV-3 and 85% of the interviewed farmers reported clinical signs of BTV in 2024.

CONCLUSIONS: All calves with corneal edema tested bluetongue virus and antibody positive. The mothers who were not vaccinated also tested bluetongue virus and antibody positive. There is a possible association between BTV-3 infection and the clinical ocular signs. There was a clear increse in congenital brain abnormalitys in Royal GD necropsies. The etiology was not definitively established. An association with BTV-3 seemes likely.

Keywords: BTV, Bluetongue, Hydrocephalus, Blue Eyes, Calves,

Intramammary mRNA Therapy Expressing an Antiviral Protein Complex Can Suppress Replication of Highly Pathogenic H5N1 Influenza Virus and Associated Pathology in the Bovine Mammary Gland

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BACKGROUND and OBJECTIVE: Bovine viral infections induce morbidity and production loss, but effective antiviral therapies are not available. Our objective was to conduct a pilot test of mRNA-based antiviral therapy for cattle with intramammary (IM) infection by the H5N1 avian influenza that caused a U.S. epizootic in 2024.

MATERIALS and METHODS: Healthy late lactation Holstein cows (n = 4) received intramammary (IM) challenge with 2 x 102 pfu of H5N1 influenza per quarter. Cows were treated IM with either 5 mg of mRNA encoding a Cas13a-based nuclease complex + guide RNAs + lipid delivery vehicle at 24 hours after challenge (n = 1), or 24 hours before and after challenge (n = 1), compared to IM 0.9% NaCl (negative control, n = 2). Clinical signs and milk production were assessed every 24 hours then cows were euthanized 48 - 72 hours post infection for assessment of mammary histopathology. Milk viral genome copy numbers were quantified by RT-qPCR. Viral protein in mammary tissue was assessed by immunofluorescent antibody (IFA) testing and histopathology was assessed with semiquantitative scoring by blinded pathologist. Histologic lesions were graded on a 1–5 scale based on estimated percentage of mammary tissue affected by acinar epithelial degeneration, epithelial necrosis and neutrophilic infiltrates (Grade 1: 0–10%; Grade 2: 11–25%; Grade 3: 26–50%; Grade 4: 51–75%; Grade 5: 76–100%).

RESULTS: Clinical signs of decreased milk production, low body temperature, and depressed activity occurred in cows after H5N1 challenge. Two antiviral mRNA treatments decreased milk viral genome copies by 10-fold and mammary viral protein expression by 2-fold, compared to negative control. The severity of histologic changes varied by treatment group, treatment schedule, and histologic section. In cows receiving a single treatment 24 h post-challenge, both Cas13a-treated and control animals exhibited similar changes characterized by marked to severe (Grades 4–5) acinar epithelial necrosis and intra-acinar neutrophilic infiltrates. In contrast, in cows receiving treatment 24 h before and 24 h after challenge, the Cas13a-treated animal developed only minimal degenerative changes (Grade 1), whereas the control animal exhibited moderate to severe (Grades 3–5) acinar epithelial necrosis with neutrophilic inflammation. These findings suggest that Cas13 mRNA treatment may reduce acinar epithelial necrosis and associated neutrophilic inflammation following H5N1 influenza infection.

CONCLUSIONS: Intramammary treatment with mRNA-expressing Cas13a nuclease complex can decrease viral load and microscopic pathology following IM H5N1 influenza infection. mRNA therapy should be investigated further for treatment of bovine mammary infections and viral diseases.

Keywords: virus, antiviral, mastitis, dairy cows

Immune and Metabolic Status in Extensive Cattle Following Vaccination Against Neonatal Diarrhea: Relationship Between Metabolic and Immunological Markers

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BACKGROUND and OBJECTIVE: Bovine Neonatal Diarrhea (ND) is a critical factor limiting profitability in suckler cow enterprises due to increased morbidity and mortality. In extensive cattle (Bos taurus), this problem is aggravated by scarce epidemiological literature and extensive systems that hinder sanitary control. Maternal immunization, together with hygiene measures, is the primary strategy, although vaccine efficacy depends on the female's metabolic state at administration. This study evaluated the serological response to ND after administering a commercial vaccine in a fighting bull farm, characterizing metabolic markers before and after immunization.

MATERIALS and METHODS: During the 2025 calving season, 323 cows were included from 382 on a farm with ND-history in 2024 and no vaccination record for this pathology in the last decade. An experimental study was designed with a control group (n=54, unvaccinated) and a vaccinated group (n=269). Blood samples were collected from 158 randomly selected cows at vaccination (M1) and four weeks later (M2). Antibodies against rotavirus, coronavirus, and E. coli were estimated using commercial ELISAs (BioxDiagnostic Monoscreen, Teknocroma). Metabolic markers analyzed at M1 and M2 included glucose (GLU), lactate (LAC), β-hydroxybutyrate (BHB), non-esterified fatty acids (NEFAs), triglycerides (TG), cholesterol (CHOL), lipoproteins (HDL, LDL), and urea (Konelab-20, Thermo-Fisher-Scientific). Statistical analysis employed Mann-Whitney U, repeated measures GLM, and Spearman correlation (IBM SPSS v.30).

RESULTS: At M1, antibody levels were similar for all pathogens (rotavirus: 71.85%, IQR: 49.57–83.93%; coronavirus: 76.66%, IQR: 56.93–89.18%; E. coli: 5.15%, IQR: 1.80–7.35%), indicating previous viral positivity but absolute negativity for E. coli. At M2, vaccinated cows showed significantly higher than unvaccinated cows: Rotavirus (83.61% vs. 73.83%, P=0.045), Coronavirus (95.57% vs. 81.07%, P<0.001), E. coli (32.96% vs. 5.60%, P<0.001). Metabolic evolution differed between groups for urea (P=0.074), cholesterol (P<0.001), lactate (P=0.002), and glucose (P=0.041); values increased in controls but remained stable in vaccinated cows. Correlation analysis showed a negative relationship between rotavirus absorbance and CHOL at M1 (ρ=-0.315; P=0.002). For coronavirus, absorbance correlated negatively with HDL-M1 (ρ=-0.277), HDL-M2 (ρ=-0.269), GLU-M1 (ρ=-0.215), and GLU-M2 (ρ=-0.256), and positively with TG-M1 (ρ=0.219). E. coli antibodies at M2 showed the strongest correlations, being direct with GOT and negative with urea-M1 (ρ=-0.282), cholesterol M1-M2, triglycerides M1-M2 (ρ=-0.335 at M2), HDL-M1, BHB-M2, and especially GLU (M1: ρ=-0.340; M2: ρ=-0.440; P<0.0001).

CONCLUSIONS: Maternal vaccination induces adequate humoral response before calving. The absence of basal immunity against E. coli classifies the herd as high-risk without vaccination. This study confirms the close relationship between metabolic status and humoral immune capacity in extensive cattle.

Keywords: Antibodies, neonatal death, cattle,

Summer Mountain Pasture (Transhumannce) as Possible Risk for the Transmission of Infectious Diseases in Cattle and Sheep

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BACKGROUND and OBJECTIVE: In many mountainous areas of the world animals are kept on mountain pastures during the summer. This transhumance also is part of the traditional way of animal livestock keeping in the Austrian alps, were

cattle, sheep and goats are kept on high altitude mountain pastures during summer. Often, animals from many different farms mingle on these pastures for the summer season, with the possible distribution of infectious dieses between animals and herds. This risk of disease transmission on shared alpine pastures was studied in the mountains of Tyrol/Austria.

MATERIALS and METHODS: In the course of different scientific studies, the transmission of *Staphylococcus* (*Staph.*) *aureus*,*Mycobacterium a vium* ssp. *paratuberculois* (MAP), *Salmonella* (*S.*) Dublin, *Dichelobacter* (*D.*) *nodosus*, and Bovine Viral Diarrhea Virus (BVDV) was studied. Samples from animals were collected before, during and after the summer season, as well as environmental samples. Furthermore, existing data and questionnaires were used to determine the risk for infection, according to the different specific study designs.

RESULTS: As suspected, the results varied depending on the disease studied. No increased risk for the transmission of MAP and foot rot (*D. nodosus*) during commingled summer pasturing of cattle and sheep could be determined. For *S.* Dublin the alpine pastures, especially contaminated surface water, was identified as possible risk for disease transmission. Austria is free of BVDV, but data analysis showed, that one single positive animal caused a severe outbreak in the northern part of Italy, near the Austrian border, infecting many other farms during the shared summer pasture. The dynamics of *Staph. aureus* in milk samples varied greatly between alpine pastures, suggesting transmission to quiet a substantial number of animals in some premises.

CONCLUSIONS: The use of mountain summer pasture has a long tradition in the Austrian alps, contributing to a sustainable, regional and resilient agricultural system. Nevertheless, the risk for the possible transmission of infectious disease between animals and herds has to be considered. Therefore, the measures in place to prevent disease transmission by testing animals prior to the grazing season have to be followed consistently, or have to be implemented where missing.

Keywords: Alpine pasture, transhumance, disease transmission

Photodynamic Therapy as a Non-Antibiotic Approach for Digital Dermatitis, Interdigital Dermatitis and Papillomatosis in Dairy Cattle

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BACKGROUND and OBJECTIVE: Digital dermatitis (DD) and interdigital dermatitis (DID) are major infectious causes of lameness in dairy cattle, leading to economic losses due to reduced milk production and impaired animal welfare. Papillomatosis is a proliferative skin infection with worldwide distribution, caused by different types of bovine papillomavirus (BPV), associated with variable clinical outcomes and limited therapeutic options. Photodynamic therapy (PDT) has emerged as a promising non-antibiotic approach with antimicrobial and antineoplastic effects.

MATERIALS and METHODS: To evaluate the efficacy of methylene blue PDT (M-PDT) in DD and DID lesions and its effect on papilloma regression, a total of 66 dairy cows were included, 34 with DD and DID in a paired contralateral design, in which lesions (n = 68) were randomly assigned to M-PDT or conventional treatment with oxytetracycline and hydrocortisone spray. 32 cows with papillomatosis were used to compare M-PDT to a topical ointment containing petroleum jelly and zinc oxide. M-PDT consisted of the topical use of 0.5% methylene blue solution followed by a red laser irradiation (660 nm; 4 J/cm²; 27 s). Cows were evaluated weekly for six weeks, and lesion area (cm²) was measured using ImageJ on days 0, 7, 14, 21, 28, and 35.

RESULTS: Healing rates for DID were significantly higher in the M-PDT group (94%) than in the control (54%; p = 0.00057). For DD, more lesions healed under M-PDT (nine vs. five), although without statistical significance (p = 0.2372). Papilloma lesions showed progressive area reduction under M-PDT, whereas control lesions remained stable. By day 28, the lesion area was markedly lower in the M-PDT group (0.15 ± 0.14 cm²) than in the control (0.65 ± 0.12 cm²).

CONCLUSIONS: M-PDT demonstrated comparable efficacy to that of antibiotics in DD, superior outcomes in DID, and significant papilloma regression, reinforcing the evidence for its use as a

non-antibiotic therapeutic strategy for these infectious diseases.

Keywords: dermatitis digitalis, claw lesions, infectious disease, Photosensitizer, methylene blue, treatment

Fifty-Five Years of Bovine Tuberculosis Research in Multi-host Ecosystems: A Systematic Review Within a One Health Perspective

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BACKGROUND and OBJECTIVE: Bovine Tuberculosis (bTB), primarily caused by Mycobacterium bovis, remains a widespread infectious challenge for animal health worldwide. The disease results in significant economic losses in livestock production and represents an important zoonotic threat to human populations. Transmission dynamics are complex, involving interactions between domestic and wildlife reservoirs, which hinder eradication efforts, particularly in endemic regions. In Mediterranean contexts such as Sicily, communal grazing systems and shared environmental resources facilitate interspecies contact, sustaining pathogen circulation and creating persistent health challenge. This study aimed to analyze the global scientific production on bTB over the past fifty-five years to understand the evolution od research trends, with a focus on multi-host ecosystems and the wildlife-livestock interface, framed within a "One Health" approach.

MATERIALS and METHODS: A systematic review was conducted following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. The study identifies prevailing research topics, summarizes their temporal trends, and highlights potential gaps in the literature. Out of 1,940 records initially retrieved from international databases, 560 abstracts were deemed eligible for analysis. The methodology combined descriptive statistics, Text Mining (TM), and Topic Analysis (TA) to process large volumes of unstructured textual data. This computational linguistic approach allowed the identification of recurring thematic structures and the quantification of shifts in scientific focus over time.

RESULTS: The analysis revealed a steady increase in scientific publications, with peaks in 2013, 2020, and 2021, reflecting growing global concern over zoonotic spillovers and the implementation of updated international regulatory frameworks. TM identified high-frequency terms such as "vaccin," "deer," "wild," "isol," "test," and "badger," indicating strong interest in diagnostic accuracy and wildlife reservoirs. TA highlighted five key research areas: diagnostic protocols, molecular epidemiology, wildlife management, vaccination strategies, and host-pathogen interactions. All areas showed a rising temporal trend, reflecting sustained scientific interest in complex dynamics of bTB and the need for integrated surveillance systems.

CONCLUSIONS: This systematic review demonstrates a growing global focus on advanced diagnostic and vaccination strategies for bTB control. The volume of scientific output underscores the challenge posed by multi-host ecosystems where domestic and wild animals share ecological niches. The findings provide an evidence-based foundation for future One Health research, emphasizing the need for targeted interventions in endemic settings. The effective eradication programs should integrate environmental, veterinary, and public health data to disrup transmission cycle in complex rural landscapes.

Keywords: Bovine tuberculosis, machine learning, husbandry, text mining, topic analysis

Seroprevalence and Risk Factors Associated with Bovine Herpesvirus-1 in Eastern Anatolia Region of Türkiye

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BACKGROUND and OBJECTIVE: Bovine Herpesvirus-1 (BoHV-1) is a major viral agent associated with respiratory, reproductive, and neurological diseases in cattle. The presence of the pathogen in a herd or its carrier status causes significant economic losses. The virus can establish a lifelong latent infection and reactivate in the presence of factors such as stress, which complicates the dynamics of infection within a herd. Therefore, seroepidemiological surveillance in herds, the identification of risk factors, and the investigation of the mechanisms of infection transmission are crucial for controlling and eradicating the disease. The aim of this study was to determine the serological prevalence of BoHV-1 and identify potential risk factors in the Eastern Anatolia region of Türkiye, where the cattle population is concentrated.

MATERIALS and METHODS: In this study, blood serum samples were collected from 592 cattle older than six months of age, selected from 49 farms across 10 provinces in the Eastern Anatolia region of Türkiye. The presence of BoHV-1 was serologically investigated using the gB-ELISA method. Farmers were asked about colostrum intake, herd size and type, animal procurement, and quarantine practices in the event of disease. Information was also gathered about sex, feeding system, calf rearing method, breed, air quality, farm milk yield, age, infertility, and milking method. Additional factors surveyed included farm type, co-rearing with other animal species, distance from other cattle farms, and visitor access to the farm. Calf housing units, body condition score, vaccination status, breeding method, presence of respiratory findings, and farm hygiene conditions were also covered. Comparative statistical analysis of the epidemiological data was performed using the Generalised Estimating Equations (GEE) model.

RESULTS: According to the results of the gB-ELISA test, BoHV-1 antibodies were detected in 49.48% (293/592) of the 592 cattle examined. Statistical analyses revealed that the variables of province ($p=0.002$), age ($p=0.003$), and animal procurement ($p<0.001$) had a statistically significant effect on seropositivity. It was found that the seropositivity rate was particularly higher in cattle aged 1–5 years. Furthermore, it was determined that purchasing locally sourced animals poses a higher risk of BoHV-1 seropositivity compared to purchasing imported animals.

CONCLUSIONS: This study shows that BoHV-1 infection circulates endemically in Eastern Anatolia. The identified risk factors provide data for infection monitoring and for planning control programmes. These findings support the development of regional control and surveillance strategies.

Keywords: Bovine Herpesvirus-1, Cattle, Risk factors, Seroprevalence, Türkiye

Bovine Coronavirus-Associated Winter Dysentery in Cattle: Clinical, Molecular, Hematological, and Some Biochemical Findings from a Herd Outbreak

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BACKGROUND and OBJECTIVE: Winter dysentery is an acute and highly contagious disease of adult cattle associated with bovine coronavirus (BCoV), and it usually involves the digestive system and sometimes the respiratory tract. This study aimed to evaluate the clinical, molecular, hematological, and biochemical findings observed during a BCoV-associated winter dysentery outbreak in a cattle herd and to assess the potential supportive value of serum iron and selected laboratory parameters in affected animals.

MATERIALS and METHODS: The animal material of this study consisted of 23 cattle from the same herd, including 14 clinically affected animals and 9 clinically healthy controls. Blood samples (10 mL) were collected from the vena coccygea of all animals. One portion was transferred into K3-EDTA tubes for hematological analysis, whereas the remaining portion was placed into gel and clot activator tubes for serum biochemical analyses, including serum iron measurement. In addition, nasal swabs and stool samples were taken from clinically affected animals and sent to a virology laboratory for molecular diagnosis. They were analyzed by PCR for the coronavirus nucleoprotein gene region.

RESULTS: Clinically affected cattle predominantly exhibited bloody, mucoid diarrhea, while some also showed cough, nasal discharge ranging from serous to mucopurulent, and anorexia. Of the 14 clinically affected animals, 9 (64.3%) were positive for BCoV by PCR in at least one sample type. A total of 12 samples tested positive, and 3 animals showed concurrent positivity in both respiratory and digestive samples, suggesting involvement of both systems in some cases. Hematological analyses showed that white blood cell, lymphocyte, eosinophil, and basophil counts were significantly lower in affected cattle than in healthy controls, whereas mean platelet volume and plateletcrit values were significantly higher. Biochemical analyses revealed significantly lower serum iron and total protein concentrations in affected animals ($p<0.05$).

CONCLUSIONS: The findings support a herd-level outbreak of BCoV-associated winter dysentery and indicate that BCoV may be present in both digestive and respiratory samples of clinically affected cattle. The high proportion of PCR-positive animals and the concurrent positivity in both sample types in some cases suggest that BCoV played an important role in the outbreak and may be associated with multisystemic involvement. The observed hematological and biochemical alterations further indicate systemic effects of the disease. Decreased serum iron may represent a supportive laboratory finding, although its diagnostic value should be confirmed in larger studies including PCR-confirmed cases.

Keywords: Cattle, coronavirus, cow, diagnosis, serum iron, winter dysentery

Bovine Coronavirus-Associated Winter Epidemiological Survey of Bovine Leukemia Virus in Beef Cattle in the Tohoku Region of Japan, 2022-2024

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BACKGROUND and OBJECTIVE: The incidence of enzootic bovine leukosis (EBL), which is due to bovine leukemia virus (BLV), has been increasing in Japan. However, large-scale surveys have not been conducted since 2009 to 2010, and the recent impact of cattle harboring high BLV proviral loads (PVLs) on within-farm BLV prevalence remains unclear. To address this, a BLV prevalence survey was conducted in the Tohoku region, Japan from 2022 to 2024.

MATERIALS and METHODS: A questionnaire survey, qPCR and serological testing for BLV, were conducted on 95 beef breeding farms (3,082 cattle) and 9 Japanese Black fattening farms (140 cattle) in the Tohoku region, Japan. For BLV provirus detection, real-time PCR analysis was performed by using a Bovine Leukemia Virus qPCR Detection Kit (RC202A; Takara Bio) on a StepOne Plus Real-Time PCR System (Thermo Fisher Scientific). For antibody detection, an ELISA kit (IDEXX Leukosis Serum X2 Ab Test) was used. We also examined the effects of various factors that potentially affected BLV prevalence within farm.

RESULTS: The proportions of qPCR-positive and seropositive cattle were 34.3% and 35.9% in beef breeding cattle, whereas 15.7% and 15.0% in Japanese Black fattening cattle, respectively. Among the 74 BLV-positive farms, the proportions of qPCR-positive cattle were significantly higher on farms with at

least one animal with a high PVL (≥400 copies/10 ng DNA) than on farms without animals with high a high PVL. Similarly, farms where high-PVL cattle accounted for ≥10% of the herd had significantly higher proportions of qPCR-positive cattle compared to those with <10%. In addition, farms that used public pastures, used on-farm paddock, or had experienced EBL within the previous 5 years had significantly higher proportions of qPCR-positive cattle.

CONCLUSIONS: Our findings suggest that the rapid increase in BLV prevalence among beef cattle in the Tohoku region, Japan may have plateaued. Moreover, the results suggest that the presence of high-PVL cattle influences BLV prevalence at the farm level and that farms using public pastures, used on-farm paddock, or having experienced EBL cases tend to have higher BLV prevalence.

Keywords: bovine leukemia virus, BLV, enzootic bovine leukosis, EBL, proviral load, regional survey

Histologic and Immunologic Characterisation of the Tuberculous Granuloma in Bcg-vaccinated, Mycobacterium bovis-Experimentally Infected Cattle

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BACKGROUND and OBJECTIVE: Bovine tuberculosis is a notifiable, zoonotic disease caused by *Mycobacterium bovis*, (*M.bovis*) affecting cattle and characterised by the formation of granulomas in the lungs, regional lymph nodes and other organs. Bacille Calmette Guerin (BCG) is an antituberculous vaccine that has been used for more than 100 years in both livestock and humans, although its efficacy is controversial. The mechanisms by which it allows animals to better control the spread of the bacterium, and the development of lesions have not been fully characterised. We hypothesised that granulomas from M. bovis exposed, BCG vaccinated animals would have histological/immunological features different from M. bovis exposed unvaccinated animals. These differences could then explain why BCG vaccinated animals are more efficient at controlling the spread of the bacteria and the development of lesions.

MATERIALS and METHODS: In the present study, we stained formalin-fixed, paraffin-embedded sections of tracheobronchial (n=22) and mediastinal (n=19) lymph nodes from BCG vaccinated (n=21) and unvaccinated (n=20) animals with haematoxylin and eosin and Masson's trichrome, and quantified extent of granuloma, extent of necrosis, and fibrosis within the granuloma and the density of multinucleated giant cells, to highlight the histological differences in the granulomas between treatment groups and anatomical locations.

RESULTS: Unvaccinated animals had a larger cut surface area of granuloma lesion than vaccinated animals in the mediastinal lymph nodes ($p<0.05$) and regardless of the anatomical location ($p<0.01$), but no statistically significant differences were seen in the area of fibrosis or necrosis. No statistically significant differences were noted in the numbers of multinucleated giant cells/area, although the medians of these were always higher in the non-vaccinated group. Semiquantitative scoring of lesion severity was performed, which showed that lymph nodes from unvaccinated animals were more severely affected than BCG vaccinated animals, regardless of the anatomical location. This also showed that tracheobronchial lymph nodes had relatively more severe lesions than mediastinal lymph nodes, regardless of whether animals were vaccinated or not; however, no significant statistical differences were seen. We are currently pursuing characterization of the histiocytic component of the granuloma by using antibodies against ADGRE1, a pan-macrophage marker, and CD163, to distinguish between CD163+ and CD163– histiocytes.

CONCLUSIONS: The results suggest that vaccinated animals may be able to better contain or delay the progression of the mycobacterial granulomas than unvaccinated animals, based on the smaller size of their granulomas compared to unvaccinated animals.

Keywords: pathology, tuberculosis, cattle, immunology, vaccine,

Bovine Leukemia Virus Infection and Fertility Decline in Dairy Cows: The Potential Role of Subclinical Mastitis

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BACKGROUND and OBJECTIVE: Reproductive performance is a key determinant of profitability in dairy herds. Subclinical mastitis is common and linked to poorer reproductive performance, and Bovine leukemia virus (BLV) is a widespread infection implicated as a potential risk factor for subclinical mastitis—particularly in cows with high proviral load (H-PVL)— yet the causal pathway whereby BLV may impair time-to-pregnancy via mastitis has not been evaluated. This study aimed to assess the causal relationship between BLV infection and time-to-pregnancy, including the mediating role of subclinical mastitis.

MATERIALS and METHODS: We conducted a retrospective cohort study of Holstein cows from 9 commercial herds in Nemuro and Kushiro regions of Hokkaido (April 2015–March 2018) using DHI records and routine veterinary BLV testing. BLV status was based on antibody testing and/or proviral DNA detection; infected cows were categorized as low (L-PVL) or H-PVL using previously established assay-specific qPCR cutoffs. Follow-up for conception began at 60 DIM (voluntary waiting period). Subclinical mastitis was defined as linear SCC score ≥5 (≈283,000 cells/mL). Pregnancy was the event of interest and culling the competing event, and cause-specific hazards were estimated using a case-base sampling approach with herd-level random effects, adjusting for parity and delivery season. Causal mediation decomposed total effects into natural direct and indirect effects through mastitis using a logistic mediator model, mediation weights, and weighted cause-specific Cox models adjusted for parity and calving season.

RESULTS: At enrollment, 85.0% of the cows were BLV-noninfected, 11.8% had L-PVL, and 3.2% had H-PVL. Subclinical mastitis was significantly associated with a lower cause-specific hazard of pregnancy (cHR = 0.60). Cows with H-PVL experienced a significantly cHR of culling compared to BLV-noninfected cows (cHR = 1.96). In the mediation analysis, H-PVL cows showed a 27% lower total pregnancy hazard (Total Effect HR = 0.73) compared with noninfected cows. The natural indirect effect operating through subclinical mastitis was associated with a lower hazard of pregnancy for both L-PVL (HR = 0.91) and H-PVL (HR = 0.85) cows. The proportion mediated was 0.51 for H-PVL cows, indicating that approximately half of the reduction in pregnancy hazard in this group is attributable to the mastitis-mediated pathway.

CONCLUSIONS: BLV infection, particularly at high proviral loads, negatively impacts reproductive performance and increases the hazard of culling in dairy cows. Subclinical mastitis acts as a significant mediating factor, explaining approximately half of the reduced conception hazard observed in H-PVL cows.

Keywords: Bovine leukemia virus, Proviral load, Reproductive performance, Subclinical mastitis, Cohort study

High-Mortality Pneumonia Outbreaks in Dairy Cows Linked to *Mannheimia haemolytica*

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BACKGROUND and OBJECTIVE: Pneumonia is a multifactorial disease that frequently affects cattle. *Mannheimia haemolytica* has classically been considered a secondary pathogen following viral respiratory infections. However, recent reports have identified it as a primary pathogen in adult dairy cows, associated with high mortality. Scientific evidence on pneumonia outbreaks in adult cows remains limited. This study aims to describe eleven acute pneumonia outbreaks with elevated

mortality in dairy cows, to elucidate the role and serotypes of M. haemolytica, and to identify key clinical patterns and herd-level risk factors to support earlier diagnosis and preventive strategies.

MATERIALS and METHODS: From October 2024 to January 2026, eleven acute pneumonia outbreaks in dairy farms in Spain were investigated. Farm visits were conducted to collect epidemiological data, including housing conditions, ventilation, stocking density, biosecurity, and recent animal introductions. Deep nasopharyngeal swabs from affected cows and lung samples from necropsies were submitted for bacteriological culture, ELISA detection of respiratory viral antigens (BoHV-1, BVDV, BRSV, PI-3), and real-time PCR for BRSV, PI-3, BoHV-1, *M. haemolytica, M. bovis, H. somni, and P. multocida. Lung tissues were examined histologically, and M. haemolytica isolates were capsular-serotyped (A1, A2, A6) by multiplex PCR.*

RESULTS: A total of 282 cows were treated, with a mean morbidity of 21.7% (range: 5.2–62.9%). Eighty animals died, and an additional 25 cows were culled due to irreversible production losses, resulting in a mean herd impact of 37.2% (range: 17–60%). Affected cows showed marked depression, severe dyspnoea, and a sudden drop in milk yield. In four herds sudden deaths were recorded. M. haemolytica was detected by PCR and/or culture in all samples from deep tracheal swabs (n=11) and/or lung tissues (n=14), while no bovine respiratory viruses were identified. Co-infections were detected in six samples (five with P. multocida and one with M. bovis). The predominant serotypes were A6 (67%), A1 (22%), and A2 (11%). Histopathology revealed exudative fibrinonecrotic bronchopneumonia consistent with acute mannheimiosis. Most outbreaks (73%) occurred in autumn–winter. Six herds were overstocked, and all farms lacked effective natural ventilation. Seven herds had recently introduced animals. In two farms, the vaccination protocol included BRSV and PI-3. None of the herds vaccinated against Mannheimia haemolytica.

CONCLUSIONS: Mannheimia haemolytica was confirmed as a primary pathogen causing severe pneumonia and high mortality in dairy cows. Preventive strategies should focus on improving housing design, ventilation and biosecurity, as well as implementing vaccination programs that include M. haemolytica antigens.

Keywords: Pneumonia, Dairy, Cows, Mannheimia haemolytica, Mortality, Outbreaks

Mapping the Distribution of Mycoplasma bovis Infections in Brazilian Dairy Cattle

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BACKGROUND and OBJECTIVE: Mycoplasma bovis is an emerging pathogen in Brazilian dairy systems, but herd-level prevalence remains poorly characterized. This study mapped its distribution and herd-level associations across Brazilian states.

MATERIALS and METHODS: Mycoplasma bovis was screened in 190 farms from six major milk-producing states: Minas Gerais (MG, n = 60), Paraná (PR, n = 39), Rio Grande do Sul (RS, n = 27), Santa Catarina (SC, n = 11), São Paulo (SP, n = 19), and Goiás (GO, n = 34), which together account for 83.7% of national milk production. Screening used bulk tank milk samples collected in triplicate at 30-day intervals and at least six nasal swabs from calves presenting respiratory disease per herd. Detection was carried out by conventional PCR targeting the vsp genomic region (319 bp amplicon). Milk samples were centrifuged before DNA extraction, and PCR products were visualized by agarose gel electrophoresis. Descriptive statistics and comparative tests were performed. Prevalence was calculated overall and by state, and differences were evaluated using the chi-square test. A matched case–control design (1: 2) was applied to compare PCR-positive and PCR-negative herds. Continuous variables were analyzed using the Mann–Whitney U test and categorical variables using the chi-square test ($p < 0.05$).

RESULTS: Mean herd size ranged from 259 ± 218 lactating cows (RS) to 460 ± 307 (MG). Milk yield varied from 33.2 ± 3.7 L/cow/day (SC) to 38.9 ± 4.3 (RS). Somatic cell count ranged from 180 ± 18 $\times 10^3$ cells/mL (GO) to 217 ± 9 (RS), and total bacterial count ranged from 5.5 ± 4.7 $\times 10^3$ CFU/mL (PR) to 18.3 ± 11.3 (SC). Overall, 25 of 190 herds (13.2%) tested positive. State-level prevalence varied substantially: Goiás 4/34 (11.8%), Minas Gerais 7/60 (11.7%), Paraná 7/39 (17.9%), Rio Grande do Sul 1/27 (3.7%), Santa Catarina 4/11 (36.4%), and São Paulo 2/19 (10.5%). Differences in prevalence among states were not statistically significant ($p = 0.137$). No significant differences

were observed between PCR-positive and PCR-negative herds in milk yield, somatic cell count, total bacterial count, herd size, number of pre-weaned calves, or feeding strategy ($p > 0.34$ for all comparisons). Waste milk was the predominant feeding practice for pre-weaned calves in both groups.

CONCLUSIONS: Mycoplasma bovis was detected in all investigated Brazilian states. In addition, herd productivity and milk quality indicators routinely used on dairy farms did not reliably distinguish between infected and non-infected herds, reinforcing the need for active surveillance and targeted diagnostic screening.

Keywords: Bovine respiratory disease, pneumonia, herd-characteristics, prevalence

Spatiotemporal Distribution of Peste des Petits Ruminants Virus: A Dramatic Turn to Lineage IV Dominance During Last Decade

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BACKGROUND and OBJECTIVE: Morbillivirus caprinae (PPRV) shows marked temporal and geographical variation in lineage distribution. Historically, lineages I, II, and III were reported mainly from different parts of sub-Saharan Africa, whereas lineage IV was more closely associated with Asia and the Middle East. Over the last decade, however, the global epidemiological picture has changed substantially, with lineage IV becoming increasingly dominant across several endemic regions and extending toward Europe. This study aimed to summarize the spatiotemporal distribution of Morbillivirus caprinae (Peste des petits ruminants virus) in the last decade and create a comparison to the pre-2015 period and to evaluate recent European outbreaks within a phylogenetic framework.

MATERIALS and METHODS: A descriptive spatiotemporal assessment was performed using lineage information compiled from publicly available outbreak notifications, sequence records, and published studies. Two distribution maps were generated to compare the pre-2015 and post-2015 periods using data derived from WAHIS, PubMed, GenBank Nucleotide, and published articles. For phylogenetic analysis, complete genome sequences were downloaded, and a Maximum Likelihood tree was constructed using MAFFT and IQ-TREE. The final tree was visualized in iTOL.

RESULTS: Before 2015, lineage distribution was more geographically compartmentalized, with lineages I, II, and III largely confined to different parts of Africa and lineage IV occurring mainly in Asia and the Middle East. In contrast, post-2015 data indicated a clear expansion and growing dominance of lineage IV across North and East Africa, South Asia, and regions closer to Europe. This pattern suggests a shift from historically localized lineage circulation to broader transboundary dissemination dominated by lineage IV. Phylogenetic analysis supported this transition and showed that recent European strains clustered within lineage IV. Isolates reported from Greece, Bulgaria, and Romania in 2024 formed a closely related subgroup, consistent with recent spread linked to a common or closely connected source.

CONCLUSIONS: The global distribution of Morbillivirus caprinae lineages has changed substantially since 2015, with lineage IV emerging as the predominant lineage across a broad transcontinental range. Recent European outbreaks appear to represent an extension of this broader epidemiological shift rather than an isolated event. Integrating spatiotemporal mapping with phylogenetic analysis may help clarify lineage replacement, track transboundary spread, and support regional surveillance strategies. (This study is performed under concept of BUÜ-BAP Project: TGA4-2025-2359)

Keywords: Peste des Petits Ruminants, Morbillivirus caprinae, Lineage distribution, Phylogeny, European Outbreaks

Histopathological and Diagnostic Features of Ulcerative Stephanofilariasis Lesions in Holstein Cows

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BACKGROUND and OBJECTIVE: Stephanofilariasis is a parasitic disease caused by nematodes of the genus Stephanofilaria, transmitted by flies, and characterised by chronic dermatitis affecting the udder of lactating cows. The objective of this study was to evaluate the histopathological characteristics of ulcerative lesions associated with stephanofilariasis in naturally affected lactating Holstein cows.

MATERIALS and METHODS: Thirty cows from the same farm were selected, all presenting circular skin lesions located on the udder and/or adjacent areas, accompanied by crusts and serosanguineous exudate. Diagnosis was confirmed through direct microscopic examination of material obtained by punch biopsy of the lesions. At the time of biopsy, two additional tissue samples were collected from the borders of each lesion for histopathological evaluation. Tissue fragments were fixed in a 10% buffered formalin and submitted to a reference laboratory for routine processing. For sample collection, animals were restrained in a stall, the affected area was disinfected with iodised alcohol, and local infiltrative anaesthesia was administered using 0.5–1 mL of 2% lidocaine.

RESULTS: All lesions examined in this study were located in the udder region. Histological analysis of the tissue fragments did not reveal larval or adult forms of "Stephanofilaria" spp. Microscopic examination showed perivascular infiltration composed of mononuclear inflammatory cells (lymphocytes, neutrophils, and histiocytes) and eosinophils. Granulation tissue was observed in 15 samples, characterised by newly formed blood vessels surrounded by mononuclear inflammatory infiltrates. These histopathological features are consisted of chronic inflammatory reactions associated with persistent parasitic infection.

CONCLUSIONS: In conclusion, histopathological examination may not demonstrate the presence of Stephanofilaria spp. in affected tissues, despite the presence of chronic inflammatory changes with mononuclear and eosinophilic infiltrates. Therefore, direct microscopic examination of lesion sediment remains the recommended method for confirming the diagnosis.

Keywords: Stephanofilaria spp., Dairy cattle, Udder lesions, Histopathology.

Surveillance of *Coxiella burnetii* in Aborted Cattle Fetuses and Milk Samples in Finland

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BACKGROUND and OBJECTIVE: The aim of this study was to assess causes of bovine abortions in Finland during 2024-2025, with the special emphasis on detecting *Coxiella burnetii*, the causative agent of Q fever. OH4Surveillance project aims to enhance disease preparedness and building laboratory capacity in the detection of new emerging diseases, which is why *Coxiella* was chosen, as it holds the potential of being an emerging pathogen with its zoonotic nature and wide range of hosts. Domestic ruminants act as main reservoir for *C. burnetii*. Despite the majority of infections being asymptomatic, Q fever does hold the potential to cause reproductive issues, abortions, stillbirths or weak offspring. All fetuses sent for necropsy in Finland are tested for possible *Coxiella* infection using Stamp staining on fetuses' liver, abomasal contents and cotyledons.

MATERIALS and METHODS: A full necropsy was performed to the aborted bovine fetuses and the

placenta (if available), while also collecting samples for histology, bacteriology and virology. Conventional bacteriological methods were used to detect bacterial and fungal infections, while enrichment culture and PCR were used to detect *Mycoplasma bovis* and *Ureaplasma diversum*. Stamp staining was performed to detect *C. burnetii*, *Chlamydia abortus* and *Brucella* spp., as well as PCR to detect *C. burnetii* in fetal and placental samples. All samples were analyzed for BVD and IBR viruses (PCR), from March 2025 onwards also for BTV (RT-PCR). In total 250 samples were examined. 116 fetuses (49,1%) had placental tissue available. 202 samples (80,8%) originated from dairy farms and 48 (19,2%) from suckler herds. 43 of the herds sent 166 milk samples with the aborted fetuses. Milk samples were examined for *C. burnetii* antibodies with ELISA.

RESULTS: All 250 samples were negative in *C. burnetii* PCR. Out of 43 herds that sent milk samples, one herd (2,3%) was positive for *C. burnetii* antibodies. None of the samples were positive for BVD or IBR viruses, *Brucella* spp., *Chlamydia abortus* or *Mycoplasma bovis*. Most common findings included bacterial infections (66 being 26,4%) with *Trueperella pyogenes* (25) and *Ureaplasma* (16) being most prevalent findings. Other common infections included parasitic *Neospora caninum* (12), fungal infections (4) and one Schmallenberg virus. Most samples (138 being 55,2%) had no specific pathological findings. 28 of the fetuses had congenital malformations.

CONCLUSIONS: Clinical disease caused by *C. burnetii* bacteria was not detected and prevalence of *C. burnetii* antibodies is low in Finland. Bovine abortions were due to other causes, most common finding being other bacterial infection.

Keywords: bovine abortion, Coxiella burnetii, Q fever, fetal and perinatal pathology, infectious diseases of cattle, zoonosis

A Latent Viral Agent in Dairy Farms: Molecular Characterization of Bovine Herpesvirus 4 in Türkiye

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BACKGROUND and OBJECTIVE: The livestock industry plays a crucial role in serving global demand for animal protein. Early detection and monitoring of cattle diseases (Herpesviral infections ie.), as well as sustainable health policies, contribute to minimizing economic losses. Bovine Herpesvirus-4 (BHV-4) is a significant viral pathogen associated with genital and respiratory system infections in cattle, and its potential for latent infection, often presenting as subclinical, poses a considerable risk within the herd. Because of this silent circulation, it may pose a risk to economically sustainable cattle farming. The aim of this study was to determine the presence of BHV-4 in Erzurum Province using molecular methods and to identify the strains through phylogenetic analysis.

MATERIALS and METHODS: In this study, samples were taken from four family cattle farms located in the Eastern Anatolia Region of Türkiye. A total of 40 blood samples were collected from cows, and the clinical conditions were recorded. Viral nucleic acid was extracted from blood samples. Conventional PCR analysis was performed using primers specific to the BHV-4 glycoprotein B (gB) gene region. The amplicons identified as positive were sequenced. The sequence data obtained were compared with BHV-4 reference sequences registered in GenBank to perform a phylogenetic analysis.

RESULTS: Based on the PCR analysis, 4 of the 40 cows in the study (10%) tested positive for the BHV-4 gB gene. The one of the positive animals had a history of abortion, while another exhibited enlarged retropharyngeal and prescapular lymph nodes. No specific clinical findings were observed in the other two positive animals. Sequence analysis revealed that Genotype II (n=3) and Genotype III (n=1) strains were circulating in the Eastern Anatolia region. Phylogenetic analysis revealed that strains from this study shared close genetic relationships with

previously reported BHV-4 strains from Türkiye, Germany, Iran, USA and, Argentina.

CONCLUSIONS: This study has showed that BHV-4 can circulate asymptomatically in herds and may also play a role in certain clinical cases. While previous studies in Türkiye have reported the presence of three BHV-4 genotypes, Genotype I is indicated as the predominant genotype. In this study, Genotype II and Genotype III strains were detected in the eastern region of Türkiye. The presence of frequent animal movements, shared grazing land, and widespread animal trade in this region may facilitate the spread of BHV-4 between regions. These findings highlight the importance of molecular-level monitoring and strain typing of BHV-4 in cattle herds both in regional and worldwide.

Keywords: Bovine herpesvirus 4, Cattle, Genotyping, Molecular detection, Phylogenetic analysis

Emergence of Antibiotic-resistant Bacteria in Sheep and Their Environment: Insights from a Cross-Sectional Study

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BACKGROUND and OBJECTIVE: Antibiotic resistance is a widespread natural phenomenon with significant zoonotic implications, presenting major challenges for both human and veterinary medicine. The misuse and overuse of antibiotics in livestock promote the emergence and spread of resistant bacteria, which can be transmitted to humans through direct contact or via environmental contamination. Sheep, as commonly farmed ruminants, harbor diverse bacterial communities in their oro-nasal cavities, serving as potential reservoirs of resistance genes.

MATERIALS and METHODS: This cross-sectional observational study evaluated the prevalence and antibiotic resistance profiles of bacterial isolates from the oro-nasal cavities of sheep (n=20) and their surrounding environment, including total flora analysis and MAR index assessment. Samples were analyzed using classical microbiological methods, with MALDI bacterial identification, and susceptibility testing was performed using the Kirby-Bauer method according to CLSI guidelines.

RESULTS: A diverse range of bacterial species was isolated, including Staphylococcus lentus, S. sciuri, S. capitis, S. xylosus, Aerococcus urinae, A. viridans (types 1 and 3), Moraxella lacunata, Enterobacter amnigenus, Pantoea spp., and Bacillus subtilis. Resistance profiling revealed high resistance to penicillin, enrofloxacin, tylosin, and streptomycin among the isolates, while total flora testing indicated elevated resistance to methicillin and tulathromycin. Over half of the isolates exhibited a multiple antibiotic resistance (MAR) index below 0.5, suggesting susceptibility to several antimicrobials.

CONCLUSIONS: These findings demonstrate the presence of resistant bacteria in both sheep and their environment, emphasizing the public health importance of antimicrobial resistance. Targeted antibiotic use is critical to prevent the emergence and spread of resistance, highlighting the need for integrated surveillance of antimicrobial resistance in livestock and their surroundings

Keywords: sheep, antibiotic resistance, MAR, environment

INTERNAL MEDICINE AND TOXICOLOGY

Big Five Clinical Reasoning: a Comprehensive Method for Undergraduates and Beyond to Reduce Diagnostic Errors in First-line Practice

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BACKGROUND and OBJECTIVE: In veterinary medicine, diagnostic errors are a major cause of therapeutic failure, unnecessary animal suffering, economic losses and legal disputes. A diagnostic error is defined as a missed, wrong or delayed diagnosis that could reasonably have been made earlier or more accurately. This abstract describes "Big Five Clinical Reasoning", a structured diagnostic reasoning method that focuses on the diagnostic work-up of the five most relevant explanations for a given clinical complaint.

MATERIALS and METHODS: The Big Five Clinical Reasoning methodology was initiated during the 2020 COVID-19 pandemic and refined annually thereafter. It builds on the assumption that the average human brain can effectively consider only about five hypotheses simultaneously for a given problem. For each common (and, in the ruminant track, also selected less common) clinical complaint, students are provided with a "Big Five" list of differential diagnoses. Inclusion criteria for the list are: disease prevalence, feasibility of diagnosis with first-line resources, and the consequences of missing the disease. The latter category, termed "must-haves/don't miss", includes conditions with high mortality if missed (e.g. botulism), public health risks (e.g. Salmonella) or major economic consequences, such as loss of herd status (e.g. IBR, BVDV). The method follows an 8-step procedure: (1) compiling a problem list; (2) selecting the key clinical sign to elaborate; (3) consulting the Big Five list; (4) weighing each differential in an "in favour/against" table to derive one or more probable diagnoses; (5) testing whether the probable diagnosis explains all items on the problem list; (6) designing a diagnostic plan; (7) re-evaluating the diagnosis once new information is obtained; and (8) formulating a therapeutic plan and communicating the prognosis.

RESULTS: The Big Five principles are now taught in the fifth year of the veterinary curriculum at Ghent University. Worked-out Big Five lists and completed argument tables are available for 15 complaints in cattle, 14 in goats, 14 in sheep and 10 in camelids. Implementation has led to a more clearly structured diagnostic landscape, greater awareness of cognitive bias and reasoning errors (through systematic self-checking and maintaining an open mind), and improved skills in designing diagnostic and therapeutic plans that account for practical and economic constraints.

CONCLUSIONS: Big Five Clinical Reasoning is a comprehensive, easy-to-grasp, stepwise method with a short learning curve. Consistent use during clinical rotations appears to habituate students to the approach and contributes to substantially improved day-one competencies across multiple species.

Keywords: Veterinary education, clinical reasoning, diagnosis

Renal Failure Treated with Intermittent Peritoneal Dialysis in a Llama

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BACKGROUND and OBJECTIVE: Renal failure in camelids is uncommon compared to small animals. In most cases, fluid therapy is sufficient to restore normal renal function. However, if renal function is difficult to restore with fluid therapy, dialysis is indicated. The objective of this case report is to present the procedure and outcome of intermittent peritoneal dialysis performed in a llama with renal failure that did not respond to fluid therapy.

MATERIALS and METHODS: A 2-year-old llama presented with an abnormal gait and was diagnosed with cystitis and pyelonephritis caused by Enterococcus gallinarum, secondary to bladder paralysis suspected to result from parasitic myelitis. Despite one week of fluid therapy, renal function

continued to decline. Intermittent peritoneal dialysis was necessary to attempt restoration of renal function. For this, a ventrally placed catheter (Mila, 28 FR × 34 cm) was used. Dialysis was performed using a Ringer's lactate (LRS) solution supplemented with 1.5% dextrose at 10 mL/kg for the first session (dialysis #1), increased to 20 mL/kg for sessions two and three (dialysis #2 and #3). The dwell times were 4 hours, 2 hours, and 1 hour for dialysis sessions 1, 2, and 3, respectively. All the session was performed the same day.

RESULTS: Only 200 mL of fluid was recovered during each session, despite the increased instilled volume and the reduced dwell time. Blood creatinine concentration decreased from 1,381 µmol/L pre-dialysis to 845 µmol/L after session #1, 648 µmol/L after session #2, and 550 µmol/L after session #3. Creatinine concentrations in the dialysate were similar to blood creatinine levels, measuring 845 µmol/L, 631 µmol/L, and 552 µmol/L during sessions #1, #2, and #3, respectively. Dialysis was discontinued at that time due to the rapid decrease in urea concentration—from 58.95 to 23.20 mmol/L within 7 hours—and the associated risk of cerebral osmotic edema. Renal parameters continued to improve over the following days, with creatinine decreasing to 289 µmol/L one week post dialysis.

CONCLUSIONS: This case report demonstrates the feasibility and high efficacy of performing intermittent peritoneal dialysis in a llama.

Keywords: New world camelids, kidney, treatment

Investigation Of the Relationship Between Motilin, Leptin and Ghrelin Levels on the 7th Day Postpartum and Displacement of Abomasum

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BACKGROUND and OBJECTIVE: Displacement of abomasum (DA) is a disease defined as the presence of the abomasum outside its normal position in the abdomen. Motilin is a hormone that physiologically regulates gastric motility and accelerates gastric emptying and decreased abomasal motility is an etiological factor for DA.

MATERIALS and METHODS: The animal material of the presented study consists of 45 Holstein cattle from the same farm. Serum samples were collected from the cattle on the 7th postpartum day and the animals were observed for 60 days. During this period, DA was diagnosed in 11 cattle in the early period (EDA group) (postpartum days 7-14) and 11 cattle in the late period (LDA group) (postpartum days 14-21). 23 healthy cattle with similar yields and lactation numbers to the cattle in the study group constituted the control group (CON). Serum Motilin, Ghrelin and Leptin hormone levels in the 45 cattle included in the study were measured with commercial ELISA kits, and serum Alkaline Phosphatase (ALP), Aspartate Transaminase (AST), calcium, phosphorus, magnesium, betahydroxybutyric acid, BUN, total protein, creatinine, cholesterol and gamma glutamyl transferase levels were measured with a biochemistry autoanalyzer.

RESULTS: Motilin was significantly ($p<0.05$) higher in the EDA group (48.14±5.63 ng/l) compared to the LDA group (35.82±2.40 ng/l) and the CON group (35.58±1.49 ng/l); It was determined that leptin tended to be higher in the EDA (0.92±0.35 ng/ml) and LDA group (0.90±0.32 ng/ml) compared to the CON group (0.39±0.12 ng/ml) ($p=0.068$). No significant difference was found between the groups in ghrelin levels. Among the measured biochemical parameters, ALP concentration was significantly higher in the EDA group (73.8±9.1 IU/l) and LDA group (75.0±9.6 IU/l) compared to the CON group (47.6±3.5 IU/l) and AST concentration was significantly higher in the EDA (141.8±17.8 IU/l) and LDA group (132.1±15.8 IU/l) compared to the CON group (87.9±6.4 IU/l).

CONCLUSIONS: Motilin is a hormone that physiologically regulates gastric motility and accelerates gastric emptying. Decreased abomasal motility is an important etiological factor in DA. The results showed that motilin levels increased in dairy cattle before DA formation, but this increase occurred only days before DA. This shows that motilin may play a role in the pathophysiology of DA, and future studies may allow animals at high risk of DA to be identified before DA formation. In addition, increased AST and ALP activities in cows with displacement may be associated with hepatocellular damage resulting from hepatic lipidosis during the transition period.

Keywords: Cattle, Abomasum Displacement, Motilin, Leptin, Ghrelin

Significance Of Milk Lactate Dehydrogenase Activity Changes for Early Detection of Liver Damage in Dairy Cows

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BACKGROUND and OBJECTIVE: Liver function has a significant impact on the productivity and metabolic stability of dairy cows. Early diagnosis of liver damage remains challenging because routine blood biomarkers (AST, ALB, GGT, LDH) require invasive sampling. Milk LDH could be a non-invasive alternative, but its diagnostic value compared to liver function is not well known. This study evaluated changes in milk LDH activity, relationships with blood liver enzymes (AST, ALB, GGT, LDH), and the potential of milk LDH as an early indicator of liver damage in dairy cows.

MATERIALS and METHODS: Clinically healthy cows across lactations were sampled for milk and blood. LDH activity was measured in milk; blood parameters included AST, ALB, GGT, LDH, and BHB. Cows were grouped by lactation number; correlations between milk LDH and blood biomarkers were analyzed. Significance was set at $p < 0.05$.

RESULTS: Lactation number markedly affected milk yield and enzyme activity. Third-lactation cows produced 42% more milk, had 44% higher milk LDH, and showed 3.1% higher blood AST than first-lactation cows. A significant correlation between blood LDH and AST was observed across lactations. In second-lactation cows, a 1% rise in AST and LDH associated with a 0.18% rise in ALB. Milk LDH was about 40% higher in third-lactation cows than in first-lactation cows. However, correlations between milk LDH and blood liver enzymes were weak or non-significant.

CONCLUSIONS: Lactation is a key physiological risk factor for hepatic and tissue enzymatic changes. While milk LDH increases with lactation intensity, it did not show meaningful correlations with main hepatic enzymes, thus cannot be regarded as a specific or reliable standalone biomarker for early liver damage. Nevertheless, due to its non-invasive nature and responsiveness to physiological changes, milk LDH may serve as a supportive parameter for overall metabolic monitoring, especially during postpartum risk periods.

Keywords: Dairy cows, Milk lactate dehydrogenase (LDH), Liver enzymes, Non-invasive biomarkers, Liver function

Acute Renal Failure in a Heifer: Case Report

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BACKGROUND and OBJECTIVE: Diagnosis of acute renal failure (ARF) is uncommon in ruminant practice. Causes include intoxications; severe dehydration; bacterial infections; metabolic and nutritional disorders; urinary obstruction; neoplasia and congenital or hereditary diseases affecting the urinary system. This study reports a case of ARF in a heifer.

MATERIALS and METHODS: An elite Nelore heifer was referred to a hospital with suspected ARF. The animal had been diagnosed with bovine parasitic tristeza and pneumonia and treated with diminazene diaceturate (5 mg/kg, single dose), oxytetracycline (25 mg/kg, single dose), meloxicam (0.5 mg/kg every 24 h for 3 days), and ceftiofur (4.4 mg/kg every 24 h for 5 days). Laboratory tests the day before admission revealed azotemia. On physical examination, the heifer was apathetic, anorexic, tachypneic (40 breaths/min), with pale mucous membranes and absence of ruminal motility. Laboratory evaluation at admission showed anemia (hematocrit 17.6%) and severe azotemia (urea 310 mg/dL; creatinine 12.8 mg/dL). Catheters were placed in the jugular and auricular veins and aggressive fluid therapy with lactated Ringer's solution was initiated. The animal received 40 L of fluids during the first 8 hours of hospitalization and urinated

approximately every two hours after the first micturition following administration of 12 L of fluid. Urinalysis the following day showed decreased urine specific gravity (1.004), granular casts (++), and glycosuria (+). During hospitalization, the animal developed fever on days 2 and 3; tests for hemoparasites and serology for leptospirosis were negative. Treatment included daily fluid therapy (30–40 L), a penicillin combination (30,000 IU/kg IM every 48 h, four doses), dipyrone during febrile episodes, iron supplementation (2 mg/kg IM every 72 h, three doses), and oral mineral and propylene glycol supplementation.

RESULTS: After four days, appetite improved and azotemia decreased (creatinine 7.5 mg/dL; urea 86.2 mg/dL), although urine specific gravity remained low (1.002) with cylindruria and glycosuria. Urea normalized six days after admission, but creatinine remained above reference and hyperphosphatemia was detected. After 12 days, the heifer was clinically stable but presented elevated creatinine (2.6 mg/dL), isosthenuria (1.010), cylindruria, and hyperphosphatemia. The owner elected to continue treatment on the farm, and protein-restricted diet with phosphorus binder was recommended.

CONCLUSIONS: Although the exact cause of ARF could not be confirmed, decreased renal perfusion from dehydration and anemia, combined with nephrotoxic drugs such as meloxicam and diminazene diaceturate, was suspected. Persistent azotemia with isosthenuria and hyperphosphatemia suggested progression to chronic kidney disease. Laboratory testing is essential for diagnosing renal disorders in cattle, likely underdiagnosed.

Keywords: Creatinine, kidney disease, urinalysis.

Congenital Cervical ectopia Cordis in a Calf: A Multimodal Diagnostic and Management Case Report

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BACKGROUND and OBJECTIVE: A 10-day-old male Whitebred Shorthorn cross calf was presented with a large, firm, pulsatile cervical mass extending from the mandible to the thoracic inlet. Palpation revealed regular rhythmic pulsations consistent with cardiac activity, reported to be present since birth. A diagnosis of cervical ectopia cordis was made, and the calf was hospitalized to evaluate the clinical significance of the condition with respect to milk intake, growth rate, and overall viability. The aim of this case report was to describe the clinical and pathological findings of a 10-day-old calf with cervical ectopia cordis.

MATERIALS and METHODS: Daily clinical examinations were performed during a 27-day hospitalization period. Milk intake was recorded daily and body weight weekly. A diagnostic investigation was undertaken, including haematology and serum biochemistry (at three time points), cardiac and thoracic ultrasonography, contrast and non-contrast cervical radiographs, and thoracic radiographs. These imaging modalities have not been routinely described in previously reported cases of bovine ectopia cordis.

RESULTS: On initial examination, the calf was bright, alert, and responsive. Vital parameters were as follow: rectal temperature 39.3 °C, heart rate 120 beats/min, and respiratory rate 60 breaths/min, with a positive tracheal pinch reflex, eliciting intermittent coughing. During hospitalization, the calf remained frequently tachycardic (100-140 beats/min) and tachypnoeic (40-80 breaths/min). These clinical signs were exacerbated during milk feeding and exercise and were accompanied by intermittent coughing. No abnormal haematological and biochemical values were found. Cervical radiographs revealed a large, rounded soft-tissue

structure occupying the ventral cervical region, resulting in dorsal displacement of the trachea. Contrast radiographs displayed a tortuous and dorsally displaced common carotid artery in the caudal cervical region. The calf developed comorbidities and was euthanized. Post-mortem examination confirmed cervical ectopia cordis and identified additional congenital abnormalities, including patent ductus arteriosus, absence of the left coronary ostium, a carnivore-like aortic arch configuration, and congenital malformations of the liver and spleen.

CONCLUSIONS: This case demonstrates that calves with cervical ectopia cordis, in the absence of severe primary cardiac dysfunction, may survive with sustained supportive care and maintain near-normal physiological parameters. Increased susceptibility to secondary disease and impaired growth, render such animals unsuitable for conventional farm rearing systems.

Keywords: Cervical {ectopia cordis}, calf, imaging diagnosis

Artificial Intelligence-Assisted Pulmonary Auscultation in Cattle: Development and Preliminary Evaluation

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BACKGROUND and OBJECTIVE: Bovine respiratory disease (BRD) complex is a major contributor to morbidity mortality and economic losses in cattle. Early detection is key for outcomes and prudent antimicrobial use. Pulmonary auscultation is a routine and low-cost field tool but is limited by inter-operator variability and subjectivity of interpretation, environmental noise and recording conditions. The objective of this study was to develop and evaluate an artificial intelligence (AI) model to assist bovine pulmonary auscultation with automatic classification of respiratory sound patterns.

MATERIALS and METHODS: Respiratory sounds were recorded with a connected stethoscope (Littman Core Digital) during routine clinical examination in veal and feedlot calves with or without BRD. Expert clinician diagnosis was recorded at time of recording and offline and classified as 4 different lung sounds types or features: normal (N), wheezes (S), bronchial sounds (B) and crackles (C). Then, the audio files were preprocessed, segmented, and converted to spectrotemporal representations. A training and validation set were sequentially used. Feature extraction was performed using machine learning approaches (random forest, gradient boosting) and neural networks. The model selection rule was based on the best overall validation score and imbalance or accuracy metrics (performance, recall, area under the receiver operating characteristic curve (AUC)and F1-score) accounting for class imbalance.

RESULTS: The training set consisted in 216 audio samples with N=119 (55.1%), S=71 (32.9%), B=14 (6.5%), and C=12 (5.6%), whereas the validation set contained 43 samples with N=30 (69.8%), S=8 (18.6%), B=4 (9.3%) and C=1 (2.3%). When applying the model at the validation set, the N recall was 100% indicating that the model adequately detected normal lung sounds. The optimal W, B and C detection models were observed using gradient boosting (F1=0.55), ensemble learning (F1=0.67) and neural networks (AUC=0.929).

CONCLUSIONS: This study supported the proof of concept that normal lung sounds can be detected automatically in calves. There was a moderate discrimination of abnormal lung sounds. However, the small simple size and class imbalance should be taken into consideration.

Keywords: bronchopneumonia, diagnosis, machine learning

Mass Mortality of Preweaned Dairy Calves Following Suspected Doxycicline Intoxicacion: A Case Report

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BACKGROUND and OBJECTIVE: One strategy to control calf disease is metaphylactic oral antimicrobial therapy, despite its potential adverse effects. Doxycycline is a bacteriostatic tetracycline commonly administered in milk to control respiratory disease. This practice poses two main risks: underdosing in sick calves with reduced milk intake and overdosing due to calculation errors or higher milk consumption by healthy calves. Doxycycline intoxication has been reported in beef calves receiving three to ten times the recommended dose; however, experimental reproduction of toxicosis has been inconsistent, suggesting the involvement of additional unknow predisposing factors. Furthermore, to our knowledge, no reports of doxycycline intoxication have been reported in dairy calves. The objective of this case report is to describe a hyperacute mortality outbreak in preweaned dairy calves, attributed to doxycycline intoxication via medicated milk.

MATERIALS and METHODS: The case occurred on a 700-cow dairy farm with low rearing calf mortality (1.7% 2024). Calves were housed individually for the first three weeks of life and subsequently grouped into pens before transferring to two calf rearing centers. The feeding program was pasteurized waste milk supplemented with skimmed milk powder (125 g/L), administered at 2 L/meal in hutches and 4 L/meal in group pens. On 29 January 2025, following the onset of cough, 450 g of oral doxycycline hyclate (500 mg/g) was administered in 290 L milk (1.5 g/L for 90 calves), and the treatment was repeated once the following afternoon. Clinical signs, mortality, necropsy findings, histopathology, serum biochemistry, and toxicological analysis (urinary doxycycline quantification) were evaluated.

RESULTS: On January 31st, the calves began to die after showing signs of depression, apathy, cold extremities, hypersalivation, dyspnoea. Assuming an outbreak of pneumonia, all calves were treated parenterally and vaccinated against BRSV-PI3. A total of 40 calves died within five days (45% mortality rate), predominantly affecting high-intake groups. Necropsy revealed hepatic congestion, pulmonary and renal oedema, consistent with circulatory failure. Histopathology confirmed multifocal left ventricular myocardial necrosis and pulmonary congestion. Elevated serum creatine kinase in two calves indicated severe myocardial and muscular damage. Toxicology confirmed high urinary doxycycline concentrations (16 µg/mL) three days post-after last medicated feeding.

CONCLUSIONS: The clinical presentation, pathological and histopathological findings, together with toxicological evidence, strongly support acute oral doxycycline intoxication as the cause of this hyperacute mortality outbreak in preweaned dairy calves. Dosing errors in group medicated milk feeding precipitated severe cardiotoxicity and multiorgan failure, underscoring the importance of prudent antimicrobial use.

Keywords: Doxycicline, intoxication, dairy calves

Fibrosarcoma in a Dairy Cow: A Case Report

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BACKGROUND and OBJECTIVE: Fibrosarcomas are malignant tumors of mesenchymal tissue. Microscopically, they are characterized by the presence of bundles of immature fibroblasts with irregular arrangement and varying amounts of collagen fibers. The tumor has a low tendency to metastasize, and its degree of malignancy is influenced by the degree of cell differentiation, collagen fiber synthesis capacity, and mitotic activity. Fibrosarcomas are mainly described in small animals and are rare in cattle. Due to the low incidence of this tumor, there are few scientific reports describing the morphology of fibrosarcomas in cattle. The aim of this study is to present a case of fibrosarcoma in a cow, its macroscopic and microscopic appearance and immunomarkers relevant for diagnosis.

MATERIALS and METHODS: A case of a cow with a large, rapidly growing pedunculated tumor located in the skin behind the mandibular angle was reported. Due to the discomfort associated with the large size of the tumor and the deteriorating clinical condition of the animal, a decision was made to remove the lesion. The tumor was surgically resected in the field. After the procedure, samples were taken for histopathological examination, fixed in 10% buffered formalin solution, and sent to the laboratory. The preparations were prepared using the paraffin technique with an automated tissue processor (ASP6025 S, Leica, Germany). The tissues were embedded in paraffin (HistoCore Arcadia H+C, Leica, Germany) and sectioned on a microtome (HistoCore MULTICUT, Leica, Germany). The 4 µm thick sections were stained with hematoxylin and eosin (H&E; ST Infinity Kit, Leica, Germany), using the Masson method and immunohistochemical tests were performed for the presence of Ki67, vimentin, and desmin. Microscopic analysis was performed using a light and fluorescence microscope.

RESULTS: After resection, the tumor weighed 2490 g. Macroscopically, it was covered with exfoliating epidermis, completely hairless, with numerous ulcers on its surface and hemorrhagic foci under the epidermis. In the central part of the tumor there was a richly cellular parenchyma with poorly differentiated cells and a poor connective tissue stroma. Numerous mitotic figures were also noticeable. Masson's staining and immunohistochemical tests for Ki67, vimentin, and desmin provided the basis for the diagnosis and determination of the mitotic activity of malignant fibrosarcoma.

CONCLUSIONS: The results of histopathological examination, supported by histochemical and immunohistochemical analysis, confirmed the diagnosis and emphasized the key role of these methods in the diagnosis of spindle cell tumors of soft tissues.

Keywords: Fibrosarcoma, Tumour, Cow

Türkiye's Calf-Rearing Practices: A Survey-Based Evaluation of Herd Size and Regional Differences

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BACKGROUND and OBJECTIVE: This study was conducted to evaluate calf-rearing and management practices in cattle farms across Türkiye and to determine the potential effects of these practices on calf health.

MATERIALS and METHODS: The study material consisted of data obtained from questionnaires administered to the responsible veterinarians working in 93 cattle farms of varying herd sizes located in seven different geographical regions of Türkiye. The questionnaire comprised 44 items and included questions designed to assess the structural characteristics of the farms, calving and neonatal care practices, housing and hygiene conditions, feeding management, and the incidence of disease and mortality in calves. Data were analyzed using SPSS (IBM SPSS Statistics 25.0), and the farms were divided into two groups according to herd size (≤2000 head and >2000 head). Descriptive statistics, the independent samples t-test, the chi-square (x^2) test, and multiple correspondence analysis were used for data evaluation.

RESULTS: Of the farms included in the study, 69.9% had a herd size of ≤2000 head, whereas 30.1% had a herd size of >2000 head. Geographically, 32.3% of the farms were located in the Marmara region, 25.8% in the Aegean region, 19.4% in Central Anatolia, 12.9% in the Mediterranean region, 6.5% in the Black Sea region, 2.2% in Eastern Anatolia, and 1.1% in Southeastern Anatolia. Colostrum quality control was found to be more common in large-scale farms

(>2000 head, 96.4%; ≤2000 head, 75.4%), and the most frequently used method was the Brix refractometer (>2000 head, 67.9%; ≤2000 head, 47.7%). When mortality and morbidity rates were evaluated according to herd size, mortality rates below 10% were observed in 85.7% of farms with more than 2,000 animals, while morbidity rates below 10% were recorded in 42.9% of such farms. In contrast, among farms with 2,000 or fewer animals, these rates were 90.8% and 67.7%, respectively. In the comparison between groups, the amount of colostrum administered was significantly higher in farms with >2000 head than in those with ≤2000 head ($p<0.001$). In addition, colostrum pasteurization ($p<0.001$), the use of scoring systems for monitoring calf diseases ($p=0.001$), and dry-period vaccination ($p=0.008$) were found to be more common in farms with >2000 head than in those with ≤2000 head. The results of the multiple correspondence analysis revealed distinct clustering patterns among herd size, rearing practices, and calf health indicators.

CONCLUSIONS: The findings indicate that calf-rearing and management practices in cattle farms in Türkiye may vary according to herd size, and that colostrum management and preventive health practices are implemented more systematically in large-scale farms.

Keywords: Cattle, calf rearing, herd size, survey, colostrum management, calf health

Pentalogy of Fallot with Double Outflow from the Right Ventricle in a One-Week-Old Beef Crossbred Calf: A Case Report

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BACKGROUND and OBJECTIVE: Congenital cardiac malformations in calves are uncommon but clinically significant due to their impact on survival and welfare. Pentalogy of Fallot is a rare and complex congenital heart defect characterized by ventricular septal defect (VSD), pulmonary stenosis, overriding aorta, right ventricular hypertrophy, and atrial septal defect (ASD). The objective of this report was to describe the clinical and echocardiographic findings of pentalogy of Fallot with features consistent with double outlet right ventricle (DORV) in a neonatal beef crossbred calf.

MATERIALS and METHODS: A one-week-old male beef crossbred calf from a cow–calf production system was presented due to inability to stand unassisted since birth. The calf was born at term and exhibited a normal suckling reflex. Clinical examination revealed recumbency and suspected malformation of the cranial cervical vertebral region. A loud cardiac murmur was detected during auscultation. Echocardiographic examination was performed using two-dimensional (2D) imaging and color Doppler. Computed tomography (CT) of the whole body was additionally performed to further evaluate the suspected cervical vertebral malformations.

RESULTS: Two-dimensional echocardiography showed subjectively adequate systolic function and marked right ventricular remodeling due to both volume and pressure overload. A large perimembranous ventricular septal defect (VSD) was identified, along with an overriding aorta receiving blood from both ventricles. The pulmonary trunk was hypoplastic, and a primum-type atrial septal defect (ASD) was also present. Color Doppler imaging revealed bidirectional blood flow across the VSD and severe pulmonary stenosis. Postmortem examination of the heart confirmed the echocardiographic findings. Given the severity of the cardiac and vertebral abnormalities and the poor prognosis, euthanasia was performed

CONCLUSIONS: This case highlights the importance of thorough clinical evaluation in neonatal calves with suspected congenital abnormalities. The combination of severe cardiac and vertebral malformations resulted in a grave prognosis, ultimately leading to euthanasia.

Keywords: Pentalogy of Fallot, congenital cardiac malformation, double outlet right ventricle, neonatal calf

Atlanto-Occipital Joint Malformation in Two Neonatal Calves: Clinical Presentation and Diagnostic Approach

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BACKGROUND and OBJECTIVE: Atlanto-occipital joint malformation (AOJM) is an uncommon cause of cervical spinal cord dysfunction in neonatal calves. The clinical presentation may overlap with other congenital, inflammatory, or traumatic disorders, which can delay recognition and diagnosis. The objective of this case report was to describe the clinical findings and diagnostic approach in two neonatal calves from different production systems presenting with inability to stand.

MATERIALS and METHODS: Two neonatal calves from separate farms were referred for further diagnostic work-up. The first calf was a one-week-old crossbred calf raised in a cow-calf production system. The second calf was a three-week-old dairy calf. Both calves were born at term and had been unable to stand since birth, while remaining alert with a strong suckling reflex. A thorough clinical and neurological examination was performed. Cerebrospinal fluid was collected, and the cervical spine was evaluated using radiography and computed tomography.

RESULTS: Both calves were bright and responsive but tetraplegic. Neurological findings were consistent with a lesion affecting the cranial cervical spinal cord segments (C1–C5). In both calves, lateral flexion of the head and neck resulted in audible crepitation and palpable joint instability in the cranio-cervical region, without evidence of pain response. In addition to AOJM, the one-week-old calf was diagnosed with Pentalogy of Fallot. Due to the severity of neurological deficits and poor prognosis for functional recovery, euthanasia was performed in both cases.

CONCLUSIONS: AOJM should be considered in neonatal calves that are alert yet unable to stand. Careful neurological localisation combined with appropriate diagnostic procedures is essential for accurate diagnosis and responsible, welfare-oriented clinical decision-making.

Keywords: Atlanto-occipital joint, malformation, tetraplegia, neonatal calves

Poisoning by Complex Mineral Fertilizers in Beef Cattle

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BACKGROUND and OBJECTIVE: Beef cattle production plays a key role in food security and the rural economy in Colombia. The aim of this study was to describe a fatal poisoning by complex mineral fertilizers in beef cattle in the Colombian Orinoquía region.

MATERIALS and METHODS: A total of 65 bovines died over a 20-day period in a herd of 324 commercial zebu and Bos taurus crossbred beef cattle. Clinical and epidemiological data were collected. A necropsy was performed on a heifer and a dying cow that was euthanized. Tissue samples were fixed in 10% buffered formalin for histological evaluation. Blood, cerebrospinal fluid (CSF), vitreous humor, and rumen contents were collected for

biochemical and toxicological evaluation, including the mineral components nitrogen (N), phosphorus (P), potassium (K) and sulfur (S). Samples of fertilizers collected from agricultural fields were chemically analyzed. Differential diagnoses were investigated using parasitological tests, pasture and paddock inspections, and water analysis.

RESULTS: The mortality outbreak occurred between August 25 and September 15, 2024, in a herd of 324 beef cattle managed in an extensive system with natural pastures in the department of Vichada, Colombia (4.624535 N, -70.954204 W). Mortality was 20.1% (65/324) in 20 days. Clinical signs developed between 6 and 48 hours and included anorexia, bloat, hypersalivation, dehydration, abortions, ataxia, hyperexcitability, prolonged recumbency, and death. The relevant macroscopic findings were found in the rumen: approximately 4 kg of heterogeneous granular mineral of yellow and black color were found in the lumen, transmural caustic lesions affected approximately 70% of the ruminal mucosa, along with severe mural hemorrhage and fibrin precipitation. Histologically, the predominant finding was severe transmural ulcerative, fibrinous-neutrophilic necrotizing rumenitis with microthrombosis, acute fibrinous peritonitis, and severe hypoxic brain changes. Rumen contents pH was markedly alkaline (9–11). Hematologic findings included neutrophil-to-lymphocyte ratio inversion, hyperfibrinogenemia (600–800 mg/dL), and severe hypoalbuminemia (2.1 g/dL). High concentrations of P (6.40 – 9.7 mg/dl) and urea (60.5 – 177.2 mg/dl) were recorded in serum. High concentrations of P and nitrogenous products were found in CSF and vitreous humor. Cyanogenic glycosides and nitrates were negative in ruminal content and drinking water. Chemical analysis confirmed elevated P and S concentrations in both fertilizer samples and rumen contents.

CONCLUSIONS: An outbreak of mortality due to poisoning by complex mineral fertilizers is described in beef cattle in the Colombian Orinoquía region. In countries where the agricultural frontier is expanding, it is necessary to prevent accidental poisoning with fertilizers.

Keywords: Beef cattle, Colombia, mortality.

Sudden Mortality of Dairy Cattle Associated with *Clostridium perfringens* Infection: A Case Report

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BACKGROUND and OBJECTIVE: Sudden mortality in dairy cattle presents a major challenge for veterinarians and dairy producers because of the rapid disease progression and difficulties in establishing a diagnosis prior to death. *Clostridium perfringens* is an opportunistic pathogen that produces toxins responsible for severe intestinal damage and systemic effects. The rapid progression and nonspecific clinical signs frequently result in death before therapeutic intervention is possible. This case report seeks to improve understanding of the pathological changes associated with *Clostridium perfringens* infections in dairy cows and to compare these findings with data from a herd management program.

MATERIALS and METHODS: Three Holstein–Friesian dairy cows from a commercial dairy farm in Lithuania were included in this report. All animals died suddenly and exhibited limited, nonspecific clinical signs. Data about activity, eating time, daily production and the composition of milk, such as fat-to-protein ratio, were recorded and collected using the Lely herd management system (Maassluis, Netherlands). Post-mortem examinations focused on the gastrointestinal tract due to a suspected involvement of enteric pathogens based on clinical symptoms and the progression of the disease. Laboratory analysis of intestinal content samples confirmed the presence of *Clostridium perfringens*.

RESULTS: Necropsy revealed severe pathological changes, including acute hemorrhagic inflammation of the intestinal wall and substantial accumulation of fluid in the abdominal cavity. Gas bubbles were present in the subcutaneous tissue. These lesions were consistent with acute clostridial enteritis and indicated extensive tissue damage likely resulting from toxin production. Data from the herd management program showed rapid and pronounced changes in activity and milk yield within the first 24 hours, correlating with the known incubation period of the pathogen. The milk yield of two affected cows decreased by 35–47%. The fat-to-

protein ratio exceeded 1.3, strongly suggesting the toxins' impact on rumen balance.

CONCLUSIONS: These findings underscore the need to consider Clostridium perfringens infection as a potential cause of sudden death in dairy cattle. The results demonstrate the diagnostic value of comprehensive post-mortem examinations and laboratory confirmation when clinical signs are limited or nonspecific. Enhanced awareness, early detection, and preventive herd management strategies may reduce cases of sudden mortalities associated with acute infections in dairy herds.

Keywords: Clostridium perfringens, sudden death, dairy cattle, necropsy, heard management program.

A Comparison of Itraconazole and Paromomycin on Oocyst Shedding in Diarrheic Neonatal Calves with Cryptosporidiosis

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BACKGROUND and OBJECTIVE: Cryptosporidiosis is one of the most important causes of diarrhoea in neonatal calves, leading to impaired growth, dehydration and economic losses in dairy systems. Despite its high prevalence, effective and practical therapeutic options remain limited under field conditions. This study aimed to compare the efficacy of itraconazole and paromomycin in reducing oocyst shedding and improving clinical outcomes in diarrhoeic neonatal calves naturally infected with *Cryptosporidium*.

MATERIALS and METHODS: The study was conducted on a commercial dairy farm under field conditions. A total of 40 Holstein calves aged between 7 and 14 days, presenting with diarrhoea and confirmed *Cryptosporidium* infection using a rapid antigen test based on enzyme-linked immunosorbent assay (ELISA), were enrolled consecutively over a three-week period. Calves were assigned sequentially into two treatment groups: the first 20 calves received oral itraconazole (10 mg/kg once daily for five days), while the subsequent 20 calves received oral paromomycin (100 mg/kg once daily for five days). Treatment efficacy was evaluated on days 0 and 5 using oocysts per gram (OPG), oocyst scoring and faecal consistency scoring

RESULTS: Both treatment protocols were associated with significant reductions in oocyst shedding and improvements in faecal consistency. However, the magnitude of reduction appeared more pronounced in calves treated with itraconazole ($p<0.001$) compared with those treated with paromomycin ($p<0.05$). By day 5, 90% of calves in the itraconazole group tested negative for *Cryptosporidium* antigen, whereas this proportion was 40% in the paromomycin group. Clinical improvement, as reflected by faecal scores, followed a similar trend in both groups.

CONCLUSIONS: Under field conditions, itraconazole appeared to provide a greater reduction in oocyst shedding compared with paromomycin in neonatal calves with cryptosporidiosis. These findings suggest that itraconazole may represent a promising alternative therapeutic option; however, further studies involving larger populations and comprehensive safety evaluations are warranted to confirm its broader applicability.

Keywords: itraconazole, paromomycin, neonatal calf, cryptosporidium, diarrhea

Evaluation of the Relationship Between NEFA, the NEFA/Cholesterol Ratio, and Metabolic and Inflammatory Parameters in Transition Dairy Cows

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BACKGROUND AND OBJECTIVES: The transition period in dairy cows, spanning from three weeks prepartum to three weeks postpartum, involves significant metabolic and endocrine adaptations. Increased energy demands combined with reduced feed intake lead to negative energy balance,

enhanced lipolysis, and elevated NEFA concentrations, increasing the risk of ketosis and fatty liver. Therefore, this period requires metabolic monitoring. The aim of this study was to investigate the relationship between serum NEFA, the NEFA/TCHO ratio, and selected metabolic, inflammatory, and production parameters during the transition period.

MATERIAL AND METHODS: Serum samples were obtained from 40 multiparous dairy cows under identical management. Blood was collected at 7 days prepartum and 7 days postpartum; serum was stored at -20°C until analysis. Postpartum day 7 samples were analyzed for NEFA and TCHO, and the NEFA/cholesterol ratio was calculated. Based on postpartum values, cows were classified into two groups: experimental (n=10; NEFA >0.6 mmol/L, ratio >0.2) and control (n=10; NEFA <0.5 mmol/L, ratio <0.2). Corresponding prepartum samples were also evaluated. Additional parameters measured included BHBA, AST, GGT, GLDH, triglycerides, TBil, albumin, glucose (by spectrophotometric), insulin, and haptoglobin (by ELISA). LDL and VLDL were calculated. BCS was recorded at -7 days, calving, and at +7, +14, and +28 DIM. Milk yield was recorded at +7, +14, +28, and +50 DIM. Between-group comparisons used independent t-tests, and within-group comparisons used paired t-tests.

RESULTS: Within-group comparisons revealed significant prepartum–postpartum changes in the experimental group for AST, GLDH, glucose, TCHO, albumin, NEFA, triglycerides, TBil, and NEFA/TCHO, whereas the control group showed significant changes in TCHO, BHBA, NEFA, TG, HDL, and NEFA/TCHO ($p<0.05$). But, on postpartum day 7, NEFA, TBil, and the NEFA/TCHO ratio were significantly higher in the experimental group ($p<0.01$). Insulin concentration changed significantly only in the experimental group ($p=0.033$). Haptoglobin (HP) showed significant differences both within groups and between groups in prepartum and postpartum comparisons ($p<0.05$). Milk yield differed between groups on postpartum days 28 and 50 ($p<0.05$), whereas no significant difference was detected for BCS ($p>0.05$).

CONCLUSIONS: NEFA and particularly the NEFA/TCHO effectively discriminated metabolic status in multiparous transition cows. Higher NEFA, TBil, and NEFA/TCHO, together with changes in insulin, indicated greater metabolic stress in the experimental group, while haptoglobin supported inflammatory involvement. More limited changes in the control group suggested better hepatic adaptation. Overall, combined evaluation of NEFA/TCHO, insulin, and haptoglobin may be useful for monitoring metabolic stress, although validation in larger populations is required.

Keywords: Bovine, fatty liver disease, NEFA/TCHO ratio

Dose-Dependent Effects of Inhaled Salbutamol in Kangal Ewes

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BACKGROUND and OBJECTIVE: Respiratory diseases are among the most important health problems affecting ruminants, contributing substantially to decreased productivity, economic losses, and impaired animal welfare. Bronchodilator agents such as salbutamol, a selective β_2-adrenergic agonist, are widely used in human medicine to relieve bronchoconstriction and improve pulmonary function. Although salbutamol is commonly administered via inhalation due to its rapid bronchodilatory effects and lower systemic side effects, information regarding its systemic biochemical and metabolic effects in small ruminants remains limited. Therefore, this study aimed to evaluate the dose-dependent effects of inhaled salbutamol on clinical parameters, biochemical variables, and venous blood gas parameters in Kangal ewes.

MATERIALS and METHODS: A total of 24 clinically healthy Kangal ewes, aged 8–12 months and of similar weight, were included in the study. Animals were randomly assigned to four groups (n=6 per group): control (5 mL saline), normal-dose salbutamol (0,05 mg/kg), medium-dose salbutamol (0,2 mg/kg), and high-dose salbutamol (0,5 mg/kg). Salbutamol was administered via nebulization using a modified mask for small ruminants. Venous blood samples were collected from the jugular vein at multiple time points (−1, 0, 5, 15, 30, 60, 120, 180, 240, and 360 minutes) for blood gas analysis. Serum biochemical analyses were performed at −1, 180, and 360 minutes. Biochemical parameters including aspartate transaminase (AST), alkaline transaminase (ALT), blood urea nitrogen (BUN), creatinine (CRE), triglyceride (TG), total cholesterol

(TCHOL), creatine kinase-myocardial band (CK-MB) and Troponin T were analyzed using an automated veterinary biochemical analyzer. Clinical parameters were also monitored during the experimental period.

RESULTS: Statistical analysis revealed a significant difference in glucose levels among the groups ($P<0.05$). In contrast, Troponin T, CK-MB, potassium, AST, ALT, TCHOL, TG, BUN, and CRE concentrations did not show statistically significant differences between groups ($P>0.05$). These findings indicate that inhaled salbutamol administration did not produce detectable alterations in cardiac enzymes, hepatic enzymes, lipid profile, or renal function parameters.

CONCLUSIONS: Inhaled salbutamol administration in Kangal ewes appears to have no systemic biochemical effects and does not significantly influence cardiac, hepatic, or renal biomarkers. However, the significant change observed in glucose levels suggests that β_2-adrenergic stimulation may influence responses at energy metabolism. Further studies investigating the dose-dependent and long-term metabolic effects of inhaled bronchodilators in ruminants.

Keywords: Kangal Ewes, CK-MB Inhalation, Salbutamol, Troponin T, Side Effect

Successful Internal Surgical Fixation of an Oblique Tibial Fracture in a One-day Old Holstein-Friesian Calf in Commercial Veterinary Practice

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BACKGROUND and OBJECTIVE: Causes of post-parturient tibial fractures in newborn calves include maternal trauma and slurry-scraper injury. Such cases present technical/economic challenges. The objective of this report was to prove that internal surgical fixation is possible in such calves and economically feasible in commercial veterinary practice.

MATERIALS and METHODS: A one-day old calf was presented with a fractured hindleg. The fracture site was simple/closed with soft tissue bruising. The calf was treated with penicillin and meloxicam. The farmer requested best quality care and agreed to surgery. Previous experience of external coaptation (Thomas extension splints) had given less than 50% success. Hence surgery was performed. The calf was brought into the veterinary clinic by the farmer on the day of detection of the fracture. An x-ray revealed a mid-shaft, oblique, diaphyseal, tibial fracture. The calf was anaesthetised with 2% xylazine (Chanazine 2%, Chanelle) and ketamine (Ketamidor, Chanelle) to effect. Maintenance fluid therapy was provided during surgery and recovery with Hartmann's solution (Aqupharm 11, Ecuphar). The site was clipped and scrubbed with chlorhexidine surgical scrub (Vetscrub, Duggan Veterinary). The distal limb was bandaged with sterile cohesive bandage. The fracture site was exposed with a 12cm incision on the medial aspect. The fracture fragments were aligned, secured with bone-holding forceps and four 3.5mm diameter, hexagonal head, variable length cortical bone screws (Veterinary Instrumentation) were placed perpendicularly into the medial border of the tibia in a lag screw effect, with the screws protruding 2 mm from the lateral border. The skin was sutured using EP7 USP 5 in a Ford interlocking suture pattern. The animal was treated with a 5-day course of penicillin and meloxicam.

RESULTS: The wound was monitored daily for 28 days with the stitches removed after 14 days. No evidence of wound infection was observed. The surgical implants cost €60 and the medicines €120. The calf was confined to a crate (2.5 m2) for 2-weeks, then in a small group pen (9m2) for 4-weeks before moving to a group pen (45m2) for 7-weeks with 15 other calves of similar age. The calf was turned out to pasture at 13-weeks of age. The heifer returned to a normal gait and growth rate at final examination at 7-months of age and was retained in the breeding herd.

CONCLUSIONS: This case demonstrates that bovine orthopaedic surgery is economically feasible in commercial veterinary practice. Careful case selection, good surgical skills and excellent post-operative care are critical to a successful case resolution.

Keywords: Tibial fracture, Internal fixation, Successful recovery

Longitudinal Description of Biomarker Profiles in an Experimentally Induced Bovine Respiratory Disease Model

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BACKGROUND and OBJECTIVE: Bovine Respiratory Disease (BRD) is among the top diseases affecting both dairy and beef cattle. Despite BRD's prevalence, ante-mortem diagnosis and prediction of disease severity remain challenging. The association of physical exam (PE) parameters and blood biomarker (BB) profiles with disease severity have not been concurrently described over time. The objectives of this study are to describe PE parameters and BB over time in an experimentally induced BRD model and to identify which are associated with disease severity. We hypothesize that PE parameters and BB will differ from baseline and between disease-severity groups.

MATERIALS and METHODS: This study was approved by the university's Institutional Animal Care and Use Committee. This study was conducted from May-July 2024 and 2025. No control group was included as the study animals were part of a concurrent pharmacokinetic study. Forty-eight, six to eight-month-old dairy-beef cross calves underwent transportation stress and were inoculated with *Mannheimia hemolytica* (MH) to induce BRD. All calves were sourced from commercial dairies or institutionally affiliated herds. Twenty-two biomarkers were collected prior to transportation, upon arrival (baseline, time= 0 hours), and every 24 hours post-inoculation throughout the study. Animals were randomized for euthanasia at pre-determined pharmacokinetic time points. Necropsy was the gold standard for confirming MH BRD and determining disease severity (mild vs. severe) based on the percentage of total lung consolidation. The results of a non-parametric repeated-measures analysis to compare disease severity groups over time are pending. A logistic regression model using biomarker parameters to predict disease severity is pending.

RESULTS: Study animals were clinically healthy prior to inoculation. All animals were negative for Bovine Viral Diarrhea virus. Forty-five animals were included in the final analysis (14 females, 31 castrated males). Total percent lung consolidation was categorized as mild (0-20%) or severe (27-58%). Differences from baseline were calculated for each biomarker at each time point. Numeric differences were identified in all biomarkers tested (rectal temperature, heart rate, respiratory rate, depression score, respiratory score, thoracic ultrasound score, neutrophil count, basophil count, fibrinogen, packed cell volume, glucose, total protein, creatinine, albumin, total bilirubin, gamma-glutamyl transferase, sorbitol dehydrogenase, lactate, interleukin-6, serum amyloid A, and haptoglobin). Further analysis to determine which biomarkers predict disease severity is pending.

CONCLUSIONS: Together, PE parameters and BB have the potential to more accurately predict disease severity in calves with BRD.

Keywords: cattle, pneumonia, biomarkers

Alopecia Areata in Related Holstein-Friesian Cattle: Insights from Clinicopathological and Whole-Genome Analysis

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BACKGROUND and OBJECTIVE: Alopecia areata (AA) is a chronic, non-scarring inflammatory condition causing hair loss in humans and several animal species, and is considered an autoimmune disorder targeting active hair follicles. Although it appears clinically non-inflammatory, its pathogenesis involves immune mechanisms along with genetic and neuroendocrine factors. This study aims to describe and characterize AA in two Holstein-

Friesian cattle through clinical, histological, and genetic investigation.

MATERIALS and METHODS: Two purebred Holstein-Friesian cows (a 9-year-old dam and her 6-year-old daughter) were clinically evaluated for hair coat abnormalities. Both animals underwent complete clinical examination, skin scrapings for parasitological analysis, and skin biopsies collected from affected black-pigmented areas. Histological evaluation was performed to assess hair follicle morphology and inflammatory patterns. Immunohistochemistry was used to characterize the inflammatory infiltrate. Short-read whole-genome sequencing (WGS) was performed in the two cases. Variants were filtered under the hypothesis of recessive or a dominant inheritance and compared with >6000 control genomes.

RESULTS: Both cows showed non-pruritic hair thinning predominantly affecting black-pigmented areas, with progression to focal alopecia more severe in the dam. White-pigmented areas were largely spared. Histologically, most hair follicles were in catagen or telogen phases, with few anagen follicles. Abnormal melanin clumping within follicular structures and pigmentary incontinence were consistently observed. Follicular changes included reduced hair shaft size and evidence of mural cell apoptosis. A mild to moderate lymphocytic infiltrate targeting hair follicles and superficial dermal vessels was present, and immunohistochemistry confirmed a predominance of CD3-positive T lymphocytes. The daughter showed similar but milder clinical and histological changes, with more evident mural folliculitis. Parasitological examinations were negative. WGS did not identify any shared protein-altering candidate variants between the two cases. Therefore, a candidate gene approach was subsequently applied, also taking potential modifier variants into account. This analysis led to the identification of two private intergenic variants located in the regions IL1RAPL1–TRNAV-CAC_22 and LOC132344340–IL13RA2. Additionally, two heterozygous intronic variants, present exclusively in the cases and both absent in controls, were identified in IL1R2 and IL15.

CONCLUSIONS: The clinical, histopathological, and immunohistochemical findings support a diagnosis of alopecia areata, characterized by pigment-associated follicular alterations and T-cell–mediated inflammation, with preferential involvement of pigmented areas. The occurrence in related animals suggests a possible genetic predisposition. Although no shared protein-altering variants were identified, the presence of variants in immune-related loci highlights potential regulatory mechanisms that warrant further investigation.

Keywords: cattle, alopecia areata, immunohistochemistry, genetics

Determination of Post-Mortem Interval in Bovine Carcasses Using Entomology in Humid Tropical Climates

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BACKGROUND and OBJECTIVE: This study analyzes thanatocronodiagnosis and forensic entomology as essential tools for estimating the post-mortem interval (PMI) in veterinary medicine, filling the technical void regarding the decomposition of large species in tropical climates. Historically, forensic research has relied heavily on human or porcine models, which are often inadequate for representing the biochemical and biological processes of large ruminants. Therefore, the objective of this work was to document the degradation phases and the complex arthropod succession in bovine carcasses in the humid tropics to establish precise methodological references for forensic investigations.

MATERIALS and METHODS: A population of 26 bovine carcasses was evaluated under average temperatures ranging from 24-32°C and relative humidity exceeding 70%. The methodology involved constant monitoring with meticulous inspections every 6 hours for three weeks. Stages were identified based on specific internal and external morphological changes recorded through extensive photography and the collection of arthropods using manual methods and sweep nets. Data were rigorously processed using Pearson correlation analysis, Student's T-tests, and measures of central

tendency to validate the chronology of these cadaveric phenomena.

RESULTS: Through the five identified stages—fresh, emphysematous, active, advanced, and bone reduction—the results revealed specific quantitative parameters, such as a muscle pH drop from 8.5 to 5.6 due to anaerobic glycolysis. While algor mortis (cooling) proved unreliable due to rapid environmental equilibration, rigor mortis recorded an average (promedio) duration of 6 hours and 36 minutes, showing a strong positive correlation (r=0.819) with tissue dehydration. Biologically, the entomofauna was dominated by the families Muscidae (72.4%) and Calliphoridae (26.9%), with a strong negative correlation (r=-0.79) between the arrival of the genus Chrysomya and the days post-mortem. Additionally, statistical testing regarding scavenging activity (T=0.076) demonstrated no significant differences between the summer and autumn seasons, confirming that the relentless activity of raptors, such as the black vulture (Coragyps atratus), along with high humidity and heat, accelerates bone reduction, completing the entire process in just six days.

CONCLUSIONS: We conclude that, because cadaveric cooling loses its diagnostic precision in warm climates—as the body temperature quickly reaches equilibrium with the surrounding environment—the succession of cadaveric fauna, led by calliphorids and muscids and followed by specialized beetles like Necrobia rufipes, represents the most scientifically valid tool. When combined with the consistent impact of local scavengers, these biological indicators constitute the most reliable parameters for estimating the post-mortem interval in bovines within this tropical ecosystem.

Keywords: Bovine, Entomology, Post-Mortem, Carcasses

Determination of Cadaveric Entomofauna on Goats and Its Relationship with the Post-mortem Interval in the Central Altiplano, Mexico

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BACKGROUND and OBJECTIVE: Forensic entomology is a key tool for estimating the post mortem interval (PMI) by analyzing the arrival and development of arthropods on decomposing bodies. However, in Mexico, there is a technical gap regarding the successional patterns of cadaveric fauna in large livestock species within specific biomes, such as the Mexican Central Altiplano. This study aimed to fill that void by characterizing the cadaveric entomofauna on goats across different stages of decomposition, evaluating the influence of seasonal and environmental conditions on PMI estimates.

MATERIALS and METHODS: The research was conducted in Ezequiel Montes, Querétaro, during the spring–summer transition of 2024. The study area represents an arid highland habitat with temperatures reaching 45 °C and low relative humidity. Five goat carcasses from natural mortalities were monitored daily, with no human intervention, to simulate natural decay. Sampling was carried out twice daily (morning and afternoon) using direct collection methods (manual tweezers and sweep nets) and indirect trapping techniques (pitfall, light, and Malaise traps). Environmental variables such as ambient temperature, wind speed, and relative humidity were recorded during each visit to establish statistical correlations with arthropod abundance and composition.

RESULTS: A total of 3,553 organisms were identified. Diptera was the dominant order, representing 76.3% of direct samples, followed by Hymenoptera (15.5%) and Coleoptera (6.2%). Within the family

Calliphoridae, the genera *Cochliomyia* (45.6%) and *Chrysomya* (16.1%) were the most prevalent. Statistical analysis using Chi square tests showed highly significant differences in all comparisons ($p<0.001$). The entomofauna exhibited a strong preference for the carcasses compared with artificial lures ($p<0.001$) and for afternoon activity, which accounted for 72.8% of the total collection ($p<0.001$). Furthermore, 88.35% of the arthropods were collected during late decomposition stages (decay and dry), showing a significant preference over early phases ($p<0.001$). Environmental factors were also decisive, as arthropod presence was significantly higher during periods of low humidity ($p<0.001$), high wind speed ($p<0.001$), and high temperatures ($p<0.001$).

CONCLUSIONS: In conclusion, this study confirms a definitive correlation between environmental parameters and the successional patterns of cadaveric fauna in the Central Altiplano. These findings highlight the sanitary risks posed by unattended animal carcasses in livestock production units, as they attract significant populations of disease transmitting dipterans. The results provide a foundational successional model for estimating the PMI in veterinary forensic investigations within semi arid highland environments, contributing to more accurate PMI estimations and improved management of carcass disposal in Mexican ruminant production systems.

Keywords: Goat, Entomology, Postmortem

NUTRITION AND METABOLIC DISEASES

Niacin Status Indicators and Their Relationship with Metabolic Parameters in Dairy Cows During Early Lactation

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BACKGROUND and OBJECTIVE: The aim of this study was to determine the influence of niacin administration in periparturient period in dairy cows on NAD, NADP and the NAD: NADP ratio and to determine relationship between these indicators of an active form of niacin with blood metabolic parameters.

MATERIALS and METHODS: The study included 90 healthy cows: 45 cows receiving niacin and 45 cows were negative control. The niacin group was treated with nicotinic acid for two weeks before, as well as two weeks after parturition. Blood samples were collected a day before the experiment and a day after experiment for the determination of NAD, NADP and NAD: NADP, glucose (GLU), nonesterified fatty acid (NEFA), beta-hydroxybutyrate (BHB), triglycerides (TG), cholesterol (CHOL), total bilirubin (TBIL), aspartate aminotransferase (AST), gamma-glutamiltransferase (GGT), total protein (TPROT), albumin (ALB) and urea. Kits from manufacturers Abcam and Biosystems were used and results were read from Rt-2100c ELISA microplate reader and standard colorimetric assay (Rayto, Shenzhen, China). The revised quantitative insulin sensitivity check index (RQUICKIBHB) of insulin resistance and glucose to insulin ratio was calculated. SPSS statistics software (IBM, USA) was used.

RESULTS: In cows receiving niacin, there was a significantly higher ($P<0.01$) concentration of NAD and NADP, but the NAD: NADP ratio did not differ ($P>0.05$) compared with control. NAD and NADP are good indicators of the availability of niacin from

additional sources. The NAD: NADP ratio is a good indicator of the biological effect of applied niacin on metabolites in cows due to its positive correlation ($P<0.05$) with glucose, insulin, glucose to insulin ratio, RQUICKIBHB, TG and cholesterol, and a negative correlation with NEFA, BHB, GGT and urea in cows receiving niacin. The same amount of added niacin in feed can produce different concentrations of NAD, NADP and NAD: NADP in the blood and this was not related to their concentration before the addition of niacin. The change in the concentration of the active form of niacin (NAD, NADP and NAD: NADP) further correlates with the concentration of metabolic parameters. Under basal conditions active forms of niacin that already exist in the blood do not show significant correlations ($P>0.05$) with metabolic parameters.

CONCLUSIONS: We concluded that NAD and NADP are good indicators of the ability of an additional niacin source to create functional cofactors due to their concentration changes, while the NAD: NADP ratio is a good indicator of the biological effects of additional niacin due to correlation with many metabolites.

Keywords: dairy cows, transition period, niacin status, metabolites

3-Methylhistidine (3-mh) in Dairy Cows

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BACKGROUND and OBJECTIVE: 3-Methylhistidine (3-MH) is a non-reutilizable amino acid found exclusively in the myofibrillar proteins actin and myosin. During muscle protein breakdown, 3-MH is released into the bloodstream. In dairy cows, circulating concentrations of 3-MH typically increase during early lactation, a period characterized by skeletal muscle mobilization to support milk protein synthesis and gluconeogenesis. Because 3-MH can be quantified in blood and urine, it is widely used as a biomarker of increased proteolysis and body protein loss in cattle. Muscle mobilization is closely linked to negative energy balance, during which adipose tissue reserves are mobilized, resulting in elevated plasma concentrations of non-esterified fatty acids (NEFA) and increased hepatic production of ketone bodies, such as β-hydroxybutyrate (BHB). The primary objective of this thesis was to measure blood concentrations of 3-MH in dairy cows and to assess its suitability as an indicator of muscle mobilization.

MATERIALS and METHODS: The study included 105 clinically healthy Holstein-Friesian dairy cows from six farms; all managed under standardized housing conditions (free-stall systems with lying boxes) and fed an identical total mixed ration (TMR) twice daily. Annual milk production of the cows ranged from 8,143 to 11,686 kg. Animals were allocated to three groups: (1) prepartum cows (10–20 days before calving), (2) early postpartum cows (10–20 days after calving), and (3) mid-lactation cows (approximately four to six months after calving). Venous blood samples were collected from the jugular vein of all animals. Serum concentrations of BHB and NEFA were determined using commercial assay kits (Randox Laboratories Ltd.) and analysed with an Alizé spectrophotometer (Lisabio, France). Concentrations of 3-MH were measured using commercial 96-well ELISA kits. Statistical analysis was performed using analysis of variance (ANOVA), with differences considered significant at $p<0.05$.

RESULTS: The highest mean concentration of 3-MH was observed in cows approximately four months postpartum ($\bar{x}$=167 nmol/mL); however, no statistically significant differences in 3-MH concentrations were found among the groups ($p>0.05$). In contrast, the highest concentrations of BHB and NEFA were recorded in early postpartum cows ($p<0.01$).

CONCLUSIONS: Under the conditions of this study, blood concentrations of 3-MH were not a reliable indicator of the degree of muscle protein degradation in dairy cows.

Keywords: 3-Methylhistidine, 3-MH, dairy cows

Co-Ensiling White Mulberry Pomace with Meadow Grass Improves Silage Fermentation and Digestibility: Determination of an Optimal Ratio

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BACKGROUND and OBJECTIVE: During ensiling, water-soluble carbohydrates are primarily converted into lactic acid and, to a lesser extent, acetic acid by lactic acid bacteria (LAB), which rapidly reduce pH and inhibit spoilage microorganisms. Limited availability and high costs of conventional feed have increased interest in utilizing agro-industrial by-products as sustainable ruminant feed. White mulberry pomace (WMP), a by-product of mulberry juice production, is rich in fermentable carbohydrates, but its high moisture content poses preservation challenges. This study aimed to determine the optimal WMP–meadow grass (MG) ratio for silage based on fermentation quality, microbial safety, digestibility, and practical applicability.

MATERIALS and METHODS: Five silage formulations were prepared: 100% WMP (WMP100), 75% WMP + 25% MG (WMP75), 50% WMP + 50% MG (WMP50), 25% WMP + 75% MG (WMP25), and 100% MG (WMP0). Each treatment was ensiled in 1 L laboratory mini-silos with five replicates and stored for 60 days. Silages were analyzed for chemical composition, fermentation characteristics, microbial populations, and in vitro digestibility, including in vitro true dry matter digestibility (IVTDMD), in vitro true organic matter digestibility (IVTOMD), and in vitro neutral detergent fiber digestibility (IVNDFD). Group comparisons were performed using the least-square method with Tukey's post-hoc test. Spearman rank correlations were calculated within and across groups, and significance was set at $P<0.05$.

RESULTS: Incorporation of WMP markedly improved fermentation characteristics compared with WMP0 silage, evidenced by lower pH, higher lactic acid concentrations, and favorable lactic-to-acetic acid ratios. WMP75 consistently demonstrated the best balance between fermentation efficiency and stability, showing low pH, high lactic acid concentration, and no detectable enterobacteria or yeast-mold populations. Higher MG inclusion increased dry matter content but reduced fermentative intensity and digestibility, whereas WMP100 achieved the highest digestibility but presented practical limitations due to excessive moisture. All WMP-containing silages had significantly higher IVTDMD, IVTOMD, and IVNDFD than WMP0 silage ($P < 0.001$). WMP75 achieved digestibility comparable to WMP100 while maintaining superior handling characteristics and microbial safety. Correlation analysis revealed strong positive associations between WMP proportion, lactic acid concentration, and digestibility, and negative associations with pH, acid detergent fiber, and neutral detergent fiber.

CONCLUSIONS: Considering fermentation quality, microbial safety, digestibility, and practical handling, a 75%WMP+25%MG mixture (WMP75) represents the optimal silage formulation. This strategy improves roughage quality and provides a sustainable approach for recycling mulberry pomace, promoting efficient and environmentally responsible ruminant feeding.

Keywords: Co-ensiling, white mulberry pomace, silage fermentation, digestibility, microbial safety, ruminant nutrition

Comparison of a Yeast, Mineral, and Antioxidant Bolus Versus Calcium-only Boluses at Calving: Effects on Early-Lactation Milk Yield and Health on a Highly Productive Spanish Dairy Farm

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BACKGROUND and OBJECTIVE: Transition dairy cows experience substantial metabolic and physiological changes that increase nutrient demands while limiting dry matter intake. These factors predispose cows to disorders such as

hypocalcemia, impaired rumen function, and postpartum disease. Oral calcium supplementation is commonly used to mitigate early lactation challenges, yet its effects on health or performance remain inconsistent. Alternative strategies that support rumen microbial activity, such as live yeast supplementation, may complement calcium by enhancing feed utilization. This study evaluated the effect of an oral bolus containing live yeast, minerals and antioxidants on milk yield and health outcomes in transition dairy cows.

MATERIALS and METHODS: A total of 202 Holstein dairy cows from a commercial dairy farm located in Barcelona (Spain) were enrolled at calving and assigned to a control group, receiving two oral calcium boluses in multiparous cows (CTL; n=100); or to a treatment group receiving one dose of an oral fresh cow bolus containing live yeast, minerals and antioxidants in all parities (BOL; n=102). Health events such as mastitis and postpartum disorders were recorded for 60 days after calving. Daily milk yield was recorded during this period in cows that completed at least 90% of the study (CTL: n = 88; BOL: n = 56). Average daily milk yield data was analyzed using a one-way ANOVA with treatment as the fixed effect. Incidence of health disorders was analyzed using chi-square or Fisher's exact tests. Statistical significance was declared at $P \leq 0.05$.

RESULTS: Cows receiving the fresh cow bolus produced more milk during early lactation, averaging 2.37 kg per day greater yield than control cows over the first 60 DIM (51.5 vs 49.1 Kg; $P < 0.001$). The incidence of postpartum disorders other than mastitis was low and did not differ between groups (control, 6 cases; treated, 5 cases; $P = 0.97$). In contrast, mastitis incidence was greater in control cows (22/100; 22.0%) compared with treated cows (5/102; 4.9%; $P < 0.001$). Mild, moderate, and severe mastitis cases were numerically higher in control cows than in treated cows.

CONCLUSIONS: Administration of an oral bolus containing live yeast, minerals and antioxidants at calving was associated with increased milk yield during early lactation and a reduced incidence of mastitis. These results suggest that combined supplementation strategies targeting mineral balance and rumen function may support performance and udder health in transition dairy cows.

Keywords: Transition cows, live yeast, antioxidants, postpartum diseases

Effect of Different Non Fiber Carbohydrate Levels on Fattening Performance in Lambs

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BACKGROUND and OBJECTIVE: The Non Fiber Carbohydrate (NFC) content, particularly the starch content, of ruminant rations is of great importance in determining the source of energy provided to the animals. Investigating this topic is not only a step towards protecting animal health but can also serve as a benchmark for determining the quality of commercial feeds. The objective of this study was to evaluate the effects of different dietary NFC levels on body weight, body weight gain, dry matter intake, FCR, hot carcass weight and yield, cold carcass weight and yield.

MATERIALS and METHODS: The experiment was lasted 60 days and in this research 30 weaned male Merino lambs, which were 90 days old, were used. There were 3 experimental groups which were created for different dietary NFC levels and 10 lambs were assigned for each group. Dietary NFC levels for group 1, 2 and 3 were 34%, 37% and 40%, respectively.

RESULTS: It was observed that increasing NFC level in the first 15 days and 0-60 days period statistically decreased the body weight gain of lambs ($P<0.05$). Dry matter intake and FCR increased just numerically. Hot and cold carcass yields were found statistically higher in 34% NFC group ($P<0.001$). According to the different level of NFC, the ruminal pH was not affected ($P=0.128$).

CONCLUSIONS: It was concluded in the experiment that reduction in the dietary NFC levels generally improved body weight gain and especially hot and cold carcass yield. Further research not only on NFC level but also NFC source would determine the actual reason of changes observed in this experiment.

Keywords: Lamb, Non Fiber Carbohydrates, Performance, Starch

Does a Novel Prepartum Bolus Add Benefit Beyond At-Calving Supplementation Only? Evidence From a Field Trial

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BACKGROUND and OBJECTIVE: Supplementing dairy cows at calving with a bolus containing live yeast, minerals, and antioxidants has been shown to effectively enhance health status and productivity postpartum. Nonetheless, prepartum nutritional strategies based on feed supplementation can be challenging due to inconsistent intake during the final weeks before calving. Therefore, implementing new strategies to ensure a stable supply of proven additives during the final three weeks prior to calving may enhance the effectiveness of the transition protocol. This study evaluates whether combining a novel sustained-release prepartum bolus (given 21 days before calving) followed by a proven bolus at calving improves milk yield and animal health in Holstein cows during early lactation.

MATERIALS and METHODS: A total of 100 Holstein cows were blocked by trimester of gestation, previous 305-d milk yield (multiparous cows), and parity, and then randomly assigned to: a group that received one bolus 21 days before the expected calving date, and a different bolus at calving (PBCB, n= 53); and a second group that received only the calving bolus (CB, n=47). The prepartum bolus contained B-vitamins, live yeast, and trace minerals. The calving bolus contained minerals, live yeast, and antioxidants. All cows were managed under the same conditions and fed the same daily ration. Daily milk yield (kg) from 1 to 60 DIM was analyzed using a linear mixed model with treatment and DIM as fixed effects, and cow as a random effect. Postpartum diseases, including mastitis, metritis, retained placenta (RP) and hypocalcemia, were monitored during 60 DIM and analyzed using chi-square test. Significance was declared at $P < 0.05$.

RESULTS: Results showed that PBCB cows produced 2.6 kg/d more milk at 60 DIM compared with CB (46.8 vs. 44.2 kg/d; P = 0.099), indicating a trend towards improved early-lactation yield. The overall incidence of postpartum disorders was low and did not differ between groups, with 11.3% (6 cases) in PBCB cows and 12.8% (6 cases) in CB cows (P = 0.824). A similar numerical pattern was observed for retained placenta, where 3 cases (5.7%) occurred in PBCB cows versus 4 cases (8.5%) in CB cows (P = 0.577).

CONCLUSIONS: Providing cows with a prepartum sustained-release bolus before the calving bolus was associated with higher milk yield through 60 DIM relative to administering the calving bolus alone. Although not statistically significant, the directional response indicates that ensuring more consistent nutrient delivery during late gestation may benefit transition performance.

Keywords: Prepartum sustained-release bolus, milk yield, transition cow health

Milk Production During Early Lactation and Rumination Time in Holstein Cows Receiving a Sustained-Release Prepartum Bolus Three Weeks Prior to Calving

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BACKGROUND and OBJECTIVE: The prepartum period is crucial for dairy cows, as metabolic and physiological changes during this time impact health, feeding behavior and milk production after calving. This trial aimed to determine if a novel prepartum sustained-release oral bolus could improve early lactation milk yield and rumination time in Holstein cows.

MATERIALS and METHODS: A total of 50 Holstein cows (primiparous and multiparous) were enrolled approximately 21 ± 5 days before expected calving date and randomly allocated to one of two treatments: a control group that received no bolus (CTL, n = 25) or a treatment group that received one intraruminal, sustained-release bolus at the enrollment (TRT, n = 25). Randomization was stratified by parity, previous 305-day milk yield or genomic prediction in primiparous cows, and sire type (Stratum). All cows were managed under the same commercial conditions and received the same

daily ration. Daily milk yield (kg) was recorded from day 1 to day 30 in milk (DIM). Health status was classified based on the occurrence of metritis, mastitis, retained placenta, displaced abomasum, or hypocalcemia, and cows were stratified accordingly. Rumination time (RT; min/d) was recorded using neck sensors for the first 7 DIM. Milk yield and RT were analyzed using a linear mixed-effects model including the fixed effects of treatment, health status, DIM, and their interactions; stratum was included as an additional fixed effect. Cow was included as a random intercept. Significance was declared at $P < 0.05$.

RESULTS: Cows receiving the TRT bolus tended to produce 2.7 kg/d more milk than CTL cows (44.7 vs. 42.0 kg/d; $P = 0.087$) during the first 30 DIM. A similar tendency was observed in cows with a post-calving disease, where those receiving TRT produced 6.8 kg/d more milk than CTL cows (47.0 vs. 40.3 kg/d; $P = 0.058$). Rumination time did not differ between treatments ($P>0.10$). Within cows with post-calving disease, rumination time was numerically greater in TRT than in CTL cows (350.3 vs. 339.6 min/day), but this difference was not significant ($P > 0.10$).

CONCLUSIONS: The administration of a prepartum sustained-release intraruminal bolus did not significantly increase early lactation milk yield in Holstein cows under the conditions of this study. While cows receiving the bolus showed a numerical increase in milk production compared to controls, the difference was not statistically significant, suggesting that further research with larger sample sizes may be needed to fully assess the potential benefits of this intervention.

Keywords: Transition Cow Health, Sustained-release, Prepartum Bolus, Postpartum Disorders

Evaluation of An Injectable Mineral Supplement on Weight Gain in Weaned Calves

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BACKGROUND and OBJECTIVE: Weaning and management changes represent periods of high stress in cattle production, often resulting in a reduction in daily weight gain. Parenteral administration of macro and micro minerals ensures the complete bioavailability, meeting the high metabolic demand during critical periods. This study aimed to assess the impact of supplementation with the injectable mineral product on the daily weight gain (DWG) of weaned calves, comparing it against an untreated control group.

MATERIALS and METHODS: One hundred and eighty female weaned calves of British breeds (6 to 8 months old) were selected and systematically randomized into two groups of 90 animals each: Control (sterile saline solution) and Treated (Fosfosan, Virbac Uruguay S.A., a multimineral supplement containing phosphorus, selenium, copper, potassium, and magnesium). Both groups received two 10 mL subcutaneous doses 29 days apart (D0 and D29). The animals were maintained under the same management and feeding conditions (pastures of Avena and Festuca) for the 88 days of the trial. Body weight was recorded on days 0, 29, 53, and 88 and average daily weight gain (ADWG) was calculated. Statistical comparisons were performed using the non-parametric Mann-Whitney test, analyzing data within the 10th–90th percentile.

RESULTS: At the start of the study, the average weight was similar between the groups (Control: 163 kg; Treated: 164 kg). The Treated group demonstrated a significantly higher ADWG compared to the control group at both day 53 ($p=0.020$) and day 88 ($p=0.027$). The higher difference in ADWG was recorded at day 53, 48 g/day greater than the control group. By the study's conclusion (D88), the Treated group's ADWG was 351.3 ± 96.6 g/day, which was 36.3 g/day greater than the control group's ADWG (315.1 ± 105.0 g/day). The total weight gain was 31 kg for the Treated group and 27 kg for the Control group; this difference

resulted in an additional 4 kg of cumulative live weight gain per animal throughout the trial period.

CONCLUSIONS: The administration of Fosfosan was linked to an improvement in the ADWG of weaned female calves. These findings suggest that injectable mineral supplementation during stressful periods, such as weaning, provides a direct positive biological and economic benefit for beef production systems.

Keywords: Micronutrition, Cattle, Calves, Minerals

Evaluation of a New Sustained Release Prepartum Bolus: Effects on Milk Production and Postpartum Health in a Commercial Holstein Dairy Herd in Spain

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BACKGROUND and OBJECTIVE: Ensuring consistent delivery of key nutrients during the close-up period is difficult due to management factors such as changes in grouping, variable feed intake, and limited control over individual consumption before calving. Variability in prepartum nutrient intake is associated with increased risk of metabolic and infectious disorders after calving and may compromise early lactation performance. Nutritional approaches that ensure continuous delivery before calving may help to reduce these risks. The objective of this study was to assess the effects of administering a single sustained-release bolus 21 days before expected calving on postpartum health and early lactation milk production in Holstein dairy cows managed under commercial conditions in Spain.

MATERIALS and METHODS: The study was conducted on a commercial Holstein dairy farm in Spain. A total of 39 cows (heifers and multiparous) were enrolled and randomly allocated to a control (n=20) or treatment (n=19) group. All cows were housed together and fed the same close-up diet. Treated cows received a single sustained-release bolus approximately 21 days before calving. After calving, control heifers received no bolus, while control multiparous cows received a standard calcium bolus according to routine farm practice. Milk yield was tracked daily for the first 100 days in milk (DIM), and postpartum health events were monitored during the initial 60 DIM. Differences in milk yield between groups were analyzed using one-way ANOVA, while the incidence of postpartum health events (clinical milk fever, retained placenta, metritis, mastitis, etc.) was compared using chi-square test, with statistical significance set at $P<0.05$.

RESULTS: Milk yield over the first 100 DIM was significantly higher in treated cows compared with control cows (50.6 vs. 48.3 kg/day), representing an absolute difference of +2.3 kg/day ($P<0.001$). Regarding animal health outcomes, the incidence of cows with at least one postpartum disorder was lower in bolus supplemented group than control group (14% vs. 30%; -52%), although this difference was not statistically significant ($P>0.05$).

CONCLUSIONS: In this study, prepartum administration of a sustained-release nutritional bolus was associated with increased milk yield during early lactation period. Although variations in postpartum health indicators were noted, these differences did not achieve statistical significance, likely due to the limited sample size of cows. Considering the limited sample size, further research involving larger cow populations and extended physiological evaluations is required to validate these preliminary findings and elucidate the underlying mechanism of action.

Keywords: Prepartum bolus, Milk production, Metabolic disease

Effects of Forage Source and Corn Grain Physical Form in Starter Diets on Ruminal Volatile Fatty Acids Concentrations in Pre-weaning Simmental Calves

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BACKGROUND and OBJECTIVE: Forage source, physical form of starter feed (ground, pelleted and textured) and milk feeding level are essential factors resulting in the different degrees of effect on rumen fermentation of young calves. This study aimed to evaluate the effects of different forage sources (wheat straw or alfalfa hay) and corn physical forms (ground or whole corn) on ruminal volatile fatty acids concentrations in pre-weaning Simmental calves offered total mixed ration under the condition of the high milk feeding level.

MATERIALS and METHODS: Forty eight calves at 3 days of age were randomly assigned to one of the four treatments (n=12 calves per treatment; 7 female, 5 male): (1) 90% pelleted calf starter (P; 34.60% ground corn) and 10% wheat straw (PS); (2) 70% pelleted calf starter (15.18% ground corn) plus 20% whole corn (PWC) and 10% wheat straw (PWCS); (3) 90% pelleted calf starter (P; 34.60% ground corn) and 10% alfalfa hay (PA); (4) 70% pelleted calf starter (15.18% ground corn) plus 20% whole corn (PWC) and 10% alfalfa hay (PWCA). In PS and PA treatments, all of the corn was within the pelleted starter. All calves were weaned at 68 days of age (the end of the study). The calves had free access to starter diet and water throughout the study (from 3 to 68 days of age). Rumen fluid samples were collected for volatile fatty acid measurements at 68 days of age.

RESULTS: Ruminal acetate concentration was higher for PWCA than PWCS ($p<0.05$). There was a tendency for the interaction between forage source and corn physical form on ruminal acetate level ($p=0.06$). Ruminal propionate concentration was greater in PWCS than PA ($p<0.05$). The interaction between forage source and corn physical form was significant for ruminal propionate concentration ($p<0.01$). Ruminal butyrate concentration was higher in PWCA than PS ($p<0.05$). An interaction was observed between forage source and corn physical form for ruminal butyrate concentration ($p=0.04$). Total volatile fatty acids concentration did not differ among the treatments ($p>0.05$).

CONCLUSIONS: Individual ruminal volatile fatty acid concentrations at weaning differed in Simmental calves depending on the forage source and/or corn physical form in the starter diets offered as a total mixed ration. These differences were likely related to variaıtions in particle size distribution of the starter diets and palatability between forage sources, potentially leading to feed sorting.

Keywords: wheat straw, alfalfa hay, corn grain physical form, ruminal volatile fatty acids, preweaning calves

The Effects of Sampling Location on Some Metabolic Parameters in Dairy Cows

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BACKGROUND and OBJECTIVE: Due to the increased energy and nutrient demands of high-yielding dairy cows, strategic programs for monitoring dairy herds for metabolic disorders are meaningful. To implement screening protocols with reliable, repeatable results, information on factors that may influence the measurement is essential. It was shown that the blood sampling location affects, e.g., the BHB concentration, since the mammary gland can utilize BHB as an energy source. This study, therefore, aimed to evaluate plasma concentrations of glucose, BHB, NEFA, AST, and total Ca in samples collected from different sampling locations of high-producing dairy cows to assess any systematic differences among these locations.

MATERIALS and METHODS: Blood samples were taken from 85 Holstein Friesian dairy cows (in DIM 1-64). Three samples were taken from each animal into heparinized tubes, one from the jugular vein (v. jugularis), one from the caudal vein (v. caudalis), and one from the mammary vein (v. epigastrica cranialis superficialis). We expected that jugular and caudal vein measurements would be equivalent, and that glucose, BHB, and NEFA concentrations measured from the mammary vein would be consistently different. Differences between jugular and caudal vein concentrations, and between jugular and mammary vein concentrations, were assessed for clinical relevance, dependence on the measurement range, and the basis for adjusting in reference to the jugular concentration, assigned as the gold standard.

RESULTS: Based on analysis of 255 blood samples, we found no clinically or statistically significant differences in glucose, BHB, NEFA, AST, or Ca concentrations between the jugular vein and caudal vessels. However, concentrations of metabolites measured in the mammary vein were significantly different (average difference (95%CI), all $p<0.05$) from jugular concentrations for all five metabolites (glucose: 0.75 (0.65; 0.84) mmol/L; NEFA: 0.037 (0.025;0.050) mmol/L; BHB: 0.17 (0.12; 0.22) mmol/L; AST: 1.75 (0.83; 2.67) U/L; Ca: 0.126 (0.095;0.156) mmol/L).

CONCLUSIONS: The differences in metabolite concentrations are presumably dependent on actual milk yield or lactation phase, which makes it difficult to establish mammary vein-specific thresholds for diagnosing metabolic impairments. We concluded that thresholds for the caudal or jugular veins cannot be automatically applied to mammary vein measurements. Further studies with a larger sample size are needed to determine lactation-phase-specific thresholds for metabolic profiling based on mammary vein sampling.

Keywords: dairy cow, metabolic profile, sampling, mammary vein, caudal vein, jugular vein

Blood Biomarkers and Transition Health Impact in Dairy Cows Supplemented with a Prepartum Oral Bolus

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BACKGROUND and OBJECTIVE: During the transition period, dairy cows face significant metabolic, hepatic, and inflammatory stress, increasing their risk of postpartum diseases and reduced performance early in lactation. Changes in energy metabolism and inflammation biomarkers signal poor adaptation, with higher levels linked to negative energy balance, systemic inflammation, and greater disease risk. This study aimed to assess the impact of a prepartum oral bolus on blood serum biomarkers at −21, +1, +7, and +14 days around calving, as well as on postpartum health outcomes.

MATERIALS and METHODS: A total of 50 Holstein cows were enrolled three weeks prior to their expected calving date and allocated to two treatment groups: Control (CTL; no bolus; n = 25) and Prepartum Bolus (PCB; one oral bolus administered 21 days before calving; n = 24). Assignment used stratified randomization based on parity, prior 305-day milk yield, and sire category. Blood samples were collected at −21, +1, +7, and +14 days relative to calving to determine serum concentrations of BHB (mmol/L), glucose (mg/dL), AST (IU/L), GGT (IU/L), cortisol (µg/dL), Hp (g/L), SAA (mg/L) and NEFA (mmol/L). Changes from baseline were calculated as delta values (Δ; post-calving values minus baseline at −21 d) and analyzed using linear mixed-effects models including treatment, time, their interaction and pair as fixed effects, with cow as a random effect. Postpartum health disorders (metritis, mastitis, retained placenta, displaced abomasum, or hypocalcemia) were recorded during the first 30 DIM, and compared between treatments using chi-square test. Differences were declared significant at $P < 0.05$.

RESULTS: The prepartum bolus had no detectable effects on changes in most metabolic, hepatic, or inflammatory biomarkers ($P > 0.10$). However, a tendency for treatment effect was observed for ΔHp ($P = 0.099$), with numerically lower values in PCB cows than in CTL (0.26 vs. 0.65 g/L). Results indicated that ΔHp was highest at 1d (CTL, 1.08 vs PCB, 0.72 g/L), decreased at 7d (0.62 and 0.35 g/L, respectively),

and was lowest at 14d (0.32 and −0.29 g/L, respectively). Postpartum disease occurred in 28% of PCB cows and 40% of CTL cows. Although overall morbidity did not differ statistically between treatments, the odds of disease were numerically lower in the PCB group (OR = 0.58; 95% CI: 0.17–1.89; $X^2 > 0.10$).

CONCLUSIONS: Prepartum administration of a sustained-release oral bolus was associated with lower postpartum haptoglobin and numerically reduced disease incidence, suggesting potential benefits for transition cow health. Further studies are needed to confirm these effects.

Keywords: Inflammatory response, Blood biomarkers, Transition cow metabolism

Determination of Nutrient Composition of Azolla Plant Cultivated Under Pond-growing Conditions as An Alternative Feed Source for Ruminants

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BACKGROUND and OBJECTIVE: Azolla, a free floating aquatic plant on water surface, is abundantly available in stagnant water in many regions of the world. It has been established that Azolla can be used as a feed ingredient for ruminants. This study aimed to determine the nutrient composition of Azolla produced under different pond-growing conditions and to develop a model for its sustainable cultivation in pond systems.

MATERIALS and METHODS: Azolla was cultivated in two experimental ponds with surface area of 1m2 located in a greenhouse, each maintained at a constant water depth of 20cm. The pond bottoms were prepared with a substrate layer consisting of dry soil (3kg) and dried cow manure (150g) prior to inoculation. At the beginning of the study, 150g of Azolla was introduced into each pond. During the cultivation of Azolla in pond 1 (Group 1), one-third of pond substrate was renewed every 30 days; 60g of dried cow manure was added to the pond every 7 days; and the accumulated dried manure was removed every 3 months. During the cultivation of Azolla in pond 2 (Group 2), only one-third of pond substrate was renewed every 30 days. The study lasted for one year, and throughout the study, one-third of the pond surface area was harvested every seven days. Azolla harvested every seven days was weighed and dried in an air-forced oven. The samples collected over a one-month period were then pooled and stored at −20°C until analysis.

RESULTS: The mean dry matter, crude protein, NDF, ADF, ADL, ether extract, crude ash, and starch contents of Azolla harvested throughout the study were 5.40 and 5.45%, 23.46 and 18.62%, 46.75 and 50.37%, 34.53 and 39.90%, 13.43 and 14.18%, 2.84 and 2.46%, 17.56 and 16.03%, and 4.77 and 5.71% in Group 1 and Group 2, respectively. Crude protein level was higher in Group 1 than in Group 2 ($p<0.01$). NDF content was lower in Group 1 than in Group 2 ($p<0.05$). The average monthly harvest amount of Azolla was higher ($p<0.01$) in Group 1 (1.175kg) than in Group 2 (0.463kg).

CONCLUSIONS: Although the monthly harvest amount varied depending on the season, air temperature, and humidity inside the greenhouse, sustainable Azolla production was achieved under the conditions of this study. The Azolla cultivation method applied in pond 1 resulted in a higher crude protein content. The relatively high crude protein content of Azolla suggests that it may be considered an alternative protein source for ruminant diets.

Keywords: Azolla plant, nutrient composition, sustainable cultivation in pond systems

Impact of Starch Versus Fat as Energy Sources on Hoof Health in Holstein and Simmental Bulls

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BACKGROUND and OBJECTIVE: To achieve high body weight gain in beef cattle, starch or fat sources are utilized as energy sources. However, because starch sources degrade rapidly in the rumen, they can lead to complications such as ruminal acidosis and laminitis. This trial was aimed to investigate the effects of using starch or fat as energy sources in the Holstein and Simmental bull diets on dry matter intake, feed conversion ratio, fat pad thickness, foot health.

MATERIALS and METHODS: The study was conducted at Bomonti Dairy Farm (Kırklareli, Türkiye). Fourteen Holstein friesian beef cattle (405±50 kg) and fourteen Simmental beef cattle (450±50 kg) were randomly divided into four groups and each group consisted of 7 animals. All animals were fed with fat or starch-based total mixed ration (TMR) based on energy sources. The experimental groups of the study were designed as follows: Simmental beef cattle group fed with fat (SF, n: 7) or starch (SS, n: 7) and Holstein beef cattle fed with fat (HF, n: 7) or starch (HS, n: 7) based ration as energy source. After the animals were slaughtered, 4 feet of each animal were collected. The fat pad of each claw was removed from each foot. The fat pads on the collected from feet were visualized by ultrasonography and the digital cushion fat pad thickness was measured. The fat sample was taken from the cranial part of the footpad and using a microtome device were stained with hematoxylin and eosin dye. The stained samples were examined under a microscope for mononuclear cell infiltration, brown fat tissue, and bleeding.

RESULTS: The dry matter intake of Holstein beef cattle fed based fat (HF, 11.9 kg/d) higher than fed starch based diet for Holstein (HS, 11.2 kg/d) and Simmental (SS, 10.9 kg/d). The feed conversion ratios of SS (0.14±0,003) and HS (0.13±0,006) groups were found to be higher than SF (0.10±0,008) and HF (0.11±0,01) groups ($P<0.05$). Fat pad thickness of animals, HF (1.2104) was higher than those fed HS (1.0993) and SS (1.1293) ($P<0.05$). Fat pad thickness in the SF group (1.2289); It was found to be higher than the HS (1.0993) and SS (1.1293) groups ($P<0.05$). There was no difference between the groups in terms of the relationship between mononuclear cell infiltration and bleeding ($P>0.05$).

CONCLUSIONS: According to the results of this study, it was concluded that feeding with fat based TMR may have a potential positive effect on foot health.

Keywords: Beef cattle, fat, starch, simmental, holstein friesian

Effects of a Complementary Feed on Milk Production and Robot Efficiency Under Heat Stress Conditions in Early Lactation Dairy Cows

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BACKGROUND and OBJECTIVE: Heat stress significantly impairs dairy cow performance and well-being. Physiological responses such as reduced dry matter intake, immune activation, and increased oxidative stress, can lead to marked declines in productivity, particularly during early lactation. Considering emerging dairy-producing countries and ongoing climatic changes, effective heat stress management is expected to become a key success factor in dairy industry. The study aimed to evaluate the effects of a complementary feed on milk production and robot efficiency in early-lactation dairy cows.

MATERIALS and METHODS: The trial was conducted on a german dairy farm. An on-off experimental design was used, including data from 77 cows during the off-period (OFP) and 75 cows during the on-period (ONP). Cows between lactation day 10 and 120 were analyzed. OFP spanned from 09.07.2025 until 04.08.2025, while ONP lasted from 05.08.2025 till 08.09.2025. During ONP, cows received additional 100 g of a complementary feed mixed into TMR, containing a long-lasting rumen buffer, betaine, coated grape extract, chili extract, and a betaine–zinc-chelate-complex. Performance data and robot efficiency (visits/day and milk production/visit) were collected using a Lely A5 robot. Climate data was recorded using a KESTREL data logger. THI was calculated as THI=dry bulb temperature+0.36×dew point+41.2 and classified as no heat stress (<68), light heat stress (68–71,) and moderate heat stress (72–79).

RESULTS: Energy-corrected milk yield (ECM) averaged 0.8 kg higher during ONP compared to OFP. While with increasing THI a decline in milk yield was observed during OFP, it increased during ONP. When data was subdivided by heat stress classification, differences in milk yield became visible. Comparing OFP vs. ONP results were as follows: No heat stress: THI: 63.4 vs. 65.7, ECM; 44.7 kg vs. 45.2 kg; Light heat stress: THI: 69.5 vs. 69.0, ECM: 44.7 kg vs. 45.4 kg; Moderate heat stress: THI 75.2 vs. 74.6, ECM: 44.0 kg vs. 45.6 kg. Resulting in 1.6 kg higher ECM during ONP considering moderate heat stress. Cows visited the robot 0.2 times more often during OFP compared to ONP (2.7 vs. 2.5 visits/day) whereas milk yield per visit was 1.6 kg/cow higher during ONP (17.2 kg vs. 18.8 kg).

CONCLUSIONS: In summary, it can be presumed that supplementation positively affected early-lactation cows by increasing milk yield and flow, with benefits becoming more pronounced under heat stress, indicating support during periods of elevated metabolic demand. Additionally, robotic milking efficiency improved during ONP, with fewer visits and higher milk yield/visit making supplementation economically interesting.

Keywords: Heat stress, milk performance, robot efficiency

Reticular pH of Dairy Cows on Grass Silage vs. Grass-maize Silage Feeding and the Effect of Forage Change on Reticular pH

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BACKGROUND and OBJECTIVE: Feeding has a crucial effect on the ruminal pH, which is an important factor for the overall health of the dairy cow. Sudden changes in feed and starch content might affect ruminal pH and disturb the microbial community of rumen. The aim was to study the effect of a change between grass (G) and grass-maize (G-M; 50: 50) silage on the reticular pH. The hypothesis was that due to the increased starch intake, feeding a mixture of maize silage and grass silage will decrease reticular pH compared to grass silage-based feeding.

MATERIALS and METHODS: A feeding trial was conducted at a research farm of Helsinki University. Twelve multiparous Nordic Red cows were admitted to a feeding experiment that was arranged using cyclic change-over model. There were six different feeding types, two six animal blocks and three 21-day periods. During the experiment, each cow had 1-2 changes in the silage type (between G and G-M), altogether 9 cows for both changes. We had pH data for each cow for 7 d before and 10 d after each silage change. Reticular pH, representing rumen pH, was measured at 10 minutes intervals using wireless boluses (SmaXtec Animal Care, Graz, Austria). For each cow, the average of 10-minute measurements was calculated for three different 8-hour periods: 00-08, 08-16 and 16-00. New feed was delivered at 09 every morning. We used linear mixed-effects model with cow as a random effect to account for repeated measurements, to study the association between average pH or minimum pH and silage type and day.

RESULTS: Average pH varied between 6.11 and 6.44, and minimum pH was 5.87. The number of 10-minute periods when reticular pH was below pH 6 during the day varied, on average, between 10 and 20. After a change from G to G-M, the average reticular pH

during day hours was slightly higher and there was a temporary decrease in the pH on the day of the feeding change. After a change from G-M to G, the reticular minimum and average pH during the evening hours was slightly higher. There seemed to be more variation on individual pH values few days after feeding changes.

CONCLUSIONS: As a conclusion, the effect of the silage type on the reticular pH was minimal, but the change in forage increased between-cow variation few days after the change, suggesting that some cows are more sensitive to the change.

Keywords: rumen pH, silage change, dairy cow

Effects of *Myrtus communis* L. Extract Supplementation on Performance, Metabolic Status, and Immune Response in Holstein Calves and Transition Dairy Cows

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BACKGROUND and OBJECTIVE: Natural plant extracts have increasingly been investigated as potential nutritional supplements to improve animal health and productivity in livestock systems. For this purpose, this study was conducted to determine the effects of Myrtus extract, added to the rations of suckling calves and transitioning dairy cows, on performance and metabolism.

MATERIALS and METHODS: The study consisted of two phases. In the first study, 50 one-week-old female Holstein calves were allocated to five groups: a control group and four treatment groups receiving myrtle leaf extract (MPEL) or root extract (MPER) at 25 or 50 mL/head per day. Extracts were administered with milk for 60 days and continued for 12 days post-weaning. The second study examined the effects of dietary myrtle extract in 40 Holstein cows during the transition period. Cows were assigned to a control group or a treatment group receiving 1.5 mL/kg body weight per day.

RESULTS: Supplementation significantly increased feed intake and live weight. Higher leukocyte counts were observed in calves receiving 50 mL/day, and IgG concentration was greatest in the MPER-50 group, indicating enhanced immune response. Serum non-esterified fatty acids (NEFA) decreased significantly in the high-dose groups, while beta-hydroxybutyric acid (BHBA) increased and glucose decreased with supplementation, suggesting improved adaptation to energy metabolism. Overall, administering 25 or 50 mL/head per day for 72 days positively influenced growth performance, immune function, and compensation of negative energy balance without reported adverse effects. Supplementation increased dry matter intake during prepartum and early-to-mid lactation and elevated prepartum glucose levels. Treated cows produced significantly more milk in early (44.56 vs. 40.72 kg/day) and mid-to-late lactation (42.57 vs. 37.19 kg/day). NEFA and BHBA concentrations were lower in supplemented cows, indicating reduced metabolic stress. Although prepartum inflammatory markers were similar between groups, treated cows had higher TNF-α concentrations at calving. Cortisol and ALT levels were also elevated around calving, warranting further investigation. Reproductive performance was largely unaffected; however, treated cows had fewer ovarian cysts.

CONCLUSIONS: In summary, myrtle extract supplementation in calves and dairy cows improved feed intake, growth, milk yield, immune indicators, and energy metabolism. These findings suggest that Myrtus communis L. extract may serve as a natural feed additive to support metabolic balance and performance during early life and the transition period, though some metabolic and stress-related responses require additional study.

Keywords: Myrtus, calf, cows, performance, feed, additive

How Useful If the Oesophageal Bloat Line in Diagnosing Bloat in Ruminants? - Expert Opinion from International Pathologists

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BACKGROUND and OBJECTIVE: The bloat line is a border between proximal congestion and distal pallor of the oesophagus proximal to the thoracic inlet. It occurs due to bloat-induced increased intra-abdominal pressure causing compression of the abdominal vasculature with consequent redistribution of blood towards the cervical region. The relevance of the oesophageal 'bloat line' in the diagnosis of bloat is equivocal. Hence, the objective of this study was to elicit the opinions of veterinary pathologists (VPs) worldwide on the topic.

MATERIALS and METHODS: A search of PubMed, Google Scholar and CAB databases failed to find quantitative data on the sensitivity or specificity of the oesophageal bloat line. We therefore conducted a VP survey to generate qualitative data on the extent of its pathological relevance. An email was sent to the listserv of the Davis-Thompson Foundation in October 2025 containing the online survey link in SurveyMonkey. A reminder was sent out two weeks later and the poll closed one week after that. The survey consisted of nine questions: seven with closed multiple option answers and two with free text answer options.

RESULTS: In total 192 pathologists responded. Approximately twice as many respondents (44.8%) consistently found a bloat line in cases where they diagnosed fatal bloat, compared to those who rarely or never did so (19.3%). More than ten-times more respondents (41.1%) rarely or never consistently found a bloat line in cases where they diagnosed non-bloat causes of death, compared to those who did always or usually found it (3.1%). The majority of respondents (82.7%) stated that the presence of a bloat line alone was not sufficient to diagnose fatal bloat, and a majority (93.7%) of respondents stated that the absence of a bloat line was not sufficient to rule out fatal bloat. The majority (91.6%) of respondents considered a bloat line helpful for diagnosing bloat in cattle; fewer did so in sheep (29.8%) or goats (22%). Over two thirds of respondents (68.3%) stated that the bloat lines fade with increasing postmortem interval. There was an even split between respondents who stated that a bloat line can be caused by postmortem bloat (49.2%) versus those who disagreed (50.8%).

CONCLUSIONS: It is concluded that a bloat line is observed more commonly in bloat than non-bloat causes of death, and while most pathologists consider its presence alone is not sufficient to diagnose fatal bloat its absence does not rule out fatal bloat.

Keywords: Bloat, bloat line, expert opinion

The Effect of Organic and Inorganic Selenium Supplementation to the Feed of Sheep During the Late Stages of Pregnancy on Colostrum and Blood Selenium Levels of Sheep and Their Lambs

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BACKGROUND and OBJECTIVE: This study was conducted to determine the effects of organic and inorganic selenium supplementation added to the diets of pregnant ewes on selenium levels in colostrum and blood serum of the ewes and their newborn lambs.

MATERIALS and METHODS: Animal material of the study consisted of a total of 36 pregnant sheep of Kıvırcık X Karacabey Merino F1 hybrid and the lambs born from these sheep. They were weighed and their blood was taken, they were randomly divided into 3 groups, each containing 12 ewes, one control and two treatment groups, and placed in individual feeding paddocks. Feed material of the experiment consisted of wheat straw and sheep

pellet feed containing 19% crude protein. Control group is which no additive added in sheep pellet feed. However, in Group I (GI) and Group II (GII), inorganic and organic selenium supplements were added to the diets at a level of 5 g/day using the on top of control group diet. The selenium content in the 5g premixes included is 0.135g for each form.

RESULTS: At the beginning of the experiment, no difference was observed in the blood serum selenium levels of the ewes. However, in the blood serum samples taken immediately after parturition, the mean selenium concentrations were determined as 165.2, 257.8, and 182.5 ppb for the Control, GI, and GII respectively, and the difference among the groups was statistically significant ($p<0.05$). In the blood serum samples collected from the lambs immediately after birth, selenium levels in the GI and GII groups were found to be higher than those in the Control group ($p<0.05$). Similarly, selenium levels in the colostrum samples taken immediately after birth were determined as 342.14, 388.55, and 393.81 ppb in the Control, GI, and GII groups, respectively, and the differences among the groups were statistically significant ($p<0.05$).

CONCLUSIONS: In conclusion, according to the results of this study, the addition of selenium to the diets of pregnant ewes resulted in significantly higher selenium levels in colostrum compared to the control group ($p<0.05$). Selenium levels in the blood serum of newborn lambs were also higher than control group ($p<0.05$). However, the addition of minerals to the diets did not have a significant effect on colostrum quality or live weight gain. This may be attributed to the use of a commercially purchased pelleted sheep feed containing 19% crude protein, which was fed at a level of 700 g/day per ewe in all experimental groups.

Keywords: Colostrum, organic and inorganic selenium, pregnant sheep, lamb, blood serum selenium level

The Effect of Heat Stress on Serum Calcium Concentration in Periparturient Simmental Cows

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BACKGROUND and OBJECTIVE: Global warming and severe heatwaves greatly affect dairy industry. Dairy cows are particularly vulnerable to heat stress, especially during periparturient period, which compromises energy metabolism of cows. Calcium availability is highly required during transition period, particularly in the early lactation for milk production. Increased calcium withdrawal by the cow's udder could lead to subclinical and even clinical hypocalcemia with consequences of appetite loss, decreased milk production and more severe health disorders including puerperal paresis or milk fever. The aim of this study was to evaluate the effect of summer heat on calcium (Ca) concentration during periparturient period in Simmental cows.

MATERIALS and METHODS: The Simmental cows (n=24) were assigned into two groups according to season: summer (S group, N=12) and autumn (A group, N=12). Blood samples were taken on days -21, -7, 8, 16, 24, 32 and 40 relative to parturition. Serum Ca concentration was assayed spectrometrically by commercial kits (Randox, UK).

RESULTS: Serum Ca concentration was significantly lower ($p<0.05$) in the S group on days 16 (2.24 mmol/L) and 32 (2.23 mmol/L) postpartum compared to the A group at the same sampling points (2.39 mmol/L and 2.32 mmol/L, respectively).

CONCLUSIONS: The results indicated that heat stress affected calcium metabolism of dairy cows in the early lactation leading to its decreased concentration what can influence both milk production as well as cow's health and performance.

Keywords: dairy cows, heat stress, transition period, metabolic disorders, calcium metabolism, negative energy balance

Comparison of Serum 25-Hydroxyvitamin D Concentrations Between Indoor-Supplemented and High-Altitude Grazing Dairy Cows

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BACKGROUND and OBJECTIVE: Vitamin D plays an important role in calcium and phosphorus metabolism, bone health, and immune function and is essential for the health and productivity of dairy cows. Although cattle are capable of synthesizing vitamin D in the skin following exposure to solar UVB radiation, sunlight alone may be insufficient to meet vitamin D requirements, as cutaneous synthesis is influenced by factors such as season, latitude, weather conditions, and hair coat coverage. Cattle housed indoors have limited exposure to solar UVB radiation and therefore rely primarily on dietary supplementation to ensure an adequate vitamin D supply.

MATERIALS and METHODS: Serum 25(OH)D was measured by ELFA (enzyme-linked fluorescent assay) on a MiniVIDAS analyzer (bioMérieux, France) in blood samples collected from lactating cows on commercial dairy farms (n = 9) during routine metabolic profiling. Eight farms kept cows indoors (n = 130), where they received dietary vitamin D_3 supplementation according to current feeding recommendations for dairy cows (25,000–30,000 IU vitamin D_3 per day). One farm was pasture-based, where cows (n = 47) grazed for approximately three months, with the last five weeks on high-altitude pasture at Planina Sleme (1448 m) without vitamin D supplementation.

RESULTS: Indoor-housed dairy cows showed serum 25(OH)D concentrations ranging from 8.4 to 32.8 ng/mL, with 60 out of 130 animals (46.2%) having concentrations below 20 ng/mL. In contrast, cows grazing on high-altitude pasture showed values ranging from 15.6 to 49.0 ng/mL, with 14 out of 47 cows (29.8%) below 20 ng/mL, and no statistically significant difference in serum 25(OH)D concentrations between indoor-housed and grazing cows ($p > 0.05$). When individual farms were analysed, serum 25(OH)D concentrations varied considerably between farms ($p < 0.001$), with mean values ranging from 14.60 ± 1.65 ng/mL on the farm with the lowest concentrations to 25.37 ± 2.20 ng/mL on the farm with the highest concentrations.

CONCLUSIONS: The results indicate that current dietary vitamin D_3 supplementation in indoor dairy systems provides a vitamin D status comparable to that observed in cows grazing on high-altitude pasture. Marked differences were observed between individual indoor farms, suggesting variability in vitamin D supply under practical feeding conditions. A considerable proportion of cows in both systems also showed serum 25(OH)D concentrations below 20 ng/mL, indicating vitamin D insufficiency. Notably, these values were observed even in cows grazing on high-altitude pasture during periods of peak solar exposure, suggesting that vitamin D supplementation may also be required in grazing systems.

Keywords: Vitamin D, Dairy Cattle, High-altitude pasture

Selenium Deficiency in Aberdeen Angus Beef Cows and Calves During the Grazing Season

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BACKGROUND and OBJECTIVE: Selenium (Se) is an essential trace element involved in antioxidant defense, immune function, and thyroid metabolism through several selenoproteins, including glutathione peroxidase (GPx). Selenium deficiency in cattle may result in impaired growth, increased morbidity and mortality, and nutritional myodegeneration in calves. In the Czech Republic, selenium deficiency is frequently reported in beef cattle due to low selenium concentrations in soils

and pasture forage. The aim of this study was to evaluate selenium status in Aberdeen Angus cows and calves during the grazing season using whole blood selenium concentration and glutathione peroxidase activity.

MATERIALS and METHODS: The study was conducted in three beef cattle herds located in a foothill region. Selenium concentration in pasture forage ranged from 0.032 to 0.074 mg/kg dry matter. In herds A and B, animals had free access to mineral licks containing 30 mg Se/kg in the form of sodium selenite. In herd C, animals received a mineral mixture containing 30 mg Se/kg in an organic form (selenium yeast). During the grazing season, blood samples were collected from 10 cows and 10 calves in each herd. Whole blood selenium concentration was determined by atomic absorption spectrometry, and glutathione peroxidase activity was measured as an indicator of selenium status.

RESULTS: In herds A and B, whole blood selenium concentrations in cows were below the recommended range, varying from 37.55 to 54.72 µg/L, whereas cows in herd C showed concentrations between 72.65 and 112.32 µg/L. Glutathione peroxidase activity in cows from herds A and B was markedly lower than in herd C. Calves in herds A and B also showed deficient selenium concentrations (32.27–46.12 µg/L), while calves in herd C had values between 88.16 and 122.47 µg/L. Glutathione peroxidase activity was significantly higher in calves from herd C compared with calves from herds A and B. Selenium deficiency in herds A and B was associated with reduced growth performance and increased morbidity and mortality. In two calves that died in herd A, nutritional myodegeneration and cardiomyopathy were diagnosed. Calves in herd C showed higher weight gains, minimal disease incidence, and no mortality during the grazing season (May–September).

CONCLUSIONS: Selenium deficiency may occur in beef cattle during the grazing season despite access to mineral licks containing inorganic selenium. A free-access mineral mixture containing organic selenium provided adequate selenium supplementation for cows and calves and improved selenium status, health, and growth performance.

Keywords: beef cattle, selenium status, cows, calves, organic selenium, inorganic selenium

Combined Effects of Vitamin D3 and An Oral Calcium Bolus on Calcium Status, Milk Yield, and Metabolic Health in Transition Dairy Cows

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BACKGROUND and OBJECTIVE: Subclinical hypocalcaemia is a common metabolic disorder in transition dairy cows and is associated with impaired health, reduced productivity, and increased risk of postpartum disease. Strategies that improve calcium homeostasis after calving are therefore of practical interest. The objective of this study was to evaluate the effects of postpartum supplementation with an oral calcium bolus alone or in combination with 25-hydroxyvitamin D3 on calcium status, vitamin profile, milk yield, and selected metabolic indicators in transition dairy cows.

MATERIALS and METHODS: Thirty late-gestation Holstein cows from a commercial dairy herd in the Czech Republic were randomly assigned to three groups: control (no supplementation, n = 10), calcium bolus (oral bolus containing calcium chloride and calcium sulphate, n = 10), and calcium plus vitamin D3 bolus (n = 10). Boluses were administered within 15 minutes after calving and again 12 hours postpartum. Blood samples were collected at calving and at 12, 48, and 72 hours postpartum. Serum concentrations of total calcium, phosphorus, 25-hydroxyvitamin D3, vitamins A and E, non-esterified fatty acids, beta-hydroxybutyrate, and additional biochemical variables were determined. Milk yield, somatic cell count, and body condition score were monitored during early lactation. Animal procedures followed national and European legislation for animal welfare.

RESULTS: Cows receiving calcium plus 25-hydroxyvitamin D3 had higher serum total calcium concentrations at 72 hours postpartum compared with control cows and cows receiving calcium alone ($P < 0.01$). Serum 25-hydroxyvitamin D3 concentrations were also higher in this group at 48 and 72 hours postpartum ($P < 0.05$). The combined treatment was associated with higher vitamin A and vitamin E concentrations at selected sampling

times. When subclinical hypocalcaemia was defined as total calcium below 2.0 mmol/L, its incidence was lower in cows receiving calcium plus 25-hydroxyvitamin D3 compared with controls. Average daily milk yield during early lactation was higher in the calcium plus 25-hydroxyvitamin D3 group than in controls (39.35 vs 37.65 kg/day; P = 0.0067). Body condition score and somatic cell count were not affected by treatment.

CONCLUSIONS: Postpartum supplementation with a bolus containing calcium and vitamin D3 improved calcium status and increased milk yield in early lactation. These results indicate that combined supplementation may help reduce subclinical hypocalcaemia and support metabolic adaptation in transition dairy cows.

Keywords: dairy cow, transition period, subclinical hypocalcaemia, calcium bolus, 25-hydroxyvitamin D3, milk yield

Herd-Level Prevalence of High Fat-to-Protein Ratio and Associated Factors During Early Lactation in Irish Spring-Calving Dairy Herds

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BACKGROUND and OBJECTIVE: An elevated milk fat-to-protein ratio (FPR >1.4) is widely used as a proxy for negative energy balance (NEB) in dairy cows. However, herd-level prevalence and associated risk factors in seasonal pasture-based systems are not well characterised. This study aimed to assess the herd-level prevalence of high FPR in early lactation and identify associated risk factors in Irish spring-calving dairy herds.

MATERIALS and METHODS: A retrospective observational study was conducted using 10 years (2014–2023) of national milk recording data from the Irish Cattle Breeding Federation (ICBF; Co. Cork) from 11,051 Irish dairy herds, comprising 327,257 milk records. Herd-level prevalence of high FPR (>1.4) was calculated for cows <30 days in milk (DIM) and ≥30 to <60 DIM. Multivariable binomial mixed-effects regression models were used to identify associations with different factors such as month of the year, parity, 305-day-yield parameters and predicted transmitting abilities (PTA).

RESULTS: The mean median herd-level prevalence of high FPR was 14.57% for <30 DIM and 8.10% for ≥30 to <60 DIM, with substantial inter-herd and annual variation. In 2018, prevalence peaked at 20.0% (<30 DIM) and 12.9% (≥30 to <60 DIM). February was associated with increased odds of high herd-level FPR (OR = 1.237 and 1.272 respectively), while April–June showed reduced odds. Higher mean 305-day milk yield was associated with reduced odds of high herd-level FPR (OR = 0.636 (<30 DIM); OR = 0.543 (≥30–<60 DIM) per 1000 kg increase). Increased genetic PTA for fat kg was associated with increased odds of a high herd-level FPR (OR = 1.058 and 1.040, respectively). A 10% increase in primiparous cows slightly increased risk (OR ≈ 1.015).

CONCLUSIONS: This study showed that there was variable prevalence for high herd-level FPR and identified certain risk factors. Month of the year had a significant impact with February representing the highest-risk period, likely reflecting housing and transition management challenges. Higher-yielding herds had lower risk, while genetic selection for fat production contributed to increased prevalence. Herd-level FPR provides a practical tool for monitoring metabolic status and identifying herds at risk of NEB in pasture-based seasonal-calving systems.

Keywords: fat-to-protein ratio, seasonal calving system, negative energy balance

Effect of Grape Seed Extract on Growth Performance and Oxidative Stress in Holstein Calves Under Heat Stress

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BACKGROUND and OBJECTIVE: The aim of this study was to investigate the effects of grape seed extract (GSE) supplementation on growth performance, antioxidant activity, inflammation, blood metabolites, fecal volatile fatty acids (VFA), hematological and physiological parameters in Holstein calves under heat stress.

MATERIALS and METHODS: In the study, 60 Holstein female calves (3 days old) were divided into four similar groups according to live weight (40.6 ± 2.17 kg) and hair cover. GSE was given orally to the calves with milk daily at doses of 0 (CON, n=15), 25 (GSE1, n=15), 50 (GSE2, n=15) and 100 (GSE3, n=15) mg/kg body weight. Calves were weighed at 15-day intervals to determine weight gain. Blood samples were taken on days 3, 33 and 63, while fecal samples were taken on days 33 and 63 to assess fecal VFA. Rectal temperatures and respiratory rates were measured three days a week. Temperature humidity index (THI) values varied between 73,2 and 87,4 throughout the study, and the study was terminated on day 63.

RESULTS: Calves were fed GSE2 had the highest average daily gain (ADG) between days 3-18 and 33-48 of the study, while the GSE3 group had the highest ADG between days 18-33 and 48-63 (P<0,01). Moreover, calves were fed GSE3 had the highest feed intake over the entire study period (P<0,01). Plasma malondialdehyde (MDA), tumor necrosis factor alpha (TNF-α) concentrations and respiratory rate were decreased in all groups supplemented with GSE (P<0,01). Plasma total antioxidant capacity (TAC) concentration increased (P=0,02) in calves were fed GSE3 on day 33 compared to the CON group. Plasma superoxide dismutase (SOD) concentrations increased in calves were fed GSE2 and GSE3 compared to the CON group, while plasma leptin, insulin and cortisol concentrations decreased (P<0,01). The hematocrit percentage, hemoglobin value and red blood cells (RBC) count increased in all groups supplemented with GSE (P<0,01). In addition, fecal total VFA concentration increased in calves were fed GSE3 (P<0,01).

CONCLUSIONS: It was concluded that supplementation with 50 mg/kg BW/d GSE could alleviate the effects of heat stress by creating beneficial effects on the health parameters and performances of calves under heat stress.

Keywords: calf, heat stress, grape seed extract, antioxidant, leptin

in vitro Evaluation of Organically Produced Corn Silage and Alfalfa Hay Combinations

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BACKGROUND and OBJECTIVE: Under the legal framework of organic dairy farming regulations, it is mandatory to maintain the forage ratio at a minimum of 60% on a dry matter (DM) basis. This requirement necessitates that organic dairy enterprises maximize the utilization of available forage sources and accurately determine their nutritional values for efficient production. This study aimed to determine the in vitro gas production (IVGP) parameters, organic matter digestibility (OMD), and metabolizable energy (ME) levels of various mixtures of organically produced corn silage (CS) and alfalfa hay (AH), which are primary forage sources in dairy cattle nutrition.

MATERIALS and METHODS: Five experimental groups were established: 100% corn silage (S1), 75% CS + 25% AH (S2), 50% CS + 50% AH (SA), 25% CS + 75% AH (A1), and 100% AH (A2). In vitro gas production was determined using the AnkomRF Gas Production System. Rumen fluid was obtained from three Holstein cows fed a diet consisting of 40% concentrate and 60% forage. Feed samples from each group were incubated in triplicate in 250 mL modular glass bottles containing a mixture of rumen fluid and buffer solution at 39°C. Gas production values were recorded at 3, 6, 9, 12, 24, 48, 72, and 96 hours of incubation. OMD and ME values were calculated

using the gas production data and specific equations.

RESULTS: At 24 hours of incubation, the highest IVGP value and the calculated OMD and ME values were obtained in the S1 group (100% corn silage) ($P < 0.001$). Conversely, the lowest IVGP, OMD, and ME values were determined in the A2 group (100% alfalfa hay) ($P < 0.001$). In the mixture rations, a statistically significant increase in OMD and ME values was observed as the proportion of corn silage increased, while an increase in the alfalfa hay ratio led to a decrease in these parameters.

CONCLUSIONS: The results of the study indicate that corn silage plays a critical role in increasing energy density in organic dairy cattle diets. Especially in organic systems where high forage inclusion is mandatory, balancing corn silage and alfalfa hay combinations in favor of maize silage for optimum digestibility would be beneficial for production performance.

Keywords: organic, alfalfa, maize silage, gas production, digestibility

Effects Of Feeding Different Levels of Rice Bran on Performance, Ultrasonic Carcass Traits and Blood Parameters of Finishing Bulls

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BACKGROUND and OBJECTIVE: Reduced corn productivity under drought conditions, together with its substantial water requirements, has intensified the search for sustainable alternative feed resources. Owing to its favorable energy and protein profile, this study was designed to assess the effects of substituting corn with increasing levels of rice bran (RB) on growth performance, blood parameters, and ultrasonic carcass traits in finishing bulls.

MATERIALS and METHODS: Forty-four Holstein bulls (initial BW 434.86±8.52 kg, average of 12 months old) were used to study the effects of feeding different levels of RB in finishing diets on performance, blood parameters and ultrasonic carcass traits. Bulls were assigned to 4 groups with dietary treatments of 0 (Control; C), 12.7 (R1), 21.2 (R2), 30.3 (R3) % RB dietary inclusion (DM basis). Corn was partially replaced by RB in corn based experimental diets. Animals were fed ad-libitum during the experiment after 21 day of adaptation period. Feed offered and orts were recorded weekly, live body weights were taken on 0, 30, 66 and 95 d. Blood sample collection and ultrasonic measurements of rib fat thickness (RFT), longissimus muscle depth (LMD) were performed on 0, 66, 95d. Ultrasound intramuscular fat % (IMF) of longissimus muscle was measured at 95d. Images were obtained by ultrasound equipped with a 5-7.5 MHz linear probe and collected and analyzed for stated carcass traits with an image analysis software (ImageJ). Statistical analysis was performed using one-way analysis of variance (ANOVA). Differences among groups were determined by Duncan's multiple range test ($p < 0.05$).

RESULTS: No significant differences were observed for overall performance and ultrasonic carcass traits (dry matter intake, average daily gain, gain to feed ratio, RFT, LMD and IMF %) between groups from 0d to 95d. Ultrasonic RFT values were 6.45±0.18, 6.44±0.22, 6.31±0.24, 6.42±0.19 mm, LMD values were 55.88±0.75, 57.20±1.46, 57.75±0.56, 54.30±0.71 mm, IMF (%) values were 4.11±0.51, 4.48±0.73, 4.36±0.80, 4.37±0.63 % at 95d for C, R1, R2 and R3, respectively. Serum glucose and triglyceride levels did not differ between groups during the experiment. Serum total protein (STP) levels in C and R3 were higher than R1 and R2 at d 0. STP levels in R3 were higher than C, R1 and R2 at d 66. No significant difference was observed in STP levels between groups at 95d.

CONCLUSIONS: In conclusion, corn can be effectively replaced as an alternative with RB up to 30% in finishing bull diets without any adverse effects on performance, blood parameters, or ultrasonic carcass traits.

Keywords: rice bran, performance, finishing bulls, alternative feed, ultrasonic carcass traits

Lyophilized Adapted Rumen Microorganisms Attenuate Subacute Ruminal Acidosis: *in vitro* Analysis and *in vivo* Efficacy in Heifers

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BACKGROUND and OBJECTIVE: Subacute ruminal acidosis (SARA) is a critical metabolic challenge in cattle during transitions to high-concentrate diets, often requiring rapid microbial stabilization to prevent health and production losses. This study evaluated the efficacy of lyophilized adapted rumen microorganisms (LM) in preventing SARA through both in vitro fermentation kinetics and in vivo trials in heifers.

MATERIALS and METHODS: Rumen fluid for LM was collected from two cannulated heifers adapted to SARA conditions (1.85 x Maintenance ME). Microorganisms were cultured in a SARA-inducing medium (40 g/L diet) at 39 ^{0}C for 14 hours under CO2. The pellet was mixed with 10% milk powder and 5% sucrose and lyophilized. In Experiment 1 (in vitro), LM stored at room temperature or 4 ^{0}C for up to 12 months was tested using the in vitro gas technique with a SARA-inducing substrate. In Experiment 2 (in vivo), four rumen-cannulated heifers were used in a crossover design. SARA was induced by increasing concentrate levels (20%, 40%, and 60%) over 7 days. Treatment animals received 4 x 1010 LM via cannula on days 1 and 3 of the 40% and 60% concentrate phases, while controls received a placebo.

RESULTS: In vitro results showed that LM stored for up to 6 months at both room temperature and 4 ^{0}C did not negatively affect fermentation kinetics. LM significantly decreased the acetate-to-propionate ratio and increased butyrate proportions ($P < 0.0001$). In the in vivo trial, while rumen pH dropped from 6.23 to 5.81 during rapid adaptation ($P < 0.004$), LM inoculation-maintained pH approximately 0.4 units higher than the control group ($P < 0.0001$). Protozoa count nearly doubled with LM ($P < 0.0001$), and propionic acid proportions increased significantly during the 60% concentrate phase ($P < 0.006$).

CONCLUSIONS: Lyophilized adapted rumen microorganisms maintain their efficacy for at least 6 months at both room temperature and refrigeration (4 ^{0}C). LM inoculation effectively attenuates the drop in ruminal pH and can be recommended to stabilize rumen fermentation in transition cows and stressed ruminants.

Keywords: Lyophilized rumen microorganisms, Grain adaptation, Subacute ruminal acidosis, Rumen fermentation.

Effects of Using Different Starch Level Rations and Adding Live Yeast to the Ration on Fattening Performance in Beef Cattle

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BACKGROUND and OBJECTIVE: Beef cattle farming plays a significant role in world meat production. Achieving maximum yield in beef cattle fattening is crucial for sustainability. The aim of this study was to observe how adding live yeast to beef cattle rations and changing the starch levels of the rations affects fattening performance.

MATERIALS and METHODS: For this purpose, 576 male Brangus beef calves (averaging 12 months old) were divided into 6 main groups, each containing 96 animals, fed with 6 different rations. The main groups had 8 subgroups, each containing 12 animals. The main groups were divided into low starch (LS) group fed a ration containing 30% starch, low starch + yeast (LS+Y) group fed a ration containing 30% starch and *Saccharomyces cerevisiae* supplementation, medium starch (MS) group fed a ration containing 33% starch, medium starch + yeast (MS+Y) group fed a ration containing 33% starch and yeast supplementation, high starch (HS) group fed a ration containing 36% starch, and high starch + yeast (HS+Y) group fed a ration containing 36% starch and yeast supplementation.

All groups were given a base ration consisting of concentrate feed mixture and corn flakes as the concentrate feed source, and corn silage, alfalfa hay, and wheat straw as the forage source. Throughout the feeding period, the base ration was formulated to include 58% concentrate feed mixture, 18.5% corn silage, 6.5% wheat straw, and 17% alfalfa hay. Rations containing medium to high levels of starch were prepared by using corn flakes to replace a portion of concentrate feed mixture. The ration was given twice daily as a total mixed ration (TMR). Yeast supplementation was top-dressed to the ration at a rate of 3 g/head/day.

RESULTS: At the end of the fattening period, hot carcass weight, daily live weight gain, carcass yield, dry matter intake, and feed conversion ratio (FCR) were compared between the groups using the one-way ANOVA method with IBM SPSS Statistics (v. 20). Dry matter intake was lower in the groups fed yeast-supplemented rations with medium (MS+Y) and high starch (HS+Y) content compared to the low starch (LS) group (P<0.05). The best FCR values were obtained from the medium and high starch groups to which yeast was added (MS+Y and HS+Y) (P<0.05).

CONCLUSIONS: In conclusion, it is believed that increasing starch levels in beef cattle rations, along with the use of live yeast, has a positive effect on fattening performance.

Keywords: Live yeast, Beef cattle, Starch, Fattening performance, Brangus

Same Carbon Backbone, Different Ruminal Footprints: Effect of HMBi and HMBA on *in vitro* Rumen Fermentation and Fatty Acid Biohydrogenation Under a High-PUFA, High-Starch MFD Model

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BACKGROUND and OBJECTIVE: An in vitro 24-h rumen batch culture evaluated two methionine hydroxy analogs, HMBi (isopropyl ester, KESSENT™ MF, Kemin Animal Nutrition and Health, Belgium) and HMBA (free acid form), under a high-starch, high-PUFA diet model designed to be highly susceptible to milk fat depression (MFD).

MATERIALS and METHODS: Treatments included Control (no additive), HMBi, and HMBA (12 mg/50 mL).

RESULTS: Early fermentation (0-6 h) gas production did not differ significantly among treatments (Control: 50.1 mL, HMBi: 49.4 mL, HMBA: 50.9 mL; P > 0.05), though HMBA showed the highest value, suggesting earlier fermentation onset. From 6-24 h, HMBi increased gas production (68.2 mL) compared to Control (64.9 mL; P < 0.05), indicating delayed but sustained fermentation. Total 24-h gas production was similar across treatments (P > 0.05). Rumen pH (6.33-6.36), concentrations of lactate and 6 h total volatile fatty acid (VFA) showed no significant differences among the three treatments. At 24 h, HMBi significantly increased total VFA compared to Control (87.8 vs. 84.1 mM, respectively; P < 0.05), with HMBA intermediate (85.9 mM, P > 0.05). Molar proportions of VFA and ammonia-N levels at 24 h were not significantly affected. At 6 h, trans-10, cis-12 CLA was elevated in HMBi versus Control (0.062% vs. 0.040%, P < 0.05), while HMBA (0.049%) remained similar to Control (P > 0.05). Cis-9, trans-11 CLA was lower in HMBA (0.187%) than Control (0.348%, P < 0.05). From 6-24 h, HMBA slowed cis-9, trans-11 CLA degradation (-0.067%) compared to Control (-0.193%)

and HMBi (-0.196%, P < 0.05). Final 24-h FAME profiles were not significantly different (P > 0.05).

CONCLUSIONS: In conclusion, HMBi and HMBA exhibited distinct ruminal modes of action. HMBi influenced fermentation output and showed a delayed fermentation pattern without affecting biohydrogenation, which may relate to changes in nitrogen utilization rather than solely to rumen escape, aligning with reports of altered NH_3-N dynamics with HMBi. In contrast, HMBA was readily fermented, enhancing early fermentation, redirecting fatty acid biohydrogenation to suppress the formation of trans-10 CLA, and may help mitigate MFD risk at rumen level by favorably altering lipid metabolism. The high-PUFA, high-starch control diet effectively induced an MFD-prone fermentation profile, against which HMBA demonstrated a clear advantage in limiting harmful trans-10 CLA accumulation.

Keywords: HMBi, HMBA, rumen fermentation

Trends in Elevated Milk Beta-Hydroxybutyrate from Portuguese Dairy Herd Improvement Records, 2015–2025

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BACKGROUND and OBJECTIVE: As milk β-hydroxybutyrate (BHB) testing has been incorporated into Dairy Herd Improvement (DHI) programs, records now provide population-level insight into cows' metabolic health. This study describes ten-year trends (2015–2025) in the prevalence of elevated milk BHB and examines concurrent changes in milk yield.

MATERIALS and METHODS: Milk samples from routine DHI test-days were analyzed for BHB by Fourier-transform infrared spectrometry in the laboratories of ANABLE (Portugal). The study included all cows with DHI test-day records between 5 and 35 days in milk (DIM). Mixed-effects logistic regression —with fixed effects for period, parity, calving month, and DIM, and a herd-level random intercept— was used to estimate period adjusted prevalence of elevated milk BHB (≥0.10 mmol/L); and comparisons between consecutive periods were Sidak-adjusted. Test-day milk yield was analyzed using mixed-effects linear regression with fixed effects for period, parity, calving month, DIM, and BHB status, and herd level random intercept, to obtain adjusted period means and the overall difference between high- and low-BHB cows.

RESULTS: A total of 689394 records from 361899 cows in 2260 herds were analyzed. Adjusted prevalence of elevated milk BHB fluctuated across the study period, ranging from 21.2% (95% CI 20.6–21.9) in May 2015–April 2016 to 24.9% (95% CI 24.1–25.6) in May 2020–April 2021, decreasing to 15.6% (95% CI 14.9–16.2) in May 2023–April 2024 and rising again to 19.6% (95% CI 18.8–20.3) in May 2024–April 2025. Year-to-year differences included increases of +1.6 percentual points (pp) in 2016–2017 (95% CI 1.2–1.9), +3.4 pp in 2019–2020 (3.1–3.8) and +2.6 pp in 2020–2021 (2.2–3.1), followed by declines of −5.4 pp, −3.0 pp, and −0.8 pp from 2021–2022 to 2023–2024. In the final period, prevalence increased again by +4.0 pp (95% CI 3.5–4.5). Adjusted test-day milk yield increased from 31.36 kg (95% CI 31.17–31.55) in May 2015–April 2016 to 33.50 kg (95% CI 33.29–33.71) in May 2024–April 2025, with significant increases in most consecutive periods. Relative to the baseline, adjusted milk yield was 2.14 kg higher in the final period ($p < 0.001$). Across the full dataset, cows with elevated milk BHB had lower adjusted test-day milk yield: 31.51 kg (95% CI 31.32–31.70) compared with 32.39 kg (95% CI 32.21–32.58) in cows with low BHB, corresponding to a reduction of 0.88 kg (*$p < 0.001$*).

CONCLUSIONS: DHI records over a ten year period revealed substantial year to year variation in the adjusted prevalence of elevated milk BHB, including periods of decline followed by a recent increase.

During the same timeframe, adjusted test day milk yield showed a consistent upward trend.

Keywords: Beta-hydroxybutyrate (BHB), Dairy Herd Improvement (DHI), Metabolic health, Milk yield, Portugal, Prevalence

Effects of Parity on Colostrogenesis, Calf Passive Immunity, and Early Lactation Energy Metabolism in Dairy Cattle

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BACKGROUND and OBJECTIVE: Transition period nutritional management in dairy cattle forms the foundation of herd health concerning mammary colostrogenesis, transfer of passive immunity (TPI) in calves, and the control of negative energy balance (NEB) during early lactation. This study was conducted to investigate the effects of parity on colostrum yield and quality in periparturient cows, the success of passive immunity transfer in calves, and the interactions between cellular energy metabolism (lipomobilization) and udder health during early lactation.

MATERIALS and METHODS: This research was conducted using data sourced from the Ergin Tarım dairy farm in Kırklareli, Türkiye. In this study, colostrum yield (n=2,819) and quality (Brix %, n=2,768) obtained from cows, along with blood serum IgG absorption levels of calves (Brix %, n=2,978), were measured. To evaluate the energy status of early lactation cows (n=1,467), blood beta-hydroxybutyrate (BHBA) concentrations were analyzed. These data were integrated with California Mastitis Test (CMT) results and parity records to conduct statistical analyses.

RESULTS: The herd mean for colostrum quality was 28.26% Brix, with an average yield of 4.69 L. The "dilution effect" associated with high yield, frequently a concern in dairy nutrition, was not observed. Colostrum yield drastically decreased to 2.21 L in older cows (7th parity). Calves achieved successful TPI with a mean of 9.97% Brix; however, this success was found to be completely independent of the dam's colostrum production. During early lactation, the mean herd BHBA concentration was 0.78 mmol/L, remaining safely below the subclinical ketosis threshold. The highest metabolic load was observed in 3rd and 4th parity cows. Furthermore, BHBA levels in cows with healthy udders (CMT-Negative; 0.78 mmol/L) were statistically higher than those in CMT-Positive cows (0.66 mmol/L) (P=0.047).

CONCLUSIONS: The farm's prepartum (close-up) diets are highly successful in optimizing colostrogenesis and controlling lipomobilization. The dam-independent success in calf parameters indicates the implementation of a standardized colostrum bank management protocol on the farm. The relatively higher BHBA in healthy cows is not pathological; rather, it is a physiological consequence of the high glucose demand driven by increased milk secretion and adaptive body fat mobilization (physiological NEB). Supplementing calves born to older cows with banked colostrum and increasing glucogenic precursors in the fresh cow diets of 3rd and 4th parity cows will further optimize overall herd health.

Keywords: Colostrogenesis, Calf Passive Immunity, Transition period, blood beta-hydroxybutyrate, California Mastitis Test

Metabolic Interactions Among Parity, Blood BHBA Levels, and Early Lactation Milk Yield in Holstein Cows

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BACKGROUND and OBJECTIVE: In dairy cows, the inability to meet the increasing energy requirements at the onset of lactation due to inadequate dry matter intake leads to negative energy balance (NEB) and the rapid mobilization of body fat reserves (lipomobilization). Beta-hydroxybutyric acid (BHBA), which elevates in the blood as a result of the incomplete oxidation of non-esterified fatty acids exceeding hepatic capacity, is the primary indicator of subclinical ketosis and a critical parameter directly affecting the metabolic health and milk yield of cows. This study aimed to investigate the metabolic interactions among parity, subclinical ketosis status monitored by blood BHBA levels, rectal temperature, and early lactation milk yield parameters.

MATERIALS and METHODS: A total of 360 Holstein cows constituted the animal material of the study. The Pearson correlation test and Independent Samples t-test were utilized for the statistical analysis of the obtained data.

RESULTS: Analysis revealed significant positive correlations between parity, blood BHBA levels ($r = 0.150$, $P = 0.020$), and the first 60-day milk yield ($P < 0.001$) in the general population. In multiparous cows, while BHBA correlated positively with peak yield ($r = 0.189$, $P = 0.019$) due to severe lipomobilization triggered by reaching peak lactation, elevated BHBA levels significantly decreased the first 60-day cumulative milk yield ($r = -0.280$, $P < 0.001$). This paradoxical situation indicates that multiparous cows achieving high peak yields enter a severe energy deficit, which suppresses their appetite (dry matter intake) and leads to cumulative milk losses during early lactation. On the other hand, the absence of a statistical relationship between BHBA levels and yield parameters in primiparous cows still undergoing physical development ($P > 0.05$) demonstrates that these young animals are protected from excessive metabolic load and the risk of ketosis by partitioning dietary energy and nutrients not only toward milk production but also toward their own somatic growth. Regarding clinical findings, due to the non-febrile nature of subclinical ketosis as a metabolic disorder, no significant relationship was detected between BHBA levels and rectal temperature; only a weak tendency for a temperature increase ($P = 0.079$), potentially related to elevated metabolic stress, was recorded in the multiparous group.

CONCLUSIONS: In conclusion, despite the well-known strong interaction between peak lactation and ketosis in multiparous cows, it was concluded that making precise predictions regarding early-stage milk yield based solely on blood BHBA levels is not possible, and further studies are needed to elucidate this complex metabolic profile.

Keywords: Beta-hydroxybutyric acid (BHBA), subclinical ketosis, Holstein, negative energy balance

Evaluating Parity, Colostrum Dynamics, and Calf Weaning Performance Under Standardized Anionic Transition Diets in A Large-Scale Dairy Farm

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BACKGROUND and OBJECTIVE: In dairy farming, dry and transition period feeding strategies are critical for optimum colostrogenesis and adequate transfer of passive immunity in neonatal calves. Nutritional imbalances in dry period diets reduce colostrum quality, increasing the risk of Failure of Passive

Transfer (FPT) and associated neonatal diseases. This study aims to examine the effects of parity and sex on colostrum quality (Milk Brix), passive immunity (Blood Brix), birth weight, and weaning weight in a 2500-head dairy herd.

MATERIALS and METHODS: Data from 2500 lactating cows (primiparous and multiparous) and their calves were analyzed retrospectively (Bomonti Dairy Farm, Kırklareli, Türkiye). At this dairy farm, cows in the close-up period were fed an anionic diet. Dam colostrum quality (Milk Brix, %) and calf serum IgG levels (Blood Brix, %) were measured via refractometer. Data were evaluated using descriptive statistics, ANOVA, Pearson correlation, and independent sample t-tests.

RESULTS: Overall averages were 25.32 ± 3.99% for Colostrum Brix and 8.87 ± 0.80% for Blood Brix. No significant difference existed between primiparous and multiparous cows in these immunological parameters ($P > 0.05$). Reflecting fetal nutrient partitioning, calves of multiparous cows (40.43 kg) were significantly heavier at birth ($P < 0.001$) than those of primiparous cows (38.90 kg). A strong positive correlation existed between sex and birth weight ($r = 0.357$, $P < 0.001$). Despite birth weight disadvantages, weaning weights did not differ between the offspring of primiparous (89.54 kg) and multiparous (88.00 kg) cows ($P = 0.12$). Additionally, Blood Brix weakly correlated with birth weight ($r = 0.041$, $P = 0.039$).

CONCLUSIONS: Low Milk Brix variation (SD: 3.99) and values above the ≥22% target indicate optimally formulated dry period diets, successfully achieved dietary cation-anion difference (DCAD), and eliminated metabolic disease risks (e.g., subclinical hypocalcemia) that suppress colostrum quality. Effective colostrum management and successful maternal antibody transfer (Blood Brix ≥8.4%) prevented FPT in calves. Furthermore, primiparous offspring closing their birth weight gap by weaning through compensatory growth reveals that the farm's liquid feeding and calf starter programs optimally stimulate early rumen papillae development.

Keywords: Colostrogenesis, Failure of passive transfer, dietary cation-anion difference

Effects of Dietary Beta-Carotene and Vitamin E on the Reproductive Performance of Dairy Cows Synchronized with Ovsynch

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BACKGROUND and OBJECTIVE: In dairy farming, metabolic load and cellular oxidative stress developing due to high milk yield are among the primary factors negatively affecting reproductive performance; therefore, enriching diets with specific antioxidants, beta-carotene, and vitamin E, to support ovarian functions and embryonic development is a common feeding strategy. In this context, the present study aimed to investigate the effects of beta-carotene and vitamin E supplementation to the diets of lactating cows subjected to the Ovsynch estrus synchronization protocol on pregnancy rates and parity (primiparous/multiparous) dynamics.

MATERIALS and METHODS: Within the scope of the research, a total of 407 cows were divided into two groups; while the control group (n=205) was fed a standard basal diet, beta-carotene and vitamin E were supplemented to the diet of the experimental group (n=202). In this beta-carotene study, data analysis and group comparisons were performed using the Chi-Square test

RESULTS: According to the findings, the overall pregnancy rate was determined as 34.7% (70/202) in the supplemented group and 31.2% (64/205) in the control group; the 3.5% numerical increase in favor of the supplementation was not found to be statistically significant ($P = 0.46$). Furthermore, no statistical interaction of the supplementation was observed on the primiparous and multiparous parity distributions of both the pregnant ($P = 0.56$) and non-pregnant ($P = 0.27$) cows.

CONCLUSIONS: In conclusion, although supranutritional beta-carotene and vitamin E supplementation to the diet created a numerical

improvement trend in pregnancy rates supporting cellular antioxidant capacity, it did not create a statistical difference. This situation indicates that rather than the marginal benefit of specific micronutrient supplementations in herd-level reproductive success, the existing vitamin/antioxidant adequacy of the farm's basal diet (TMR) and the lactation-related negative energy balance (NEB) of high-yielding cows (especially multiparous ones) play a much more decisive role.

Keywords: Dairy cow, Beta-carotene, Vitamin E, Ovsynch

Prepartum Blood and TMR Sampling to Determine Efficacy of Phosphorus Binding Strategy to Prevent Hypocalcemia

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BACKGROUND and OBJECTIVE: Hypocalcemia in dairy cows is both a metabolically and economically weighty disorder occurring at the onset of lactation. Both clinical and subclinical hypocalcemia negatively impacts cow health and productivity. Various prepartum nutritional strategies exist to mitigate this risk, including the use of phosphorus-binding agents. Aluminum-based binders reduce dietary phosphorus availability, lowering circulating phosphorus and enhancing calcium homeostasis at calving. Thus, our objective was to develop a practical prepartum blood and total mixed ration (TMR) sampling methodology to evaluate the efficacy and optimization of phosphorus-binding strategies for hypocalcemia prevention.

MATERIALS and METHODS: Ten commercial dairy farms were evaluated, including two herds with repeated sampling, yielding a total of thirteen herd-level observations of farms feeding an aluminum-based phosphorus binder. Blood samples were collected from a minimum of 10 close-up cows per farm and analyzed for blood phosphorus concentration. Concurrently, duplicate TMR samples were collected and analyzed via wet chemistry for dietary phosphorus and aluminum content. The relationship between dietary phosphorus-to-aluminum (P/Al) ratio and blood phosphorus concentrations was assessed.

RESULTS: Blood phosphorus concentration was highly predicted by the dietary P/Al ratio (R^2 = 0.8731) of the TMR. A dietary P/Al ratio ranging from 0.8: 1 to 1.1: 1 corresponded to periparturient blood phosphorus concentrations between 1.8 and 3.3 mg/dL. As the dietary P/Al ratio decreased, blood phosphorus concentrations decreased, while increasing P/Al ratios resulted in higher blood phosphorus concentrations. These findings demonstrate a consistent and predictable relationship between dietary P/Al ratio and circulating phosphorus status.

CONCLUSIONS: This study establishes a novel and practical monitoring approach combining TMR and blood sampling to predict blood phosphorus concentrations in prepartum dairy cows. The dietary P/Al ratio can be used as a reliable tool to optimize phosphorus-binding strategies, supporting improved calcium homeostasis during the transition period. This approach provides a scalable, field-applicable method to monitor and optimize phosphorus binding programs.

Keywords: phosphorus binder, TMR, phosphorus, hypocalcemia, periparturient

Body Condition Score Loss, Hepatic Lipidosis, and Selected Blood Metabolites in Holstein Cows During the Transition Period

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BACKGROUND and OBJECTIVE: The aim of this study was to evaluate the relationship between body condition score loss (ΔBCS), energy balance (EB), hepatic lipidosis and blood serum concentration of non esterified fatty acids (NEFA), glucose, triacylglycerol (TG) and total bilirubin (tBIL) in healthy dairy cows during transition period.

MATERIALS and METHODS: Twenty healthy Holstein cows were included and categorized into two groups based on BCS loss (ΔBCS) between dry period and early lactation, (low ΔBCS <0.75, n=9, and high ΔBCS ≥0.75 BCS, n=11,). BCS, ΔBCS and EB were determined using Elanco Animal Health and NRC standards. Blood was sampled twice during the late dry period - in week 1 (7±2 days) and - week 2 (15±2 days) relative to parturition, and twice during early lactation period +1(7±2 days) and +2 weeks (15±3) days after parturition. The blood concentrations of NEFA, glucose, TG, tBIL., were measured by different colorimetric techniques using spectrophotometer (COBAS MIRA). Shortly after blood collection, the liver was sampled through percutaneous biopsy at day 15 after calving (15±3 days) and contents of lipid (%) in the liver were determined. The liver lipid contents were semi-quantified through computer image analysis (Software Q Win) made using the appliance (LEICA Q 500 MC). The statistical software Statgraphics Centurion (Statpoint Technologies Inc. Warrenton, USA) were used for statistical analysis. The experiment was done in compliance with Serbian Law on Animal Welfare (Official Gazette of the Republic of Serbia No 41/09).

RESULTS: Significant differences between groups ($p<0.05$) were observed for blood serum NEFA, glucose and tBIL. Cows with high ΔBCS (≥0.75) between dry period and early lactation showed increased hepatic lipidosis, blood serum NEFA and tBIL concentrations and lower blood serum TG and glucose concentrations during transition period, compared to the low ΔBCS cows (<0.75). Metabolic profiles of cows during transition period indicate negative EB, high level of lipid mobilization from adipose tissue, possible decreased liver cells TG export and gluconeogenic ability and impaired bilirubin metabolism if ΔBCS is increased by more than 0.75 points. ΔBCS was in relation with energy balance (EB) in transition period.

CONCLUSIONS: Dairy cows with high ΔBCS (≥0.75) show a significantly higher degree of negative EB, lipomobilization, hepatic lipidosis and reduced functional capacity of liver cells compared to the low ΔBCS cows (<0.75) during transition period.

Keywords: dairy cows, transitonal period, body condition score, metabolites, fatty liver

Blood Metabolic Profile Differences Associated with Milk Lactose Concentration in Early-Lactation Dairy Cows

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BACKGROUND and OBJECTIVE: Milk lactose concentration is increasingly recognised as a useful, non-invasive indicator reflecting metabolic adaptation in dairy cows during early lactation. Significant physiological and metabolic changes occur during this time, and variations in milk lactose concentration may be linked to changes in blood biochemical indicators pertaining to energy balance, protein metabolism, mineral status, and liver function. The objective of this study was to assess variations in the blood metabolic profile of early-

lactation dairy cows grouped according to milk lactose concentration.

MATERIALS and METHODS: Clinically healthy early-lactation dairy cows (n = 28) were classified into two groups based on milk lactose concentration: cows with lactose <4.70% (n = 14) and cows with lactose ≥4.70% (n = 14). Blood samples were collected once per cow and analysed for a broad panel of biochemical indicators reflecting metabolic status. The evaluated parameters included glucose (GLUC), urea (UREA), magnesium (Mg), iron (Fe), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), calcium (Ca), triglycerides (TRIG), non-esterified fatty acids (NEFA), albumin (ALB), and C-reactive protein (CRP). The lactose groups' mean blood parameter values were compared, and $p < 0.05$ was considered statistically significant.

RESULTS: Significant differences in several blood biochemical parameters were observed between cows differing in milk lactose concentration. Cows with lactose ≥4.70% exhibited a 21.63% higher UREA concentration compared with cows with lower lactose levels ($p < 0.001$). ALT activity was also markedly higher in the higher lactose group, with an increase of 22.96% ($p < 0.001$), suggesting differences in hepatic metabolic activity. In addition, Fe concentration was 13.41% higher ($p < 0.05$), GLUC concentration increased by 4.75% ($p < 0.05$), and Mg concentration was 8.25% higher ($p < 0.05$) in cows with higher lactose concentration. No statistically significant differences were detected between lactose groups for AST, ALP, GGT, Ca, TRIG, NEFA, ALB, or CRP.

CONCLUSIONS: In conclusion, early-lactation dairy cows differing in milk lactose concentration exhibit distinct differences in selected blood biochemical parameters, particularly those related to protein metabolism, energy availability, mineral status, and liver function. These results suggest that milk lactose concentration could be a useful marker for detecting changes in metabolic condition in the early stages of lactation and may support more targeted metabolic monitoring during early lactation. All experimental procedures were conducted in accordance with national legislation on animal welfare and the principles of laboratory animal care.

Keywords: milk lactose concentration, blood biochemical parameters, metabolic profile, early lactation dairy cows

Relationship Between NEFA, NEFA/Total Cholesterol Ratio, and Liver Function Index in Early Postpartum Dairy Cows

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BACKGROUND AND OBJECTIVES: Inflammatory processes during the postpartum period may disrupt metabolic homeostasis by increasing lipolysis and impairing hepatic synthetic function. NEFA is an important parameter in fatty liver. Under proinflammatory stimulation, positive acute-phase proteins increase while negative acutephase proteins decrease, reflecting reduced hepatic capacity. The Liver Function Index (LFI), calculated from albumin, total cholesterol (TCHO), and total bilirubin (TBil), is proposed as a composite indicator of hepatic adaptation and subclinical metabolic stress. This study aimed to evaluate the relationship between NEFA, NEFA/TCHO ratio and LFI in dairy cows during the early postpartum period.

MATERIALS AND METHODS: Serum samples were collected from 20 multiparous dairy cows on postpartum days 3, 7, and 28. NEFA and TCHO were measured on day 7, and the NEFA/TCHO ratio was calculated. Based on these values, cows were classified as experimental (n=10; NEFA >0.7 mmol/L, ratio >0.3) or control (n=10; NEFA <0.4 mmol/L, ratio <0.2). Albumin, TCHO, and TBil were analyzed on days 3 and 28, and LFI was calculated using a standardized composite formula. All parameters were measured by spectrophotometric methods. Group differences were assessed using independent and paired t-tests, and correlations were evaluated with Spearman's analysis.
Results
TCHO increased significantly from day 3 to 28 in both groups ($p < 0.001$) and TBil was higher in the experimental group than in the control group on day 3 ($p=0,01$). There was also a significant difference ($p=0,04$) in LFI between the experimental (-5,66±1,05) and control groups (-1,99±1,36).

In both groups, no significant correlation was observed between NEFA and LFI, and between the NEFA/cholesterol ratio and LFI. However, a significant correlation was found between NEFA and TBil (r= -0.693, p=0.02) and NEFA/cholesterol ratio and TBil (r= -0.681, p=0.02) in the experimental group. Milk yield on day 7 and 28 did not differ between groups.

CONCLUSIONS: LFI was significantly different between the experimental and control groups; however, no significant correlation was found between the NEFA and LFI, and NEFA/TCHO ratio and LFI. These findings suggest that although liver function scores differed between groups, early postpartum lipolysis was not directly reflected in LFI values in clinically healthy cows. Nevertheless, the observed intergroup difference in LFI may indicate the presence of subclinical hepatic alterations during the postpartum period.

Keywords: Dairy cows, Early Postpartum, Liver Function Index (LFI)

Effects of a Complementary Feed on Energy Metabolism, Milk Production, and Metabolic Health in Transition Dairy Cows

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BACKGROUND and OBJECTIVE: Optimal lactation performance in dairy cows depends on a well-managed transition from the dry period to early lactation, a phase characterized by pronounced metabolic adaptations and elevated energy requirements. The resulting lag in dry matter intake can lead to negative energy balance, predisposing cows to metabolic disorders and reducing milk yield. Ensuring sufficient dry matter intake and a stable energy supply is therefore essential. This study aimed to evaluate whether targeted supplementation with a complementary feed can support the energy metabolism by providing glucogenic precursors and increasing feed intake due to improved palatability during transition period.

MATERIALS and METHODS: The trial was conducted in summer 2023 at the University of Ankara with 40 Holstein-Friesian cows, all in second lactation, randomly assigned to a control group (CG; n = 20) and a trial group (TG; n = 20). All cows received a total mixed ration (TMR); TG cows were supplemented with 200 mL/day of a complementary feed during the close-up period (21 days prepartum) and 300 mL/day during the fresh period (21 days postpartum). The supplement contained a combination of fast- and slowly digestible glucogenic energy sources, glycerine, and betaine. Blood samples were collected at 21 and 7 days prepartum, on the day of calving, and 7, 14, and 21 days postpartum. Serum concentrations of non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHBA) were measured. Individual milk yield was recorded. Group differences were analyzed using a two-sided Welch's t-test ($\alpha = 0.05$).

RESULTS: Days in milk did not differ between groups (CG: 87 ± 0.57 d; TG: 88 ± 0.41 d). Mean milk yield was significantly higher in TG than CG (38.5 vs. 32.3 L/day; $p < 0.001$). Subclinical ketosis occurred in five CG cows and two TG cows (BHBA ≥ 1.0 mmol/L). NEFA and BHBA concentrations remained within reference ranges but differed significantly between groups. Prepartum NEFA levels were lower in CG, whereas TG showed lower NEFA at calving and on days 14 and 21 postpartum ($p = 0.007$). A similar pattern was observed for BHBA, with TG showing lower concentrations from day 7 postpartum onwards.

CONCLUSIONS: In conclusion, the complementary feed presumably supported the energy balance of dairy cows during the transition period. This was reflected by increased milk yield, lower postpartum NEFA and BHBA concentrations, and a reduced incidence of subclinical ketosis, indicating an improved energy metabolism and overall health status of the cows.

Keywords: Milk yield, Transition period, Complementary feed, NEFA, BHBA

Nutrient Distribution of White Corn Silage and Dispersion Analysis of Samples Analyzed with Nir

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BACKGROUND and OBJECTIVE: White corn silage (WCS) is the main forage used in the feeding of dairy cattle in Mexico. Although its energy contribution is essential, there is very little information on WCS. It is very important to know the distribution of each nutrient and the possible correlations that may exist among nutrients and forage dry matter to understand the relationships between variables. The statistics of 8,679 samples from the past 5 years of WCS offer unique information relative to the Central area of Mexico.

MATERIALS and METHODS: The data used for this study are the results of NIR analysis of Corn Silage by Laboratorio Servicios GAQ S.A de C.V., an affiliate of CVAS (Cumberland Valley Analytical Services of UE). The information is from 2020 to 2024. The statistical analyses focus on Dry Matter (DM), crude protein (CP), Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF), and Digestibility of the NDF at 30 hr. The were 8,679 corn silage samples analyzed, with the results almost completely from WCS. Statistical analyses were performed using the Minitab Workspace software.

RESULTS: The values for main analytes of WCS from central Valley of Mexico 2020-2024, dry matter basis, in 8,679 samples, Average and Estandar Deviation in each nutrient; Dry Matter (DM) 32.96 % and 5.69 %, CP 7.93 % and 1.11 %, ADF 29.47 % and 3.34 %, NDF 46.53 % and 5.05 %, Digestibility of NDF 30 hrs. % of NDF 57.85 % and 4.18 % and Starch 22.54 % and 6.24 % respectively. Dispersions were carried out among the WCS nutrients; CP, ADF, NDF, Dig. NDF 30 h % NDF and Starch. Statistical result of the value of R^2. Values > 20% in R-square were DM - ADF 26.8%, DM - NDF 25.76%, DM - Starch 28.7%, ADF - NDF 88.67%, ADF - Starch 74.85% and NDF - Starch 79.25%.

CONCLUSIONS: The analysis done in this study shows a confidence interval of 95% on the mean, median and standard deviation on the data for nutrient distribution. The precision of this data will offer a greater certainty on the formulation of diets for dairy cows in Central Mexico. The R^2 results are accurate because of the large sample sizes in the corn silage analysis; the high R^2 values (>70%) in the dispersions of two WCS nutrient variables lead to better hybrid selection and forage quality in the ration.

Keywords: Nutrient distribution, white corn silage, dispersion analyzed

Housing and Lipopolysaccharide (lps)-induced Inflammation Effects on Lying Behavior of Heat-stressed Dairy Cows

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BACKGROUND and OBJECTIVE: Lying time and bout patterns may be altered by heat stress as well as by inflammatory or infectious diseases. The study aimed to evaluate the effect of housing and LPS infusion on lying behavior in heat- stressed dairy cows.

MATERIALS and METHODS: Multiparous Holstein cows (649 ± 51 kg BW; 2.8 ± 0.19 BCS; 195 ± 31 days in milk; n=32) housed in a compost barn (CB) or an open sky pen (OP) were intravenously infused with LPS (Escherichia coli O55: B5; 62.5 ng/kgBW) or sterile saline (CTRL). The experiment was conducted in summer under heat stress conditions; the mean temperature-humidity index (THI) was >72. Cows were milked twice a day and fed a total mixed ration (47: 53 forage: concentrate). Infusion with LPS reduced dry matter intake (DMI) by 41% in CB and 49% in OP cows and CTRL cows were pair-fed to DMI of LPS cows. The inflammatory response was confirmed by increased rectal temperature (mean maximum of 39.7°C vs. 38.2°C at 4.5 h post-infusion for LPS vs. CTRL cows). Lying behavior was recorded using three-axis accelerometers (HOBO pendant G, Onset Corp.) attached to the hind leg, for 3 days (day -1, 0 and +1 relative to the infusion) recording at 1 min-intervals. Data were analyzed in a split-plot design with housing, treatment within housing, day and their interactions as fixed effects, cow as a random effect and daily THI as a covariate. Means differed when P<0.05.

RESULTS: Housing did not affect daily lying time (554±19 min/d) but CB cows had more lying bouts than OP cows (10.6 vs. 9.2±0.5 bouts/d) with no differences in lying bout duration (55 vs. 61±3 min/bout). From day -1 to +1, LPS cows spent less time lying and the reduction in lying time was greater for OP than CB cows (-149 vs. -67 min/d). In contrast, on day 0, lying time increased in CTRL-OP but not in CTRL-CB cows. On the infusion day, lying time was lower for LPS than CTRL cows (481, 686, 512 and 624±38 min for LPS-OP, CTRL-OP, LPS-CB, CTRL-CB, respectively). Lying bouts decreased after infusion (day 0 and 1) for all cows whereas lying bout duration increased only for CTRL-OP (+20 min/bout).

CONCLUSIONS: In conclusion, LPS-induced inflammation reduced lying behavior, particularly in cows with greater environmental exposure (OP).

Keywords: dairy cattle, postural behavior, immune challenge, animal welfare

Temporal Physiological and Metabolic Responses to Intravenous Lipopolysaccharide Challenge in Early-Lactation Dairy Cows

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BACKGROUND and OBJECTIVE: Inflammatory processes in early-lactation dairy cows markedly alter nutrient partitioning and metabolic homeostasis. Intravenous lipopolysaccharide (LPS) challenge is a well-established experimental model to induce acute systemic inflammation and evaluate its physiological and metabolic consequences under controlled conditions. The aim of this work was to characterize the temporal physiological and metabolic responses to an intravenous LPS challenge in early-lactation dairy cows.

MATERIALS and METHODS: Sixteen multiparous Holstein cows (639±68 kg BW; 3.0±1.4 BCS) at 15±8 days postpartum were assigned to receive either LPS (Escherichia coli O55: B5; 62.5 ng/kg BW; n=8) or sterile saline (CTRL; n=8). Cows were fed a TMR meeting 100% of requirements and milked twice daily. Control cows were pair-fed to match intake of LPS cows. Blood samples were collected at −24 h, 0 h, hourly from 1 to 8 h, and at 24 and 48 h post-infusion; plasma was harvested for metabolite analyses. Data were analyzed using a generalized linear mixed model (PROC GLIMMIX, SAS), including treatment, time, and their interaction as fixed effects and cow as a random effect. To account for baseline differences between treatments, β-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA) concentrations were expressed as the change from the pre-challenge value (−24 h). Significance was declared at P≤0.05.

RESULTS: Intravenous LPS induced a clear inflammatory response, evidenced by increased rectal temperature, heart rate, and respiratory rate (treatment x time, P≤0.05). When expressed as the change relative to baseline (−24 h), BHB increased in LPS cows whereas control cows initially decreased and later recovered. In contrast, NEFA increased in pair-fed control cows but remained lower in LPS-infused cows throughout most of the sampling period. Calcium decreased following LPS administration (treatment x time, P≤0.05), consistent with a negative acute-phase response. Urea increased significantly in LPS cows (treatment effect, P≤0.05), with mean concentrations of ~38mg/dL, suggesting altered nitrogen metabolism. Glucose was affected by the interaction treatment x time, reaching its nadir 24 h post-infusion in both treatments (55 vs. 61mg/dL) but no overall treatment effect (P>0.05), reflecting tight glycemic regulation. Aspartate aminotransferase (AST) showed both time effects and treatment x time interactions (P≤0.05), with transient increases (AST~110U/L) consistent with hepatic metabolic stress rather than structural damage.

CONCLUSIONS: The dissociation between BHB and NEFA dynamics further supports that these changes reflect immunometabolic adaptation rather than metabolic responses solely associated with negative energy balance.

Keywords: Inflamation, transition, healt

Effects of Feeding Different Levels of Bakery Waste By-Products on Performance, Ultrasonic Carcass Traits and Blood Parameters of Finishing Bulls

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BACKGROUND and OBJECTIVE: Rising feed costs have increased interest in using bakery waste by-products (BWB) as alternative energy sources in feedlot diets; however, information about high inclusion levels is limited. This study evaluated the effects of replacing corn with increasing levels of BWB on performance, blood parameters, and ultrasonic carcass traits in finishing bulls.

MATERIALS and METHODS: Forty-six Holstein bulls (initial BW 434.86±8.52 kg, average of 12 months old) were used to study the effects of feeding different levels of BWB in finishing diets on performance, blood parameters and ultrasonic carcass traits. Bulls were assigned to 4 pens with dietary treatments of 0 (Control; C), 13.72 (B1), 22.9 (B2), 42 (B3) % BWB dietary inclusion (DM basis). BWB were obtained from pasta and biscuit industry (42% macaroni waste + 20.5% biscuit waste). Corn was partially replaced by macaroni by-product in B1 and B2 and totally replaced with BWB in B3. Animals were fed ad libitum during the experiment after 21 d of adaptation period. Feed offered and orts were recorded weekly, live body weights were taken on d 0, 30, 66 and 95. Blood sample collection and ultrasonic measurements of rib fat thickness (RFT), longissimus muscle depth (LMD) were performed on d 0, 66 and 95. Images were obtained by ultrasound equipped with a 5-7.5 MHz linear probe and collected and analyzed for stated carcass traits with an image analysis software (ImageJ). Statistical analysis was performed using one-way analysis of variance (ANOVA). Differences among groups were determined by Duncan's multiple range test ($p < 0.05$).

RESULTS: No significant differences were observed for dry matter intake, average daily gain, gain to feed ratio and LMD between groups from d 0 to d 95. Ultrasonic RFT values were not different between groups at d 0 and 66. However, ultrasonic RFT of B3 (5.88±0.17mm) was significantly lower than C (6.38±0.15mm), B1 (6.53±0.16mm) and B2 (6.49±0.18mm) at d 95 ($P<0.05$). Ultrasonic LMD values were 58.49±0.58, 57.85±0.97, 57.56±0.98, 55.12±1.12 mm at d 95 for C, B1, B2 and B3, respectively. Blood glucose and amylase levels were not different between groups during the experiment.

CONCLUSIONS: In conclusion, corn can be successfully replaced with bakery waste by-products up to 42% in finishing bull diets without negative effects on performance or LMD, while high inclusion levels may reduce rib fat thickness during the finishing period.

Keywords: beef cattle, bakery waste by-products, ultrasonic carcass traits, performance, alternative feed.

Effects of Different Calcidiol (25(OH)D_3) Doses on Performance and Functional Dairy Product Production in Lactating Cows

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BACKGROUND and OBJECTIVE: The objective of this study is to holistically evaluate the effects of different vitamin D doses (1 mg/day and 3 mg/day) supplemented in the form of calcidiol (25(OH)D_3) to dairy cattle diets on lactation performance, milk composition, and the bioactive richness of the products.

MATERIALS and METHODS: In the study, 30 lactating Holstein cows were randomly allocated into 3 groups and fed a Total Mixed Ration (TMR) ad libitum using an Automated Individual Feeding System (AIFS). The statistical analysis of the obtained data was performed using the SPSS 29.0 software package; One-Way Analysis of Variance (One-Way ANOVA) was used as the analysis method, and Duncan's post-hoc multiple comparison test was applied to determine the differences among means, with the significance level set at $p < 0.05$.

RESULTS: No statistically significant difference was observed among treatment groups regarding dry matter intake (DMI). However, calcidiol supplementation positively affected lactation performance; the average milk yield significantly increased from 34.50 in the control group to 35.85 in D1 and 34.84 in D2 groups (P=0.001). Regarding milk composition, lactose percentage increased significantly in both treatment groups compared to the control (P=0.007). Milk fat percentage peaked in the D2 group (3.70%, P=0.05), whereas milk protein in the same group (3.24%) exhibited a marked decrease compared to others (P=0.001). Milk urea nitrogen (MUN), an indicator of rumen nitrogen metabolism and protein-energy synchronization, was significantly higher in D1 (7.65) than other groups (P=0.001). Preliminary data reflecting a 2-week period showed calcidiol supplementation increased vitamin D transfer into dairy products. Consistent with its lipophilic nature, the highest (25(OH)D_3) accumulation occurred in cheese curd (20.9-22.3 ng/mL in D groups), followed by raw milk (12.4-14.0 ng/mL), while whey values remained quite low. Notably, (25(OH)D_3) accumulation in milk and cheese under the 1 mg/day (D1) treatment was numerically higher than the 3 mg/day (D2) dose.

CONCLUSIONS: Supplementing dairy cattle diets with (25(OH)D_3) stimulated milk yield and lactose synthesis without altering the dry matter intake (DMI) of the cows. The obtained preliminary findings indicate that calcidiol supplementation enriches the vitamin D content of milk and cheese, thereby providing a strong foundation for the production of "functional foods". These dose-dependent changes observed in milk composition (fat, protein, MUN) should be taken into consideration in diet formulations, and the definitive bio-transfer dynamics should be evaluated upon the completion of the project.

Keywords: Dairy cattle, Lactation performance, Calcidiol, Functional dairy products

PARASITIC DISEASES

Prevalence and Seasonal Variation of Cryptosporidiosis (cryptosporidium Spp.) in Young Calves from Romania

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BACKGROUND and OBJECTIVE: Cryptosporidiosis (caused by Cryptosporidium spp.) is one of the most important enteric diseases of young ruminants worldwide. Cryptosporidium parvum damages the microvilli of the small intestine, leading to osmotic and malabsorptive diarrhea. Oocysts, of which millions are shed daily, are immediately infective, highly resistant in the environment, and zoonotic. Economic losses arise from medication costs, labor, reduced daily gain, and mortality. OBJECTIVES: To analyze laboratory records from 2018–2024 and determine the prevalence and seasonality of Cryptosporidium parvum infections in Romanian calves.

MATERIALS and METHODS: Between 2018 and 2024, 1,277 fecal samples and rectal swabs were submitted to Synevo Vet Laboratory, a private veterinary reference laboratory in Romania, by field veterinarians from dairy and beef farms located across ten counties. Samples were collected on-farm from calves with diarrhea within 48 hours of outbreak onset. Routine parasitological techniques were used, and Cryptosporidium parvum was identified using direct fluorescent microscopy. None of the farms included had implemented vaccination against cryptosporidiosis during the study period; therefore, results reflect natural infection pressure in non-vaccinated herds.

RESULTS: Of the 1,277 samples analyzed, 436 (34.1%) were positive for Cryptosporidium parvum. Yearly prevalence ranged from 10% (lowest year, 2018) to 71% (peak year, 2021), confirming substantial annual variation. Prevalence in 2022–2023 was stable (both 37%). Seasonality was evident: spring (35%) and autumn (32%) together accounted for 67% of all positive findings. No positive samples were detected in July, suggesting reduced environmental survival of oocysts during hot, dry summers.

CONCLUSIONS: This 7-year dataset demonstrates a consistently high and fluctuating burden of cryptosporidiosis in Romanian calves (average 34%). Spring and autumn were identified as recurrent high-risk periods across all years. The absence of positive samples in July likely reflects environmental inactivation of oocysts during peak summer conditions. Given the high infection rate of samples for Cryptosporidium parvum, vaccination of dams against this pathogen seems indicated in Romanian herds. Routine surveillance, strict hygiene, reduced stocking density, and age segregation remain essential measures to mitigate economic losses—especially in non-vaccinated herds.

Keywords: cryptosporidiosis, neonatal diarrhea, calves, field diagnostics, disease prevention

Metabolic Response to Deworming in Beef Cattle with Subclinical Gastrointestinal Nematodes Infection

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BACKGROUND and OBJECTIVE: Gastrointestinal nematodes (GINs) remain a major constraint to beef cattle production due to their predominantly subclinical nature, which limits diagnosis and leads to cumulative productivity losses. Increasing anthelmintic resistance highlights the need for complementary approaches to better understand host–parasite interactions and support more precise control strategies. Metabolomics offers a powerful framework to investigate systemic metabolic alterations, identify diagnostic biomarkers, and assess treatment responses. This study aimed to characterize the serum metabolome of beef cattle with subclinical GIN infection by comparing

fenbendazole-treated animals with untreated controls.

MATERIALS and METHODS: Twenty Nellore male beef cattle in the growing phase, raised under extensive pasture-based conditions, and confirmed to have natural infection with gastrointestinal nematodes (GINs), were divided into two groups, fenbendazole treated (TRAT, n = 10) and untreated controls (CON, n = 10). The treated group (TRAT) received weekly oral doses of fenbendazole. Fecal samples were collected for parasitological analyses, animals were periodically weighed, and on day 42, blood samples were obtained for NMR-based metabolomic analysis.

RESULTS: Treated (TRAT) animals showed significantly higher weight gain on days 35 and 42 compared to controls (CON). Treatment efficacy was confirmed by fecal egg count reduction, and pooled fecal cultures indicated predominance of Cooperia spp. Metabolomic analysis identified 43 metabolites, of which 11 differed significantly between groups. Metabolites related to protein metabolism and aerobic energy pathways were increased in TRAT animals, particularly methionine and aspartate. Pathway enrichment analysis supported modulation of tryptophan metabolism and mitochondrial electron transport chain activity. In contrast, CON animals showed higher levels of metabolites associated with lipid mobilization, including glycerol and carnitine, with enrichment of peroxisomal phytanic acid oxidation pathways. Volcano plot analysis confirmed significant differences in glycerol and carnitine (higher in CON) and methionine (higher in TRAT). Although ethanol and succinate had very low p-values, their $\log_2$ fold changes were small. Variable importance analysis identified aspartate, methionine, and glycerol as the main discriminant metabolites.

CONCLUSIONS: The TRAT group exhibited a metabolic profile consistent with higher protein anabolism and greater use of aerobic energy pathways, whereas the CON group showed increased mobilization of lipid reserves. These results provide relevant insights into the physiological effects of subclinical infection, highlight metabolites with potential to be used as biomarkers, and reinforce the importance of integrated approaches for the diagnosis, monitoring, and management of GIN infections in beef cattle production

Keywords: Gastrointestinal nematodes, fenbendazole, metabolomic analysis, beef cattle

Effect of An Oral Fluralaner Program on Tick Infestation and Milk Production in Dairy Cattle

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BACKGROUND and OBJECTIVE: The effect of a strategic control program using orally administered fluralaner at a dose of 0.5 mg/kg (T01) on Rhipicephalus microplus infestation and milk production of 7/8 Holstein × 1/8 Gyr cows was compared with the conventional treatment routinely adopted on the farm, based on a spray formulation containing chlorpyrifos (825 ppm) and high-cis cypermethrin (99 ppm) (T02).

MATERIALS and METHODS: The study was conducted from October 2024 to May 2025. Each group included 29 cows allocated in a balanced manner according to initial tick counts, milk production, number of lactations, and days in milk. The first treatment of the T01 group was administered on study day 0. Subsequent retreatments were based on weekly visual assessments and were only administered when ticks <4 mm were observed in ≥30% (9/29) of the animals. In the T02 group, treatments followed the routine farm management practices to treat the animals based on the discretion of the farm staff. The entire group received the spray formulation.

RESULTS: After day 0, tick counts and milk production measurements were performed every 7 and 14 days, respectively, until day 244. During the study, the T01 group received seven oral fluralaner treatments at intervals ranging from 21 to 49 days, whereas the T02 group received nine treatments at intervals of 7 to 49 days. Oral fluralaner treatment maintained effective tick control over 244 days, as mean tick counts were below one tick per animal in 25 of the 33 evaluation time points. In the spray-treated group, this same level of control was observed in 7 of the 33 evaluations. At the beginning of the study, mean milk production did not differ between groups (p = 0.9877). From day 140 onward,

however, the fluralaner-treated group showed significantly higher milk production ($p \leq 0.05$). During this period, mean production was 4.14 L per cow greater than that of the spray-treated group.

CONCLUSIONS: These results demonstrate the efficacy of fluralaner for the strategic control of R. microplus under field conditions and indicate a potential positive impact on milk production in response to fluralaner treatment in naturally infested grazing dairy cows maintained in a tropical climate.

Keywords: Rhipicephalus microplus, Milk, fluralaner

Efficacy of Orally Administered Fluralaner Against Rhipicephalus Microplus in Experimentally Infested Cattle

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BACKGROUND and OBJECTIVE: The objective of the present study was to evaluate the acaricidal efficacy of orally administered fluralaner at a dose of 0.5 mg/kg body weight in the treatment of cattle experimentally infested with Rhipicephalus microplus larvae.

MATERIALS and METHODS: The study, conducted in accordance with animal welfare principles and approved by an Institutional Animal Care and Use Committee, was performed as a stall test in southeastern Brazil. Fourteen clinically healthy cattle that had not received antiparasitic treatment within the 45 days prior to the start of the study were used. Animals ranged between 4 and 36 months of age and weighed 81–160 kg. Prior to treatment, animals were experimentally infested with approximately 5,000 unfed tick larvae (0.25 g), aged 7–21 days post-hatching, on Days −25, −23, −21, −19, −17, −15, −11, −9, −7, −5, −3, and −1. Animals were enrolled after counting detached engorged female ticks (teleogynes) on Days −3, −2, and −1. They were then homogeneously allocated, based on teleogyne counts, into two experimental groups: fluralaner (0.5 mg/kg) or saline solution (0.9% NaCl), both administered orally. After treatment, detached teleogynes were counted daily from Day 1 to Day 41. In addition, up to 10 teleogynes per animal were randomly selected for incubation and evaluation of reproductive parameters. Reinfestations were performed on Days 4, 7, 11, 14, 18, 21, 25, 28, 32, 35, and 39.

RESULTS: Efficacy results exceeded 95% between Days 3 and 34 and reached 100% from Days 6 to 33. A decline in efficacy was observed thereafter, with values below 80% between Days 37 and 41, when the study was concluded. Mean teleogyne counts in the treated group were significantly lower ($p \leq 0.05$) than those of the negative control group from Days 1 to 39 post-treatment.

CONCLUSIONS: Based on these findings, it can be concluded that orally administered fluralaner at a dose of 0.5 mg/kg body weight is effective for the treatment and control of experimental R. microplus infestations in cattle.

Keywords: Fluralaner, Rhipicephalus microplus, cattle

Is The Treatment of Diarrhoeic Calves with Paromomycin Sulphate, at the Height of *Cryptosporidium Parvum* Infection, Safe and Effective?

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BACKGROUND and OBJECTIVE: *Cryptosporidium parvum* infection is one of the major causes of neonatal diarrhoea in calves worldwide. A study has shown that clinical cryptosporidiosis in beef calves

resulted in a reduction in growth of 34kg, when animals went to market at 6 months of age. Another study looking at the economic costs of cryptosporidiosis in dairy calves in three European countries attributed losses of between 43.83 to 60.62 Euro per affected calf, depending on country. Currently there are different products licensed for the prevention and/or treatment of cryptosporidiosis in neonatal calves, but their licensed application is restricted to use prior to or 24 hours after the onset of diarrhoea. Therefore, the are no products licensed to treat severely affected calves. The objective of this study was to address if treatment of diarrhoeic calves, at the height of *Cryptosporidium parvum* infection, with Parofor® CRYPTO is safe and effective?

MATERIALS and METHODS: The experimental infection study involved 2 groups of 10 calves each, where each calf was infected with 1x106 *C. parvum oocysts*, at ~4 days old. Calves were monitored twice daily for the duration of the study (demeanour, body condition, diarrhoea, skin tent, appetite and oocyst shedding) and were weighed at several timepoints during the study. When animals reached peak of infection, treatment was administered, either Parofor® CRYPTO (2.5ml per 10kg body weight) or with PBS, for a duration of 7 days. Animal groupings were blinded for the duration of the study. Ethical approval was obtained from an Animal Welfare Ethical Review Body (AWERB) and followed Home Office legislation in the United Kingdom.

RESULTS: Some neonatal calves, which were brought onto the study farm were already infected with *Cryptosporidium*. There were no significant differences between the groups for demeanour, body condition, skin tent and appetite. However, there were differences in diarrhoea scores, oocyst shedding and weight gain during the treatment period.

CONCLUSIONS: The study has shown that Parofor® CRYPTO treatment of diarrhoeic calves at the height of *C. parvum* infection did not have any negative health impacts and that treatment resulted in better growth during the treatment period, reduced *C. parvum* oocyst shedding and lower diarrhoea scores, indicating that the product is safe and effective to use in diarrhoeic calves. A higher infectious dose may have increased the difference between the groups as none of the calves were severely affected.

Keywords: Cryptosporidium, Parofor® CRYPTO, Treatment, Diarrhoea,

Improved Management of Cattle Tick Infestations Using a Strategic Oral Fluralaner Protocol

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BACKGROUND and OBJECTIVE: The objective of the present study was to evaluate the effect of a strategic control program using orally administered fluralaner at a dose of 0.5 mg/kg against Rhipicephalus microplus parasitizing naturally infested dairy cows.

MATERIALS and METHODS: Thirty-six cows were allocated into two groups based on mean tick counts recorded on Days −3, −2, and −1. In group T01, 18 cows received oral fluralaner as part of a strategic control program. The first treatment was administered on study Day 0, and subsequent retreatments were based on weekly visual assessments and administered when ticks <4 mm were observed in ≥30% (6/18) of the animals. Group T02 received rescue treatment with a spray formulation containing chlorpyrifos (825 ppm) and high-cis cypermethrin (99 ppm) whenever the group mean tick count exceeded 30. After Day 0, counts of female R. microplus measuring 4.5–8 mm were performed on Days 7, 14, 21, and 28, and subsequently every seven days until Day 133.

RESULTS: During the study, both groups received four treatments, which were carried out according to tick challenge. Treatment intervals ranged from 28 to 35 days in the fluralaner group and from 21 to 28 days in the spray-treated group. The rescue-treated group showed high mean tick counts, reaching 47.4 on Day 14, 53.2 on Day 49, 50.7 on Day 84, and 64.9 on Day 112, indicating a high level of parasitic challenge. Mean tick counts in fluralaner-treated cows were significantly lower ($p \leq 0.05$) than those of the rescue-treated group in 14 of the 19 post-treatment evaluations. Oral fluralaner demonstrated high efficacy throughout the experimental period, achieving 100% tick elimination on Days 7, 14, 21, 28, 42, 49, 56, 77, 84, 91, 105, 112, 119, and 126 after the first treatment.

CONCLUSIONS: These findings show that oral fluralaner was highly effective in controlling R.

microplus in naturally infested cows, producing significant reductions in tick counts and complete parasite elimination at multiple time points. The longer interval between the fluralaner treatments indicates a prolonged residual effect, supporting the potential of fluralaner as an efficient tool for strategic tick control in dairy herds.

Keywords: Tick, Fluralaner, cow

Complete Efficacy of Fluralaner and Lack of Efficacy of Doramectin in the Prevention of Umbilical Myiasis in Brazilian Newborn Calves

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BACKGROUND and OBJECTIVE: Myiasis caused by Cochliomyia hominivorax larvae is a re-emerging challenge in the Americas due to the recent reappearance of this species in areas where it had been eradicated and reports of doramectin inefficacy, despite its recognition as the most effective avermectin for prevention in cattle. As an alternative, 5% fluralaner administered via pour-on has shown high efficacy in the treatment and prevention of myiasis. Therefore, this study aimed to evaluate the efficacy of different antiparasitic drugs in preventing umbilical myiasis caused by C. hominivorax in newborn calves.

MATERIALS and METHODS: The study was conducted on a commercial beef cow–calf farm in Mato Grosso do Sul, Brazil, in September 2025. On day 0, during umbilical care, 200 newborn calves were randomly allocated to four groups: control; 1% doramectin subcutaneously (200 µg/kg); 5% fluralaner pour-on (2.5 mg/kg); and 1% fluralaner orally (0.5 mg/kg-experimental formulation). All umbilical stumps were disinfected with 10% iodine tincture (10–30 s), without larvicidal compounds. Umbilical sites were evaluated on days 3, 7, and 14 for the presence of C. hominivorax larvae and lesion scores (0–3). Calves with active myiasis were treated with an organophosphate-based larvicide and excluded from the study

RESULTS: The results showed marked differences among treatments in the prevention of umbilical myiasis. In the control group, myiasis occurrence was 26% (95% CI: 15–39), confirming the high field risk of the disease. Treatment with 1% doramectin did not significantly reduce myiasis occurrence (22%; 95% CI: 12.7–35.2), showing a relative risk reduction of only 15.4%, an odds ratio close to unity (OR = 0.80; 95% CI: 0.31–2.01), and no statistical significance (P = 0.8153). Pour-on fluralaner reduced myiasis occurrence to 4% (95% CI: 1.1–13.4), corresponding to a relative risk reduction of 84.6% and a statistically significant association (OR = 0.12; P = 0.0038). Incidental myiasis was probably due to the maternal licking which might have led to removal of the pour-on product from the newborn calves. On the other hand, orally administered fluralaner showed 100% preventive efficacy (0%; 95% CI: 0–7.1). Umbilical lesion scores corroborated these findings, with a predominance of score 0 in fluralaner-treated groups.

CONCLUSIONS: Doramectin was ineffective in preventing umbilical myiasis in newborn calves, whereas orally administered fluralaner showed 100% efficacy.

Keywords: umbilical myiasis, fluralaner, doramectin

Evaluation of the Acaricidal Efficacy of Orally Administered Fluralaner Against Rhipicephalus Microplus in Naturally Infested Cattle

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BACKGROUND and OBJECTIVE: The objective of this study was to evaluate the acaricidal efficacy of a single dose of 0.5 mg/kg of orally administered

fluralaner in the treatment of cattle naturally infested with Rhipicephalus microplus in the Southeast and Midwest regions of Brazil. The study followed animal welfare principles in accordance with national ethical regulations and was approved by an Institutional Animal Care and Use Committee.

MATERIALS and METHODS: In the Southeast region, 20 cattle aged 12–33 months and weighing 121.0–322.0 kg were used. In the Midwest region, 20 cattle aged 6–8 months and weighing 80.0–161.5 kg were included. To be eligible for the study, animals were required to present an average count of at least 20 R. microplus ticks on one side of the body, measuring 4.5–8.0 mm, on Days −3, −2, and −1. Additionally, animals had to be in good health and free from antiparasitic treatment for at least 45 days prior to the study. Animals were allocated into two experimental groups according to the average tick count: one group of 10 animals treated with single dose oral fluralaner and one negative control group of 10 animals receiving single dose the saline solution (0.9% NaCl), both administered at 0.5 mg/kg. Tick counts were performed on Days 1, 3, 5, 7, 10, 14, 21, 23, 26, 28, 30, 32, and 35 in the Southeast region, and up to Day 32 in the Midwest region.

RESULTS: In the Southeast region, efficacy was 97.41% on Day 1, 100% from Days 3 to 23, 99.44% on Day 26, and 98.24% on Day 28. After Day 28, efficacy declined to below 90%, reaching 65.71% on Day 35, the final evaluation day. At all post-treatment counts, the treated group showed significantly lower mean tick counts ($p \leq 0.05$) than the negative control group. In the Midwest region, efficacy was 13.63% on Day 1, 87.89% on Day 3, and 95.33% on Day 5, reaching 100% from Days 7 to 23. On Day 26, efficacy was 99.36%, decreasing to 90.83% on Day 28 and 56.96% on Day 32, the last evaluation day. The treated group had significantly lower mean counts ($p \leq 0.05$) than the negative control group at all post-treatment assessments, except on Day 1.

CONCLUSIONS: These results demonstrate the high efficacy of single dose orally administered fluralaner at a dose of 0.5 mg/kg against R. microplus in cattle maintained under field conditions in different regions of Brazil.

Keywords: fluralaner, tick, naturally infested

Efficacy of Oral Fluralaner Against Dermatobia Hominis Larvae in Naturally Infested Cattle

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BACKGROUND and OBJECTIVE: The objective of this study was to evaluate the therapeutic efficacy of orally administered fluralaner at a dose of 0.5 mg/kg for the control of Dermatobia hominis larvae in naturally infested cattle. The protocol strictly followed the principles of Veterinary Good Clinical Practices (VGCP) and was approved by an Institutional Animal Care and Use Committee.

MATERIALS and METHODS: Twenty female cattle (482–662 kg) presenting a natural infestation of at least 10 live larvae were selected. Animals were randomized into two groups of 10 experimental units each: one group treated with a single dose of oral fluralaner (0.5 mg/kg) and a negative control group receiving saline solution (0.9% NaCl). All animals were maintained in the same pasture under extensive grazing conditions with free access to water and mineral supplementation in southeastern Brazil. Efficacy evaluations were performed on Days 1, 3, 5, 7, 14, 21, 28, and 35 post-treatment using masked tactile and visual inspections. For statistical analysis, raw count data were transformed using $\ln(x+1)$, and group means were compared using Tukey's test ($p \leq 0.05$).

RESULTS: The results demonstrated a larvicidal efficacy of 80.3% on Day 1, reaching 100% from Day 3 through the end of the study on Day 35, with complete elimination of larvae in treated animals. Mean larval counts in the treated group were significantly lower ($p \leq 0.05$) than those of the control group at all post-treatment evaluations. Clinical assessments performed at 6 and 24 hours after treatment, in addition to daily observations, confirmed the absence of adverse events or signs of systemic toxicity.

CONCLUSIONS: It is concluded that orally administered fluralaner at a single dose of 0.5 mg/kg is safe and highly effective for the treatment of D. hominis infestations in cattle.

Keywords: Dermatobia hominis, fluralaner, cattle

Large-scale Field Evaluation of the Anthelmintic Efficacy of a Fixed-Dose Doramectin–Levamisole Combination Compared with Long-Acting Avermectins in Brazilian Beef Cattle

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BACKGROUND and OBJECTIVE: Although the efficacy of a fixed-dose injectable combination of doramectin and levamisole has been demonstrated in controlled trials, the advanced scenario of anthelmintic resistance in Brazil demands field-based evaluations. Considering the regional diversity of gastrointestinal nematode populations and resistance profiles, this study aimed to evaluate fecal egg count reduction in cattle treated with a fixed-dose doramectin–levamisole combination (DRM+LEV) compared with long-acting avermectins (LA-AVMs) in a multiregional field study in Brazil.

MATERIALS and METHODS: Fecal egg count reduction tests (FECRT) were performed on 46 cattle farms across all geographical regions of Brazil: South (7), Southeast (5), Midwest (25), Northeast (2), and North (7). Farms were selected by convenience and included at least 75 post-weaning calves with no anthelmintic treatment in the previous 90 days. On each farm, ≥35 animals per group, homogeneous for breed, sex, and age, were allocated in a completely randomized design to receive either a fixed-dose injectable DRM+LEV combination or a LA-AVM formulation selected according to farm history; animals with zero pre-treatment fecal egg counts were excluded, and three farms were removed due to an insufficient number of egg-positive animals at baseline. Individual fecal samples were collected before treatment and 14–20 days post-treatment, and FECR was estimated using a Bayesian hierarchical model. Anthelmintic efficacy was classified as Susceptible (FECR ≥95% and lower 95% CI ≥90%), Inconclusive (either FECR <95% or lower 95% CI <90%), or Resistant (FECR <95% and lower 95% CI <90%).

RESULTS: The proportion of farms with anthelmintic efficacy classified as Susceptible, Inconclusive, and Resistant for the DRM+LEV combination was 69.8%, 11.6%, and 18.6%, respectively. In contrast, for LA-AVMs, 0%, 4.7%, and 95.3% of farms were classified as Susceptible, Inconclusive, and Resistant, respectively. The mean efficacy across all evaluated farms was 96.6% for DRM+LEV and 44.2% for LA-AVMs. When stratified by efficacy classification, the mean FECR values for DRM+LEV were 98.8% (Susceptible), 96.4% (Inconclusive), and 88.6% (Resistant). For LA-AVMs, no farms were classified as Susceptible; the mean FECR values were 95.9% in Inconclusive farms and 41.7% in Resistant farms. These results demonstrate that, unlike LA-AVMs, even when resistance is detected by the FECRT, the mean efficacy percentage remains satisfactory for the DRM+LEV combination.

CONCLUSIONS: Therefore, this large-scale field survey demonstrates that, even in a scenario of advanced anthelmintic resistance to avermectins, the DRM+LEV combination maintained high efficacy and a low proportion of farms classified as resistant.

Keywords: Efficacy, Doramectin-Levamisole, Avermectins, Antihelmintic resistence, Fecal egg reduction

Efficacy of a Fixed-Dose Doramectin + Levamizole Combination Against Gastrointestinal Nematodes and Effects on Weight Gain in Beef Cattle

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BACKGROUND and OBJECTIVE: Drug combinations are a field-validated strategy to broaden the spectrum of activity and extend the useful lifespan of existing anthelmintics. The efficacy of a fixed-dose injectable combination containing 0.2 mg/kg doramectin and 6.0 mg/kg levamisole hydrochloride has been demonstrated in controlled anthelmintic efficacy trials; however, its effects on cattle productivity remain to be fully elucidated. The objective of this study was to evaluate the efficacy of this drug combination in reducing gastrointestinal

nematode fecal egg counts and improving weight gain in grazing beef calves during the growing phase under tropical conditions.

MATERIALS and METHODS: A randomized complete block design was used, with blocking based on body weight at Day −7. A total of 231 male Nellore cattle, aged 12 to 22 months, were allocated to either a control group (n = 76) or a treated group (n = 155). Throughout the study period (Day −7 to Day 84), all animals were maintained in a single communal paddock under identical environmental and management conditions, with the individual animal considered the experimental unit. On Day 0, animals in the control group received 1.0 mL of saline solution per 25 kg of body weight by subcutaneous injection, whereas animals in the treated group received 1.0 mL per 25 kg of body weight of a fixed-dose injectable combination containing 0.2 mg/kg doramectin and 6.0 mg/kg levamisole hydrochloride, administered subcutaneously. Fecal egg counts, coprocultures, and body weight measurements were performed on Days −7, 28, 56, and 84.

RESULTS: Treated calves showed significantly higher body weight gain than the control group as early as Day 28 post-treatment, and this difference persisted until Day 84. Differences between groups in average daily gain were 122 g (Day −7 to Day 28), 50 g (Day −7 to Day 56), and 42 g (Day −7 to Day 84). Anthelmintic efficacy was high at Day 14 (99.1%; 95% CI: 97.5–99.8) but decreased to 75.7% (95% CI: 68.6–81.4) at the subsequent evaluation on Day 28. At both time points, mean fecal egg counts differed significantly between the two groups. Pre-treatment coprocultures showed a predominance of Haemonchus spp. and Cooperia spp., with lower proportions of Oesophagostomum spp.; following treatment, only Cooperia spp. larvae were recovered.

CONCLUSIONS: The fixed-dose combination of doramectin and levamisole was effective in reducing fecal egg counts and was associated with improved weight gain in growing beef calves between 14 and 28 days after treatment

Keywords: Efficacy, Doramectin-Levamisole, Drug combination, Anthelmintics efficacy, Weight gain

The Importance of Procalcitonin and Neopterin in The Disease Course of Lambs with Cryptosporidiosis and Their Prognostic Evaluation in Different Treatment Groups

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BACKGROUND and OBJECTIVE: Cryptosporidium spp. is a zoonotic protozoan causing diarrhea, growth retardation, and economic losses in neonatal lambs, and monitoring the inflammatory response is important for clinical management. This study evaluated inflammatory biomarkers (serum amyloid A (SAA), haptoglobin (HPT), procalcitonin (PCT), neopterin (NPT)) in cryptosporidiosis in Kıvırcık lambs under 30 days, and assessed two treatment protocols' effects on clinical findings and growth.

MATERIALS and METHODS: The study material consisted of 45 Kıvırcık lambs from the same farm raised under identical management conditions, including 30 lambs with diarrhea and 15 healthy controls. Fecal samples were examined microscopically using carbol fuchsin staining; infection was confirmed with ≥1.2×10^5 oocysts per gram. Only lambs testing positive via rapid antigen test were included in the diarrheic group. Diarrheic lambs were split into two treatment groups. Group-P received paromomycin (100 mg/kg, orally, daily), while Group-H received halofuginone lactate (0.1 mg/kg, orally, daily) for 7 days. Lambs showing severe dehydration or requiring intensive fluid therapy were excluded from the study. Blood samples were collected on days 0, 3, and 7 for biomarker analysis using ELISA kits. Body weights were recorded on days 0, 7, 15, and 30. Statistical analyses used appropriate tests based on data distribution, with significance at $p < 0.05$.

RESULTS: Serum SAA, NPT, and PCT levels were significantly higher in diarrheic lambs versus healthy controls across sampling points. SAA differences were significant on days 0 and 3, but not

day 7. NPT and PCT differences remained significant through day 7. While HPT levels showed no differences between treatment groups, significant differences emerged when comparing healthy versus diarrheic animals on days 3 and 7. Fecal oocyst counts decreased in both groups, with no oocysts detected by day 7. On day 3, oocysts were detected in 3 lambs in Group-P and 6 lambs in Group-H. Though initial oocyst counts were similar, they were higher in the Group-H on day 3 (10.69±4.71 vs. 1.93±1.54). The overall mean oocyst burden was higher in the Group-P. Body weights were initially comparable, but by day 30, the control group showed higher weights (16.35±0.63 kg) versus Group-H (12.90±0.81 kg) and Group-P (12.09±0.88 kg) ($p<0.001$). The lowest weight gain was observed in Group-P.

CONCLUSIONS: SAA (early phase) and NPT/PCT may serve as sensitive systemic inflammation biomarkers in lambs with cryptosporidiosis. Their combination with clinical scoring, oocyst shedding, and growth performance could help monitor disease progression and treatment efficacy, though validation in larger multi-herd studies is needed.

Keywords: Cryptosporidiosis, diarrhea, procalcitonin, neopterin, lamb health

Control Of Neonatal Cryptosporidiosis in Large Dairy Farms Using Maternal Gp40 Vaccination and Extended Transition Milk Feeding: A Potential Alternative to Metaphylaxis?

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BACKGROUND and OBJECTIVE: Cryptosporidium parvum (C. parvum) is a primary cause of neonatal diarrhea, especially in high-risk large dairy farms. Metaphylaxis is widely used as a control strategy to delay oocyst shedding and reduce excretion intensity; reducing clinical disease and mortality. A novel vaccine (Bovilis® Cryptium®, MSD) (C) has demonstrated safety and efficacy in preclinical and field studies. In this study, its performance in large dairy herds and its impact on oocyst shedding dynamics was evaluated.

MATERIALS and METHODS: Two blinded, randomized field trials were conducted on two Spanish dairy herds (Herd1: 420 cows; Herd2: 700 cows). A total of 250 pregnant cows (5–12 weeks pre-partum) were allocated to either rotavirus-coronavirus-E. coli vaccination (Bovilis® Rotavec®Corona, MSD A.H.) (R) or R plus C. parvum Gp40 vaccine (R+C). Overall, 207 calves were included, housed individually (direct contact was possible in Herd1), received 3.5L of colostrum within 6 h of birth and were fed pooled transition milk for 5 days. Gp40-specific antibodies (Gp40-Ab) were quantified in pre-partum dam serum, colostrum, transition milk and calf serum (1–3d) by in house ELISA. Calves were monitored for 3 weeks for Wisconsin health and fecal score (FS), diarrhea-related mortality, and growth (0-21±3d). In sentinel calves, five sequential fecal samples were collected to quantify oocyst excretion.

RESULTS: Across both herds, R+C cows and calves showed significantly higher Gp40-Ab titers in all matrices compared with R. Herd1 showed inferior colostrum quality, passive transfer, and Gp40-Ab levels compared to Herd2. As result, in Herd1, overall FS was higher than in Herd2 (median FS R+C: 36.3 % vs 21.1%). In Herd1, R+C calves had lower diarrhea-related mortality (3.7% vs. 13.5%), higher average daily gain trend (483 vs. 400g/d), and fewer severe FS than R. In Herd2, mortality and growth were similar between study groups; however, R+C calves showed lower FS at 10–12d and fewer recurrent diarrhea at FS>2, consistent with a milder and shorter clinical course. In both farms, R+C sentinel calves demonstrated a delayed onset of diarrhea and decreased intensity of oocyst shedding compared to R calves (ee.g., Herd2: onset delayed from 7 to 9 days, and peak shifted from 10-12 to 16-18 days), consistent with previous findings with metaphylaxis.

CONCLUSIONS: Maternal vaccination against C. parvum Gp40, combined with extended transition milk feeding, is an effective alternative to metaphylaxis for controlling neonatal cryptosporidiosis in large dairy herds. Observed differences support a biologically relevant effect of

maternal immunization, while improved colostrum management and lower environmental oocyst contamination enhance vaccine performance.

Keywords: Cryptosporidiosis, vacunación materna, anticuerpo Gp40, leche de transición, terneros

Impact of an Extended Booster-to-Calving Interval on Anti-GP40 *Cryptosporidium parvum* Antibody Levels in Colostrum of Primiparous Holstein Heifers

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BACKGROUND and OBJECTIVE: Cryptosporidium parvum is a major cause of neonatal calf diarrhoea. Vaccination of pregnant heifers with Gp40 based vaccines aims to enhance Gp40 antibodies concentrations in colostrum and transition milk, providing passive protection to newborn calves. The vaccine SPC recommends completing vaccination in the third trimester of pregnancy. This pilot study evaluated whether the booster to calving interval influences Gp40 antibody levels in colostrum and second milking of primiparous Holstein heifers.

MATERIALS and METHODS: Thirty primiparous Holstein heifers from a commercial Spanish dairy herd received a two dose Gp40 vaccination protocol (primary + booster, 28 day interval with Bovilis Cryptium®, MSD Animal Health). Twenty two unvaccinated heifers served as negative controls. Colostrum (C1; n=28) and second milking (C2; n=29) was collected. Gp40 IgG levels were quantified by in house ELISA (Center for Diagnostic Solutions, Boxmeer). Vaccinated heifers were grouped by booster to calving interval: G1 ≤50 days (n=4), G2 51–80 days (n=6), G3 81–110 days (n=4), G4 111–130 days (n=5), and G5 >131 days (n=11). Data analysis included Kruskal–Wallis, one way ANOVA with Tukey HSD, Spearman correlation, and comparison with controls using Mann–Whitney U tests ($p<0.05$).

RESULTS: All vaccinated groups showed significantly higher C1 Gp40 IgG levels than controls (all $p<0.01$). Vaccinated heifers showed a 45.5% to 57.4% increase in antibody levels over controls, confirming strong benefits at all intervals. Vaccination consistently increased colostral antibody concentrations across all intervals. Within vaccinated groups, significant differences were detected for both C1 and C2 (Kruskal–Wallis $p=0.025$). Mean C1 values were highest in G1–G3 and progressively declined in G4 and G5. For C2, a significant difference was observed between G1 and G5 ($p=0.027$). Spearman analysis showed a moderate negative correlation between interval length and antibody levels (C1 $r=-0.529$; C2 $r=-0.601$; both $p\leq0.003$), although values remained well above those of unvaccinated controls.

CONCLUSIONS: Vaccination against C. parvum Gp40 significantly elevates colostral Gp40 antibody levels in primiparous heifers across all booster to calving intervals studied. These results provide robust field evidence that even heifers vaccinated 4–5 months before calving achieve colostral antibody concentrations far superior to unvaccinated animals. Even long intervals (>4 months) result in antibody levels markedly higher than unvaccinated heifers. Nevertheless, administering the booster 2–4 months (61–120 days) before calving maximizes antibody transfer and is recommended to optimize passive protection of newborn calves. However, to remain within the labeled use, the booster should be administered during the third trimester of pregnancy, as giving it earlier constitutes off-label use.

Keywords: Cryptosporidium parvum, colostrum, vaccination, booster-to-calving interval, antibody levels

Medium-Term Follow-Up of Neonatal Cryptosporidiosis Control in Beef Cattle Through Dam Vaccination: Impact on Calf Health, Paromomycin Use and Growth

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BACKGROUND and OBJECTIVE: Neonatal cryptosporidiosis is an important cause of diarrhea, economic losses, and antimicrobial use in beef herds. Control has traditionally relied on therapeutic or metaphylactic antimicrobial treatment. However, prepartum vaccination of dams against Cryptosporidium parvum has recently been proposed as a more sustainable alternative to reduce the clinical signs caused by C. parvum. The objective of this study was to evaluate, in a commercial beef herd, the medium term impact of vaccinating cows against C. parvum on morbidity and mortality due to neonatal diarrhea, paromomycin use, and calf growth, by comparing five prevaccination calving seasons with the first three vaccinated seasons.

MATERIALS and METHODS: The study was conducted in a Limousin beef herd in Zamora (Spain), with 48 breeding cows and one bull, with laboratory confirmed high prevalence of cryptosporidiosis. Two periods were compared: (1) Prevaccination: spring and winter 2022, 2023 and spring 2024 calvings, during which control relied on therapeutic paromomycin (50 g/day orally for 5 days) administered to clinically affected calves; and (2) Vaccination period: three consecutive calving seasons (winter 2024, spring 2025 and winter 2025), cows were vaccinated against C. parvum (Bovilis® Cryptium®, MSD). For each period, numbers of births, treatments for neonatal diarrhea and diarrhea related deaths were recorded. Additionally, individual calf weights at birth, 120 and 210 days, and average daily gain (ADG) were obtained. Outcomes before and after vaccination were compared descriptively and t-student statistics to compare body weights.

RESULTS: During the prevaccination period (1), neonatal cryptosporidiosis was a recurrent problem, with diarrhea requiring treatment in 81–100% of calves, resulting in a high dependence on paromomycin. However, in the second period after vaccination, only 2 of 60 calves (3.3%) required paromomycin and no diarrhea related deaths occurred. Thus, dam vaccination led to a marked and sustained reduction in neonatal diarrhea and paromomycin use without compromising clinical control. Calves from vaccinated dams showed significantly improved performance despite the near elimination of therapeutic paromomycin. At 120 days (p=0.031), mean weight increased from 185.17 kg to 199.81 kg (ADG 1.13 vs 1.18 kg/day); at 210 days (p=0.039), mean weight increased from 312.35 kg to 329.61 kg; (ADG 1.27 vs 1.30 kg/day).

CONCLUSIONS: These findings support prepartum vaccination of beef cows against C. parvum as an effective and sustainable alternative to systematic paromomycin use for controlling neonatal cryptosporidiosis, while improving calf health, performance and contributing to antimicrobial use reduction goals.

Keywords: cryptosporidiosis, vaccination, neonatal diarrhea, paromomycin, calf growth

Herbal–Linseed Preparation as a Natural Strategy for Parasite Control in Cattle: A Field Study

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BACKGROUND and OBJECTIVE: Parasitic infections negatively affect the health and productivity of cattle, leading to impaired growth, weight loss, and reduced milk yield. In recent years, increasing attention has been given to phytobiotics as potential natural alternatives to support animal health. Their biological activity may extend beyond the digestive tract, contributing to the modulation of the gut environment and support the control of parasitic infections. The aim of the study was to evaluate the effects of dietary supplementation with a herbal mixture and linseed on rearing performance and health status of calves and young fattening cattle,

with particular emphasis on reducing mortality rates and the incidence of parasitic infections.

MATERIALS and METHODS: The study included 28 calves aged 2–12 weeks and 30 fattening cattle aged 6–12 months, maintained under organic farming conditions. The same animals were assessed before and after the introduction of the herbal–linseed supplementation. Animals received a daily dietary supplement consisting of a herbal mixture and linseed at body weight–adjusted doses (30 g and 50 g/100 kg BW, respectively). Clinical observations, growth performance, feed intake, mortality, fecal consistency, selected haematological, biochemical, immunological and antioxidant blood parameters, and parasitological status were evaluated. The obtained data were analysed statistically using Statistica software.

RESULTS: Dietary supplementation with the herbal–linseed preparation improved growth performance and feed utilisation in both calves and fattening cattle. A reduction in the incidence of clinical symptoms, particularly gastrointestinal disorders (including diarrhoea), was observed after the introduction of the supplementation. Parasitological analysis revealed a marked decrease in parasite burden, including reduced prevalence, intensity, and abundance of Eimeria spp., Buxtonella sulcata, and gastrointestinal nematodes. Additionally, improvements in selected haematological, immunological, and antioxidant parameters were noted, including increased total antioxidant status (TAS), enhanced activity of antioxidant enzymes (SOD, CAT, GPx), and modulation of immune indicators.

CONCLUSIONS: Herbal–linseed supplementation demonstrated a pronounced antiparasitic effect, reducing the prevalence and intensity of Eimeria spp., Buxtonella sulcata, and gastrointestinal nematodes. The observed improvements in haematological, immunological, and antioxidant parameters were consistent with reduced parasite burden and enhanced host resilience.

Keywords: cattle, herbs, nutrition, parasites, health

Case study: The Influence of Climatic Conditions on the Seasonal Trends of Gastrointestinal Nematode Infections in Sheep

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BACKGROUND and OBJECTIVE: Gastrointestinal nematodes (GINs) represent a major health challenge in grazing ruminants, significantly affecting animal productivity and farm sustainability. The epidemiology of GIN infections is influenced by multiple factors, including farm management system, breed susceptibility, and climatic conditions. The latter play a crucial role in the survival and transmission of GIN infective larvae in pastures, affecting the seasonal dynamics of infestations. The aims of this study were to: (i) assess breed- and category-related differences in GIN faecal egg counts (eggs per gram of faeces - EPG) over time under identical management conditions; (ii) evaluate the influence of climatic variables (temperature and relative humidity) on seasonal dynamics; and (iii) characterise the distribution of infective third-stage larvae (L3) across genera.

MATERIALS and METHODS: A longitudinal study was conducted from April 2024 to March 2025 on 44 sheep (Lacaune and Bagnolese) stratified by physiological category and reared under identical semi-extensive conditions. Monthly faecal sampling was performed and analysed using the Mini-FLOTAC technique at the laboratories of the Regional Center for Monitoring Parasitic Infections (CREMOPAR). Temperature and relative humidity were continuously recorded using dataloggers. A repeated measures ANOVA assessed the effects of breed, category, and time on GIN EPG. Climatic influence

was evaluated using a lagged linear model (t-1). L3 genus distribution was analysed using a Bayesian multinomial GLMM.

RESULTS: Results showed a clear seasonal trend, with lowest EPG values in summer and a peak in autumn (Bagnolese: 310 EPG; Lacaune: 214 EPG). Higher values were observed in Bagnolese sheep, particularly in specific physiological categories. ANOVA confirmed a significant effect of season ($p < 0.05$) and a Group × Season interaction ($p < 0.05$), while breed effects were not significant. Dynamic regression revealed temperature (lag -1) as a significant predictor of EPG ($p < 0.05$), whereas humidity had no effect, highlighting temperature as a key environmental driver. The Bayesian GLMM identified distinct seasonal patterns in larval genus composition, with significant effects of breed and physiological category.

CONCLUSIONS: These findings highlight the role of host factors and seasonality in shaping the distribution of infective larvae on pasture. The study aims to provide insights into the adaptation of different sheep breeds to helminth infections and environmental conditions, supporting the implementation of targeted helminth management programs. This allowed the evaluation of climatic influences on larval survival and helminth transmission risk. Understanding how climate and management strategies interact with breed-specific resistance to GINs is essential for developing predictive models and sustainable helminth control strategies.

Keywords: gastrointestinal nematodes, sheep, monitoring, epidemiology, datalogger.

Associations Between Faecal Balantidium Spp. Findings, Milk Parameters, and Welfare-associated Indicators in Dairy Cows

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BACKGROUND and OBJECTIVE: Gastrointestinal health is important for dairy cow welfare and productivity. Parasite infections transmitted through contaminated feed, water, or the farm environment may cause subclinical gastrointestinal disorders that can be reflected in milk and behavioural parameters. Therefore, integrating parasitological findings with herd monitoring data may improve the interpretation of biologically significant changes in dairy cows. The aim of this study was to perform a parasitological evaluation of coprological samples from dairy cows and to determine their associations with routinely recorded milk parameters and animal welfare indicators.

MATERIALS and METHODS: The study was conducted in January 2026 on a large dairy farm and included 164 lactating cows (2nd–4th lactation; 20–100 days in milk). Faecal samples were collected per rectum or from freshly voided faeces in accordance with animal welfare and biosafety principles. Body condition was assessed using a five-point BCS scale. Coprological examinations were performed at the Lithuanian University of Health Sciences (Veterinary Academy) using sedimentation and McMaster methods. Herd monitoring and milk production data were obtained from the LELY Astronaut 3 automatic milking system. Cows were classified into three groups based on faecal Balantidium spp. counts (negative, low, and higher counts).

RESULTS: Milk lactose content was significantly associated with daily milk yield ($r = -0.227$, $p = 0.004$), SLS readings ($r = -0.208$, $p = 0.007$), and faecal Balantidium spp. count ($r = 0.224$, $p = 0.004$). No significant correlations were observed between milk lactose content and total eating time, rumination time, fat-to-protein ratio, or body condition score ($p > 0.05$). One-way ANOVA across Balantidium-based groups showed significant between-group differences in lactation days ($F(2,161) = 6.190$, $p =$

0.003), milk lactose content ($F(2,161) = 8.800$, $p < 0.001$), and faecal Balantidium spp. count ($F(2,161) = 227.895$, $p < 0.001$). No statistically significant between-group differences were found for total eating time, rumination time, daily milk yield, fat-to-protein ratio, SLS readings, or body condition score ($p > 0.05$). Descriptive analysis demonstrated a marked increase in mean faecal Balantidium spp. counts across groups (0.00, 9.44, and 39.45 in groups 0, 1, and 2, respectively).

CONCLUSIONS: The presence of Faecal Balantidium spp. was found to be associated with variations in specific routinely recorded parameters, particularly the milk lactose content. These findings suggest that milk lactose may be a relevant complementary parameter when interpreting parasitological findings in dairy cows. Further studies are required to confirm these associations and to elucidate their biological significance.

Keywords: dairy cows, herd monitoring, animal welfare indicators

Subclinical Theileria Buffeli/orientalis infection on São Miguel Island (azores): Preliminary Evidence of Herd-level Effects on Nitrogen Metabolism and Milk Yield

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BACKGROUND and OBJECTIVE: The Azores archipelago represents one of Portugal's most favourable regions for dairy cattle production and contributes substantially to the national milk supply. Production is predominantly semi-intensive, with cattle maintained on pasture year-round. We previously identified the presence of *Theileria buffeli/orientalis* in the region, specifically the Chitose and Buffeli genotypes, and in the present study, we assess its impact on herd-level production parameters. Historically, infections within the *T. buffeli/orientalis* agent were regarded as largely benign. However, recent reports have called this paradigm into question, describing outbreaks associated with high morbidity and mortality rates. Infection with this agent can compromise host health status through haemolytic anaemia and has been associated with reductions in milk yield and milk solids, posing a potential threat to pasture-based dairy systems.

MATERIALS and METHODS: Given the regional economic importance of dairy production, a longitudinal assessment of production consequences was initiated. Here we report preliminary findings from one herd in which 71 cows were screened by PCR, with 64 PCR-negative and 7 PCR-positive samples. Monthly milk records were analysed using linear mixed-effects models with random intercepts for cow and fixed effects for infection status, continuous time (months) and their interaction, complemented by per-cow aggregated comparisons.

RESULTS: Preliminary analyses reveal consistent and biologically plausible perturbations in nitrogen metabolism among PCR-positive cows, together with a numerical reduction in milk production. Estimated differences in the monitored herd included a reduction in daily milk yield of approximately 3.22 kg ($p = 0.105$) and a decrease in energy-corrected milk of 2.61 kg ($p = 0.172$). Milk urea concentration was lower in PCR-positive cows (estimated -28.05 mg/kg, $p = 0.054$), and the milk Urea/Protein ratio was significantly reduced in positives (-8.65; $p = 0.0486$). The TB/Urea ratio was higher in positive cows ($p = 0.0016$). No significant differences were detected for milk fat % ($p = 0.462$) or protein % ($p =$

0.776) nor for somatic cell count on the log scale (p = 0.37).

CONCLUSIONS: The limited number of PCR-positive cows in the monitored herd constrains statistical power and precision, however, the results highlight the potential subclinical burden of *T. buffeli/orientalis*, with detectable metabolic shifts and a trend towards reduced milk output. Ongoing monitoring of additional farms and combined multi-herd analyses adjusted for lactation stage, parity and management factors are under way to quantify production and inform regional control strategies.

Keywords: Azores, Cattle, Production losses

Risk Factors Associated with Babesia Spp. Infection in Dairy Cattle from Southern Brazil

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BACKGROUND and OBJECTIVE: Tick-borne hemoparasites remain an important challenge for dairy production in Brazil, particularly in regions characterized by enzootic instability. This study aimed to determine the molecular occurrence of *Babesia* spp. in dairy cattle from southern Brazil and to identify management-related risk factors associated with infection.

MATERIALS and METHODS: A cross-sectional study was conducted in 76 dairy farms, and 509 cattle were sampled. DNA was extracted from whole blood and analyzed by polymerase chain reaction (PCR) targeting *Babesia* spp. Epidemiological data were collected through structured questionnaires. Variables with biological relevance were evaluated using multivariable logistic regression to estimate adjusted odds ratios (OR) and 95% confidence intervals (95% CI).

RESULTS: PCR detected *Babesia* spp. DNA in 22.0% (112/509) of the animals. In the multivariable model, higher milk production level (OR = 1.52; 95% CI: 1.27–1.82; p < 0.001), increased tick infestation intensity (OR = 1.39; 95% CI: 1.07–1.81; p = 0.014), and greater frequency of acaricide application (OR = 1.32; 95% CI: 1.13–1.53; p < 0.001) were significantly associated with *Babesia* spp positivity. Production system was not significantly associated after adjustment. Additionally, *Anaplasma platys* DNA was detected in a small proportion of animals (2.7%), although no significant associations were identified for this agent.

CONCLUSIONS: These findings indicate that *Babesia* spp. infection in dairy cattle from southern Brazil is influenced not only by vector presence but also by production intensity and management practices. The association between infection and increased frequency of acaricide use suggests complex herd-level dynamics that warrant further investigation, particularly in enzootically unstable regions.

Keywords: PCR, tick-borne pathogens, epidemiology, {Anaplasma platys}

Field Evidence for High Efficacy of Pour-On Macrocyclic Lactones Against Bovine Lungworm in Sweden

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BACKGROUND and OBJECTIVE: Dictyocaulosis caused by Dictyocaulus viviparus leads to respiratory disease and production losses in grazing cattle. As treatment failure after use of macrocyclic lactones is increasingly suspected, field data are needed to assess treatment outcome under Swedish conditions. This study evaluated the apparent

efficacy of pour-on macrocyclic lactones in cattle herds with suspected lungworm infection in Sweden.

MATERIALS and METHODS: From July 2024 to November 2025, 19 farms comprising 20 herds in 11 Swedish counties were recruited. Farmers sampled 10-20 affected or in-contact cattle immediately before treatment and again 14 days later from the same animals. Animals were treated with a pour-on macrocyclic lactone according to label directions. Faecal samples were examined qualitatively for first-stage (L1) D. viviparus larvae using the Baermann technique.

RESULTS: A total of 272 cattle were sampled, and 39/272 (14.3%) were Baermann-positive before treatment. At follow-up, 38/39 (97.4%) previously positive cattle were negative, whereas one animal remained positive. Six cattle were positive at follow-up, including five that had tested negative at the first sampling.

CONCLUSIONS: Pour-on macrocyclic lactones showed high apparent field efficacy in this dataset, suggesting no widespread loss of efficacy under current Swedish conditions. The single persistent case and the occurrence of cattle that were positive at follow-up highlight the importance of timely diagnostics, correct dosing, and continued surveillance. Single-time-point Baermann testing may miss some infections under field conditions.

Keywords: Dictyocaulus viviparus, bovine lungworm, macrocyclic lactones, pour-on treatment, anthelmintic efficacy

PHARMACOLOGY AND THERAPEUTICS

Phytochemical Characterisation and Evaluation of the Antibacterial Activity of Schinus Molle Resin

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BACKGROUND and OBJECTIVE: Resins occupy a special place among natural plant products. They are secreted by certain plants in response to external aggressions or injuries, and are composed of complex mixtures of terpenic, phenolic and aromatic compounds. The aim of our present work is to promote a medicinal plant, Schinus molle or false pepper tree, which grows wild in the Blida region (Algeria), by phytochemically characterising its resin extract through a qualitative analysis of its main chemical components and an evaluation of its antibacterial activity.

MATERIALS and METHODS: This study is a scientific contribution to the determination of certain phytochemical compounds and the study of the in vitro antibacterial activity of the ethanoic extract of false pepper resin. The alcoholic extract of Schinus molle resin was prepared using Soxhlet extraction. The antibacterial activity was evaluated using the agar diffusion method, known as 'aromatogram'.

RESULTS: Qualitative analysis of this extract using colorimetric tests revealed the presence of phenolic

compounds, tannins, coumarins and alkaloids. Antibacterial activity testing using the disc diffusion method showed that the ethanolic extract of Schinus molle resin has moderate activity against the pathogenic germs tested.

CONCLUSIONS: Through this study, we aim to highlight a locally available natural resource, using both scientific and ecological approaches, with a view to promoting its use in the pharmaceutical and biological fields.

Keywords: Resin, false pepper tree, Phytochemical screening, Antibacterial activity.

Study of Hepatoprotective Activity of Medicinal Plant Mediterranean buckthorn *Rhamnus alaternus* Rhamnaceae

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BACKGROUND and OBJECTIVE: Mediterranean buckthorn *Rhamnus alaternus* is a medicinal plant generally used in traditional medicine in Algeria to treat liver diseases in general and jaundice in particular. The objective of our study is to make a botanical analysis of the plant, a qualitative and quantitative chemical study, and a study of the hepatoprotective activity of this plant.

MATERIALS and METHODS: The plant material was collected from the Wilaya of Blida (Algeria) in March 2021. Botanical identification was carried out based on macroscopic and microscopic characteristics. The aqueous extract of the plant was prepared according to conventional extraction procedures described in the bibliography. Hepatotoxicity was induced in Wistar rats using allyl alcohol. The hepatoprotective effect of the aqueous extract was assessed by measuring serum transaminase activities (ASAT and ALAT) and evaluating the hepatic necrosis index. Biochemical and histological parameters were analyzed according to standard statistical methods, and results were expressed as indicators of hepatoprotective activity.

RESULTS: The plants has the botanical and microscopic characteristics listed in the bibliography. The study of the hepatoprotective activity of the aqueous extract of the plant in wistar rats after allyl alcohol poisoning revealed a significant regression of the biochemical parameters of the ASAT and ALAT transaminases and of the hepatic necrosis index.

CONCLUSIONS: hese findings support the hepatoprotective activity of the plant and justify further studies to identify the active compounds and elucidate the underlying mechanisms.

Keywords: Mediterranean buckthorn, characterisation, hepatoprotecive activity.

Effect of Injectable Trace Mineral Supplementation on Antioxidant Status and Health of Dairy Cows and Calves

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BACKGROUND and OBJECTIVE: The transition period in dairy cows, the pre-calving period in heifers, and the neonatal phase in calves are critical windows marked by oxidative stress and increased disease susceptibility. Trace minerals like selenium, zinc, copper, manganese, and chromium are essential for immune function, energy metabolism and antioxidant enzyme activity. This study aimed to evaluate the effects of a single subcutaneous injection of trace minerals (ITMS) on the antioxidant status, morbidity and mortality rates, metabolic health, and performance of transition cows, pre-calving heifers, and newborn calves.

MATERIALS and METHODS: Two experiments were conducted at the Dookie Dairy Farm of the University

of Melbourne, Australia. In Experiment 1, 55 Holstein Friesian cows and heifers received either ITMS (containing Zn, Mn, Se, Cu, and Cr) or a saline control (CNTRL) 1 mL/75 kg for heifers and 1 mL/100 kg for cows 60 days before expected calving. In Experiment 2, 30 female calves were injected with ITMS or saline on their day of birth (1 mL/50 kg at Day 0). Blood samples were collected at fixed intervals (60 and 21 days before and 7 and 14 days after calving for cows and heifers; days 0, 15, and 35 for calves) to analyse plasma trace minerals, non-esterified fatty acid (NEFA), beta hydroxybutyric acid (BHBA), erythrocyte glutathione peroxidase (GSHPx) and superoxide dismutase (SOD) activity. Health status of the calves was recorded daily during the first 50 days of their life.

RESULTS: In cows, ITMS significantly increased plasma selenium concentrations and enhanced GSHPx activity compared to CNTRL ($p < 0.05$). Heifers in the ITMS group showed lower post-partum NEFA concentrations ($p < 0.05$), while multiparous ITMS cows exhibited reduced BHBA levels on day 7 post-calving ($p = 0.018$). In calves, ITMS significantly improved GSHPx activity on days 15 and 35 compared to CNTRL but no difference in SOD was observed. While the calf study was underpowered for health statistics, the overall disease incidence was lower in the ITMS group (13.3%) compared to the CNTRL group (33.3%), with no mortality in ITMS calves versus 13.3% in CNTRL.

CONCLUSIONS: A single injection of trace minerals effectively enhances the antioxidant status in both transition dairy cows, pre-calving heifers, and newborn calves. The reduction in energy metabolism markers (NEFA, BHBA) and improved GSHPx antioxidant enzyme activity suggest that ITMS is a valuable tool for supporting health and performance during high-stress physiological periods in dairy production.

Keywords: Injectable Trace Minerals, Trace Minerals, Transition Period, Calves, Cows, Health

Update; the Impact of Tolfenamic Acid on Significant Inflammatory Mediators

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BACKGROUND and OBJECTIVE: Thromboxane B2 (TxB2) and prostaglandin E2 (PGE2) are eicosanoid mediators of inflammation, and they are both acknowledged for their role in the inflammatory process. Moreover, TxB2 and PGE2 are considered reliable and quantifiable biomarkers. The objective of this analysis was to assess the pharmacodynamic effects of tolfenamic acid and meloxicam on inflammation biomarkers (TxB2 and PGE2).

MATERIALS and METHODS: This study was performed in 6 ruminant juvenile cattle, 4 males and 2 females, 13 to 15 months old, and weighing 255 to 382 kg. Each animal received an injection at recommended doses: tolfenamic acid 2 mg/kg IM (0.05 mL/kg), meloxicam 0.5 mg/kg SC (0.025 mL/kg), after a 5-day washout period. Pre-dose samples were taken for each individual before NSAID injections. Ionophore induced *ex vivo* synthesis of TxB2 and PGE2 in whole blood was selected as a marker of the pharmacologic effect for both tolfenamic acid and meloxicam. Venous blood samples (~5 mL each) from the jugular vein, were taken following this hourly schedule after nonsteroidal anti-inflammatory drug (NSAID) treatment: 0.5, 1, 2, 4, 6, 10, 24, 32, and 48. PGE2 and TXB2 results were obtained by LCMSMS assay.

RESULTS: Maximum TxB2 and PGE2 synthesis inhibition values were obtained in all animals for both NSAIDs. The maximum TxB2 synthesis inhibition values had a mean of 80.82 % (sd = 8.27%) for tolfenamic acid and 53.24% (sd = 9.24%) for meloxicam. Maximum inhibition of PGE2 synthesis had a mean value of 68.64% (sd = 5.98%) for tolfenamic acid and 27.12% (sd = 7.85%) for meloxicam. Differences in maximum synthesis inhibition in both biomarkers were found to be statistically significant between the two NSAIDs through paired samples t-tests (Bonferroni adjusted *p*-values <0.01 and <0.001 for TxB2 and PGE2 respectively). The previous occurred for TxB2 at the mean time (Tmax_inhib) of 0.75 h (sd = 0.61 h) and 4.17 h (sd = 1.83 h) respectively for tolfenamic acid

and meloxicam. Maximum inhibition of PGE2 occurred at the mean time (Tmax_inhib) of 1.75 h (sd = 1.75 h) and 3.25 h (sd = 2.09 h) for tolfenamic acid and meloxicam respectively.

CONCLUSIONS: Comparable to past peer-reviewed publications, these new results once again underline the effectiveness of tolfenamic acid to inhibit TxB2 and PGE2. These results emphasize that tolfenamic acid provides a quick and potent anti-inflammatory response, befitting for rapidly developing conditions.

Keywords: TxB2, PGE2, Inflammatory, Mediators, Tolfenamic-acid

Strategic Modulation of Hepatocellular Integrity and Biliary Dynamics Using Parenteral Genabilic Acid in Elite *Bos Indicus* Cattle Under Metabolic Challenge

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BACKGROUND and OBJECTIVE: In tropical livestock systems, elite *Bos indicus* cattle selected for maximum phenotypic expression are subjected to hyper-caloric nutritional planes. This intensification imposes severe metabolic loads on the liver, often exceeding its physiological capacity for detoxification and energy partitioning. Subclinical hepatic insufficiency acts as a silent rate-limiting factor in animal performance. Menbutone, a potent choleretic agent, stimulates hepato-digestive secretions. This study aimed to validate the efficacy of Genabilic acid, as a metabolic modulator to restore hepatic homeostasis and mitigate cellular stress in high-genetic merit cattle prepared for premier exhibition events.

MATERIALS and METHODS: This study adhered to strict ethical standards for animal welfare. A cohort of 12 *Bos indicus* animals (Gyr, n=8; Nelore, n=4) managed in the Colombian tropical medium-altitude (953–1035 m) conditions was stratified by nutritional intensity. Nelore subjects received a basal growth concentrate (1 kg/day) with mineral supplementation, while Gyr subjects underwent a hyper-intensive finishing protocol comprising high-density concentrate (2 kg/day), *ad libitum* hay, and phenotypic finishing additives (high-energy cores and vitamin-phosphorus complexes). The experimental protocol was divided into three distinct phases: Phase 1 (Baseline) involved serum collection to establish a baseline metabolic profile; Phase 2 (Treatment) consisted of menbutone administration (50 mg/mL, 1 mL/20 kg BW in a cycle of three consecutives injections, spaced 72h apart; Phase 3 (Post-treatment) involved follow-up serum collection to evaluate biochemical response. The primary markers analyzed were Aspartate Aminotransferase (AST) for hepatocyte structural integrity, L-gamma-glutamyl transferase (GGT) for biliary canaliculi health, and Alkaline Phosphatase (ALP) for metabolic and bone development status.

RESULTS: Therapeutic intervention resulted in a marked restoration of hepatic biomarkers despite the heavy nutritional load. A 100% (12/12) clinical improvement in ALP levels was observed, indicating a rapid alleviation of cholestatic pressure. Furthermore, 91.6% (11/12) of subjects exhibited normalization or significant reduction of AST, confirming the restoration of hepatocellular structural integrity. GGT levels stabilized or improved in 58.3% of the cohort. The singular non-responsive outlier for AST was associated with exogenous handling stress rather than therapeutic failure.

CONCLUSIONS: Parenteral menbutone proves to be a robust tool for reversing hepatic strain in cattle under intensive supplementation. The data confirms its dual capacity to resolve cholestasis and protect hepatocyte integrity. Consequently, menbutone should be considered a critical prophylactic agent to unlock full genetic potential and ensure metabolic resilience in elite livestock production systems.

Keywords: genabilic acid, hepatic insufficiency, detoxification

Milk Production Response Following Varied Anthelmintic Regimes in Naturally Infected Dairy Cattle in Victoria, Australia

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BACKGROUND and OBJECTIVE: Gastro-intestinal parasitism remains a primary challenge for grazing dairy cattle globally, with studies indicating that effective anthelmintic treatment can increase milk production by a median of 0.63kg/cow/day. In Australia, there is significant variation in the timing and type of treatments used, alongside emerging resistance to macrocyclic lactones and triclabendazole. The objective of this study is to compare milk yield (L) and milk components (fat and protein) among adult dairy cattle treated with three distinct drenching programmes: a standard ivermectin/clorsulon regime, a regime with improved worm control (moxidectin/levamisole), and a regime with combined improved worm and liver fluke control (moxidectin/levamisole plus triclabendazole/oxfendazole).

MATERIALS and METHODS: A randomized clinical interventional field study is being conducted in a commercial dairy farm in Victoria, Australia, involving approximately 1,600 Holstein Friesian cows. Animals were randomly allocated into three treatment groups, blocked by dry-off dates. Group 1 (control) received ivermectin/clorsulon at dry-off and again 42 days later. Group 2 received ivermectin/clorsulon at dry-off followed by moxidectin/levamisole 42 days later. Group 3 received ivermectin/clorsulon and triclabendazole/oxfendazole at dry-off, followed by moxidectin/levamisole 42 days later. Milk volume and milk solids (fat + protein) are being collected via daily milk meters and routine herd tests throughout the lactation season. Linear mixed models will be used to assess treatment effects, accounting for repeated measures and covariates such as age and breed.

RESULTS: Data collection is currently ongoing, with final herd test analysis scheduled for completion by June 2026. The study is powered at 80% to detect a biologically important difference in milk solids (0.03kg/day overall or 0.06kg/day at the farm level. It is anticipated that the groups receiving enhanced worm and fluke control (Groups 2 and 3) will demonstrate superior milk productivity and constituent yields compared to the standard treatment group, reflecting the higher efficacy of the moxidectin/levamisole and triclabendazole combinations against resistant or varied parasite burdens.

CONCLUSIONS: The findings of this study will help define the production benefits of modern, multi-active anthelmintic programs in pasture-based dairy systems. By comparing standard practices against protocols designed for broader parasite coverage, this research aims to provide veterinarians and producers with evidence-based drenching strategies to optimize the health and productivity of adult dairy cattle

Keywords: Anthelmintic, dairy cattle, liver fluke, nematodes

Comparative Evaluation of the Clinical Efficacy of Tulathromycin and Commercial Tilmicosin, and the Use of Enrro-c as Rescue Therapy, in Bovine Respiratory Disease in An Intensive Feedlot in the Tropics

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BACKGROUND and OBJECTIVE: Bovine respiratory disease is one of the main health problems in intensive feedlot systems, since its multifactorial origin and the difficulty of early detection affect both animal health and productive performance. The

objective of the study was to compare the clinical efficacy of tilmicosin and tulathromycin as initial treatments, as well as to evaluate the use of recrystallized enrofloxacin (Enrro-C) as a rescue treatment in cattle with respiratory disease under commercial conditions in the Mexican tropics.

MATERIALS and METHODS: The study was conducted on 84 feedlot cattle from different sources, which underwent individual clinical evaluation and were classified according to severity, selecting animals with moderate to severe respiratory disease. The cattle were assigned to two groups: Group A, treated with tilmicosin (42 animals), and Group B, treated with tulathromycin (42 animals), both administered subcutaneously according to the commercial product label instructions. Clinical and productive performance were monitored for 15 days. Animals that did not show evident clinical improvement by day five received rescue treatment with Enrro-C.

RESULTS: The results showed that tulathromycin had a higher proportion of initial clinical response (42.9%) compared to tilmicosin (28.6%). However, in both groups, a considerable number of animals did not respond to the initial treatment and required rescue therapy. Administration of Enrro-C achieved clinical recovery in all treated animals, with resolution of respiratory signs, restoration of feed intake, and improvement in overall condition. Average daily weight gain was 1.4 kg in Group A and 1.5 kg in Group B, whereas healthy animals gained 2 kg per day.

CONCLUSIONS: conclusion, the results indicate that the use of stepwise therapeutic regimens, based on timely and objective clinical evaluation, represents an effective strategy for managing bovine respiratory disease in intensive feedlot systems.

Keywords: Bovine respiratory disease, Feedlot cattle, Tilmicosin, Tulathromycin, Enrofloxacin, Clinical efficacy

Clinical Response to Intravenous Omeprazole Treatment in Dairy Calves with Signs Consistent with Gastrointestinal Hemorrhage Associated with Abomasal Ulcers Under Field Conditions

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BACKGROUND and OBJECTIVE: Abomasal ulceration is common in cattle raised under intensive production systems and is associated with stress, management changes, and nutritional transitions, which may lead to gastrointestinal hemorrhage and negatively affect animal welfare and productivity. Omeprazole, a proton pump inhibitor, has been shown to increase abomasal pH and promote recovery of the gastrointestinal mucosa; however, clinical evidence regarding its use in cattle under field conditions remains limited. The objective of this study was to evaluate the clinical response of dairy calves showing signs consistent with gastrointestinal hemorrhage associated with abomasal ulcer following intravenous omeprazole administration under field conditions.

MATERIALS and METHODS: A group of 96 Holstein dairy calves of Canadian and European genetics from an intensive dairy production system located in Torreón, Coahuila, Mexico, was evaluated. Following management procedures including weaning, regrouping, ear tagging, and an abrupt transition from a liquid to a solid diet, calves exhibited depression, reduced feed intake, loss of body condition, and the presence of dark feces in the pens. Clinical parameters were recorded, including mucous membrane coloration, capillary refill time, heart rate, respiratory rate, general demeanor, body condition, and macroscopic fecal characteristics. Fecal samples were collected for qualitative detection of occult blood, yielding positive results consistent with gastrointestinal hemorrhage. Based on these findings, omeprazole was administered intravenously at 2 mg/kg every 24 h for 1–4 days, accompanied by adjustments in nutritional management to minimize stress-related factors.

RESULTS: Following omeprazole administration, 88 of 96 calves (≈92 %) showed rapid clinical recovery,

characterized by normalization of general demeanor, increased feed intake, appropriate mucous membrane coloration, and progressive disappearance of dark feces. The remaining calves improved gradually, possibly due to progressive adaptation to management and diet. No mortalities were recorded.

CONCLUSIONS: Intravenous administration of omeprazole was associated with rapid clinical recovery and preservation of the general health status of the calves, minimizing loss of body condition. The combination of pharmacological treatment and appropriate management represents an effective strategy to maintain animal health and productivity in intensive production systems, promoting rapid recovery while sustaining productivity without compromising animal welfare.

Keywords: Abomasal ulcer, Omeprazole, Dairy calves, Gastrointestinal bleeding, Clinical response, Field conditions

Efficacy of Flunixin Transdermal for the Control of Experimentally Induced Pulmonary Inflammation by Lipopolysaccharides in Calves

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BACKGROUND and OBJECTIVE: Flunixin meglumine is a NSAID that acts as a potent, nonselective cyclooxygenase inhibitor. A transdermal formulation has been developed for pour-on administration in cattle for the reduction of pyrexia associated with bovine respiratory disease and for the control of inflammation associated with endotoxemia. This study aimed to evaluate the efficacy of flunixin transdermal (Banamine® Transdermal, MSD Animal Health) in controlling pulmonary inflammation experimentally induced by lipopolysaccharide (LPS), as assessed by clinical and hematological parameters, fibrinogen and PGE_2 concentrations.

MATERIALS and METHODS: A total of 21 calves, 10–12 months of age, with no history of BRD were enrolled into one of three treatment groups: 1) single intravenous (IV) dose of LPS at 2.5 µg/kg body weight (BW) + flunixin transdermal at 3.3 mg/kg (LPS + TF); 2) single IV dose of LPS at 2.5 µg/kg BW + 1 mL/15 kg BW of saline solution (pour-on – placebo) (LPS + PLBO); and 3) single IV administration of 10 mL of sterile saline solution per animal (sham induction) + 1 mL/15 kg BW of saline solution (pour-on – placebo) (CNTL + PLBO). Blood samples were collected and clinical scores were recorded at 0, 2, 4, 6, 8, 10, 12, 24, 48, 72, 96, 120, and 144 hours following LPS or saline solution administration. The parametric variables (physical examination findings, hematological parameters, fibrinogen levels and PGE_2 concentrations) were analyzed using analysis of variance (ANOVA), followed by Student's t-test when appropriate. For all analyses, a significant level of 5% ($p<0.05$) was adopted. Statistical analyses were performed using R software.

RESULTS: All animals inoculated with LPS were symptomatic (tachycardia, tachypnea, and apathy), including the death of one calf in group LPS + PLBO with a histopathological report of acute multifocal suppurative bronchopneumonia. Pyrexia was observed only in the LPS+PLBO group at 4 and 6 hours post-LPS induction. Changes in the erythrogram were nonspecific and sporadic. Leukopenia with lymphopenia and monocytopenia was identified in the groups that received LPS up to 10 hours post-inoculation. The LPS + TF group showed a reduction in mean PGE2 values (pg/mL) between 8 hours (334.06±103.06) and 24 hours (297.41±81.29), with a significant difference compared to LPS + PLBO and no significant difference ($p>0.05$) compared to the CNTL + PLBO group. Fibrinogen remained within the normal range in all groups.

CONCLUSIONS: The experimental model confirmed that LPS promotes acute symptomatic pulmonary inflammation, and treatment with flunixin transdermal modulated the inflammatory response as measured by PGE2 mean concentrations.

Keywords: Flunixin, transdermal, LPS, challenge, PGE2

PUBLIC HEALTH FOOD SAFETY AND ANTIMICROBIAL RESISTANCE

Prevalence and Molecular Characterization of Esbl-producing Escherichia coli in Cattle at Slaughter in Southern Chile

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BACKGROUND and OBJECTIVE: Antimicrobial resistance (AMR) is a global concern affecting both human and veterinary medicine. Among resistant bacteria, extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* are of particular relevance, as these enzymes confer resistance to third- and fourth-generation cephalosporins, which are critical for treating severe infections. Food-producing animals are recognized as important reservoirs of AMR, and fecal shedding facilitates its dissemination and potential spread along the food chain. In southern Chile, cattle production is predominantly pasture-based, contrasting with the intensive production systems typically described in the literature. This study aimed to estimate the prevalence of ESBL-producing *E. coli* in fecal samples collected at the slaughter line and to characterize the ESBL-associated genes in cattle from southern Chile.

MATERIALS and METHODS: The study was conducted at a commercial slaughterhouse in the Los Ríos Region, Chile, between July and August 2025. A total of 400 fecal samples were collected directly from the slaughter line after sacrifice. For each sample, 0.1 g of feces was inoculated into 5 mL of Mueller–Hinton broth and incubated at 37°C for 18–24 h. After enrichment, aliquots were streaked onto chromogenic ESBL-selective agar, and presumptive *E. coli* colonies were confirmed by real-time PCR. Phenotypic confirmation of ESBL production was carried out using the double-disk synergy test. A multiplex PCR was used to detect the β-lactamase genes *bla*CTX-M, *bla*TEM, and *bla*SHV.

RESULTS: Out of the 400 fecal samples, 113 were positive for ESBL-producing *E. coli*, giving an overall prevalence of 28.25% (95% CI: 24.06–32.85). All isolates were confirmed as ESBL producers by the double-disk synergy test. Among them, 109/113 (96.46%) isolates carried *bla*CTX-M, either alone or together with *bla*TEM. Specifically, 67 (59.29%) isolates harbored *bla*CTX-M alone, while 42 (37.17%) carried both genes. No isolate carried *bla*TEM alone, and blaSHV was not detected. Four isolates (3.54%) were phenotypically ESBL-positive but negative for the genes evaluated.

CONCLUSIONS: More than one quarter of the cattle sampled at slaughter carried ESBL-producing *E. coli*. The predominance of *bla*CTX-M confirms its central role in ESBL resistance. In this predominantly pasture-based production system, these findings provide insight into AMR circulation in grazing cattle. Slaughter-based surveillance offers a valuable opportunity to monitor resistance patterns and may support enhanced antimicrobial stewardship interventions at the farm level under commercial production conditions.

Keywords: Antimicrobial resistance, Food safety, Grazing systems, Slaughterhouse

High Prevalence of Extended-spectrum Beta-lactamase Producing enterobacterales in Neonatal Dairy Calves From a High-density Dairy Production Area in Northern Italy

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BACKGROUND and OBJECTIVE: Extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales represent a major One Health concern due to their role in limiting the efficacy of critically important antimicrobials. Dairy calves have been identified as

important reservoirs of these bacteria, particularly in intensive production systems. However, regional epidemiological data from high-density dairy cattle areas in Italy remain limited. This study aimed to investigate the prevalence of ESBL-producing Enterobacterales in neonatal dairy calves' feces in a high-density dairy farming area of Northern Italy, and to characterize associated antimicrobial resistance (AMR) patterns and ESBL-encoding genes.

MATERIALS and METHODS: A cross-sectional observational study was conducted between June and September 2023 on 35 dairy farms located in Lombardy, Emilia-Romagna and Piedmont. Faecal samples were collected from 437 Holstein-Friesian calves younger than 21 days that had not received antimicrobial treatment prior to sampling. A 0.1 g of faeces was enriched in 5 mL of Mueller–Hinton broth and incubated aerobically at 37 °C for 24 hours. Subsequently, 100 µL of the enriched broth were plated onto selective chromogenic media for ESBL-producing bacteria. Colonies were identified using MALDI-TOF MS. All presumptive ESBL-producing Enterobacterales were confirmed using the double-disk synergy test (DDST). Antimicrobial susceptibility testing (AST) was carried out by disk diffusion, and blaCTX-M, blaTEM, and blaSHV genes were detected by multiplex PCR.

RESULTS: Overall, 381 samples tested presumptively positive for ESBL-producing Enterobacterales. Of these, 354 were Escherichia coli, 20 were Klebsiella pneumoniae, 3 were Klebsiella variicola, 2 were Citrobacter braakii, 2 were Enterobacter spp. All 381 isolates were phenotypically positive for ESBL production at the DDST, yielding a prevalence of 87.2% of ESBL-producing Enterobacterales. Eighteen samples tested negative by multiplex PCR. Among the positive samples, the blaTEM and blaCTX-M combination was the most frequently detected pattern, identified in 157 of 308 samples. Based on the AST results, the most common antimicrobial susceptibility profile (6,2%) was susceptible to enrofloxacin and amoxicillin/clavulanic acid. The second most frequent profile (5,2%) was a multidrug-resistant (MDR) pattern, resistant to cephalosporins, ampicillin, and tetracycline. The third most frequent profile (4,5%) was pan-resistant.

CONCLUSIONS: This high prevalence is consistent with recent studies and indicates that AMR is a significant issue in newborn calves as well. Notably, the high prevalence of MDR ESBL-producing E. coli suggests that neonatal dairy calves could serve as reservoirs of resistant bacteria on farms. From a One Health perspective, these findings highlight the need for epidemiological surveillance to track and understand the spread of resistance among animals, humans, and the environment.

Keywords: ESBL, Antimicrobial resistance, Calves

Evaluation of Five Commercially Available Screening Tests for the Detection of Cefalonium Residues in Milk

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BACKGROUND and OBJECTIVE: Dairy producers are legally required to provide safe milk for human consumption. Commercially Available Screening Tests (CAST), are preliminary analytical methods used to rapidly detect antibiotic residues in milk. CAST are designed to be sensitive and rapid but not highly specific. They help identify samples that potentially exceed regulatory limits and require confirmatory analysis, usually by Liquid Chromatography–Mass Spectrometry. The limit of detection (LOD) is the lowest concentration of a residue in milk that can be detected but not necessarily quantified with acceptable certainty. This study aimed to evaluate the performance of five CAST in the detection of cefalonium residues in individual milk samples, considering their respective LOD.

MATERIALS and METHODS: Milk samples with known concentrations (0, 2.5, 3, 4, 5, 8, 10, 14, 15, 17, 18, 19, 20, 21, 22, 24, 25 and 30 µg/L) of cefalonium (LGC Lot 92224 and Sigma Aldrich BCBV1595), previously analyzed by an analytical laboratory using HPLC/MS-MS, were used as the gold standard. For

each concentration, three analyses were performed using the following CAST: Delvotest® SP NT (DV), BetaStar S Combo® (BS), IDEXX SNAPDuo® ST Plus Test (IDD), Charm® MRL Beta-lactam 1-Minute Test (CH), and IDEXX SNAP® Beta-Lactam ST Plus Test (IDB). Sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), accuracy, and Kappa coefficient were calculated for each CAST.

RESULTS: All CAST showed 100% Se and NPV, correctly identifying all positive samples above the individual kit sensitivity. Specificity was low for all CAST (33.3, 33.3, 14.3, 23.5, and 20.0%), and PPV was moderate (85.5, 85.5, 60.0, 76.4, and 73.3) for DV, BS, IDD, CH, and IDB, respectively. The low specificity negatively impacted accuracy (86.4, 86.4, 62.5, 78.0, and 75.0%) and resulted in Kappa agreement values considered weak (0.44, 0.44, 0.20, 0.30, and 0.30). Kits were not compared to each other due to differing detection limits. BS and IDD did not show cross-reactivity with terramycin at any cefalonium concentration, resulting in 100% Sp, NPV, and accuracy. The same was observed for IDD and IDB with cephalexin, and BS with desfuroylceftiofur. However, BS showed cross-reactivity with cephalexin from 17 µg/kg of cefalonium, resulting in 50% Sp and accuracy.

CONCLUSIONS: All positive milk samples yielded positive results for beta-lactam residue, regardless of concentration. Commercially available CAST for beta-lactam residue detection in milk showed very high sensitivity, including below the claimed LODs, and therefore positive results require robust confirmatory tests when violative residues (above the MRL) are suspected.

Keywords: CAST, test, milk, cefalonium, LOD

Impact of Spanish Antimicrobial Regulations on Bovine Respiratory Disease (BRD) Management in Beef Calves

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BACKGROUND and OBJECTIVE: Royal Decrees 992/2022 and 666/2023 restricted antimicrobial use in Spain. This study quantifies the impact of this legislative shift on antimicrobial consumption and field efficiency in beef finishing units.

MATERIALS and METHODS: An observational retrospective study was conducted on 3,301 calves (6,565 treatments) in a commercial feedlot in Northern Spain (2021-2025). Data from 20 months PRE-law (n=1,705 calves; 2,598 treatments) was compared to 20 months POST-law (n=1,596 calves; 3,963 treatments). Variables analyzed: treatments/calf (Tt/C), incomplete treatments (IT), BRD episodes (BRD/C), antibiotic category (by the Spanish official program PRAM; A: Avoid, B: Restrict, C: Caution, and D: Prudence). Used to balance animal health and public antimicrobial resistance.), antibiotic group (AbG), and clinical outcome (O: cure, failure, or relapse). Statistical analysis: IBM SPSS® v.30, using x^2, Kruskal-Wallis, Mann-Whitney, and logistic regression.

RESULTS: Overall BRD incidence was 83.8%. The risk of BRD increased POST-law vs. PRE-law (OR=30.04; 95%CI: 3.65-247.0; P=0.002), with 1.92±1.10 episodes/calf (88.5% animals affected) vs. 1.60±0.73 (79.4%). No significant productive differences were found except for Feed Conversion Ratio, which improved POST-law (6.14±3.90 vs. 6.54±4.02kg/kg; P<0.001). Regulations significantly shifted AbG choice: phenicols decreased (77.8% to 14.6%) while penicillins increased (0.6% to 49.3%; P<0.01). Usage of Categories B and C dropped drastically (5.2% to 4.1% and 93.6% to 45.1%, respectively; P<0.01) in favor of Category D. Total antimicrobial use rose from 1.92 to 2.81 Tt/C (P<0.01). Complete treatments decreased from 51.8% to 48.2% (P<0.01). Antibiotic failure rates

rose (16.7% to 40.4%; P<0.01), and cure rates fell (51.7% to 35.2%; P<0.01). However, relapses after successful treatment decreased (31.5% to 24.4%; P<0.01). Logistic regression confirmed that the general probability of treatment failure increased POST-law (OR=2.278; 95%CI: 1.862-3.778; P<0.001) but decreased in subsequent BRD episodes (OR=0.623; 95%CI: 0.523-0.736; P<0.001).

CONCLUSIONS: Results show strict compliance with new regulations, drastically reducing Category B and C antibiotic use. This shift is associated with higher failure rates and more incomplete treatments, suggesting a need to revise clinical protocols. Despite lower field efficacy, productive efficiency was not negatively affected. Spanish veterinarians remain committed to reducing antimicrobial resistance despite these challenges

Keywords: feedlot, antimicrobials, treatment efficiency

Effect of Copper Ion Treatment on Penicillin G and Cefoperazone Concentrations in Milk and PBS Determined by HPLC

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BACKGROUND and OBJECTIVE: Waste milk (WM) from antibiotic-treated cows frequently contains antimicrobial residues and is often fed to calves, raising concerns about antimicrobial resistance. Practical on-farm strategies to reduce antibiotic residues in WM are limited. Copper ions have antimicrobial properties and may also promote the transformation of β-lactam antibiotics. This work aimed to evaluate the effect of copper ion treatment on the concentrations of penicillin G and cefoperazone in milk and PBS using high-performance liquid chromatography (HPLC).

MATERIALS and METHODS: An in vitro experiment was conducted using PBS and commercial UHT milk spiked with target concentrations of β-lactam antibiotics (20, 200, and 1,000 ppb). Samples were exposed to an experimental copper ion treatment system, and subsamples were collected before treatment (0 min) and after 15 and 30 min. For PBS samples containing 1,000 ppb, additional early time points were included to evaluate degradation kinetics. Antibiotic concentrations were determined by HPLC, and all experiments were performed in triplicate.

RESULTS: Penicillin G concentrations in PBS and milk decreased to below the detection limit of method after 15 min of treatment at all concentrations tested. In PBS, concentrations decreased from 20.8, 196.7, and 1,115 ppb (20, 200, and 1,000 ppb targets, respectively) to non-detectable levels after treatment. In milk, concentrations were 22.3, 232.9, and 1,187 ppb, and also decreased to non-detectable levels after 15 min. In a kinetic experiment with PBS containing 1,000 ppb penicillin G, concentrations decreased from 1,115 ppb to 14.6 ppb at 3 min and to below the detection limit within 5 min. For cefoperazone, a similar pattern was observed in PBS. Mean concentrations were 22.0 ppb and 206 ppb, and no detectable concentrations were observed after treatment. In the PBS kinetic experiment (1,000 ppb target), concentrations decreased from 912.4 ppb to non-detectable levels from 3 min onward. In milk, cefoperazone concentrations also decreased after treatment but remained detectable at higher initial concentrations. At 200 ppb, concentrations decreased from 122.7 ppb to non-detectable levels after 30 min. At 1,000 ppb, concentrations decreased from 777.1 ppb to 611.4 ppb at 15 min and 251.8 ppb at 30 min, representing a 67.6% reduction.

CONCLUSIONS: Copper ion treatment reduced concentrations of two β-lactam antibiotics under experimental conditions. The effect was fast in PBS, where both antibiotics became non-detectable within minutes. In milk, reductions were observed, although higher cefoperazone concentrations persisted after 30 min. These findings suggest copper ion treatment could be a potential strategy to reduce β-lactam residues in WM.

Keywords: Waste milk, Antimicrobial residues, Electrochemical treatment, Dairy production, Antimicrobial resistance

Fecal Escherichia Coli Antimicrobial Resistance in Italian Dairy Calves From to 2024 to 2025

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Dairy Vet Services SRL STP

BACKGROUND and OBJECTIVE: Treatment of calves diarrhea needs the use of laboratory analyses as a tool to identify the correct etiology and therapy. Increasing attention to antibiotic-resistance requires close monitoring of bacteria responsible for enteric diseases. A retrospective analysis of the antimicrobial resistance of Escherichia coli strains isolated from fecal samples of dairy calves processed by Italian diagnostic laboratory during the period 2024–2026.

MATERIALS and METHODS: 509 samples in 2024 and 517 in 2025 were collected from calves presenting clinical signs of enteric disease. Fecal material was obtained using sterile rectal swabs and transported to the laboratory under refrigerated conditions. Samples were inoculated onto selective chromogenic media for the isolation and identification of coliform bacteria. Plates were incubated at 37°C/24 hours. Antimicrobial susceptibility testing was performed using the disk diffusion method on Mueller Hinton Agar, 37 °C/24 hours. Molecular characterization of isolates was carried out by RT-PCR to determine the presence of pathogenic strains of Escherichia Coli: ETEC, VTEC, EHEC and Shigella spp. The antimicrobial agents tested were selected based on the prototype molecules recommended by the National Reference Centre for Antimicrobial Resistance (CRAB). Selection criteria also included the availability of veterinary antimicrobial agents authorized in Italy for the treatment of enteric diseases, in accordance with national law.

RESULTS: In both studies antimicrobial susceptibility showed high levels of resistance, ranging from 2.3% to 98% depending on the antimicrobial agent. Compared with 2024, higher susceptibility rates were observed in 2025 for several antimicrobials, including amoxicillin, enrofloxacin, gentamicin, neomycin, and tetracycline. Conversely, an increase in resistance to paromomycin was detected.

CONCLUSIONS: The results of this study highlight the high prevalence of antimicrobial resistance among enteric bacterial isolates from calves with clinical enteric disease. The wide variability in resistance rates observed across antimicrobial agents underscores the importance of routine antimicrobial susceptibility testing to guide therapeutic decisions in veterinary practice. The increased susceptibility observed in 2025 for several commonly used antimicrobials may reflect more targeted therapeutic approaches and improved antimicrobial stewardship. However, the increase in resistance to paromomycin suggests that the intensified use of this molecule for the control of cryptosporidiosis may contribute to selective pressure, emphasizing the need for careful monitoring. Overall, these findings reinforce the importance of continuous surveillance of antimicrobial resistance in livestock pathogens, as well as the responsible and regulated use of veterinary antimicrobials, in order to preserve their efficacy and mitigate the spread of resistant bacteria.

Keywords: Escherichia coli, antimicrobial resistance, calves

High Prevalence of F17 Fimbriae in Bovine strains of *Enterotoxigenic Escherichia coli* (ETEC) and Presence of Strains Producing Extended-spectrum B-lactamases (ESBL): Diagnostic and Therapeutic Implications

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BACKGROUND and OBJECTIVE: Neonatal diarrhoea in cattle is a multifactorial and multi-etiological disease that causes significant economic losses in cattle production, both due to direct costs and its medium- and long-term effects on the performance of these animals. Neonatal diarrhoea of infectious origin can be caused by various bacterial, viral and parasitic agents that act at different stages of the calf's life. Neonatal colibacillosis caused by ETEC strains is one of the main causes of diarrhoea in calves. These strains express enterotoxins and fimbriae such as K99 (F5), F41 or F17, which are essential for adhesion to the intestinal epithelium. The aim of this study was to molecularly characterise these fimbriae in bovine clinical isolates and to detect the simultaneous presence of ESBL-producing strains.

MATERIALS and METHODS: A total of 991 clinical samples of bovine origin sent from the Animal Health and Production Laboratory of Galicia to the *Escherichia coli* Reference Laboratory (LREC) between February 2025 and February 2026 were analysed. Multiplex PCR was used to detect the K99 (F5), F41, F17, P987 (F6) and K88 (F4) fimbriae genes. At the same time, at the Agricultural Research Centre of Mabegondo, the presence of ESBL-producing strains and carbapenemases was evaluated in 170 samples following the European Union Reference Laboratory protocol. Strains with compatible morphology were identified and confirmed as *E. coli*/*E. coli* ESBL using the VITEK®MS/VITEK®2 automated systems, respectively.

RESULTS: ETEC-associated fimbriae genes were detected in 54% of the samples (539/991), with a predominance of F17 (481/539; 89%), followed by F5 (89/539; 17%) and F41 (30/539; 6%). In 56 of the positive samples, more than one fimbriae type was detected, either due to co-expression or the simultaneous presence of different ETEC strains. Typical porcine fimbriae (P987 and K88) were rare, with only five isolates testing positive for K88. In addition, 101 ESBL-producing strains (59% of the total) were identified with resistance associated with different categories of antibiotics, with 100% of ESBL strains being multidrug resistant (≥3 categories of antibiotics).

CONCLUSIONS: These results demonstrate the possible convergence between virulence factors associated with ETEC and mechanisms of resistance to critical antibiotics, posing an additional challenge for the clinical management of bovine colibacillosis. The detection of multidrug-resistant ESBL strains underscores the need to strengthen surveillance programmes, improve microbiological and molecular diagnostics, and promote prudent antibiotic use in order to optimise therapeutic decisions in animal health from a One Health approach. Funded project PID2022-143041OB-I00 MCIN/AEI, FEDER, UE.AC2025C-05, Xunta Galicia, MAPA, FEADER, UE

Keywords: {Escherichia coli}, ETEC, bovine colibacillosis, antibiotic resistance, fimbriae

Associations Between Selective Treatment Practices and the Frequency of Contagious Mastitis Pathogens in Clinical and Subclinical Samples from Alberta Dairy Herds

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BACKGROUND and OBJECTIVE: Mastitis control is a major driver of antimicrobial use (AMU) on dairy farms, primarily through the treatment of clinical mastitis (CM) and as dry cow therapy (DCT). Selective treatment practices such as selective treatment of CM (STCM) and selective dry cow therapy (SDCT) aim to reduce AMU by targeting only cows with confirmed or likely to develop intramammary infections. Importantly, implementation of these approaches should not impact udder health. This study evaluated the impact of these strategies on the frequency of contagious pathogens (e.g., *Staphylococcus* spp. and *Corynebacterium* spp.) in both clinical and subclinical mastitis cases.

MATERIALS and METHODS: Twenty-five dairy herds in Alberta, Canada, were followed for 1.5 years. Eligible herds either participated in routine milk recording or used on-farm systems enabling cow-level sampling with linked data (e.g., automated milking systems). Three visits were conducted approximately every six months. At the initial visit, current CM and DCT treatment protocols were collected through a structured questionnaire, and milk samples were collected from all cows with high somatic cell count (SCC; >200,000 cells/mL), based on the most recent assessment. Farmers were then instructed to aseptically collect and freeze (-20°C) milk samples from all CM cases during the study, which were later retrieved by the research team during farm visits. All samples were cultured on blood agar and MacConkey agar, the bacterial isolates were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and then classified as contagious or environmental. Data were analyzed using generalized linear mixed models (GLMM), with treatment protocol (blanket therapy, SDCT, STCM, or combined SDCT/STCM) as a fixed effect and herd as a random effect.

RESULTS: Questionnaire data indicated that 8 herds implemented SDCT, 6 used STCM, and 2 adopted both practices, while 11 relied on blanket treatment protocols. Overall, 336 milk samples from subclinical cases (SCC > 200,000 cells/mL) and 472 from CM cases were collected and analyzed. Non-aureus staphylococci (n = 96, 20%) were the most frequently identified pathogens in subclinical mastitis, whereas *Escherichia coli* (n = 76, 16%) was the predominant pathogen in CM. No differences in the frequency of contagious pathogens were observed between herds implementing selective approaches (SDCT and/or STCM) and those applying blanket treatment protocols.

CONCLUSIONS: The use of evidence-based SDCT and STCM protocols was not associated with a higher frequency of contagious pathogens in clinical or subclinical mastitis cases. These results support adopting selective treatment strategies to reduce AMU without increasing contagious mastitis.

Keywords: selective treatment of clinical mastitis, selective dry cow therapy, contagious mastitis-causing pathogens.

REPRODUCTION IN DAIRY AND BEEF CATTLE

Effect of Herbage Allowance of Native Grassland on Calf Weight at Weaning and Cow's Interval Between Calvings

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BACKGROUND and OBJECTIVE: Herbage allowance (HA, kg herbage dry matter [DM]/kg animal body weight [BW]) affects herbage mass and canopy height, which by his side affects herbage intake and animal productivity. The aim of this work was to assess the effect of two HA, High and Low (mean of 5 and 8 kg DM/kg BW per year) of native grassland, with seasonal variation (8, 4, 4 and 4 for Low and 12,

8, 8, 4 for High in spring, summer, fall and winter respectively) on calf BW at weaning and interval between calvings of the cows.

MATERIALS and METHODS: The experiment design was a randomized complete block with 2 blocks (4 plots per block) 60 ha each block, carried out in Experimental Station "Bernardo Rosengurtt", School of Agronomy, Uruguay, from 2017 to 2020 over three cow cycles (from April to March). We used 132 cows and their calves during the gestation-lactation cycle. Calf BW was measured without fasting period, in the morning from birth to weaning. Cow's data of calving was used to estimate the intervals between parturition, for this purpose we used only cows that calved the year before of the year of measurement and were pregnant and lactating during the year of evaluation. Calf BW at weaning and cow calving interval (days) were analyzed using mixed models of SAS software (On Demand for Academics) with HA, year and its interaction as the fixed effect and block and plot as the random effects, and sex of the calf and age in day as covariates.

RESULTS: Calf BW at weaning (162±20 days from birth) was higher (P<0.05) in High HA only during the third year (180 vs 161±3 kg in High and Low) showing non difference in the first two. However, calving interval tend to (P=0.07) decrease in High HA (363 vs 373±4 days for High and Low HA), while year greatly affect the interval.

CONCLUSIONS: The results suggest that the High HA should be implemented in commercial grazing systems to improve both, calf weight at weaning and the interval between calving which then impact also calf BW at weaning because of age plus the possible effect of HA and the whole animal productivity.

Keywords: rangeland, beef cattle, grazing, calf weight, calving interval

Adding a Progesterone Device to Double-ovsynch56 in High Producing Dairy Cows Improve Fertility and Help to Mitigate Twinning

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BACKGROUND and OBJECTIVE: Fertility in high producing dairy cows is a challenge. Some hormonal base protocols help to mitigate these challenge. The objective of this study was to evaluate if adding a progesterone device in the breeding Ovsynch part of a Double Ovsynch will improve fertility in high producing dairy cows.

MATERIALS and METHODS: The trial was perform in a tie stall farm in Canada over the period of march 2022 to march 2026. The farm milked 120 cows, twice a day and calved year round. All cows are bred between 70 and 77 day in milk. Lactation 1 and 2 are submit to a Double ovsynch56 for first breeding. The high producing cows (lactation 3 and more) are submit to a Double Ovsynch56 with an addition of a progesterone device (CIDR) in the breeding ovsynch.

RESULTS: Over this period of time, conception rate in lactation 1 and 2 were 50 % and 48 % for lactation 3 and more. Before 2022, conception rate in lactation 1 and 2 were 50 % and 40% for lactation 3 and more. The major weakness of this study is that we don't have control group, because the owner of the farme want to add progesterone device in all lactation 3+ protocol. However, a strengh of this study is his duration (4 years). Also, it could metigate the season effect. Another strength is that is the same breeder over this period of time.

CONCLUSIONS: Adding a progesterone device in the breeding Ovsynch part of a Double Ovsynch56 improve fertility by 8% of conception rate in high producing dairy cows. Moreover, twinning is reduce.

Keywords: reproduction, high producing dairy cow, twinning, Double Ovsynch56

in vitro Embryo Production in Beef Donor Cows, Superstimulated with Porcine Follicle Stimulating Hormone Prior to Ovum Pick-Up

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BACKGROUND and OBJECTIVE: The number of embryos produced by ovum pick-up (OPU) and *in vitro* culture are critical for the success of genetic improvement programs. An experiment was designed to evaluate the effect of superstimulation with a porcine pituitary extract (pFSH) prior to OPU on *in vitro* embryo production in beef donor cows.

MATERIALS and METHODS: A total of 21 non-lactating, mature and cycling Angus cows, with mean (± SEM) body condition score 3.1±0.1 (1 to 5 scale) were used in a cross-over design with two treatment groups (pFSH or Control) and two replicates performed at 30-day intervals. All cows received an intravaginal device containing 1.9 g of progesterone and a 150 µg D-cloprostenol on Day -9, followed by the administration of 0.02 mg of buserelin 7 days later (Day -2) to induce ovulation of the dominant follicle. Cows in the control group received no further treatment and OPU was performed on Day 3. In the pFSH group, treatments were initiated on Day 0; all cows received a total dose of 160 mg pFSH (NIH-FSH-P1) distributed in 4 injections 12 h apart (40 mg per injection) and OPU was performed 36-40 h after the last pFSH injection (Day 3). Cumulus-oocyte-complex (COC) recovered from each cow by OPU on Day 3 were evaluated morphologically following the recommendations of the IETS Manual, then matured, fertilized and cultured in vitro. A pool of semen from two Angus bulls was used. Data was analyzed using the General Linear Mixed Models for a Poisson distribution (Infostat, UNC). The fixed effect included in the model was treatment and cow (id) as a random effect.

RESULTS: Although the number of the COCs retrieved was greater (P=0.02) in the untreated Control group (15.6 ± 2.5), compared to the pFSH group (12.7 ± 1.4), the number blastocysts produced did not differ (P=0.19) between the Control (3.2 ± 0.8) and pFSH groups (3.9 ± 0.7). However, the proportion of blastocysts produced in relation to the number of COCs retrieved was greater (P<0.01) in the pFSH group (33.1% ± 4.4%) compared to the Control group (18.3% ± 2.8%).

CONCLUSIONS: Although superstimulation did not improve the number of COCs aspirated, it had a positive effect on the proportion of blastocysts produced from beef donors.

Keywords: pFSH, OPU, Cows

Clinical Efficacy and Practical Advantages of a Novel Adjustable-Loop Ovarian Ligation Device for Estrus Suppression in Cattle

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BACKGROUND and OBJECTIVE: Ovarian ligation is widely used in cattle to suppress estrus behavior and manage reproductive function in cull cows. This study introduces a novel adjustable-loop (AL) ligation device featuring a universal-fit, ratcheted tightening mechanism designed to simplify the procedure.

MATERIALS and METHODS: Thirty Hanwoo heifers were assigned to three groups (n=10 each): AL, fixed-ring (FR), and control. Ovarian volume was monitored by ultrasonography and expressed as a relative index (Week 0 = 100.0). Serum progesterone (P4) and cortisol concentrations were measured to evaluate functional suppression and post-operative stress.

RESULTS: The FR group demonstrated a more rapid initial reduction at week 3 compared with the AL group (54.9±14.5 vs. 62.4±16.4). However, by week 6, the AL group showed significantly greater and more consistent ovarian atrophy (23.0±4.7 vs. 39.4±14.4, P<0.05), whereas the control group exhibited

continued ovarian growth (139.1±29.7). Serum P4 concentrations in the AL group remained at basal levels (0.2±0.0 ng/mL) by week 6, indicating effective functional suppression. Serum cortisol concentrations increased immediately after surgery (AL: 5.0±0.7 ng/mL; FR: 5.1±0.8 ng/mL) but declined significantly by week 3 (AL: 4.3±0.7 ng/mL; FR: 3.6±0.8 ng/mL), indicating stable post-operative recovery.

CONCLUSIONS: In conclusion, the AL device provides a simplified and effective method for ovarian ligation in cattle, achieving consistent structural and functional suppression without increased post-operative stress. Histopathological evaluations in aged multiparous cows are ongoing to further confirm long-term safety and irreversible atrophy.

Keywords: cattle, ovary, ligation, adjustable loop, progesterone

Prolonged Epidural Analgesia with a Mixture of Lidocaine and Bupivacaine as the Part of the Treatment Plan After Severe Dystocia in Dairy Cow

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BACKGROUND and OBJECTIVE: Epidural analgesia is commonly used in large animals as easy, cheap, effective technique used to prevent or control pain during surgeries in the genital tract, gynecological and obstetrical procedures involving the tail, anus, vulva, perineum, caudal udder, scrotum, and upper hind limbs. However, local anesthetics also improve postoperative pain control and recovery significantly. Bupivacaine and lidocaine are two commonly used local analgesics but as a mixture to provide the longer action of bupivacaine and the faster onset of lidocaine also reduce the amount of postoperative pain killing drugs required.

MATERIALS and METHODS: A primiparous dairy cow with depression, apathy, decreased food intake, drop milk yield, reluctance for movement and pain was presented for check-up after severe dystocia. The vulvar wounds, vaginal and vestibular lacerations were surgically cleaned under caudal epidural analgesia with a 5 ml of 1: 1 mixture of lidocaine (0,2mg/kg) and bupivacaine (2mg/kg) without need of any extra physical restrain.

RESULTS: The clinical improvement was immediate and long lasting without need of repeates.

CONCLUSIONS: To conclude, it is close to an ideal local anesthetic mixture which provides complete sensory blockade of the caudal region without sciatic nerve motor paralysis on application and has an adequate duration of effect to include the procedure and a generous postoperative period. Lidocaine, bupivacaine epidural combination is useful in dairy cattle in cases where prolonged analgesia is beneficial and where extended postoperative pain control can enhance significantly patient comfort and recovery as well.

Keywords: Epidural, dystocia, dairy, cow

Evaluation of Dna Fragmentation of Bovine Granulosa Cells From the "cumulus Oophorus" By Chromatin Dispersion Using Fluorescence and Brightfield Microscopy

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BACKGROUND and OBJECTIVE: This study aimed to evaluate the validity of fluorescence microscopy compared to brightfield for assessing DNA fragmentation in bovine granulosa cells from the cumulus oophorus using a chromatin dispersion assay.

MATERIALS and METHODS: Ovaries were obtained post-mortem from cows and transported to the laboratory in physiological saline supplemented with gentamicin. Cumulus–oocyte complexes (COCs) were recovered by follicular aspiration and slicing, morphologically classified into three quality categories, matured in vitro, and subsequently denuded. The obtained oocytes were evaluated for

their maturation status, and the granulosa cells were processed for further analysis. DNA fragmentation of granulosa cells was evaluated by classified test (the chromatin dispersion test - Ovoselect, Halotech, Spain) and analyzed by both bright-field and fluorescence microscopy with conventional staining or classified labelling (fluoGreen), respectively. For each group, granulosa cells were classified as having non-fragmented or fragmented DNA based on chromatin halo size and morphology. Statistical analysis included t-test comparisons of DNA fragmentation percentages between imaging methods within COC classes and chi-square analysis of the distribution of fragmented versus non-fragmented cells across COC quality categories.

RESULTS: The results showed no significant differences ($p > 0.05$) in granulosa cell DNA fragmentation indices between bright-field and fluorescence microscopy, indicating that both microscopic techniques can be used interchangeably for this assay. The prolonged hyaluronidase exposure during denudation did not significantly affect cell dispersion or the rate of DNA fragmentation.

CONCLUSIONS: These findings suggest that chromatin dispersion testing of granulosa cells can be reliably performed with either microscopy technique and that granulosa cell DNA fragmentation may not directly reflect routine COC morphology in slaughterhouse-derived bovine material.

Keywords: DNA fragmentation, "cumulus oophorus", oocyte quality

In-Line Milk Lactose and Urea Concentrations as Biomarkers of Dairy Cow Health, Calving Performance and Reproductive Success

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BACKGROUND and OBJECTIVE: Automated milking systems allow routine in-line recording of milk composition parameters that can be used to monitor the physiological and health status of dairy cows. Milk lactose concentration and milk urea nitrogen (MUN) have been associated with metabolic processes, udder health and responses to calving-related stress, but their interpretation under practical farm conditions remains inconsistent. The objective of this study was to assess the relationships between in-line recorded milk lactose and urea concentrations and indicators of udder health, calving-related disorders and reproductive performance during early lactation.

MATERIALS and METHODS: The study was carried out on a commercial dairy farm equipped with Lely Astronaut® A3 automated milking systems. During early lactation, milk lactose concentration, MUN, electrical conductivity (EC), somatic cell count (SCC), milk yield and cow activity were continuously recorded. Cows were classified according to milk urea concentration (≤15, 16–30 and >30 mg/dL) and milk lactose concentration (<4.70% and ≥4.70%). Differences between groups were evaluated, and relationships among variables were analysed using correlation analysis and logistic regression ($p < 0.05$).

RESULTS: Cows with moderate milk urea concentrations (16–30 mg/dL) experienced fewer cases of dystocia and showed lower SCC and EC values, together with higher milk lactose concentrations, compared with cows with low or high MUN levels. Dystocia was associated with an increased risk of mastitis (odds ratio ≈ 1.36). Milk lactose concentration showed a clear association with udder health and reproductive performance. Cows with milk lactose concentrations below 4.70% had significantly higher SCC and EC and a more than 15-fold higher risk of mastitis. In contrast, cows with milk lactose concentrations of ≥4.70% showed

substantially higher conception success (odds ratio ≈ 26). Milk lactose concentration was also positively related to cow activity and rumen pH, and negatively related to SCC and EC.

CONCLUSIONS: In-line milk lactose concentration is closely linked to udder health, metabolic status and reproductive performance in dairy cows. Milk urea concentration during early lactation provides complementary information related to physiological adaptation and calving-associated stress. When evaluated together, these routinely recorded milk parameters can support early identification of health and fertility problems in dairy herds.

Keywords: automated milking systems, milk lactose, milk urea nitrogen, mastitis, reproductive performance

Strategic Trace Mineral Injection During the Dry Period Association with Post-partum Health Traits and Reproductive Performance in Dairy Cows

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BACKGROUND and OBJECTIVE: Trace minerals play a critical role for immunity and fertility, as they are key components of the antioxidant system. The aim of the present study was to identify potential benefits of trace mineral injections during the dry period on post-partum health traits and reproductive outcome in confined dairy cows.

MATERIALS and METHODS: Three commercial dairy herds located in Northern Italy were enrolled into the study. A total of 1,271 dairy cows were randomly assigned to a treatment (MM) and a control group (CON). MM cows (n=597) received an injection of a trace mineral solution (1 ml/100Kg bodyweight SC) containing Zinc (60mg/ml), Copper (15mg/ml), Manganese (10mg/ml) and Selenium (5mg/ml)(Multimin®, Axiota Animal Health) at dry-off and about 30 days before calving. CON cows (n=674) remained untreated. All animals were fed according to NASEM 2021 requirements for trace minerals. After a 70-day voluntary waiting period all cows were submitted to timed artificial insemination using a Double Ovsynch protocol. Treatment group differences for post-partum diseases occurrence were assessed using chi-square test. Association among treatment and time to conception and conception risk were assessed using Kaplan-Meier curves and a Cox's proportional hazard model, respectively, with censoring at 150 DIM. Statistical analysis was performed with JMP 19® (SAS) and significant differences and tendencies were set for $p \leq 0.05$ and $p < 0.10$.

RESULTS: Animals were 449 primiparous cows, 360 second calving and 462 with 3 or more calvings, without difference among treatment groups (p=0.97). MM cows received the trace minerals injections 58±8 and 30±6 days pre-calving. No significant differences were detected among treatment groups for retained placenta occurrence (MM 8.9% vs CON 7.3%; p=0.29) puerperal metritis (MM 22.4% vs CON 19.1%; p=0.15) and clinical mastitis (MM 8.2% vs CON 7.0%; p=0.4). Median time to conception was significantly lower for MM compared to CON (MM 77d vs CON 96d; p=0.03). MM cows had 1.15 greater odds for pregnancy compared to CON cows (HR 1.0-1.32; p=0.04). Treatment by farm interaction was not significant (0.74), while treatment by parity interaction tended to significance (p=0.06). Treatment was not significantly associated with the risk of pregnancy in primiparous cows (p=0.56), while multiparous MM had 1.25 greater odds for pregnancy compared to multiparous CON (HR 1.04-1.50; p=0.01).

CONCLUSIONS: In the current study, trace mineral strategic injection at dry-off and pre-calving significantly reduced time to pregnancy and increased odds for pregnancy, especially in multiparous cows but was not associated with health traits post-calving.

Keywords: Injectable trace minerals, reproduction, dairy cows, fertility, dry period, antioxidants

Fertility Performance of Dairy Cows with Clinical Endometritis After Intrauterine Treatment with Cephapirin or Self-recovery

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BACKGROUND and OBJECTIVE: Clinical endometritis (CE) is a common postpartum uterine disease in dairy cows. Intrauterine antibiotic infusions are commonly used to treat CE in cows. However, the treatment of CE in cows is controversial because the rate of spontaneous recovery is high. The objective of this field trial was to compare the effect of intrauterine cephapirin treatment or no treatment on fertility outcomes of dairy cows with CE.

MATERIALS and METHODS: The study was carried out on 271 Polish Holstein Friesian cows with CE diagnosed by vaginoscopy and ultrasonography between 21 and 28 days after parturi¬tion. In cows, CE was diagnosed if there was mucopurulent or purulent discharge in the vagina, the diameter of the cervix was greater than 7.5 cm, and uterine fluid was present. Cows with CE were randomly divided in two groups. Cows in the group 1 (n = 137) received intrauterine cephapirin, and cows in the group 2 (n = 134) did not receive intrauterine antibiotics. All cows were clinically examined three times at an interval of 2 weeks. Cows without signs of CE were considered clinically cured. The following reproductive per¬formances were calculated for cows after clinical cure of CE: first AI pregnancy rate, pregnancy rate 200 days after artificial insemination (AI), number of AI per pregnancy, interval calving to conception, preg¬nancy loss, and culling rate.

RESULTS: There were no significant differences in clinical recovery between the groups (83.9% vs. 79.1%; $p > 0.05$). The first conception rate, pregnancy rate 200 days after AI, number of AI per pregnancy, pregnancy loss and culling rate were better in cows with CE after intrauterine cephapirin treatment, but the differences were not statistically significant ($p > 0.05$). However, compared to self-cured cows, the calving to conception interval was significantly shorter in cows treated with cephapirin (132.2 ± 58.2 days vs. 151.3 ± 62.9 days; $p < 0.05$).

CONCLUSIONS: The study showed that the clinical cure rate of CE after intrauterine treatment with cephapirin or self-recovery was similar, but the treatment with cephapirin resulted in a shorter calving to conception interval.

Keywords: cows, clinical endometritis, cephapirin, self-recovery, ferility

Injectable Trace Mineral Supplementation as a Component of a Non-Cycling Cow Programme. Effect on Reproductive Outcomes in Dairy Cattle

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BACKGROUND and OBJECTIVE: Trace minerals (TM) play a critical role in cattle reproduction. Although required in minute quantities, their bioavailability is essential for optimal reproductive physiology, fertility, and overall productivity. The objective of this study was to assess the effect of a multimineral supplementation provided by injection (ITMS; MULTIMIN®, Virbac New Zealand), administered at the commencement of a routine non-cycling treatment programme. The study evaluated its impact on initial conception, six-week in-calf (6WK) risk, final pregnancy risk, and the time to conception from the planned start of mating (PSM).

MATERIALS and METHODS: A large-scale randomised controlled trial was conducted across 44 dairy farms in four regions of New Zealand (Waikato, Bay of Plenty, Canterbury, and Otago). From an initial enrolment of 4,337 animals, the final analysed dataset comprised 4,040 cows. These were evenly distributed into a treatment group receiving the ITMS (n=2,024) and a control group (n=2,016). Baseline parameters, including age, breed, days from treatment to PSM, and days-in-milk at the time of treatment, were recorded, with no significant differences observed between the two groups at enrolment.

RESULTS: The ITMS treatment had no immediate effect on the odds of conception to the non-cycling programme (p = 0.49). Additionally, the effect of the treatment on the 6WK in-calf risk varied significantly by farm (p < 0.0001), a finding that warrants further investigation. However, the treatment's effect on the days to conception was consistent across the study period; the daily hazard of conception was 1.08 times higher (95% CI: 1.01–1.15) in the treated group compared to the control group (p = 0.035). Ultimately, animals in the treatment group demonstrated a 2.2% greater chance of being pregnant at the end of the mating period compared to controls (p = 0.045).

CONCLUSIONS: The strategic use of injectable TM supplementation prior to breeding—without replacing traditional oral TM supplements—helps maintain adequate trace mineral and oxidative status during critical reproductive phases. Integrating MULTIMIN® into a non-cycling programme resulted in tangible improvements in dairy cattle reproductive outcomes. While these positive effects were not immediately apparent in initial conception rates or 6WK risk, treated animals exhibited a consistent improvement in the time to conception rate over the entire mating period, culminating in a significant improvement in final pregnancy status.

Keywords: trace minerals, anestrus, dairy cow, FTAI

Postpartum And Reproductive Disease Management Practices Among Cattle Veterinarians in 11 European Countries

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BACKGROUND and OBJECTIVE: Effective postpartum management is essential to optimize

fertility in cattle. While clinical definitions of postpartum and reproductive diseases are established, less is known about how veterinarians apply diagnostic and therapeutic strategies under field conditions. This study aimed to characterize diagnostic criteria and therapeutic approaches used by veterinarians in 11 European countries and evaluate associations with demographic and practice-related factors.

MATERIALS and METHODS: A questionnaire was distributed to veterinarians between November 2023 and January 2025. A total of 1,190 responses were included. The survey addressed postpartum monitoring, diagnosis and treatment of hypocalcemia, retained fetal membranes (RFM), uterine prolapse, puerperal and clinical metritis, endometritis, ovarian cysts, and anovulatory anestrus. Multiple correspondence analysis (MCA) was applied to categorical management variables to identify underlying patterns and their relation to demographic characteristics. Logistic regression models evaluated associations between responses and demographic variables including country, age, gender, herd size, and herd type.

RESULTS: Routine postpartum examinations were reported by 45% of respondents and were more common in dairy than beef herds and in larger herds. Respondents most frequently reported assessing cows for RFM, vaginal discharge characteristics, and body temperature, whereas determination of ketone concentrations and monitoring of automated disease indicators were less common. MCA revealed two gradients: one reflecting therapeutic intensity (particularly antimicrobial and non-steroidal anti-inflammatory drugs [NSAID] use) and another representing diagnostic intensity, including use of ultrasonography and active screening. Younger veterinarians and those working primarily with dairy herds reported more frequent use of ultrasonography and structured diagnostic criteria for endometritis and ovarian cysts, while older respondents relied more on transrectal palpation and behavioral signs. Definitions of RFM varied, with thresholds ranging from ≤6 to >48 hours postpartum. Management of puerperal metritis predominantly involved systemic antimicrobials and NSAIDs, whereas treatment of clinical endometritis and ovarian cysts showed marked variation in hormonal protocols, intrauterine therapies, and “wait and see” approaches. Country-level differences were evident for antimicrobial and NSAID use (Germany and Austria with greater adoption of these interventions), prostaglandin protocols, oxytocin administration, and screening practices. Despite this variability, used drugs were consistent across conditions, predominantly penicillins, tetracyclines, meloxicam, and ketoprofen.

CONCLUSIONS: Substantial heterogeneity exists in postpartum and reproductive disease management across Europe. Variability follows identifiable patterns influenced by herd type, herd size, age, and country. These findings highlight the need for harmonized clinical guidance and the development of consensus-based guidelines where lacking, alongside targeted education to promote consistent, evidence-based postpartum management. Work supported by FCT project UIDB/00276/2025 (CIISA) and LA/P/0059/2020 (AL4AnimalS).

Keywords: Survey, postpartum, disease, practices

Parenteral Micromineral and Vitamin Supplementation Improves Semen Quality in Bulls Subjected to Transient Testicular Heat Stress

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BACKGROUND and OBJECTIVE: Testicular heat stress is associated with transient alterations in semen quality in breeding bulls. The objective of the study was to evaluate the effects of parenteral supplementation with microminerals and vitamins involved in antioxidant defense on seminal parameters in bulls subjected to experimental testicular heat stress.

MATERIALS and METHODS: The study was carried out on a commercial farm located in Buenos Aires, Argentina. Eleven mature Angus bulls were randomly assigned on day −40 to receive saline solution (control; n = 5) or a parenteral formulation consisting of two products: one containing copper (10 mg/mL), zinc (40 mg/mL), manganese (10 mg/mL), and selenium (5 mg/mL; Adaptador® MIN, Biogénesis Bagó SA); and the other containing vitamins A (59.500 UI/mL) and E (50 UI/mL;

Adaptador® VIT, Biogénesis Bagó SA) (Min+Vit; n = 6). Treatments were repeated on day −15. Testicular heat stress was induced by scrotal insulation for 72 h (day 0–3). Semen samples were collected on days 0, 3, 15, 30, and 57. Sperm membrane integrity, morphology, motility, and lipid peroxidation were evaluated. Data were analyzed using repeated measures mixed models including treatment, time, and their interaction, with day 0 values included as a covariate.

RESULTS: Testicular heat stress produced time-dependent changes in semen quality, with the most evident alterations observed in sperm morphology on day 15 ($P < 0.05$). Mineral and vitamin supplementation significantly increased the percentage of spermatozoa with intact functional membranes (78.04 ± 1.50 vs. 83.41 ± 1.34% for control and Min+Vit, respectively; $P = 0.01$) and improved total and progressive motility (54.83 ± 5.81 vs. 74.53 ± 5.21% and 38.27 ± 5.92 vs. 56.32 ± 5.35% for control and Min+Vit, respectively; $P \leq 0.03$). Supplementation also tended to increase the proportion of morphologically normal spermatozoa, with significant differences between groups detected on day 15 following heat stress (52.32 ± 4.57 vs. 76.85 ± 4.08% for control and Min+Vit, respectively; $P < 0.01$). No effects of supplementation were observed on sperm viability, or sperm lipid peroxidation.

CONCLUSIONS: These results suggest that parenteral supplementation with selected microminerals and vitamins improves sperm functional quality and may enhance seminal resilience during transient testicular heat stress. This strategy could contribute to maintaining reproductive performance in breeding bulls exposed to short-term thermal challenges.

Keywords: Bulls, Semen quality, Microminerals, Heat stress, Reproduction

Impact of Different Puberty Induction Strategies on the Reproductive Performance of Prepubertal Nelore Heifers

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BACKGROUND and OBJECTIVE: The study objective was to evaluate the impact of different puberty induction strategies on pregnancy rates of Nelore heifers exposed to fixed-time artificial insemination (FTAI).

MATERIALS and METHODS: Heifers were examined via ultrasonography on days -31 and -24 to confirm the absence of a corpus luteum (CL). Heifers without a CL in both examinations (n=629) were randomly assigned to 1 of 3 puberty induction treatments on Day -24: 1) CTRL: control heifers receive no treatment; 2) IVD: heifers received a controlled internal drug release (CIDR; 1.9 g of progesterone) on Day -24, which was removed 12 days after insertion, concurrently with a 0.6-mg injection of estradiol cypionate; 3) iP4: heifers received a 150-mg injection of progesterone on Day -24 and a 0.6-mg injection of estradiol cypionate on Day -12. All heifers were then exposed to the same ovulation synchronization protocol, where a CIDR device was inserted concurrently with a 2 mg of estradiol benzoate on Day 0. Heifers received 12.5 mg of prostaglandin F2α, 0.6 mg of estradiol cypionate, and 200 IU of equine chorionic gonadotropin on Day 9, concurrently with CIDR removal. Artificial insemination was performed 48±2 hours later (Day 11). All CIDR devices were previously utilized for 27 days. Ultrasonography was used to determine CL presence and uterine scores (1 to 5 scale) on Day 0, follicle diameter at FTAI, and pregnancy status on Day 30.

RESULTS: Puberty induction with IVD or iP4 increased the percentage of heifers with a CL present on Day 0 compared with CTRL ($P<0.01$), with no

differences between IVD and iP4 heifers (P=0.22). Likewise, both IVD and iP4 treatments resulted in a greater proportion of heifers with uterine scores ≥4 compared with CTRL (P<0.01), and no difference was observed between the IVD and iP4 groups (P=0.44). Follicle diameter at FTAI was decreased (P≤0.01) in CTRL compared with both IVD and iP4 heifers. There were no differences in follicle diameter between IVD and iP4 heifers (P=0.24). Although there was no effect of treatment on estrus expression (P=0.99), greater pregnancy rates (P≤0.05) were observed in IVD heifers compared with both CTRL and iP4 heifers (46.1, 37.0, and 31.4%, respectively). There were no differences in pregnancy rates between CTRL and iP4 heifers (P=0.20).

CONCLUSIONS: In summary, puberty induction with either iP4 or IVD resulted in a similar percentage of heifers with a CL on Day 0; however, IVD-treated heifers had greater pregnancy rates compared with both CTRL and iP4 heifers.

Keywords: Heifers, puberty induction, progesterone, reproductive performance

Ovarian And Fertility Responses of Nelore Heifers Submitted to Different Puberty Induction and Ovulation Synchronization Strategies

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BACKGROUND and OBJECTIVE: The study objective was to determine the impact of different industry-standard puberty induction and ovulation synchronization strategies on pregnancy rates of Nelore heifers exposed to fixed-time artificial insemination (FTAI).

MATERIALS and METHODS: Nelore heifers (n=1,446) were enrolled in a completely randomized design with a 2×2 factorial arrangement of treatments. Heifers were randomly assigned receive 1 of 2 puberty induction treatments: 1) IVD+ECP: heifers received controlled internal drug release (CIDR; 1.9 g of progesterone) device on Day -24 followed by a 0.6-mg injection of estradiol cypionate (ECP) and CIDR removal on Day -12; 2) iP4: heifers received a 150-mg injection of progesterone (iP4) on Day -24. Within each puberty induction treatment, heifers were randomly assigned to 1 of 2 synchronization protocol: 1) 9-day: Heifers received a CIDR and 2.0 mg of estradiol benzoate on Day 0. Heifers then received 12.5 mg of prostaglandin F2α (PGF; dinoprost tromethamine) and 0.6 mg of ECP on Day 9, concurrently with CIDR removal; 2) 7-day: Heifers received a CIDR concurrently with 2.0 mg of estradiol benzoate and 0.5 mg of PGF (cloprostenol) on Day 2. Heifers then received a second injection of PGF, 1.0 mg of ECP, and 300 IU of equine chorionic gonadotropin on Day 9. All heifers received FTAI 48 hours after CIDR removal. Gonadotropin-releasing hormone (100 µg) was administered only to 7-day heifers at FTAI. All CIDR devices were previously utilized for 27 days. Transrectal ultrasonography was performed to evaluate corpus luteum (CL) presence on Day 0, dominant follicle diameter at CIDR removal and FTAI, and pregnancy status 30 days after FTAI.

RESULTS: A greater percentage of IVD+ECP heifers had a CL on Day 0 compared with iP4 heifers (P<0.01). However, puberty induction and the interaction between puberty induction × synchronization protocol did not influence any other response variable (P≥0.12). Heifers exposed to the 9-day protocol had greater (P<0.01) follicle diameter at CIDR removal and FTAI compared with 7-day. Compared with the 9-day protocol, the 7-day protocol increased (P<0.01) follicular growth between CIDR removal and FTAI and increased the proportion of heifers ovulating prior to FTAI (P<0.01). Heifers exposed to the 9-day protocol had greater (P<0.01) pregnancy rates compared with heifers exposed to the 7-day (38.5 vs. 31.4%).

CONCLUSIONS: Puberty induction using IVD+ECP increased the percentage of heifers with CL on Day 0. Moreover, regardless of puberty induction strategy, 9-day protocol resulted in a larger dominant follicle, decreased early ovulation, and increased pregnancy rates compared with 7-day.

Keywords: Heifers, puberty induction, protocol length, reproductive performance

Strategic Use of Prostaglandin F2α During Puberty Induction and Ovulation Synchronization Increases Fertility in Nelore Heifers

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BACKGROUND and OBJECTIVE: The aim of this study was to investigate the impact of a prostaglandin F2α (PGF)-based adaptation to current puberty induction and ovulation synchronization protocols on pregnancy rates of Nelore heifers exposed to fixed-time artificial insemination (FTAI).

MATERIALS and METHODS: A total of 2,136 heifers were enrolled in a completely randomized design where heifers were randomly assigned to 1 of 2 treatments: 1) CTRL: a controlled internal drug release (CIDR; 1.9 g of progesterone) device that had been previously utilized for 27 days was inserted on Day -24, followed by a 0.6-mg injection of estradiol cypionate (ECP) and CIDR removal on Day -12. Heifers then received a new CIDR and 2.0 mg of estradiol benzoate on Day 0. Nine days later, heifers were administered 0.5 mg of cloprostenol (PGF), 0.6 mg of ECP, and 200 IU of equine chorionic gonadotropin concurrently with CIDR removal. Exposure to FTAI occurred at 48±2 after CIDR removal; 2) PGF: Heifers received the same treatment was CTRL; however, PGF was administered on Days -12 and 7 instead of Day 9. Estrus expression was evaluated at FTAI using tail-chalk. Transrectal ultrasonography was performed on Days -12 and 0 to determine the present of a corpus luteum (CL), Days 9 and 11 to determine dominant follicle diameter, and 30 days after FTAI to determine pregnancy status.

RESULTS: There were no differences between treatments on the percentage of heifers with a CL on Day 0 (P=0.76). Although there were no differences between treatment on follicle diameter on Day 9, PGF heifers had greater (P<0.01) follicle diameter on Day 11. A treatment × CL on Day -12 interaction was observed for follicular growth (P<0.01), estrus expression (P<0.01), and pregnancy rates to FTAI (P<0.01). Heifers in the PGF treatment with a CL present on Day -12 had greater follicular growth (P<0.01), estrus expression (P<0.01), and pregnancy rates to FTAI (P<0.01) compared with CTRL heifers with CL present on Day -12. There were no differences between treatments in heifers without a CL present on Day -12 (P=0.74). The positive responses in heifers with a CL on d-12 generated an overall increase in pregnancy rates for PGF compared with CTRL heifers (P<0.01; 48.5% vs. 40.4%, respectively).

CONCLUSIONS: In summary, the strategic adjustments to PGF administration during puberty induction and ovulation synchronization evaluated in this study enhanced follicular development, estrus expression, and pregnancy rates to FTAI in Nelore heifers.

Keywords: Heifers, puberty induction, prostaglandin, protocol management, reproductive performance

Impact of Puberty Induction Strategy on Circulating Progesterone Profile in Prepubertal Nelore Heifers

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BACKGROUND and OBJECTIVE: The objective of this study was to characterize the impact of different progesterone (P4) supplementation strategies on circulating concentrations of P4 in prepubertal Bos indicus heifers exposed to puberty induction protocols.

MATERIALS and METHODS: A total of 33 Nelore replacement heifers (body weight: 270.8 ± 18.40) were examined twice via ultrasonography on Days -4 and 0 to confirm the absence of a corpus luteum. Heifers without a corpus luteum were then randomly assigned to 1 of 3 puberty induction treatments: 1) CTRL: control heifers receive no treatment; 2) IVD: heifers received an intravaginal P4 insert (CIDR; 1.9 g of P4) previously utilized for 27 days on Day 0, which was removed 11 days after insertion (Day 11); 3) iP4: heifers received a 150-mg intramuscular injection of P4 on Day 0. Blood samples were collected from all heifers daily from Days 0 to 12 to estimate circulating concentrations of P4 using electrochemiluminescence.

RESULTS: Circulating concentrations of P4 did not differ between treatments on Day 0 (P≥0.80). From Days 1 to 11, IVD-treated heifers generally had greater P4 concentrations than iP4-treated heifers, and both treatments exceeded CTRL (P≤0.03). These differences were only not detected on Day 7 for IVD vs. iP4 (P=0.10) or on Days 10 (P=0.08) and 11 for iP4 vs. CTRL (P=0.20). The number of days with circulating P4 ≥0.8 ng/mL also differed among treatments (P<0.01). Heifers treated with IVD maintained P4 above this threshold for 10.2 ± 1.0 days, compared with 7.0 ± 0.8 days for iP4 and 0.6 ± 0.2 days for CTRL. Area under the curve (AUC; Day 0 to 11) was also greater (P<0.01) for IVD and iP4 heifers compared with CTRL, and greater for IVD compared with iP4 heifers (P<0.01). One day after device removal (Day 12), P4 concentrations in IVD heifers rapidly decreased to similar levels to CTRL (P=0.83), whereas iP4-treated heifers tended to maintain greater P4 on Day 12 compared with CTRL (P=0.09).

CONCLUSIONS: Although both IVD and iP4 increased circulating concentrations of P4 compared with CTRL, IVD resulted in greater and more sustained systemic P4 concentrations than iP4. Following device removal, IVD-treated heifers rapidly returned to P4 concentrations comparable to CTRL, whereas iP4-treated heifers maintained elevated P4. These findings indicate that P4 supplementation strategy influences systemic P4 dynamics during puberty induction protocols in prepubertal Bos indicus heifers.

Keywords: Heifer, puberty induction, progesterone supplementation strategies, progesterone concentration

Effects of Progesterone Device and Equine Chorionic Gonadotropin (eCG) on Fertility of Bos indicus Heifers Developed in Drylots

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BACKGROUND and OBJECTIVE: The objective of this study was to compare the fertility of drylotted Bos indicus heifers subjected to ovulation synchronization protocols differing in progesterone device strategy and equine chorionic gonadotropin (eCG) administration.

MATERIALS and METHODS: A total of 789 Nelore heifers developed under drylot conditions were subjected to a puberty induction protocol in which heifers received 0.5 mg of melengestrol acetate (MGA) from Days −26 to −14, followed by a 0.6 mg injection of estradiol cypionate (ECP) 24 hours after MGA withdrawal. Heifers were then enrolled in a completely randomized design and assigned to 1 of 4 ovulation synchronization treatments: 1) CIDR4: Heifers received a controlled internal drug release (CIDR; 1.9 g of progesterone) device that had been previously utilized for 27 days, along with 2.0 mg of estradiol benzoate on Day 0. Nine days later, heifers received 12.5 mg of prostaglandin F2α (PGF) and 0.6 mg of ECP concurrent with CIDR removal. 2) CIDR1+eCG: Heifers received the same treatment as CIDR4; however, a new CIDR was used on Day 0 and 200 IU of eCG was administered on Day 9. 3) DIB0.5: Same as CIDR4; however, a 0.5-mg progesterone device (DIB0.5) instead of the 1.9-g CIDR was utilized. 4) DIB0.5+eCG: Heifers received the same treatment as CIDR1+eCG; however, heifers received a DIB0.5 instead of the 1.9-g CIDR. For all treatments, FTAI was performed 48 ± 2 after progesterone device removal and estrus expression was evaluated at FTAI using tail-chalk. Transrectal ultrasonography was performed on Days 9 and 11 to determine dominant follicle diameter and early ovulation.

Pregnancy diagnosis was performed 30 and 60 days after FTAI.

RESULTS: Heifers exposed to the CIDR1+eCG treatment had decreased (P≤0.05) follicle diameter on Days 9 and 11 compared with CIDR4, DIB0.5, DIB0.5+eCG heifers, which were similar among them (P≥0.18). There was a decrease in the percentage of heifers expressing estrus in the CIDR1+eCG compared with CIDR4 heifers (P=0.03), whereas DIB0.5 and DIB0.5+eCG heifers were intermediate (P≥0.21). The percentage of heifers with early ovulation was not impacted by treatment (P=0.18). Pregnancy rates 30 (P=0.51; CIDR4=56.9%, CIDR1+eCG=51.1%, DIB0.5=57.7%, and DIB0.5+eCG=53.5%) and 60 days (P=0.69; CIDR4=53.4%, CIDR1+eCG=48.2%, DIB0.5=49.2%, and DIB0.5+eCG=52.5%) after FTAI were also not influenced by treatment. Treatment did not impact pregnancy loss (P=0.54).

CONCLUSIONS: In summary, although heifers exposed to CIDR1+eCG treatment had reduced follicle diameter and estrus expression, early ovulation and pregnancy rates were not impacted by treatment in the present study.

Keywords: Heifer, Progesterone device, Equine Chorionic Gonadotropin (eCG), AI protocol strategy, Reproductive performance

Incidence of Pregnancy Loss on Ten Dairy Farms in the Usa: Setting New Parity Categories and Stage of Pregnancy-Specific Abortion Benchmarks

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BACKGROUND and OBJECTIVE: Traditionally "high abortion rate" (pregnancy loss, PL) is often defined as >2-5%. However, this does not consider the widespread adoption of early ultrasonographic pregnancy detection (PD). A higher percentage of PL occurs in the embryonic (<45 days of gestation; embryonic mortality, EM) than in the fetal stage (45 days to parturition, fetal mortality, FM) of pregnancy. However, on modern farms ultrasonographic PD tends to occur at ~30 and ~60 days of gestation. Thus, PL encompasses both late EM and FM. Given these changes we need to reevaluate how we calculate and our benchmarks for "high" abortion rates in the modern dairy herds. Hence, the objective of this retrospective analysis was to characterize the incidence and parity characteristics of late EM and FM after ultrasonographical PD on USA dairy farms.

MATERIALS and METHODS: Ten dairy farms in the southwest and midwest of the USA with good reproduction records were selected. The average herd size was 2,523 lactating cows (range 160-7753). Each farm's software database was used to extract the data on PL (PL excluded perinatal mortalities >260 days of gestation) for nulliparae and parous cows. The first ultrasonographic PD was done at ~32 days (PD1) and the second between 60-75 days of gestation (PD2). Overall PL included losses from PD1 to 260 days of gestation (i.e. only pre-term losses).

RESULTS: In lactating dairy (parous), the overall incidence of PL was 10.9% (herd range 7.8-13.9%). The incidence of PL between PD1 and PD2 was 6.5% (herd range 4.5-7.5%). Nearly 60% of all PL occurred between PD1 and PD2. In dairy heifers (nulliparae), the overall incidence of PL was 4.8% (herd range 1.7-9.9%). The incidence of PL between PD1 and PD2 was 1.7% (herd range 0.2-6.2%). Nearly 35% of all PL occurred between PD1 and PD2.

CONCLUSIONS: In this dairy cohort both early and late PL rates were more than twice as high in parous cows compared to nulliparae. These findings highlight the need for parous category- and stage of pregnancy specific benchmarks for intervention thresholds when investigating PL ("abortion") on modern USA dairy farms. It is suggested that thresholds of ~ >2 and 7% (for PD1-PD2 losses), >3 and >4%, (from PD2 to pre-term) and >5 and 10% (from PD1 to pre-term) may be used to define pregnancy stage-specific "high abortion rate" in nulliparae and parous, respectively. A larger dataset is needed to confirm or refute these preliminary conclusions.

Keywords: Abortion, dairy, Embryonic mortality, Fetal Mortality

Injectable Trace Mineral Supplementation Increases Conception Rates in Nellore Cows Submitted to Fixed-Time Artificial Insemination

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BACKGROUND and OBJECTIVE: An optimal trace mineral status is critical for reproductive efficiency in beef cattle. The objective of this study was to evaluate the impact of injectable trace mineral (ITM) administration at the beginning of a fixed-time artificial insemination (FTAI) protocol on the conception rates of Nellore cows in Brazil.

MATERIALS and METHODS: A total of 1,182 multiparous Nellore cows from two farms in Mato Grosso do Sul, Brazil, were enrolled in December 2025. Animals were maintained in an extensive grazing system on tropical pasture with ad libitum access to mineral salts. Cows were randomly assigned to one of the following treatment groups blocking by farm: ITM (n=586) or Control (CON, n=596). On Day 0, the ITM group received a subcutaneous injection (1 mL/100 kg BW) of a solution containing zinc (60 mg/mL), copper (15 mg/mL), manganese (10 mg/mL) and selenium (5 mg/mL) (Multimin90®, Axiota Animal Health). Simultaneously, the synchronization protocol was initiated with an intravaginal progesterone device (P4) and intramuscular (IM) administration of 2.0 mg of estradiol benzoate (EB). On Day 8, the device was removed, and cows received 500 µg of cloprostenol (PGF), 400 IU of equine chorionic gonadotropin (eCG), and 1.0 mg of estradiol cypionate (ECP) IM. Insemination occurred on Day 10. Pregnancy diagnosis was conducted via ultrasound on Day 41. Body condition score (BCS) differences among treatment groups were assessed using ANOVA, while differences in the proportion of pregnant cows between treatment groups were calculated using chi-square test. A logistic regression model was built to analyze the association of treatment with pregnancy diagnosis outcome (Pregnant vs Open), using treatment, farm, sire, BCS category (BCS< or ≥3), and estrus expression (Yes/No) and their interactions as fixed independent variables.

RESULTS: On a 1-5 scale mean BCS was 3.03±0.37 and didn't differ between treatment groups (p=0.23). Conception rate was significantly higher (p=0.02) in the ITM group (57.7%; n=338) compared to the CON group (50.8%; n=303). The logistic regression revealed that cows treated with ITM had 1.30 times greater odds of pregnancy compared to controls (OR=1.30; 95%CI 1.03–1.67; p=0.02). No significant interactions were observed for treatment by farm (p=0.96), treatment by estrus expression (p=0.63), treatment by sire (p=0.73), or treatment by BCS category (p=0.98), confirming the effect of ITM was consistent across all evaluated variables.

CONCLUSIONS: Administration of injectable trace minerals (Zn, Cu, Mn and Se) at the beginning of a synchronization protocol significantly increased conception rates in multiparous Nellore cows.

Keywords: Injectable trace minerals, reproduction, Brazil, Nellore, cows

Association of Estrous Expression Detected by an Ear-Attached Automated Activity Monitoring System Within 50 Days in Milk and Reproductive Performance of Lactating Holstein Cows

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BACKGROUND and OBJECTIVE: The objective of this observational study was to evaluate the association of estrous expression within 50 days in milk (DIM) using an ear-attached automated activity monitor (CowManager, AgisAutomatisering, NL) with reproductive performance in lactating Holstein cows.

MATERIALS and METHODS: A total of 2,911 cows (1,061 primiparous cows and 1,850 multiparous cows) from a commercial dairy farm were included in the statistical analyses. Activity data from the first 7 d after calving were excluded. An estrus event was defined based on a proprietary algorithm. Cows were classified according to the number of estrus events from d 7 until d 50 postpartum into 3 categories: (1) no estrus event (Estrus0); (2) one estrus event

(Estrus1), and (3) 2 or more estrus events (Estrus2). Generalized linear mixed models were used to analyze pregnancy per AI at first service. Cox proportional hazard models were used for time to event data.

RESULTS: Overall, 21.5% of cows had no estrus event detected by an automated activity monitor system from d 7 until d 50 postpartum. For Estrus0, Estrus1, and Estrus2 cows, pregnancy per first AI was 35.4 ± 2.1%, 40.4 ± 1.7%, and 44.7 ± 1.8%, respectively. There was a significant (P<0.05) difference between all groups regarding P/AI at first service. Estrous expression from d 7 until d 50 postpartum affected time to first AI and time to pregnancy. Compared with Estrus0 cows, cows in Estrus1 (hazard risk [HR] = 1.43; 95% CI 1.29 – 1.58; P <0.01) and Estrus2 (HR = 1.91; 95% CI 1.72 – 2.11; P <0.01) had an increased hazard of being inseminated within 100 days in milk (DIM). Median DIM to first AI were 80, 65, and 62 for cows in Estrus0, Estrus1, and Estrus2, respectively. There was a significant (P<0.05) difference between all groups regarding median DIM to first AI. Compared with Estrus0 cows, cows in Estrus1 (HR = 1.43; 95% CI 1.26 – 1.62: P<0.01) and Estrus2 (HR = 1.67; 95%CI 1.47 – 1.89; P<0.01) had an increased hazard of becoming pregnant within 150 DIM. Median DIM to pregnancy were 125, 96, and 86 for Estrus0 cows, Estrus1 and Estrus2, respectively. There was a significant (P<0.05) difference between all groups regarding median DIM until pregnancy.

CONCLUSIONS: In conclusion, cows with no estrous expression from 7 to 50 DIM had inferior reproductive performance compared with cows that displayed estrous activity using an ear-attached sensor system.

Keywords: Estrous expression, voluntary waiting period, reproduction, automated activity monitoring

Influence of Maternal Position on Traction Force During Calving Assistance - a Randomized, Controlled Study

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BACKGROUND and OBJECTIVE: The objective of this randomized, controlled study was to compare traction force profiles during assisted calf extraction in standing versus recumbent cows in order to generate evidence-based recommendations for bovine obstetrical practice.

MATERIALS and METHODS: This study was conducted on a commercial dairy farm milking 3000 cows, using a just-in-time calving management system. Cows are moved to the individual calving pen when signs of imminent parturition (e.g. vaginal discharge with bloody traces, lying lateral with abdominal contractions, a visible or broken amniotic sac, or feet of the emerging calf outside the vulva) are apparent. After a vaginal examination, only cows and heifers with a normal fetal presentation are included in the study. Each dam then is given 30 minutes, from the time when fetal membranes or hooves were seen in the vulva, to allow opening of the soft birth-channel, before assisted calf extraction is performed. Cows are randomly assigned to either a standing or lying position during calving assistance. Cows in the lying group are cast down with a rope prior extraction. Pulling force is measured using a digital force gauge placed between the calf and a mechanical calf puller. Linear mixed-effects models are used to assess the effect of treatment (standing vs. lying) on peak traction force (PeakForce), average traction force (avgForce) and area under the curve (AUC). Data collection is ongoing. Based on a power analysis assuming a 30% difference in force between groups, a total sample size of 126 cows, with 63 animals per group are planned for enrollment.

RESULTS: To date, 23 cows (10 primiparous and 13 multiparous; standing n = 11, lying n = 12) have been included in the preliminary statistical analyses. Maternal position during calving assistance had no effect on PeakForce (standing 1,102 ± 505 N vs. lying

888 ± 369 N; P = 0.332), avgForce (standing 340 ± 184 N vs. lying 341 ± 149 N; P = 0.278) and AUC (standing 114,623 ± 134.990 Ns vs. lying 117,602 ± 149,015 Ns; P = 0.529).

CONCLUSIONS: In this preliminary findings, maternal position had no effect on pulling force during calving assistance. Whether significant differences between groups exist will be determined after completion of data collection, as a larger sample size will allow a more reliable assessment of statistical significance and clinical relevance.

Keywords: Bovine obstetrics, Calving assistance, Maternal position, Traction force, Dairy cow

Effect of Omega-3 Supplementation on Follicle and Luteal Morphology and Pregnancy Rate in Cows under Thermoneutral Conditions: A Preliminary Study

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BACKGROUND and OBJECTIVE: Reproductive efficiency is a key factor affecting the profitability of dairy cattle production. Omega-3 polyunsaturated fatty acids are known to influence reproductive physiology by modulating prostaglandin synthesis, steroidogenesis, and follicular dynamics. However, the effects of Omega-3 supplementation on luteal and follicular morphology evaluated by ultrasonography and Doppler imaging remain unclear, particularly under thermoneutral conditions. This study aimed to investigate effect of Omega-3 supplementation on follicular and luteal morphology and pregnancy rate in dairy cows under thermoneutral conditions.

MATERIALS and METHODS: Twenty-six clinically healty Holstein dairy cows aged 3-8 years, with a mean body condition score 2.75-3.0, primiparous and 45 days postpartum, were used in te study. Cows randomly divided into two groups. On days -3 and 4, cows in the O3 group (n = 17) received Omega-3 bolus orally; whereas cows in the Control group (n = 9) received Omega-3 free bolus orally. For synchronization, all cows were treated Ovsynch protocol (Day 0 GnRH- Day7 PGF2 alpha- Day 9 GnRH), followed by timed artificial insemination16-20 h after the second GnRH injection. To evaulate changes in follicular and luteal morphology, transrectal ultrasonography was performed on Day 10 (follicular evaluation) and on Days 16, 22, and 24 (luteal evaluation). Pregnancy diagnosis was performed on Day 40.

RESULTS: According to preliminary results, luteal diameters in the O3 group were 25.36 ± 4.31 mm, 26.25 ± 4.65 mm and 27.51 ± 4.47 mm, whereas those in Control group were 24.35 ± 3.24 mm, 27.69 ± 3.95 mm, 26.79 ± 8.26 mm on Days 16 (P=0.546), 22 (P=0.346), and 24 (P=0.772), respectively. No significant differences were observed between the O3 and Control groups (P>0.05). Similar patterns were also observed for luteal area and luteal doppler area. Similarly, mean follicle diameter was 16.76 ± 3.70 mm in the O3 group and 18.28 ± 6.20 mm in the Control group (P=0.705). No significant differences were observed between the groups in terms of follicle area and Doppler area of follicular wall (P>0,05). The mean pregnancy rate was numerically higher in the O3 group (%52.9) than in the Control group (%33.3); however, this difference was not statistically significant (P=0.340).

CONCLUSIONS: In conclusion, although Omega-3 supplementation did not significantly affect luteal and follicular morphology in this preliminary study, the higher pregnancy rate observed in the O3 group suggests that further studies with larger sample sizes are warranted.

Keywords: Omega-3, Cow, Ovarian morphology, Pregnancy rate

Effects of New and Reused Intravaginal Progesterone Devices After Timed Artificial Insemination on Reproductive Performance in Dairy Cows

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BACKGROUND and OBJECTIVE: Early embryonic loss accounts for 25–30% of pregnancy failures in dairy cows and is associated with low circulating progesterone (P4) during the early luteal phase. Intravaginal progesterone-releasing devices, either new or reused, are used to improve reproductive performance. This study evaluated the effects of these devices applied after timed artificial insemination (TAI) on serum P4 concentrations, pregnancy, and return rate in dairy cows.

MATERIALS and METHODS: Ninety multiparous dairy cows, second lactation, were enrolled in Balıkesir, Türkiye. Following the Ovsynch protocol for the first insemination, cows were assigned to three groups (n = 30): control (no device), new PRID-DELTA (NP), and reused device (RP; previously used for 7 days). Intravaginal devices were used between days 5 and 12 after TAI. Blood samples were collected on days 5, 9, and 12 after TAI to determine serum progesterone (P4) concentrations. Follicle diameter at insemination, corpus luteum presence on day 9, pregnancy status on day 30, and return to estrus after insemination were recorded.

RESULTS: Mean days in milk (78.0 ± 2.63, 80.2 ± 1.70, and 83.6 ± 1.82 days) and milk yields (44.50 ± 1.07, 43.16 ± 2.00, and 40.37 ± 1.51 kg) did not differ among control, NP, and RP groups ($p > 0.05$). Similarly, there were no significant differences in follicle diameter (1.93 ± 0.11, 1.82 ± 0.10, and 1.88 ± 0.11 cm) or corpus luteum presence (93.3%, 83.3%, and 90.0%) among groups ($p > 0.05$). Serum P4 concentrations were similar among groups ($p > 0.05$) on days 5 (3.43 ± 1.27, 2.99 ± 1.07, and 1.69 ± 0.70), 9 (4.83 ± 1.07, 4.51 ± 0.38, and 3.88 ± 1.08), and 12 (4.55 ± 0.98, 4.28 ± 0.51, and 3.97 ± 0.44 ng/mL) in the control, NP, and RP groups, respectively. Pregnancy rates on day 30 (33.3%, 50.0%, and 43.3%) did not differ among groups ($p > 0.05$). However, the return rate in non-pregnant cows was higher ($p<0.05$) in the NP (53.3%) and RP (70.6%) groups than in the control group (25.0%).

CONCLUSIONS: Progesterone supplementation with new or reused devices between days 5 and 12 after TAI did not affect serum P4 concentrations or pregnancy rates but increased return to estrus in non-pregnant cows. These findings indicate that intravaginal P4 devices may improve synchronization of the subsequent estrous cycle and reproductive management efficiency in non-pregnant cows.

Keywords: Cow, embryonic loss, intravaginal progesterone, pregnancy

Is Early Insemination Too Early? Reproductive Performance of Dairy Cows in Targeted Reproductive Management Programs Using Sexed Semen

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BACKGROUND and OBJECTIVE: Targeted reproductive management programs improve reproductive efficiency by integrating estrus detection with fixed-time artificial insemination, where cows detected in estrus are inseminated and those not detected are enrolled in synchronization protocols. Because sexed semen with high genetic merit is commonly used at first service, it is important to determine whether early insemination within targeted reproductive management programs poses a risk to fertility. The aim of this study was to evaluate whether early insemination according to days in milk (DIM) within a TRM program poses a risk for pregnancy rate in Holstein cows.

MATERIALS and METHODS: A total of 1,938 cows were included in the study, consisting of 1,255 primiparous and 683 multiparous cows. Estrus detection was performed using a herd monitoring system (GEA, Germany). Cows detected in estrus were grouped according to DIM (51–60 [d50+], 61–70 [d60+], and 71–80 [d70+] DIM). Cows not detected in estrus were inseminated using a G6G synchronization protocol, with fixed-time artificial

insemination performed between 91 and 99 days in milk. All inseminations were performed using sex-sorted semen. Cows expressing estrus after the first insemination were recorded as return cases, and pregnancy diagnosis was performed at 33 ± 3 days after insemination.

RESULTS: Overall pregnancy rates were 44.1% in primiparous cows and 38.5% in multiparous cows. A total of 57.3% of primiparous cows and 60.9% of multiparous cows were inseminated after estrus detection. In primiparous cows, pregnancy rates were 38.5%, 44.4%, 47.8%, and 44.5% in the d50+, d60+, d70+, and G6G groups, respectively, and did not differ significantly among groups. In contrast, in multiparous cows, pregnancy rates were 21.5%, 34.8%, 38.7%, and 49.4% for the d50+, d60+, d70+, and G6G groups, respectively, with cows inseminated at d50+ showing significantly lower ($p<0.05$) pregnancy rates than those inseminated later in lactation. Moreover, the return-to-estrus rate among non-pregnant cows was lower ($p<0.05$) in the G6G group (42.2%, 36.3%; primiparous and multiparous, respectively) than in cows inseminated after estrus detection in the d50+ (57.8%, 53.0%), d60+ (58.3%, 50.7%), and d70+ (53.4%, 53.7%) groups.

CONCLUSIONS: Although insemination with sexed semen before 60 days in milk may compromise fertility in multiparous cows, the targeted reproductive management program remained effective because more than 50% of the cows had estrus alerts during the voluntary waiting period. Insemination based on estrus detection within TRM programs can reduce the need for reproductive hormone treatments and shorten the interval to pregnancy, thereby promoting earlier conception in lactation.

Keywords: cows, fertility, sexed semen, targeted reproductive management

The Impact of Inflammation on the Proportion of Reproductive Hormone Receptors in the Endometrium and the Dimensions of the Superficial and Glandular Epithelium in Anestrus Type II Dairy Cows

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BACKGROUND and OBJECTIVE: The extension of the interval between calving and the subsequent fertilization is a critical factor affecting the reproductive efficiency of dairy cows. In the context of anestrus type II cows, this study aimed to initially compare the levels of hormone receptors and the height of the epithelium in inflamed and healthy endometria.

MATERIALS and METHODS: Among 33 cows exhibiting anestrus type II, biopsy samples were collected prior to the insertion of progesterone vaginal inserts. The samples underwent histological processing, cutting, and staining with hematoxylin-eosin (HE) and immunofluorescent (IF). On HE-stained slides, the manual assessment of inflammatory cell infiltration (ICI) was conducted, and histometric analysis was carried out using CellSens Standard Image Analysis software. In six samples identified as endometritis (E), inflammatory cell infiltration (ICI), mainly comprising lymphocytes, was observed. Meanwhile, six additional slides were designated as the control group (C). The histometric analysis involved assessing the height of the superficial epithelium (HSE), the diameter of inactive uterine glands (DIG), and the diameter of secretory-active uterine glands (DSG). Immunofluorescent (IF) labeling on slides allowed visualization of estrogen receptors (ERα, ERβ) and progesterone receptors (PR) using specific primary mouse monoclonal antibodies. Comparative analysis of data series from the control (C) and endometritis (E) groups was conducted using an unpaired t-test with Welch's correction, and the results were presented as mean±SD.

RESULTS: No significant differences were observed in the endometrial percentage of immunopositive

cells for ERα (C: 5.95±2.37%; E: 4.54±3.34%; p=0.465), ERβ (C: 5.77±1.96%; E: 4.45±2.03%; p=0.328), and PR (C: 3.74±0.92%; E: 3.15±1.33%; p=0.441). However, histometric measurements consistently indicated higher values (HSE: p=0.025; DIG: p=0.002; DSG: p=0.021) in the endometritis group (HSE: 17.11±0.58µm; DIG: 75.15±2.35µm; DSG: 90.13±17.81µm) compared to the control group (HSE: 15.98±0.70µm; DIG: 66.86±3.17µm; DSG: 61.92±5.73µm).

CONCLUSIONS: In anestrus type II, it is suggested that, in addition to the influence of reproductive hormones, other factors related to inflammation may contribute to alterations in the height of the superficial and glandular epithelium in the bovine endometrium.

Keywords: bovine endometrium, anestrus, endometritis, hormone receptors, histometry

Evaluation of the Dynamic Interactions of Serum Macro Minerals, Trace elements, Glutathione Peroxidase (GPx) Activity, and Energy Metabolites Throughout the Gestation–Lactation Period in Holstein Cows

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BACKGROUND and OBJECTIVE: Macro minerals (Ca, P, Mg, Na, K, Cl) and trace elements (Cu, Fe, Zn) play essential roles in maintaining the biochemical functions of the body. One of the most important cellular defense mechanisms against harmful free radicals is GPx, a selenium-dependent enzyme[1]. With the onset of lactation, negative energy balance develops, leading to mobilization of body fat reserves and increased blood concentrations of non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHBA)[2]. This study aimed to evaluate the dynamic changes and interactions among serum macro minerals, trace elements, GPx activity, and metabolic stress markers (NEFA, BHBA) across eight critical reproductive stages from the pre-dry period to mid-lactation in Holstein dairy cows.

MATERIALS and METHODS: Thirty Holstein cows (BCS: 2.75–3.00; age: 5±1.5 years; average milk yield: 27±3.7 L/day) were included in the study. Blood samples were collected (V.jugularis) at eight different time points: 4–6 weeks before the dry period (PreDP4–6W), at dry period (DP–D60), early dry period (EDP–D30), 8–3 days antepartum (APD8–3), postpartum day 3 (Pp–D3), postpartum day 8 (Pp–D8), postpartum weeks 3–4 (Pp3–4W), and postpartum weeks 7–8 (Pp7–8W). Data were analyzed using repeated measures ANOVA and Pearson correlation tests.

RESULTS: Among macro minerals, P levels showed the most significant decrease at Pp-D8 compared to the dry period (5.76 ng/mL; P<0.001). Mg concentrations reach the low levels at Pp-D3 and Pp-D8. K levels significantly decreased at Pp-D3 and Pp-D8 (P<0.001). Na concentrations decreased during EDP-D30, reached the lowest level at APD8–3 (129 mmol/L; P<0.001). Cl levels showed a significant decline from Pp3–4W through Pp7–8W compared to dry period values. Regarding trace elements, Zn levels reached their lowest concentration at APD8–3 (P<0.001). Cu levels began to decline significantly at EDP-D30 and, although they increased postpartum. Fe concentrations showed a continuous decrease from APD8–3 to Pp3–4W (P<0.001). A significant increase in BHBA concentration was observed at Pp-D3 (0.615 mmol/L; P<0.05), followed by a significant decrease at Pp-D8 (0.410 mmol/L; P<0.001). NEFA levels increased significantly at APD8–3 (0.560 mmol/L) and reached the highest level at Pp-D3 (0.983 mmol/L; P<0.001). Regression analyses revealed a positive association between GPx and NEFA at APD8–3, and a negative relationship between BHBA and Cu, K, and Fe at Pp-D8.

CONCLUSIONS: In conclusion, the evaluated parameters showed significant alterations particularly during the APD8–3, Pp-D3, and Pp-D8 periods. These findings emphasize the importance of proper dry period management to support postpartum health during the transition period in dairy cows. [1]Sayiner et al.(2021)Theriogenology,159,116-122. [2]Macmillan et al.(2020)Res.Vet.Sci.,130,126-132.

Keywords: Dairy Cows, Minerals, GPx Activity, NEFA, BHBA

Effect of Anti-GnRH Vaccine on Postpartum Ovarian Activity and Milk Production in Lactating Cows During Summer

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BACKGROUND and OBJECTIVE: Suppression of ovarian activity with use of Anti-GnRH vaccine was aimed during summer in lactating Holstein cows. Moreover, effect of suppression of ovarian activity on milk yield was investigated.

MATERIALS and METHODS: Cows were randomly assigned to Control (n=24) and Anti-GnRH (n=21) groups. Physiologic saline or anti-GnRH vaccine (Bopriva, Zoetis Animal Health) was administered four weeks apart on days 3±2 and 31±2 postpartum (pp) in Control and Anti-GnRH groups, respectively. Ovarian activity was accepted as present if there was at least a follicle ≥ 10 mm and/or corpus luteum or follicular cyst on days 40±3 pp. Cows in Control group were inseminated following estrous detection, and cows in Anti-GnRH group were enrolled to Ovsynch+CIDR protocol at 120-121 days pp. In Ovsynch+CIDR protocol, GnRH (gonadorelin acetate; 100 µg; Acegon®, Zoetis Animal Health) and intravaginal CIDR (1.38 g progesteron, CIDR®, Zoetis Animal Health) were administered followed by PGF2α (dinoprost tromethamine; 25 mg; Dynolytic®, Zoetis Animal Health) injection and CIDR removal seven days later. Fiftysix hours after PGF2α administration, GnRH was injected followed by AI 16-18 hours later. Daily milk yield (L) was collected during 180 days of lactation.

RESULTS: Ovarian activity was 0.006 (0.001-0.203) times lower (P<0.01) in primiparous (9/24; 37.5%) compared to multiparous (17/18; 94.4%) cows. Ovarian activity was similar between Control (17/23; 73.9%) and Anti-GnRH (9/19; 47.4%) groups despite tended (P<0.08) to be higher in Control group. Significant (P<0.05) effect of treatment by parity revealed severe effect of Anti-GnRH treatment on primiparous cows. In this matter, ovarian activity in Control group was 6/12 (50%) and 11/11 (100%) in primiparous and multiparous cows, respectively. Whereas, ovarian activity in Anti-GnRH group was 3/12 (25%) and 6/7 (85.7%) in primiparous and multiparous cows; respectively. Pregnancy rate at 29-32 days was significantly (P<0.01) lower in Anti-GnRH group (1/20; 5%) following timed AI at 130 or 131 days and more services until 180 days pp than that in control group (14/24; 58.3%) with one or multiple services at 49-174 days pp. There was no difference between Control (37.2 ± 1.09 L) and Anti-GnRH (36.9 ± 1.24 L) groups for average daily milk yield.

CONCLUSIONS: In conclusion, anti-GnRH vaccine administration could suppress ovarian activity for longer period leading to lower fertility. Further research is warranted to examine the effects of short term supression of ovarian activity during heat stress in cows. Suppression of ovarian activity did not have any effect on milk production.

Keywords: Cow, anti-GnRH, ovarian activity, milk yield

Dose–Response Effects of Recombinant eCG on reproductive performance of Nelore Heifers Submitted to Fixed-Time Artificial Insemination in Tropical Systems

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BACKGROUND and OBJECTIVE: These studies evaluated the effects of different doses of recombinant equine chorionic gonadotropin (r-eCG; Foli-Rec®, Ceva Animal Health) compared with conventional eCG (eCG; Folligon®, MSD) in fixed-

time artificial insemination (FTAI) protocols for Nelore heifers raised under different tropical conditions.

MATERIALS and METHODS: A total of 1,165 heifers from two Brazilian regions were enrolled. In Experiment 1, 618 heifers were evaluated during the dry season in the northeastern region of Brazil. In Experiment 2, 547 heifers were evaluated during the rainy season in the central-west region. All animals were submitted to the same synchronization protocol. On Day 0 (D0), heifers received an intravaginal progesterone device (Repro-One®, GlobalGen) and 2mg of estradiol benzoate (Benzoato HC®, Ceva). On Day 8 (D8), the progesterone device was removed and animals received prostaglandin $F_2\alpha$ (Luteglan®, Ceva) and 1mg of estradiol cypionate (Cipionato HC®, Ceva). At device removal, animals were randomly assigned to treatments according to the experiment. In Experiment 1, heifers received either 200IU of eCG (FG1.0mL; n=204), 49IU of r-eCG (FR0.7mL; n=207), or 70IU of r-eCG (FR1.0mL; n=207). In Experiment 2, heifers received 35IU (FR0.5mL; n=184), 49IU (FR0.7mL; n=182), or 70IU (FR1.0mL; n=181) of r-eCG. Artificial insemination was conducted in D10, and the pregnancy was diagnosed after 30 days (D40). Data were analyzed using PROC GLIMMIX in SAS 9.4.

RESULTS: In Experiment 1, pregnancy per artificial insemination (P/AI) was 30.6% for FR0.7mL, 39.2% for FR1.0mL, and 42.9% for FG1.0mL. No difference was observed between FR1.0mL and FG1.0mL (P=0.35), whereas FR0.7mL showed lower fertility compared with FG1.0mL (P=0.02) and to tended be lower than FR1.0mL (P=0.08). Body condition score (BCS) significantly influenced fertility (P<0.001), with heifers with BCS≥3 achieving higher P/AI than those with BCS<3 (41.6% vs. 25.4%). Treatment did not affect (P>0.20) follicle diameter at device removal (FG1.0mL= 9.2mm vs. FR0.7mL=8.3mm vs. FR1.0mL=8.7mm) or at insemination (FG1.0mL=11.2mm vs. FR0.7mL=10.9mm vs. FR1.0mL=11.4mm). In Experiment 2, overall P/AI was 54.7% and did not differ among reCG doses (P>0.10), with rates of 56.0%, 51.1%, and 56.9% for FR0.5mL, FR0.7mL, and FR1.0mL, respectively. Estrus expression at the time of insemination was the strongest predictor of fertility, with heifers expressing estrus presenting higher P/AI than non-estrous animals (58.2% vs. 45.4%; P<0.01).

CONCLUSIONS: In conclusion, recombinant eCG yields reproductive outcomes comparable to conventional eCG when doses of 70IU (FR1.0mL) are used in Nelore heifers across different regions and climatic conditions. Body condition score and estrus expression were more important determinants of pregnancy success than gonadotropin doses.

Keywords: Recombinant Hormones, Reproductive efficiency, Bos indicus heifers

Effects of Recombinant eCG Dose on Follicular Dynamics and Fertility in Nelore Cows Submitted to Fixed-Time Artificial Insemination

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BACKGROUND and OBJECTIVE: Equine chorionic gonadotropin (eCG) is widely used in fixed-time artificial insemination (FTAI) protocols to stimulate follicular growth and improve fertility in postpartum beef cows. Recently, recombinant eCG (r-eCG) has been developed as a standardized alternative to conventional eCG, potentially reducing variability associated with biological extraction. The objective of this study was to evaluate the effects of different doses of r-eCG (Foli-Rec®, Ceva Animal Health) compared with conventional eCG (Folligon®, MSD) on follicular dynamics and pregnancy per artificial insemination (P/AI) in postpartum Nelore cows.

MATERIALS and METHODS: A total of 1,062 cows were used. On Day 0 (D0), all animals received 2mg of estradiol benzoate (Benzoato HC®, Ceva Animal Health) and an intravaginal progesterone device (Repro-one®, Globalgen). On Day 8 (D8), the device was removed and cows received prostaglandin $F_2\alpha$ (Luteglan®, Ceva Animal Health) and 1mg of estradiol cypionate (Cipionato HC®, Ceva Animal Health) and were randomly assigned to one of three treatments: conventional eCG (FL1.5mL; 300 IU; n = 358), r-eCG 1.2 (FR1.2mL; 89 IU reCG; n = 353), or r-eCG 1.5mL (FR1.5mL; 105 IU reCG; n = 351). Artificial insemination was performed on Day 10 (D10), and pregnancy diagnosis was conducted 30 days after AI. Follicular dynamics were evaluated in a subset of 88 cows at device removal (D8) and at the time of AI (D10). Data were analyzed using logistic regression,

including the effects of treatment, herd, category (multiparous vs primiparous), and body weight composite.

RESULTS: Overall P/AI was 53.5% (568/1062) and did not differ among treatments [FL1.5mL = 53.4% (191/358), FR1.2mL = 54.1% (191/353), FR1.5mL = 53,0% (186/351); P > 0.05). Dominant follicle diameter at the time of AI was also similar among groups (FL1.5mL = 13.1±3.3 mm; FR1.2mL = 13.0±2.5 mm; FR1.5mL = 14.4±2.6 mm; P > 0.05).
CONCLUSIONS: In conclusion, recombinant eCG produced follicular development and fertility outcomes comparable to conventional eCG in Nelore cows submitted to FTAI protocols. These findings indicate that reCG (at doses of 89 IU or 105 IU) may represent a standardized alternative to conventional eCG for reproductive management in Bos indicus beef production systems.

Keywords: Recombinant hormones, Bos indicus cows, Postpartum beef cows, Reproductive performance

Effect of Reduced D-Cloprostenol Doses at Progesterone Device Removal on Reproductive Performance in Nelore Cows Submitted to Fixed-Time Artificial Insemination

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BACKGROUND and OBJECTIVE: The aim of this study was to evaluate the effect of a reduced dose of D-cloprostenol (Luteglan®, Ceva Animal Health) administered at the time of progesterone device removal in fixed-time artificial insemination (FTAI) protocols in Nelore cows.

MATERIALS and METHODS: A total of 572 cows from two commercial farms located in the states of Minas Gerais and Tocantins, Brazil, were enrolled. On Day 0 (D0), all animals received 2 mg of estradiol benzoate (Benzoato HC®, Ceva Animal Health) and an intravaginal progesterone device (Repro-One®, GlobalGen). On Day 8 (D8), the progesterone device was removed and cows received 1 mg of estradiol cypionate (Cipionato HC®, Ceva Animal Health) and 89 IU of recombinant eCG (r-eCG; Foli-Rec®, Ceva Animal Health). At this time, animals were randomly assigned to one of two treatments: 1ML (75 µg of D-cloprostenol) or 2ML (150 µg of D-cloprostenol). On Day 10, cows received 100 µg of gonadorelin (Cevarelin®, Ceva Animal Health) and were then artificially inseminated. Pregnancy diagnosis was conducted 30 days after AI. Data were analyzed using the GLIMMIX procedure of SAS 9.4.

RESULTS: The overall pregnancy rate was 46.3% (265/572). Pregnancy per AI did not differ between treatments (1ML=45.5% [133/292] vs 2ML=47.1% [132/280], P=0.76). No interaction was observed between treatment and the presence of a corpus luteum at Day 0 (P=0.88), Body Weight Composite (P=0.65), expression of estrus at the time of AI (P=0.51), sire (P=0.19), or farm location (P=0.60).
CONCLUSIONS:In conclusion, reducing the dose of D-cloprostenol at progesterone device removal resulted in pregnancy outcomes similar to the conventional dose in Nelore cows subjected to FTAI protocols.

Keywords: Prostaglandin $F_2\alpha$, Reproductive performance, Bos indicus cows

Why Are Fewer Abortions and Stillbirths Being Reported to Veterinary Diagnostic Laboratories and Does This Affect Our Diagnostic Rate?

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BACKGROUND and OBJECTIVE: The international model of bovine abortion diagnosis is passive surveillance. While this model allows us to monitor temporal trends in submissions, it suffers from significant under-reporting bias. This under-reporting challenges our ability to diagnose the relative importance of the 'true' causes of abortion. Furthermore, it is not known if there is an interaction between changes in the reporting rate of abortions and the diagnosis rate of abortions. To address this question, we conducted a retrospective study with the dual objectives of, firstly, determining if the abortion reporting/submission rate has changed over time and secondly if it had what effect this might have on abortion diagnosis rates.

MATERIALS and METHODS: We interrogated data from the Irish Department of Agriculture, Food and the Marine's busiest Regional Veterinary Laboratory, Cork, to explore temporal trends across a decade using three time points, 2015, 2020 and 2025. These data were the number of abortion/stillbirth submissions/year and the microbiological culture findings from these foetal submissions. While we examined data from all isolates, here we report only on four abortifacients: Truperella pyogenes, Salmonella Dublin, Bacillus licheniformis and Listeria monocytogenes. Logistic regression was used to calculate odds ratios (OR) and confidence intervals (CI95). Data analysis was performed using RStudio (version 4.5.2).

RESULTS: The number of submissions/year declined annually; 1,025 in 2015, 560 in 2020 and 344 in 2025 – a reduction of 66.4% over the decade. The detection rates for T. pyogenes across the three years were 7.4, 10.4 and 6.4%, respectively. For S. Dublin this was 2.1, 3.4 and 3.5%, for B. licheniformis 2.3, 2.3 and 2.9% and for L. monocytogenes 2.5, 2.5 and 4.7%. Using 2015 as the reference year, the OR (CI95) for detection of T. pyogenes in 2020 and 2025 were 1.44 (1.00-2.06) and 0.85 (0.51-1.37), respectively. For S. Dublin this was 1.60 (0.85-2.98) and 1.65 (0.78-3.31), for B. licheniformis 0.99 (0.4901.93) and 1.25 (0.56-2.57) and for L. monocytogenes, 0.99 (0.50-1.87) and 1.87 (0.97-3.50). None of these numerical changes were statistically significant (P>0.05).

CONCLUSIONS: The key finding from this study was that despite a dramatic decline in bovine foetal submissions/year across a decade, there was no significant change in the detection rate for the four abortifacients. While this is reassuring, if the declining submission rate continues this downward trajectory there may come a point where insufficient abortion cases are being investigated to maintain adequate passive surveillance of the prevalence of infectious abortifacients.

Keywords: abortion, under-reporting, laboratory diagnoses

Parity-Targeted Timing of Human Chorionic Gonadotropin Administration After First Postpartum Insemination Improves Fertility in High-Producing Dairy Cows

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BACKGROUND and OBJECTIVE: Optimal progesterone (P4) concentration after ovulation is essential for successful pregnancy establishment in cattle. Human chorionic gonadotropin (hCG) increases luteal area, thereby increasing P4 concentration. We hypothesized that a parity-targeted timing of hCG administration would increase luteal area and improve fertility in high-producing dairy cows. Thus, we aimed to evaluate this strategy, consisting of hCG administration on Day 2 after fixed-time AI (FTAI) in cows with >2 lactations and on Day 5 in cows with 1–2 lactations, on total luteal area and pregnancy per AI (P/AI) at first postpartum FTAI.

MATERIALS and METHODS: Holstein-Friesian cows (n=1,253) were enrolled during the warm (July–September 2025; n=523) and cold (November 2025–January 2026; n=730) seasons. Within farm, cows were blocked by parity, days in milk (DIM), synchronization protocol, and body condition score (BCS) and randomly assigned to receive 2,500 IU of hCG (VeteCorH, Calier; n=614) or no treatment (control; n=639). Using ultrasonography, pregnancy was diagnosed at approximately 30 days after FTAI and, additionally, luteal area was evaluated in a subset of cows (hCG: n=165; control: n=169) on Days 7 and 14. Data were analyzed using mixed-effects models (SAS), including treatment, day of administration (lactation category), season, DIM, BCS, and their interactions, with farm as a random effect.

RESULTS: hCG-treated cows tended to have greater luteal area than controls on Day 7 (4.8 ± 0.2 vs. 4.3 ± 0.2 cm2, respectively; P=0.06) and had greater luteal area on Day 14 (6.3 ± 0.2 vs. 5.0 ± 0.2 cm2 respectively; P<0.01). Overall, hCG increased P/AI compared with control cows (41.0% vs. 36.5%; P=0.03). No treatment × lactation category interaction was detected, indicating a consistent treatment effect whether administered on Day 2 (>2-lactation cows: 41.0% vs. 33.5%) or Day 5 (1–2 lactation cows: 41.1% vs. 38.6%). However, a treatment × season interaction was observed (P=0.04). During the warm season, hCG improved P/AI (38.6% vs. 29.1%; P=0.02), whereas no treatment effect was detected during the cold season. When stratified by day of administration, no treatment × season interaction was observed in cows treated on Day 2, indicating a consistent beneficial effect across seasons (warm: 42.0% vs. 27.5%; cold: 40.5% vs. 36.1%). In contrast, a treatment × season interaction was detected in cows treated on Day 5 (P=0.01), with the beneficial effect restricted to the warm season (38.6% vs. 29.1%).

CONCLUSIONS: These findings support the use of a parity-targeted hCG strategy to improve fertility. Funding: MCIN/AEI/10.13039/501100011033 and NextGenerationEU/PRTR (RYC2021-033574-I); JuntadeAndalucia/CUII and FSE (DGP_POST_2024_00045).

Keywords: cattle, hCG, progesterone, synchronization protocol, reproduction

Exploring Letrozole as a Potential Presynchronization Strategy in Heifers: Preliminary Results

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BACKGROUND and OBJECTIVE: Letrozole, an aromatase inhibitor, increases LH concentration by reducing estradiol levels regardless of the phase of the estrous cycle in cattle. Letrozole prevents the dominant follicle in the current follicular wave from ovulating and undergoing atresia. With these properties, letrozole may be used as a presynchronization strategy. The primary purpose of presynchronization methods is to ensure the presence of a dominant follicle of a size (>8mm) that can respond to the first GnRH injection administered in Ovsynch or Cosynch protocols. For this purpose, the study aims to determine the effect of a single dose or two doses administered 24 hours apart at different intramuscular (IM) and subcutaneous (SC) doses of Letrozole on follicular development in heifers and to compare it with the conventional method.

MATERIALS and METHODS: In the study, 140 Holstein nulliparous heifers were allocated to 14 equal groups including SC and IM administrations of letrozole at doses of 0.5, 0.25, and 0.125 mg/kg, their respective double-dose protocols, a $PGF_2\alpha$ group, and a control group. The PGF2α group will receive only 25 mg dinoprost on day 1. The control group will receive no treatment. Starting from the day of Letrozole or PGF2α administration, ovarian examinations will be performed once daily by ultrasound for 10 days, and the structures on both ovaries was recorded.

RESULTS: Preliminary results suggest that letrozole markedly influences ovarian follicular dynamics compared with controls. In treated groups, the proportion of the animal has dominant follicles rapidly increased to 100% and remained stable for several days before declining, indicating a delay in follicular regression and an extension of dominant follicle lifespan. A significant group × time interaction was observed for follicle diameter ($p<0.001$). While no significant temporal change was detected in the SC, IM, and control groups ($p>0.05$), a significant time effect was found in the $PGF_2\alpha$ group ($p=0.001$), characterized by a marked decrease on day 5 followed by a return to baseline values on days 9–10. On day 5, follicle diameter in the $PGF_2\alpha$ group (7.0±2.73) was significantly lower than in the SC, IM, and control groups ($p=0.001$), and on day 6 the SC group showed the highest value (12.95±3.3) while the $PGF_2\alpha$ group remained the lowest (8.19±2.62) ($p=0.016$).

CONCLUSIONS: These preliminary findings suggest that letrozole may used for presynchronization in heifers by modulating follicular dynamics and prolonging dominant follicle lifespan. The final results of the ongoing study will provide a better understanding of its effectiveness and applicability in synchronization protocols.

Keywords: Presynchronization, Letrozole, Aromatase inhibitors, Follicular dynamics, Heifers

Effect of $PGF_2\alpha$ Dose at Device Removal in a 6-Day GnRH/P4 Protocol on Estrus Expression and Pregnancy Rate in *Bos taurus* Cattle

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BACKGROUND and OBJECTIVE: The objective of this study was to compare the effects of a single versus double $PGF_2\alpha$ dose administered at device removal on estrus expression rate (EER) and pregnancy to timed AI (P/TAI) in beef cattle.

MATERIALS and METHODS: A total of 1,644 Bos taurus females (heifers=364, lactating cows=1,104, non-lactating cows=176) from 21 herds were synchronized using a 6-day GnRH/P4 protocol for TAI. On Day −9, animals received 100 µg of a GnRH analogue and an intravaginal P4 device. On Day −3, the device was removed and eCG was administered (300 IU for heifers and 400 IU for cows). At device removal, animals were randomly assigned to receive one $PGF_2\alpha$ dose (Single, n=817; 500 µg sodium cloprostenol) or two simultaneous $PGF_2\alpha$ doses (Double, n=827; 1,000 µg sodium cloprostenol). Ovulation to the first GnRH was evaluated by ultrasonography in a subsample (heifers=226, lactating cows=355, non-lactating cows=156). Tail paint was applied at device removal. On Day 0 (72–74 h later), animals received 100 µg GnRH i.m. at TAI. Tail paint was evaluated at TAI, and estrus was defined as ≥30% paint removal. Pregnancy diagnosis was performed by transrectal ultrasonography on Day 35. Generalized linear mixed models (PROC GLIMMIX) with binomial distribution and logit link were used for EER and P/TAI. The fixed effect for EER was treatment group. For P/TAI, fixed effects included treatment group and estrus expression. Additionally, response to the first GnRH and its interaction with treatment were included in the P/TAI model. Herd was included as a random effect.

RESULTS: Overall EER was 65.4%, 64.7%, and 73.9% for heifers, lactating cows, and non-lactating cows, respectively. Treatment affected EER only in lactating cows (Single=61.3% vs. Double=67.9%; P=0.01). Overall P/TAI was 54.9%, 53.1%, and 65.5% for heifers, lactating cows, and non-lactating cows, respectively, and was not affected by treatment group in any category (P≥0.19). However, P/TAI was affected by estrus expression in lactating cows (yes=61.4% vs. no=38.1%; P<0.01), tended to be affected in non-lactating cows (yes=66.1% vs. no=52.2%; P=0.07), and was not affected in heifers (P=0.17). Response to the first GnRH and its interaction with treatment group did not affect P/TAI (P≥0.10).

CONCLUSIONS: In conclusion, administering a double dose of $PGF_2\alpha$ at device removal in a 6-day GnRH/P4 protocol increased EER in lactating cows but did not improve P/TAI in beef herds

Keywords: Beef cattle, 6-day Co-Synch protocol, PGF2α dose, Estrus expression, Pregnancy per TAI, GnRH response

A Simple and Efficient Method for the Isolation and Characterization of Bovine Endometrial Mesenchymal Stem Cells for Potential Treatment of Uterine Disorders in Cattle

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BACKGROUND and OBJECTIVE: Mesenchymal stem cells (MSCs) are multipotent adult cells characterized by their ability to self-renew, adhere to plastic surfaces, and differentiate into multiple mesodermal lineages. Due to their regenerative, immunomodulatory, and anti-inflammatory properties, MSCs have gained increasing attention in veterinary regenerative medicine. Conventional sources such as bone marrow and adipose tissue require invasive collection procedures, which may limit their practical application. The uterine endometrium represents a promising alternative source due to its natural regenerative capacity and accessibility. The objective of this study was to establish a simple and efficient protocol for the isolation, culture, and characterization of bovine endometrial mesenchymal stem cells (eMSCs) with potential application in the treatment of uterine disorders in cattle.

MATERIALS and METHODS: Bovine reproductive tracts were obtained from an abattoir shortly after slaughter. Endometrial samples (1–2 cm^2) were aseptically collected from the uterine horns and transported to the laboratory in sterile PBS supplemented with antibiotics and antifungal agents. Tissues were washed, mechanically minced into small fragments, and subjected to enzymatic digestion using collagenase type II and DNase I at 37°C for 60 minutes. The resulting suspension was filtered through a 70 µm nylon filter and centrifuged. The cell pellet was resuspended in DMEM/F12

culture medium supplemented with FCS, antibiotics, glutamine, non-essential amino acids, sodium pyruvate, and β-mercaptoethanol. Cells were seeded in culture flasks and incubated at 37°C with 5% CO_2. Non-adherent cells were removed after 72 hours. Cultures were expanded and passaged at approximately 70% confluence. Immunophenotypic characterization was performed at passage five by flow cytometry using MSC markers (CD29, CD44, CD90, CD105) and hematopoietic markers (CD34, CD45). Clonogenic potential was assessed using a colony-forming unit–fibroblast (CFU-F) assay.

RESULTS: Primary cultures demonstrated progressive adhesion and proliferation, with attachment increasing from approximately 42% after 24 hours to about 60% after 72 hours. Cells exhibited a spindle-shaped, fibroblast-like morphology typical of mesenchymal stem cells, forming a confluent monolayer after approximately 10–12 days of culture. The CFU-F assay confirmed clonogenic potential, with an average of 56.33 ± 3.05 colonies observed. Flow cytometry analysis revealed strong expression of mesenchymal markers, including CD90 (97.8%), CD44 (97.6%), and CD105 (97.5%), while hematopoietic markers CD34 and CD45 were absent.

CONCLUSIONS: The protocol represents a reliable and efficient method for the isolation and expansion of bovine eMSCs. The isolated cells exhibit typical characteristics, consistent with MSCs, supporting the potential use of bovine endometrial tissue as a valuable source of stem cells for future therapies targeting uterine disorders in cattle.

Keywords: bovine, endometrium, mesenchymal stem cells, therapy

The Use of Blood Biochemical Biomarkers and Innovative Technologies to Assess Post-Calving Diseases in Dairy Cows

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BACKGROUND and OBJECTIVE: Post-calving diseases remain one of the most important health and productivity challenges in dairy cows. These diseases negatively affect animal health, milk production and reproductive performance. Herd management programs can help detect physiological changes before clinical symptoms appear. The purpose of this study was to evaluate the diagnostic value of using biomarkers recorded by innovative technologies and blood biochemical parameters associated with post-calving diseases.

MATERIALS and METHODS: The study included 40 fresh dairy cows between 1 and 60 days in milk (DIM). Based on clinical diagnosis, cows were classified into five groups: group 1 – clinically healthy (n=21), group 2 - with metritis (n=6), group 3 - with endometritis (n=6), group 4 - with retained placenta (n=2), group 5 - with mastitis (n=5). Blood biochemical parameters analysed included glucose, aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine, calcium, magnesium, phosphorus, triglycerides, gamma-glutamyl transferase (GGT), albumin, beta-hydroxybutyrate (BHB). Data from the automated herd management system included milk yield (kg/d), somatic cell count (thousands/mL), milk fat, protein and lactose (%), activity (steps/hour), rumination (min/day).

RESULTS: The most frequently diagnosed diseases were metritis and endometritis (30%). The incidence of mastitis was 12.5% of all dairy cows while retained placenta occurred in 5% of fresh dairy cows. Higher body condition score was associated with the occurrence of endometritis ($p<0.05$). Milk yield decreased by 28% in cows with retained placenta and by 25% in cows with mastitis ($p<0.05$). Somatic cell count was significantly higher in cows with mastitis (463 thousand/mL, $p<0.05$). Cow activity decreased by 60% in cows with endometritis ($p<0.05$). Significant differences in glucose, creatinine, magnesium and BHB concentrations were observed between diseased and healthy cows ($p<0.05$). Days in milk positively correlated with glucose and triglyceride levels and negatively correlated with creatinine concentration.

CONCLUSIONS: Biomarkers recorded by the herd management system and blood biochemical parameters are valuable indicators for detecting post-calving diseases as they are sensitive to physiological changes associated with disease and strongly correlate with each other. The use of these diagnostic methods may improve early disease detection and overall herd management.

Keywords: Innovative technologies, dairy cows, reproduction, blood biochemical biomarkers

Effects of Dry Period Length on Milk Yield and Calving Interval in Holstein Cows

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BACKGROUND and OBJECTIVE: Dry period management plays a key role in optimizing milk production and reproductive performance in dairy cows. Inappropriate dry period duration may impair mammary gland regeneration and affect subsequent lactation yield. The aim of this study was to evaluate the effects of dry period length and season on milk yield parameters and calving interval in dairy cows.

MATERIALS and METHODS: A total of 2,695 lactation records were included in the analysis. Cows were classified into four groups according to dry period length as follows: 40–50 days (Group 1, n = 1564), 51–60 days (Group 2, n = 817), 61–70 days (Group 3, n = 214), and ≥71 days (Group 4, n = 99). Calving season was categorized as hot season (May to September) and cool season (October to April). The effects of dry period length and season on lactation performance traits, including average milk yield, peak milk yield, days to peak yield, 305-day milk yield, total milk yield, and calving interval, were evaluated using one-way analysis of variance (ANOVA).

RESULTS: Days to peak milk yield was 64.14 ± 0.72, 61.87 ± 0.97, 63.90 ± 1.70, and 62.96 ± 2.31 days for Groups 1–4, respectively, and was not affected by dry period length ($p > 0.05$). However, peak milk yield was significantly lower ($p < 0.01$) in Group 1 (50.96 ± 0.18 kg) compared with Groups 2 (52.88 ± 0.24 kg), 3 (53.07 ± 0.55 kg), and 4 (53.78 ± 0.65 kg). Similarly, 305-day milk yield was lower ($p < 0.05$) in Group 1 (11,376.27 ± 43.94 kg) than in Group 2 (11,586.98 ± 60.03 kg). Calving interval was significantly shorter in Group 1 (403.91 ± 1.66 days) compared with Groups 2 (416.69 ± 2.63 days), 3 (426.29 ± 5.68 days), and 4 (423.11 ± 8.35 days). Seasonal effect was significant ($p < 0.05$), with cows calving in the cool season showing higher peak milk yield (≈3 kg), higher 305-day milk yield (≈330 kg), and shorter calving interval (≈7 days) compared with cows calving in the hot season.

CONCLUSIONS: Dry period length influenced milk production parameters. A dry period of 51–60 days was associated with higher 305-day milk yield, whereas dry periods shorter than 50 days were related to lower peak and 305-day milk yield but a shorter calving interval. Seasonal effect was significant, and milk yield performance was lower in the hot season.

Keywords: calving interval, cows, dry period, milk yield

Management Practices in Spanish Beef Cattle Systems: Implications for Reproductive Efficiency and Herd Health

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BACKGROUND and OBJECTIVE: Beef cattle production encompasses diverse management systems whose characterization is essential to identify improvement opportunities. Epidemiological data is scarce. The objective was to describe management practices, herd structure, and health and reproductive indicators in Spanish beef farms, providing a useful reference for management efficiency.

MATERIALS and METHODS: Herd-annual records from five veterinary groups were assembled for 25 beef-herds over a mean of 2.5 seasons per herd (2018–2025) across eight Spanish provinces. Collected data included herd characteristics,

sanitary status, and reproductive management descriptors. Recorded outcomes included reproductive indexes. Data were analyzed using IBM SPSS Statistics v.30.0, applying Chi square tests for categorical associations, and logistic regression and decision tree models to identify predictors and classify outcomes.

RESULTS: Data comprised 11,633 cattle (mean 191±265 cattle/herd) spanning >9 breeds; 49.2% of herds bred purebreds. Limousin was predominant in cows (34.3% of herds) and bulls (70.5%), alone or combined; Charolais was second, combined with local breeds in cows (32.7%) and bulls (41.0%). Health control programs were used in 91.8% of herds; vaccination covered clostridia (CLOST) in 100%, CLOST+IBR in 29.5%, CLOST+IBR+BVD in 29.5%, CLOST+IBR+BVD+neonatal diarrhea (ND) in 23.0%, and in one herd (1.6%) bovine respiratory disease (BRD). Regarding reproduction, 91.8% controlled breeding and the calving season (mean 7.59±1.23mo). The most frequent strategy was AI with subsequent clean up bulls (85.2%); 8.2% used only natural service and 6.6% only AI. When using AI, 4.9% restricted it to heifers; 83.6% applied a mating plan to match AI bulls with cows. Natural service averaged a cow: bull ratio of 30.6±8.19, and 86.9% performed annual BBSE. Routine reproductive examinations were conducted in 91.8% of herds, averaging 1.84±0.67 checks/cow/season. Among AI herds, 68.7±35.6% of heifers and 72.8±31.3% of adult cows received AI. Observed outcomes were: AI fertility 57.5±8.44%, bull fertility 83.3±17.7%, age at first calving (AFC) 30.1±3.0mo, calving interval (CI) 427±30.82d, and weaned calves per cow present 71.9±13.25%. Sold calves weighed 201.4±35.53kg and were 185.9±49.14d at weaning. Calf mortality was 10.23±4.18%, culling rate 11.6±5.28%, and abortion rate 3.7±2.56%. 50.8% of herds reported no specific health problem; among affected herds, ND was most prevalent (29.5%), followed by besnoitiosis (8.2%), neosporosis (4.9%), campylobacteriosis (3.3%), and IBR (1.6%).

CONCLUSIONS: Spanish beef herds showed structured management, broad vaccination, and advances in reproductive and health practices, although improvement niches remain. As volunteer collaborators, these farms do not represent national averages. We acknowledge the contributing veterinarians, the COVAP-team and the Real Asociación de Criadores de Raza Avileña Negra-Ibérica for their support.

Keywords: Artificial insemination, fertility, calving seasonality, calf mortality

Comparative Clinical Approaches to Calving in Veterinary Practice

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BACKGROUND and OBJECTIVE: This study aimed to evaluate the approaches and interventions of clinicians (clinic/hospital owners) and farm veterinarians (full-time employees on farms) before, during, and after calving.

MATERIALS and METHODS: A questionnaire was sent to 490 veterinarians working in different provinces of Türkiye; 430 veterinarians from 69 provinces responded and were included in the study (n=339 clinicians; n=91 farm veterinarians). Data were collected via an online questionnaire (Google Forms®). The first section included demographic questions (n=10), while the second section evaluated clinical approaches to the calving (n=20). Associations between variables and differences between veterinarians, responses were analysed using Chi-square and Mann–Whitney U tests.

RESULTS: Among respondents, 96.3% were male and 3.7% female. The mean (SD) age was 37±8.7 years and the mean professional experience was 12.7±8.7 years. Clinicians had slightly longer professional experience than farm veterinarians (12.91±8.59 vs. 11.79±8.92 years; p=0.166). Farm veterinarians (72.5%) were significantly more likely not to perform prophylactic intervention during calving compared with clinicians (47.5%) (p=0.001). When the forelimbs and nose were visible at the vulvar lips, 69.4% of clinicians and 96.6% of farm veterinarians preferred to wait for spontaneous calving (p<0.001). Clinicians reported higher levels of stress related to calving difficulties and environmental conditions (57.8%) compared with farm veterinarians (43.3%) (p=0.049). Regarding suspending the calf to remove birth fluids, a higher proportion of farm veterinarians (34.4%) did not use this practice compared with clinicians (17.1%) (p=0.001). After eutocia, only 19.7% of

veterinarians administered analgesic or anti-inflammatory drugs, while 80.3% did not; non-use was more common among farm veterinarians (87.9%) than clinicians (80.2%) (p<0.04). Antibiotics were administered after eutocia by 38.3% of veterinarians (42.1% clinicians vs. 24.2% farm veterinarians; p<0.002). After dystocia, 86.2% administered antibiotics, with higher use among clinicians (88.4%) than farm veterinarians (78.0%) (p<0.02). Overall, 48.2% of veterinarians did not perform prophylactic practices against retained fetal membranes (RFM) after eutocia, whereas 75.8% performed prophylactic measures after dystocia. For RFM management, 39.2% preferred manual removal, 36.4% left membranes if removal failed, and 24.4% avoided manipulation. Manual removal before the third postpartum day was reported by 70.0% of veterinarians, and 99.0% administered antibiotics when fever (>39.5 °C) occurred after manual removal or when membranes were left untreated.

CONCLUSIONS: Significant differences were observed between clinicians and farm veterinarians in several aspects of calving management. Clinicians were more likely to intervene calving and to use jack, analgesia, and antibiotics than farm veterinarians. Further studies are required to clarify the reasons for these differences and their implications.

Keywords: Questionnaire, calving, eutocia, dystocia, cow, veterinary

Periodic Injections of a Copper, Zinc, and Selenium Complex Accelerate the Onset of Puberty in Angus Heifers

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BACKGROUND and OBJECTIVE: Two experiments were conducted to evaluate the effect of periodic injectable supplementation with copper (Cu), zinc (Zn), and selenium (Se) from weaning until 15 mo (Exp I) or 18 mo (Exp II) of age on puberty incidence in Angus heifers.

MATERIALS and METHODS: In Exp I, 66 Angus heifers (mean body weight [BW]: 203.9±22.3 kg) were evaluated from weaning (d0) to d149. Heifers were stratified by BW and nutritional management (pasture only or pasture plus supplementation) and assigned to receive either an injectable mineral solution (SUP; 1 mL/50 kg BW, Suplenut® Se; Biogénesis Bagó; subcutaneous) or no supplementation (CON) on d0 (weaning), 60, and 122. Presence of a corpus luteum (CL) was determined by ultrasonography on d93, 122, 135, and 149. In Exp II, 102 Angus heifers (mean BW: 198.0±23.9 kg) were evaluated from weaning to d245. Heifers, stratified by BW, were assigned to SUP (1 mL/50 kg BW; Suplenut® Se; subcutaneous) or CON; SUP heifers were injected on d 0, 61, 126, and 183. Presence of CL was assessed by ultrasonography on d126, 154, 183, 223, and 245. Puberty incidence (defined by CL presence) was analyzed using Kaplan–Meier survival curves (PROC LIFETEST; SAS®) and Cox proportional hazards models (PROC PHREG; SAS®), reporting hazard ratios (HR) and 95% confidence intervals (CI). In Exp I, the Cox model included treatment and nutritional management as fixed effects. Significance was declared at P≤0.05.

RESULTS: In Exp I, SUP heifers exhibited a greater and earlier cumulative incidence of CL presence than CON heifers. By d149, puberty incidence reached 66.7% in SUP vs. 30.3% in CON. Kaplan–Meier curves differed between treatments (log-rank x^2=7.93; P<0.01). Cox regression revealed independent effects of treatment (Wald x^2=7.63; P<0.01) and nutritional management (Wald x^2=14.93; P<0.01). SUP heifers had a higher hazard of attaining puberty than CON heifers (HR=2.90; 95% CI: 1.36–6.17), whereas heifers managed under pasture plus supplementation had a greater hazard of puberty onset than the pasture-only group (HR=4.93; 95% CI: 2.20–11.08). In Exp II, mineral supplementation increased both cumulative incidence and rate of CL presence compared with CON. By d245, CL presence was 72.5% in SUP and 45.1% in CON. Survival curves differed between treatments (log-rank x^2=9.36; P<0.01). Cox regression confirmed a treatment effect (Wald x^2=8.75; P<0.01), with SUP heifers having a greater hazard of puberty onset than CON heifers (HR=2.20; 95% CI: 1.31–3.70).

CONCLUSIONS: In conclusion, periodic injectable supplementation with Cu, Zn, and Se from weaning accelerated puberty onset in Angus heifers.

Keywords: Angus heifers, Puberty onset, Injectable supplementation, Copper zinc selenium

Seasonal Influence on Uterine Disease Prevalence in Sicilian Dairy Farms: Preliminary Results

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BACKGROUND and OBJECTIVE: The Temperature-Humidity Index (THI) is a widely used indicator to assess environmental conditions that can lead to heat stress in dairy cows. Heat stress negatively affects dairy cow health, increasing the risk of subfertility and reproductive tract diseases. Endometritis has a pronounced negative effect on bovine fertility, mainly due to its adverse impact on fertilization, conception, and embryo survival. The aim of this preliminary study was to evaluate the seasonal effect on uterine diseases in Sicilian dairy farms in cows after 27 days in milk (DIM).

MATERIALS and METHODS: A prospective observational study was conducted between July 2025 and February 2026. Three dairy farms, with a total of 490 lactating cows, were enrolled. All farms were located in the same climatic area and experienced comparable THI values, calculated daily from data recorded by on-farm cooling system control units. Inclusion criteria required farms to have year-round calvings. The farms used sand-bedded cubicles, similar ventilation and cooling systems, and fed a total mixed ration. Multiparous cows were examined between 30±3 and 45±3 DIM. Each cow underwent a routine reproductive tract examination. Endometritis was defined by cytology (≥10% polymorphonuclear neutrophils or a positive leucocyte esterase test) and/or ultrasonographic detection of mixed-echogenic intrauterine fluid ≥0.2 cm, consistent with inflammation. Statistical associations were evaluated using binary logistic regression, considering farms as confounding variables, seasons as independent variables, and uterine disease as the dependent variable.

RESULTS: A total of 316 cows from three dairy farms were enrolled (Farm 1: n=220; Farm 2: n=64; Farm 3: n=32). Mean (±SD) THI was 56±3 during the cold season and 79±8 during the warm season. Uterine diseases were diagnosed in 55/160 cows calving in the warm season and 35/156 in the cold season. In the warm season, 22 cows were cytology-positive only, and 33 were positive both on cytology and ultrasound, including 3 with purulent vaginal discharge (PVD). In the cold season, 8 cows were cytology-positive only, and 27 were positive on both methods, including 4 with PVD. Logistic regression showed that warm-season calving was associated with higher odds of uterine disease compared with the cold season (OR=1.81; p<0.02).

CONCLUSIONS: These preliminary results indicate that warm-season conditions, characterized by higher THI values, were associated with increased odds of postpartum endometritis. Optimizing heat-abatement strategies and improving cow comfort during high-THI periods may help support postpartum uterine health.

Keywords: THI, Heat stress, bovine, reproduction, endometritis

Comparative Post-Thaw CASA Evaluation of Deep-Frozen Bull Semen Stored From the 1980s to the 2010s

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BACKGROUND and OBJECTIVE: Long-term cryostorage of bull semen in liquid nitrogen is widely used in bovine reproduction. Under these conditions, sperm cells are considered biologically inactive; however, questions remain as to whether residual metabolic or structural changes may occur during prolonged storage. The aim of this study was to compare post-thaw quality of deep-frozen bull

semen from different time periods using computer-assisted sperm analysis (CASA) and cytomorphological evaluation.

MATERIALS and METHODS: A total of 32 frozen semen samples from Simmental bulls maintained under uniform housing and management conditions were allocated into four groups according to the period of semen production and storage: 1980–1989 (n=6), 1990–1999 (n=6), 2000–2009 (n=10), and 2010–2019 (n=10). Semen samples were thawed at 37°C for 30 s and analyzed using CASA for total and progressive motility, sperm subpopulations, concentration, and kinematic parameters. Cytomorphological evaluation included assessment of sperm viability and morphological abnormalities. Statistical analysis was performed using appropriate parametric or non-parametric tests depending on data distribution, with significance set at $P<0.05$.

RESULTS: Total motility did not differ significantly among groups. Progressive motility was significantly higher in semen produced in 2010–2019 than in the 1990–1999 and 2000–2009 groups, while the proportion of fast progressively motile spermatozoa was higher than in all other groups. Sperm concentration per mL and per dose were significantly lower in the 2010–2019 group compared to all earlier groups. Most CASA kinematic parameters (including VAP, VSL, and ALH) were significantly higher in the 2010–2019 group compared with all other groups, while VSL was higher only compared to the 1980–1989 and 1990–1999 groups. VCL did not differ significantly, and BCF differed only between the 2010–2019 and 2000–2009 groups. No significant differences were observed in sperm viability or morphological characteristics among groups.

CONCLUSIONS: Post-thaw semen quality differed among decades, primarily in progressive motility and CASA kinematic parameters, whereas no significant differences were observed in sperm viability or morphology. Despite these differences, semen quality remained within a range compatible with its practical use in artificial insemination, highlighting the importance of preserving functional integrity of cryostored semen for long-term conservation of valuable genetic material. These findings suggest a potential impact of long-term cryostorage on sperm functional quality, although differences in semen processing over time cannot be completely excluded.

Keywords: bull semen, cryopreservation, CASA, sperm motility, bovine reproduction

No Impact of Corpus Luteum Size on Conception Rates in Embryo Transfer Recipient Cows

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BACKGROUND and OBJECTIVE: Embryo transfer (ET) is a reproductive biotechnology used to obtain offspring from genetically superior donors, utilizing recipient cows of lower economic value. A key advantage is bypassing oocyte quality issues during hot seasons, as hyperthermia primarily affects oocytes during folliculogenesis and early-stage embryos. By the morula or blastocyst stage, embryos have already acquired thermal resistance. ET success depends on recipient selection, synchronization protocol, and embryo production method. Corpus luteum (CL) quality and activity are critical for embryonic development, and CL presence is the primary selection criterion in large-scale ET programs. While some studies consider CL size a determining factor for gestational success, others report that conception rate is more influenced by CL blood perfusion than luteal size. This study evaluated whether conception rates in recipient cows in a frozen in vitro-fertilized ET program vary according to CL size.

MATERIALS and METHODS: The study was conducted in Guerrero, Mexico (dry tropics). Recipients were *Bos taurus* × *Bos indicus* crossbred cows, body condition score 3–3.5 (scale 1–5), vaccinated, dewormed, and supplemented from 45 days pre- to 45 days post-transfer, with confirmed cyclicity. Synchronization protocol: Day 0, estradiol benzoate and intravaginal progesterone device; Day 8, intravaginal device removal, prostaglandin, equine chorionic gonadotropin (eCG), and estradiol cypionate; Day 10 designated as estrus; Day 17, embryo transfer in recipients presenting CL2 (15–25 mm) or CL3 (>25 mm).

RESULTS: Of 206 recipients transferred, 82 conceived (overall conception rate: 40%). Among 132

CL2 recipients, 53 conceived (40%) and 79 did not (60%). Among 74 CL3 recipients, 29 conceived (39%) and 45 did not (61%). No statistically significant difference was observed between groups (p = 0.7572).

CONCLUSIONS: Corpus luteum size at the time of embryo transfer does not significantly impact conception rate, provided the minimum threshold of CL2–CL3 (>15 mm) is met.

Keywords: Embryo transfer, corpus luteum, conception rate, tropical climate, recipient cows

SHEEP AND GOATS

Evaluation of Immunity Duration and Booster Response to a Phase I *Coxiella burnetii* Vaccine in Sheep

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BACKGROUND and OBJECTIVE: An inactivated Phase I *Coxiella burnetii* vaccine is authorized in several countries since 2010 for goats and cattle. Its market authorization was extended to sheep in 2023. According to the Summary of Product Characteristics, in sheep, the duration of immunity is four months after primary vaccination (PV). However, in the field the vaccine had long been used successfully in sheep with single annual boosters (B). This study aimed to evaluate how long immunity persists after primary vaccination and to compare the immune response from either a single booster or a repeated two-dose primary course administered six or twelve months later.

MATERIALS and METHODS: Sixty seronegative ewes were enrolled and randomly allocated into six groups. Five groups received a PV with two injections at days 0 and 21. One group served as a sentinel group (S). Among the five vaccinated group, animals were revaccinated later: • group PV4: new full protocol (2 doses 21 days apart) 4 months later • group B6: single booster 6 months later • group PV6: new full protocol 6 months later • group B12: single booster 12 months later • group PV12: new full protocol 12 months later Blood samples were collected regularly over 470 days and analyzed by a semi-quantitative commercial ELISA kit. Duration of immunity was assessed until revaccination, and humoral responses after booster vaccinations were compared.

RESULTS: Data were available for 53 ewes (S: n=8; PV4: n=10, B6: n=8; PV6: n=8; B12: n=10; PV12: n=9). All vaccinated ewes showed a strong antibody response after PV. Across groups, antibody decline varied slightly: a few animals in B6 (n=1), B12 (n=1) and PV12 (n=4) shifted to negative values before revaccination, while the majority (n=39) remained positive. Overall, PV generated robust serological responses, with only isolated cases of early waning immunity. Following revaccination, whether performed at 4, 6, or 12 months, all animals became strongly positive again and remained so until the end of the study, irrespective of the timing of revaccination or the use of a single dose or two-dose revaccination. Across all groups, antibody levels after revaccination were higher than after the initial primary course, confirming a strong anamnestic response.

CONCLUSIONS: Primary vaccination against *Coxiella burnetii* (Q fever) induces immunity lasting up to one year at the flock level in adult ewes. A single booster injection is sufficient to restore strong humoral immunity, with no advantage provided by repeating the full primary vaccination course.

Keywords: Vaccination, Coxiella burnetii, Q fever, sheep, immunity, duration of immunity

Association of Udder Imbalance with Age, Parity, and 350-day Milk Yield in Saanen Dairy Goats on a Dutch Farm

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BACKGROUND and OBJECTIVE: Imbalanced udders in goats, characterised by asymmetry in shape or size between udder halves, can negatively impact milk yield, milking routines, and overall health and welfare. Udder asymmetry is often associated with chronic intramammary infection, which may lead to fibrosis, tissue atrophy, and morphological alterations. These changes can result in a reduction in milk production and are a frequent reason for culling in dairy goats. This study aimed to investigate the relationship between udder asymmetry and factors such as age, lactation number, and milk production over the previous 350 days, to identify management practices that could help reduce the prevalence or severity of udder imbalance.

MATERIALS and METHODS: The study was conducted in December 2023 on a Saanen dairy goat farm in the Netherlands and included 3,164 lactating goats. Udder imbalance was classified using a three-part system: No (n=2,393), Mild (n=473), and Severe (n=298) - and a two-part system including the "No" group and grouping Mild and Severe into a single "Yes" category. Additionally, 64 culture-positive samples collected from mastitis cases and goats with udder imbalance in 2022 identified Staphylococcus spp. as the predominant pathogen (59.4%), with non-aureus Staphylococci (NAS) representing 53.1% of all isolates. Linear regression models assessed the association between udder imbalance (two-part classification), lactation number, days in milk (DIM), and milk production over the previous 350 days. Logistic regression models evaluated udder imbalance as a response variable in relation to age, lactation number, and production. All analyses were performed using R version 4.4.3.

RESULTS: Results showed a relationship between udder asymmetry and reduced milk production. Goats without udder imbalance produced an average of 1,324.9 L in the previous 350 days, compared with 1,254.1 L in goats with udder asymmetry, a statistically significant difference. Younger animals (1- 2 years) had a significantly lower prevalence of udder imbalance compared with older age groups. Across all ages, goats with asymmetry produced less milk, with the greatest production gap observed in animals aged 5- 6 years. The negative association between udder imbalance and production was most evident during the first year of lactation (first 365 DIM).

CONCLUSIONS: Udder asymmetry is associated with significantly lower milk production, likely linked to chronic mastitis and subsequent mammary tissue atrophy. Given its economic impact, particularly through reduced yield and increased culling risk, preventive mastitis control - especially against Staphylococcus spp. - is essential to maintaining udder health and productivity.

Keywords: goats, udder imbalance, Staphylococcus, vaccination, milk yield

Physical Rehabilitation in Small Ruminants: Clinical Implementation

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BACKGROUND and OBJECTIVE: Physical medicine is well established in human healthcare and increasingly used in veterinary practice for musculoskeletal, neurological, and age-related conditions. Owner expectations for adjunctive, non pharmacologic therapies in food producing species are rising. This abstract outlines feasible indications, modality choices, and species specific constraints for sheep and goats, and summarizes our clinical experience integrating physical medicine to support analgesia, reduce inflammation, improve function, and expedite recovery.

MATERIALS and METHODS: We incorporated manual therapies (massage, passive range of motion, stretching), therapeutic exercise programs (adapted to pens/pastures), low level laser therapy and

electrotherapy for analgesia and inflammation control, therapeutic ultrasound (after complete clipping of the treatment area), and extracorporeal shockwave therapy for tendinopathies and osteoarthritis (with clipping). Acupuncture and chiropractic techniques were also applied. Indications included acute and chronic musculoskeletal disease, selected neurological disorders (dependent on etiology), and wound/injury management. Practical constraints were documented: fleece related limitations in sheep (restricted thermotherapy; contraindication for underwater treadmill), and feasibility of underwater treadmill therapy in goats. Modalities were selected based on condition, tolerance, and logistics. Because these interventions carry no withdrawal time restrictions, they were available for food producing animals.

RESULTS: Across multiple clinical cases in sheep and goats, modalities were well tolerated and easily integrated with conventional care. Adjunctive physical medicine was associated with improved functional recovery and shorter time to discharge in our clinical experience; formal, prospective outcome measurements were not systematically collected.

CONCLUSIONS: Physical medicine is feasible and broadly applicable in small ruminants, complementing standard treatments for musculoskeletal, neurological, and wound related conditions without withdrawal time concerns. Species specific factors—especially fleece in sheep—limit certain modalities (thermotherapy, underwater treadmill) and necessitate clipping for ultrasound and shockwave; goats are suitable for aquatic treadmill therapy. Further controlled studies are needed to quantify efficacy, optimize protocols, and standardize outcome measures to guide evidence based implementation in sheep and goats.

Keywords: Physical medicine, Rehabilitation, Physiotherapy, Goats, Sheep

Distribution of Genotypes a and B of Small Ruminant Lentiviruses in Goat and Sheep Production Units in Mexico

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BACKGROUND and OBJECTIVE: Background. Small ruminant lentiviruses cause a persistent, chronic degenerative, multisystem disease in goats and sheep. Globally, the prevalence of SRLV can reach up to 90%, with risk factors for infection spread including the productive stage, age, breed, and production purpose as well as facility-related factors such as cohabitation with other species, population density, preventive measures, cleanliness, biosecurity, and management practices. Objective To assess the genetic diversity of SRLV in Mexico and identify factors that favor its presence in sheep and goats across different production units.

MATERIALS and METHODS: Material and methods This study was performed in total, 890 goats and sheep from 52 mixed-species and single-species production units in northern, central, and southern Mexico were analyzed. Serological and molecular diagnosis was conducted. Positive samples were further analyzed to identify genotypes A and B. Survey data were evaluated to determine the relationship between virus presence, seropositivity, and associated factors in the production units.

RESULTS: Of the 890 animals sampled (507 sheep and 383 goats), 40% (354/890) tested positive for specific antibodies to SRLV, while 78% (697/890) were positive for the viral genome. The results confirmed that genotype A circulated in 15% of infected animals, genotype B circulated in 66%, and 19% were co-infected with both genotypes.

CONCLUSION: This study highlights the circulation and spread of SRLV genotypes A and B in goat and sheep production units in Mexico. Factors such as

species cohabitation, cleanliness, and preventive measures were associated with the presence of clinical signs.

Keywords: Small, ruminant, lentivirus, Distribution

Molecular Identification of Viral Agents in Goat Production Units in Mexico

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BACKGROUND and OBJECTIVE: Background. In Mexico, are approximately three million goats raised primarily for meat and milk. Among the main causes of declining goat production are the presence of viruses that decrease animal welfare and production, consequently impacting producers economic well-being. The objective of this study was to molecularly identify the viral agents that circulate naturally in goat production units in Mexico.

MATERIALS and METHODS: Materials and Methods One thousand goats from intensive and semi-intensive milk production units were sampled. Samples obtained included nasal swabs, vaginal swabs, and scabs. DNA and RNA were extracted from these samples and used to amplify a DNA fragment using endpoint polymerase chain reaction (PCRpf) to identify Parapoxvirus (Papoxv), Lentivirus (Lenv), and Varicellovirus (Variv). The endpoint reverse transcription polymerase chain reaction (RT-PCRpf) test was used for Orthopneumovirus (Orthov), Respirovirus (Respv), and Pestivirus (Pestiv). PCRpf and RT-PCRpf products were visualized by horizontal electrophoresis on a 1.5% agarose gel.

RESULTS: Of the 1000 goats, 31.4% tested positive for Orthov, 53% for Respv, 71.4% for Variv, 77.7% for Pestiv, 84.4% for Lenv, and 94.9% for Papoxv.

CONCLUSION: Natural infection with Parapoxvirus, Orthopneumovirus, Respirovirus, Lentivirus, Varicellovirus, and Pestivirus in Mexican goats is confirmed. These results demonstrate the importance of establishing and enforcing preventive and biosecurity measures in production units.

Keywords: molecular, Viral, Goat

Claw Disorders in Sheep: Prevalence, Risk Indicators, and Preventive Management Under Practical Farm Conditions

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BACKGROUND and OBJECTIVE: Lameness caused by claw disorders represents a major challenge in sheep production, affecting animal welfare and farm profitability. Management factors such as housing conditions, hoof care routines, and biosecurity measures are considered influential, yet data from Austrian sheep farms are limited. The objective of this study was to assess the prevalence of lameness and claw disorders in sheep flocks in Styria and to evaluate potential associations with farm management and animal-related factors.

MATERIALS and METHODS: A cross-sectional study was conducted on 29 sheep farms in Styria, Austria. Data were collected through structured questionnaires completed by farm managers and through on-site clinical examinations. A total of 682 sheep aged six months or older were assessed for lameness using a standardized scoring system and

examined for claw disorders. Diagnoses included footrot, contagious ovine digital dermatitis (CODD), white line disease (WLD), hyperkeratosis, and other claw pathologies. Farm-level parameters such as housing, manure removal frequency, pasture management, hoof care practices, and biosecurity measures were recorded. Statistical analyses were performed to identify associations between management or animal-related factors and the occurrence of claw disorders.

RESULTS: Lameness was observed in 21 of 682 animals, corresponding to a prevalence of 3.1%. The most frequently diagnosed claw disorders were footrot (6.5%), hyperkeratosis (6.5%), and white line disease (5.4%). Most cases of footrot were detected in early clinical stages. No statistically significant associations were found between the occurrence of claw disorders and farm structure, pasture management, manure removal frequency, or hoof care frequency, although certain trends were observed. Frequent manure removal and the implementation of quarantine measures showed a potential preventive effect. No significant associations were detected with age or breed. However, male sheep showed a significantly higher prevalence of footrot compared to females ($p = 0.034$).

CONCLUSIONS: Claw disorders, particularly footrot, remain prevalent in Styrian sheep farms despite generally low lameness levels. Although few statistically significant risk factors were identified, the findings highlight the importance of consistent management practices and biosecurity measures. Early detection and targeted preventive strategies are essential to maintain claw health and improve animal welfare in sheep production systems.

Keywords: Lameness, farm management, footrot, hyperkeratosis, white line disease, claw health

Investigation of Respiratory Problems in Lambs in a Countrywide Investigation in Greece Through a Participatory Epidemiology Approach

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BACKGROUND and OBJECTIVE: The objectives of the present study were i) the presentation of data on the occurrence of lamb respiratory problems on sheep farms and ii) the identification of variables (including variables related to environmental factors) associated with the disorder.

MATERIALS and METHODS: A countrywide study was performed in 325 sheep flocks throughout Greece, which were visited by the researchers, in order to carry out an extensive investigation of pneumonia in lambs. An interview was carried out with all farmers, in order to obtain information regarding conditions about management conditions and health problems that prevailed on the farm.

RESULTS: The within farm annual incidence rate of lamb pneumonia varied from 0.0% to 21.4% in sheep flocks. The median (interquartile range) within farm annual incidence rate among farms was 0.0% (0.8%) and was lower in farms that applied the intensive or semi-intensive management system. Multivariable analysis for within farm

annual incidence of lamb pneumonia revealed two predictors: proximity of the farm to industrial sites and availability of separate barn for lambs. Farmers considered pneumonia as the second most important health problem of lambs, specifically, 27.4% of sheep farmers mentioned it as important health problem. Farmers who considered pneumonia as important problem, routinely administered antibiotics to lambs more often than those who did not: 29.3% versus 20.1% of respective farmers.

CONCLUSIONS: A notable finding was the association of proximity to industrial sites with a higher incidence rate of lamb pneumonia. This has similarities to results of relevant studies in people and potentially reflects that air pollution in farm environment might be a factor to consider in health management in sheep flocks.

Keywords: goat, pneumonia, respiratory problem, sheep

Lameness in Adult Sheep in Greece: Prevalence, Predictors, Treatment, Importance for Farmers

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BACKGROUND and OBJECTIVE: The study refers to an extensive investigation of lameness performed countrywide in Greece, on 325 sheep farms. The specific objectives of the study were i) the presentation of data on occurrence of the disorder on sheep farms and ii) the identification of variables (including variables related to climatic factors) associated with the disorder.

MATERIALS and METHODS: Farms were visited and animals were clinically assessed for the presence of lameness. An interview was carried out to obtain information regarding practices applied on the farm. Climatic variables at farm locations were derived from NASA research.

RESULTS: The within farm prevalence rate of lameness varied from 0.0% to 25.0% among sheep flocks. The mean (median) within farm prevalence rate among farms was 1.9% (0.0%). A multivariable analysis for within farm prevalence of lameness revealed three predictors: i) the application of vaccination against foot-rot, ii) the increased precipitation at the farm location and iii) a longer annual grazing period. Treatment of the problem involved mostly administration of antibiotics (on 86 farms); the antibiotics administered most often were lincomycin and oxytetracycline. There was a tendency for higher median within farm prevalence of lameness among farms where no antibiotic administration was practiced. Finally, 6.2% of sheep farmers considered lameness as an important health problem for the animals, specifically the third most important problem on the farms.

CONCLUSIONS: The study has provided information regarding the presence of lameness in sheep in Greece. The results indicate the importance of increased precipitation at the farm location and of extended grazing periods as predictors for the presence of the problem on farms. These findings can be useful in the health management of sheep farms.

Keywords: goat, lameness, precipitation, rainfall, sheep, vaccination

Evaluation of a Targeted Selective Treatment Protocol Based on Parity and Body Condition in Dairy Sheep in Field Conditions in France: Impact of Fecal Egg Counts and Treatment on Production

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BACKGROUND and OBJECTIVE: Refugia-based strategies to delay the appearance of resistance to anthelmintic (AH) drugs in gastro-intestinal nematodes (GIN) have been discussed in research for over 20 years, yet they need to be adapted to local constraints. In many countries, farmers mainly use eprinomectin (EPN) to control GIN infection in dairy sheep, as this macrocyclic lactone has a broad spectrum and a null milk withdrawal period. Its key role in dairy farms and the lack of new drugs on the market in the foreseeable future make it necessary to implement strategies to maintain its efficacy as long as possible.

MATERIALS and METHODS: A targeted selective treatment (TST) protocol was implemented in 5 dairy sheep farms in France during their lactation period for 2 to 3 years. Farms were recruited upon EPN efficacy, estimated by fecal egg count reduction tests at the beginning of the protocol. Treatment criteria were (i) lactation rank and (ii) body condition score as estimated by the farmer. All first lactating (primiparous) ewes and multiparous ewes in bad body condition were treated using injectable EPN according to the manufacturer's instruction.

Treatment day fecal egg count (FEC) as well as individual milk yield and body condition scores were used to evaluate the pertinence of treatment criteria. GIN species infecting primiparous and multiparous ewes were identified using Nemabiome analysis.

RESULTS: Selective treatment led to a significant reduction in parasite load in flocks, while keeping from 13 to 80% of ewes as refugia. FEC had a significant impact on pre-treatment milk yield. Ewes left untreated produced on average 8 or 9% less milk than the treated ewes. Ewes above the FEC threshold were not the animals that had a significant milk increase post-treatment. In 2 farms, non-treatment of ewes during the period leading up to mating had no significant impact upon ewe fertility. EPN efficacy was maintained over the duration of the study in farms in which efficacy was initially high, ie in 3/5 farms. In most (4/5) farms, *Haemonchus contortus* was overwhelmingly present, and was absent from the fifth farm.

CONCLUSIONS: TST protocol as implemented here was easy for the farmer to apply. The production loss, in line with other results estimating milk gain after AH treatment, needs to be balanced with the cost of AH resistance once installed. Implementation of TST is an important component of integrated parasite management.

Keywords: eprinomectin, integrated parasite management, Haemonchus contortus

Efficacy of An Ecg-like Glycoprotein on Estrus Induction and Ovulatory Response in Merino Ewes During Seasonal Anestrus

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BACKGROUND and OBJECTIVE: Seasonal anestrus is a major constraint to reproductive efficiency in sheep production systems. Merino ewes experience a deep, well-defined seasonal anestrus, with reproductive activity closely tied to photoperiod, limiting breeding to autumn and winter in the Southern Hemisphere. This study evaluated the effectiveness of an eCG-like glycoprotein to induce estrous behavior and stimulate ovulation in Merino ewes during Spring seasonal anestrus in Argentina from late October to early November 2025.

MATERIALS and METHODS: The study was conducted at the Pilcaniyeu Experimental Field (INTA Bariloche), located at −41.032° latitude and −70.59° longitude, using 60 Merino ewes in seasonal anestrus. At the beginning of the study (Day 0), body weight (BW) and body score (BCS) were recorded, and all animals received a 12-day intravaginal progesterone implant (medroxyprogesterone acetate 60 mg). On Day 12, the vaginal implant was removed, and ewes received an intramuscular (IM) injection of 125 µg of sodium cloprostenol, were randomized into two groups of 30 animals each, according to age, BW, and BCS to either a Control Group (T01) with no additional treatment, or a treated (T02) receiving 400 IU of an eCG-like glycoprotein IM. Estrus was detected twice a day (am and pm) from days 13 to 17 using four teaser rams fitted with marking harnesses. On Day 21, ovulation was assessed by laparoscopy, with the number of corpora lutea (CL) recorded per ewe and statistically compared at $P<0.05$ using Fisher's exact test for proportions and generalized linear models with a Poisson distribution in SAS.

RESULTS: All 30 (100%) ewes in T02 (eCG-like glycoprotein-treated group) displayed estrus behavior within 24-96 h interval following progesterone implant removal, while only 2 out of 30 (6.7%) ewes in T01 (control) exhibited estrus ($P < 0.001$). Ovulation occurred in all T02 ewes (mean of 1.80±0.66 CL/ewe); while none ovulated in T01 ($P < 0.0001$). On Day 12, group average BW and BCS were similar (T01: 43.3±0.94 kg, 2.47±0.04; T02: 45.2±0.80 kg, 2.50±0.04; $P > 0.14$), with no significant differences at the end of the study (Day 21; $P > 0.05$).

CONCLUSIONS: Administration of 400 IU of eCG-like glycoprotein IM effectively synchronized estrus and induced ovulation in Merino ewes during seasonal anestrus in the Southern Hemisphere, with no differences observed in BW or BCS. These findings support the use of an eCG-like glycoprotein as an effective tool for improving reproductive outcomes in sheep during the non-breeding season.

Keywords: Sheep, non-breeding season, eCG-like glycoprotein, hormonal induction, ovarian activity

Mapping the Scientific Literature on Sheep and Goat Research: General Appraisal and Significance of the Year of Publication

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BACKGROUND and OBJECTIVE: The objectives of this work were: i) the mapping of the bibliometric characteristics of publications related to sheep and goat studies internationally, ii) the comparison of publications related to each of the two animal species and iii) the comparison of the characteristics in a 55-year long time-frame from 1970 to today.

MATERIALS and METHODS: The Web of Science platform was used with search terms: [sheep OR ovine OR Ovis aries] or [goat* OR caprine OR Capra hircus]; only articles or reviews published until the end of year 2024 were included, in total, 165,052 papers related to sheep and 67,637 papers related to goats.

RESULTS: There was a progressive increase in the number of published papers annually, with a steeper slope for papers related to goats. Most papers were published in Small Ruminant Research; other journals with a large number of published papers were Journal of Animal Science and Indian Journal of Animal Science. Small Ruminant Research was the journal with most published papers for 21 (sheep) and 29 (goats) years. Most papers originated from the United States of America; other countries with a large number of papers were United Kingdom and Australia (sheep) and China and India (goats). United States of America was the country with most published papers for 52 (sheep) and 41 (goats) years. Most published papers related to sheep or to goats were classified in the Dairy & animal sciences topic-meso; other topics-meso with large number of papers were Parasitology – General (sheep) and Food Science & Technology (goats). The two predominant topics-micro were Ruminant nutrition and Livestock reproduction for published papers related to sheep and to goats. Ruminant Nutrition was the predominant topic-micro for 55 (sheep) and 53 (goats) years. Approximately one-third of papers were published under open access. Papers related to sheep had received on average 0.93 citations per paper annually and papers related to goats on average 0.73 citations per paper annually.

CONCLUSIONS: The work provided an extensive mapping and analysis of the scientific output related to sheep and goat research internationally. Work on both species shows strong thematic stability. However, changes have been detected in the origin of the published papers in recent years, as well as in the journals used for dissemination of the results. A trend emerges that potentially in the future, new fields of work can develop, for example, climate adaptation research.

Keywords: bibliometric, goat, literature, meta-analysis, One Health, sheep

Longitudinal Study of Subclinical Mastitis in Sheep in Greece: An Investigation into Incidence Risk, Associations with Milk Quality and Risk Factors for the Infection

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BACKGROUND and OBJECTIVE: The objectives of this work were i) the description of the incidence risk of subclinical mastitis in dairy sheep locks throughout the milking period, ii) the presentation of potential associations of subclinical mastitis with the quality of milk and iii) the identification of risk factors for high-incidence risk of the infection.

MATERIALS and METHODS: A longitudinal study was performed in 12 dairy sheep flocks in Greece. Mammary secretion samples from 240 ewes and samples of bulk-tank milk of the farms were collected in four repeated visits over a period of six months for bacteriological, chemical and cytological examinations.

RESULTS: Overall, the incidence risk of subclinical mastitis throughout the study period was 51.7%, and it varied among farms from 25.0% to 75.0%. The respective figure for staphylococcal subclinical mastitis was 48.8%. The incidence risk of recurrence of subclinical mastitis among ewes in the flocks was 35.4%. The most frequently identified bacteria from cases of subclinical mastitis were S. aureus and S. simulans; of the mastitis-causing staphylococcal isolates, 65.4% were biofilm-forming. Somatic cell counts in bulk-tank milk progressively increased as

the lactation period advanced, with significant increases seen on the third and fourth visits to the farms. Somatic cell counts in bulk-tank milk correlated well with prevalence of subclinical mastitis in flocks. A significant inverse correlation and a significant positive correlation were seen for prevalence of subclinical mastitis versus total protein content or added water in bulk-tank milk. During multivariable analysis, younger age of newborns when taken away from the dam and consequently delayed start of milking of ewes, omission of anti-mastitis vaccination of ewes and lack of employed staff on the farms emerged to be significantly associated (positively) with increased incidence risk of subclinical mastitis.

CONCLUSIONS: Given the significance of dairy sheep production in the agricultural sector of Greece, there is always interest in the study of mastitis and the monitoring of the situation in flocks in the country. The results have provided evidence regarding the situation in the country and have also identified factors that should be taken into ac-count for controlling the infection. The findings also present another interaction be-tween people and animals in the food-production chain, as well as the actions that farmers may take to recuperate income.

Keywords: mastitis, milk, predictor, prevalence, sheep, Staphylococcus

Recombinant Equine Chorionic Gonadotropin Enhances Out-of-Season Reproductive Performance of artificially inseminated ewes: Effects on Follicular Dynamics, Ovulation Synchrony, and Fertility in Aragonesa and Merino Ewes in Spain and South Africa

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BACKGROUND and OBJECTIVE: Artificial insemination (AI) is essential for accelerating genetic gain and improving reproductive efficiency in sheep. Traditional protocols rely on equine chorionic gonadotropin (eCG) derived from pregnant mares, raising strong welfare concerns and creating an industry need for valuable alternatives. The objective of these combined studies was to evaluate the efficacy of recombinant equine chorionic gonadotropin (reCG) on reproductive performance in two breeds of ewes managed out of the breeding season. Study 1 assessed the effect of three reCG doses on follicular dynamics, synchrony of ovulation, and pregnancy per timed AI in Aragonesa ewes. Study 2 evaluated the fertility response to a single 105 IU reCG dose compared to saline control after laparoscopic timed AI in Merino ewes.

MATERIALS and METHODS: Study 1: A total of 107 Aragonesa ewes were randomized by age, body weight, bcs, and cyclicity. All received a 12-day FGA sponge protocol, followed at removal by assignment to one of five groups: Negative Control, Positive Control (480 IU natural eCG), or reCG at 70, 105, or 140 IU. Cervical AI with fresh semen was performed 55 hours after sponge removal. Pregnancy diagnosis

was conducted 40 days later. Study 2: A blinded, parallel group trial included 151 Merino ewes synchronized with a 12-day progesterone based protocol. At device removal, ewes received either saline (Negative Control, n=75) or 105 IU reCG (Treatment, n=76). Laparoscopic AI with frozen semen was performed 52 ± 2 hours later. Pregnancy was assessed at Days 35–42 and reconfirmed at Days 60–65, with follow up until lambing.

RESULTS: Study 1: Pregnancy per AI increased markedly with hormonal treatment. The Negative Control achieved 0%, while the Positive Control reached 59.1%. The reCG groups achieved pregnancy rates of 47.6% (70 IU), 61.9% (105 IU), and 71.4% (140 IU). Higher doses were associated with improved reproductive outcomes, demonstrating dose dependent efficacy. Study 2: At pregnancy diagnosis, 24% of ewes conceived in the Negative Control group compared with 55.3% in the reCG treated group. Litter size did not differ significantly between groups, though treated ewes produced more total lambs (62 vs. 25). A single 105 IU dose effectively enhanced out of season fertility.

CONCLUSIONS: Across both studies, recombinant eCG demonstrated strong potential as an alternative to natural eCG for AI programs. The hormone improved pregnancy rates in two breeds under out of season conditions, with evidence of dose responsive effects. Recombinant eCG represents a promising, welfare friendly tool to support sustainable genetic progress in sheep.

Keywords: Sheep breeding, recombinant eCG, Timed AI, pregnancy

The Contribution of Explainable Machine Learning in the Detection of Two Zoonotic Pathogens: coxiella Burnetii and listeria spp., in the Milk of Dairy Small Ruminants

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BACKGROUND and OBJECTIVE: The objective of the present study was the development of computational models for predictions for the presence of Coxiella burnetii or Listeria spp. in the bulk-tank milk in dairy sheep and goat farms.

MATERIALS and METHODS: The presence of C. burnetii or Listeria spp. in the bulk-tank milk was defined as the target value and computational models were constructed for their prediction, through the use of independent variables (n = 11 for C. burnetii and n = 8 for Listeria spp.) related to factors prevailing at the farms. Seven machine learning tools were employed: Decision Trees (36 different models evaluated for each pathogen), Random Forest (9 models), LightGBM (24 models), XGBoost (54 models), k-Nearest Neighbours (32 models), Support Vector Machines (3 models) and Neural Networks (24 models). In all, 182 evaluations were performed for each pathogen.

RESULTS: For the prediction of the presence of C. burnetii in the bulk-tank milk, Random Forest and XGBoost were the two tools that produced the best measures of quality: the median value for four measures of quality of the predictions (accuracy, precision, recall, F1-score) evaluated was over 0.942 for each of these two tools. The results of the analysis for SHapley Additive exPlanations values for the impact of the independent variables in the prediction of the presence of the pathogen indicated that (a) the management system applied on the farm and (b) the average wind speed at the location of the farm during the 15 days prior to sampling, were the variables that mostly influenced the prediction outcome. In the case of Listeria spp., meaningful classification could not be achieved, due to the highly imbalanced and limited nature of the dataset. For this pathogen, the models heavily favoured the majority class and produced predictions that defaulted almost entirely to 'no presence' of the pathogen; for the minority class, recall and precision were as low as 0.300.

CONCLUSIONS: The findings of this study indicate that machine learning algorithms can be employed for the prediction of the presence of C. burnetii in the milk produced in dairy sheep and goat farms. These computational prediction models were developed using field data collected during countrywide studies. The models showed excellent performance and thus can be employed as adjunct tools to support clinical decisions in the health management of dairy small ruminant farms and the production of safe milk there.

Keywords: artificial intelligence, bulk tank milk, goat, health management, sheep

Comparative Evaluation of Reproductive Performance Following Two Progesterone Device-based Estrus Synchronization Protocols in Ewes During the Breeding Season

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BACKGROUND and OBJECTIVE: Objective of this work was the evaluation of two different estrus synchronization protocols in adult ewes, during the breeding season, in Thessaly region, in central Greece.

MATERIALS and METHODS: A total of 172 adult ewes, from four sheep farms, were enrolled into the study. The inclusion criteria were: a) age (>2 years and <6 years), b) BCS (2.5–3), c) clinically healthy animals, and d) individual identification tag. The animals were randomly divided into two equal groups as follows: In Group A, the progesterone - devices remained for 7 days and in Group B for 12 days. Mating in both groups started the same day (i.e. insertion of devices in Group B started 5 days earlier). At removal of the progesterone - devices, 200 IU equine Chorionic Gonadotropin (eCG) were administered. Vaginal secretions were observed and recorded, and a 0–2 scoring scale was applied based on the amount and nature of the discharge. Also, cytological examination of vaginal smears was performed and a 5- scale score was used according to the number of inflammatory cells. Rams were introduced into the ewes, 36 hours after progesterone - device removal, at a ratio of 1: 6. Pregnancy diagnosis was performed via ultrasonography 45–60 days after ram introduction. Also, lambing data were recorded. Comparisons between groups were performed using the chi-square test. Statistical significance was set at $p<0.05$.

RESULTS: In both groups, there was a 4.65% of lost progesterone - devices. Slight vaginal discharge was observed in 14.81% of Group A animals and 4.88% of Group B animals ($p=0.03$). The cytological examination of vaginal smears was not associated with the appearance of vaginal discharge and showed no differences between the groups. Ultrasonographic pregnancy diagnosis showed that 93.02% of Group A ewes and 85.37% of Group B ewes were pregnant ($p=0.11$). All ewes with lost progesterone – devices or with vaginal discharge were found pregnant. Of the ewes diagnosed as pregnant, 90.14% in Group A and 91.52% in Group B, eventually, lambed. 84.85% of Group A and 68.52% of Group B lambings, occurred during the first lambing wave ($p=0.033$).

CONCLUSIONS: Both progesterone – device-based synchronization protocols resulted in high pregnancy rates. Pregnancy rate was not associated with presence of vaginal discharge. However, the 7-day protocol led to a significantly higher proportion of births in the first lambing wave. In conclusion, the short-duration protocol can be safely incorporated into sheep reproductive management programs.

Keywords: Sheep, progesterone - device, synchronization protocols, estrus synchronization, short protocol

Investigation of the Presence of Zoonotic Pathogens in the Gastrointestinal Tract of Lambs

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BACKGROUND and OBJECTIVE: The objective of the present study was the evaluation of the presence of zoonotic pathogens in the gastrointestinal tract of lambs after birth and until 90th day of their life.

MATERIALS and METHODS: Seven newborn lambs were monitored for a period of three months. Faecal samples and samples of gastric content were collected on the day of birth (D0), as well as on D3, D10, D20, D40 and D90 (days of age). Samples were examined for zoonotic pathogens by using the 'BioFire® FilmArray® Gastrointestinal (GI) Panel', a fully automated multiplexed nucleic acid-based test for in vitro detection and identification of multiple pathogens. The panel was developed for rapid identification of a multitude of pathogens in clinical samples and could detect simultaneously 22

different gastrointestinal pathogens (13 bacteria, 4 protozoa, 5 viruses).

RESULTS: Zoonotic pathogens were detected in 67.1% of all samples; median number of pathogens was 1 per sample. Pathogens were found in three lambs on D0 and thereafter in all lambs. Pathogens were detected less often and at lower numbers on D0 and D3 (46.4% of samples, median value: 0 pathogens per sample) than on D20 and subsequently (83.3% of samples, median value: 2 pathogens per sample) ($p < 0.002$). No difference was seen between rectal samples (74.3% of samples, median value: 1 pathogen per sample) and gastric samples (62.9% of samples, median value: 1 pathogen per sample) ($p > 0.15$). Overall, 17 pathogens were detected in the samples. Campylobacter (jejuni, coli, upsaliensis), Clostridium difficile (toxin A/B), Cryptosporidium, Cyclospora cayetanensis, Enteropathogenic Escherichia coli, Giardia duodenalis, Norovirus GI/GII, Shiga-like toxin-producing E. coli stx1/stx2, stx1/stx3, stx1/stx4, stx1/stx5, stx1/stx7, and Shigella/Enteroinvasive E. coli (in total: 14 pathogens) were detected in rectal samples. Cryptosporidium, C. cayetanensis, Enteropathogenic E. coli, G. duodenalis, Norovirus GI/GII, Rotavirus A, Salmonella spp., Shiga-like toxin-producing E. coli stx1/stx2 and stx1/stx6 (in total: 9 pathogens) were detected in gastric samples.

CONCLUSIONS: The findings have indicated that all lambs can harbour a variety of zoonotic pathogens. Pathogens were identified in lambs as early as immediately after birth. However, frequency of infection and burden of pathogens increased with age. Thus, lambs must be treated cautiously in contacts with people, given also that some of these pathogens can be transmitted to people through the direct contact with animals.

Keywords: GI infection, lamb, sheep, zoonotic

Detection of Cathelicidin-1 and Cathelicidin-2 Biomolecules in the Milk of Goats and Their Use as Biomarkers for the Diagnosis of Mastitis

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BACKGROUND and OBJECTIVE: The objectives of the present work were as follows: i) the detection of cathelicidin bio-molecules in the milk of individual goats during the early stages of mastitis and their potential use for the diagnosis of mastitis at its early stage and ii) the evaluation of the presence of cathelicidin proteins in the bulk-tank milk from goat and sheep farms.

MATERIALS and METHODS: In an experimental study, after inoculation of *Staphylococcus simulans* into a mammary gland of goats, bacteriological and cytological examinations of milk samples, as well as proteomics examinations [two-dimensional gel electrophoresis analysis (2-DE) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF MS) analysis] were performed sequentially, from 4 to 48 h post-challenge. Cathelicidin-1 and cathelicidin-2 were detected consistently in milk samples obtained throughout the study, and spot optical densities obtained from PDQuest v.8.0 were recorded. Associations were calculated between the presence of mastitis in a mammary gland at a given timepoint and the detection of cathelicidin proteins in the respective milk sample.

RESULTS: All inoculated mammary glands developed mastitis, confirmed by the consistent bacterial isolation from milk samples and the increased somatic cell content therein. Spot optical density of cathelicidin proteins was higher than in samples from contralateral mammary glands. There was a significant association between the presence of mastitis in a mammary gland and the detection of cathelicidin biomolecules in the respective milk sample; the overall accuracy was 81.8% (95% confidence interval: 70.4–90.2%). In a field investigation, the presence of cathelicidin proteins was evaluated in the bulk-tank milk of 32 dairy goat

and 57 dairy sheep farms. In this part of the work, no cathelicidin proteins were detected in any bulk-tank milk sample of goat, 0.0% (95% confidence interval: 0.0–10.7%), or sheep, 0.0% (95% confidence interval: 0.0–6.3%), farms.

CONCLUSIONS: The detection of cathelicidin biomolecules can offer increased accuracy for the early detection of mastitis in goats. Post-infection, the detection of cathelicidin proteins in the milk of individual animals occurred earlier than the increase in somatic cell counts in milk from the same animals. This approach presents an advantage in that cathelicidin proteins can be used as a non-specific biomarker, as simply a 'positive'/'negative' assessment would be sufficient.

Keywords: goats, cathelicidin, biomarkers, mastitis

Characterization of Morphological Traits and Morphometric Measurements of Local Goats in Hong Kong

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BACKGROUND and OBJECTIVE: While the morphology and morphometry of commercial goat breeds are well documented, local populations (backyard goats) lack such characterization. This knowledge gap is pronounced in regions where importation and breeding records are sparse. This study aimed to characterize the morphology and morphometrics of Hong Kong local goats.

MATERIALS and METHODS: In early 2026, 212 healthy goats ≥ 1 year old were randomly selected from 17 farms across Hong Kong. For each goat, a single trained assessor recorded 15 morphological traits and 16 morphometric measurements using standardized tools, as described by the Food and Agriculture Organization (United Nations). The Sexual Dimorphism Index (SDI) assessed the relative size differences in body length, wither height, chest girth, and live body weight (LBW) between intact males and females.

RESULTS: Most goats were females (84%, 178/212). Females were commonly plain (53.4%, 95/178), black (29.2%, 52/178), with smooth hair (97.8%, 174/178) and curved horns (70.8%, 126/178). Wattles were typically absent (74.7%, 133/178). On average, females had a body length of 65.0 cm (± 7.3), a wither height of 61.2 cm (± 6.2), a chest girth of 75.5 cm (± 8.2), and an LBW of 33.4 kg (± 8.5). SDI showed that intact males (n = 14) were larger than females across the four traits compared (0.07 – 0.16).

CONCLUSIONS: This first phenotypic description of Hong Kong goats enables future comparative research with established breeds. Such comparisons are essential for elucidating their potential origins and informing targeted management strategies in local goat populations.

Keywords: Capra hircus, Body length, Wither height, Chest girth, Sexual Dimorphism Index, China

Interrater Reliability and Accuracy of Morphometric Measurements for Estimating Body Weight in Local Goats

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BACKGROUND and OBJECTIVE: Indirect methods for estimating body weight in livestock are commonly based on morphometric measurements. Their reliability and accuracy may vary between populations. This study aims to assess inter-rater reliability and identify morphometric measurements that accurately predict live body weight (LBW) in Hong Kong local goats.

MATERIALS and METHODS: Three morphometric measurements and LBW were recorded in 212 adult goats. Two independent trained assessors measured the goats' heart girth, body length, and wither height. Morphometrics and LBW were measured using a

measuring tape and a calibrated digital scale, respectively. Inter-rater reliability was analyzed using Intraclass Correlation Coefficients. Marginal and conditional R^2 from univariate mixed-effect linear models were used to assess the accuracy of LBW predictions from those three measurements and the impact of farm differences on this predictive ability.

RESULTS: Most goats were older than 4 years old (61%, 130/212) and females (84%, 178/212), amongst which 101 were open (63%, 101/160). Heart girth showed the highest inter-scorer reliability (0.80), followed by wither height (0.76), and body length (0.69). Considering the morphological measurements alone, heart girth provided the most accurate predictions of LBW (R^2_m = 0.86), while body length and wither height accounted for 53% and 47% respectively of the variance in LBW. When accounting for differences between farms, heart girth remained the best-fitted model (R^2_c = 0.87).

CONCLUSIONS: In our population, the heart girth was the most reliable morphometric measurement and provided accurate estimations of live body weight, validating its use in routine procedures.

Keywords: Capra hircus, Heart girth, Body weight

Bilateral Mastectomy in Small Ruminants: Clinical Indications, Surgical Outcomes, and Long-Term Development

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BACKGROUND and OBJECTIVE: In small ruminants, udder health is crucial for both animal health and animal welfare. This study evaluates clinical indications and long-term outcomes following bilateral mastectomy in small ruminants treated at one veterinary clinic from 2022 – 2025 with particular emphasis on survival, surgical site outcome, and owner-reported treatment perception.

MATERIALS and METHODS: Twenty-one animals were included: 17 goats and 4 sheep, aged 2–14 years. The most common indication (multiple indications possible) for bilateral mastectomy was inappropriate lactation (n=12), followed by apostematosus mastitis (n=9), cisternal rupture (n=3), gangrenous mastitis (n=1), and mammary neoplasia (n=1). In three goats with concurrent hydrometra or suspected neoplasia of the reproductive tract, mastectomy was combined with ovariohysterectomy. Surgical procedures were performed under general anesthesia using standardized techniques. Long-term follow-up was conducted through owner interviews assessing animal survival, condition of the surgical site, and willingness to repeat the procedure under similar circumstances. Descriptive statistics were calculated, and categorical data were analyzed using Fisher's exact test and exact binomial testing.

RESULTS: One goat (4.8%) died intraoperatively due to systemic deterioration; no short-term complications occurred in the remaining 20 animals (95.2%). Postoperative follow-up via owner interviews was possible in 20 out of 21 cases (95.2%). At the time of follow-up, fifteen animals (75%) were still alive, while the other five (25%) had died 2–24 months postoperatively due to systemic conditions unrelated to mastectomy. Seventeen animals (85.0%) were reported to have unremarkable wound healing without any abnormalities, corresponding to an exact 95% CI of approximately 62.1%–96.8%. Mild postoperative complications were reported in three animals (15.0%; exact 95% CI: approximately 3.2%–37.9%). All owners interviewed (100%; exact 95% CI: approximately 83.2%–100%) indicated that they would elect to have the procedure performed again under comparable circumstances.

CONCLUSIONS: Bilateral mastectomy in small ruminants was associated with favorable long-term survival, minimal procedure-related mortality, and consistently high owner acceptance. With appropriate indication and careful perioperative management, bilateral mastectomy should be regarded as a viable, welfare-oriented, and owner-acceptable therapeutic strategy in goats and sheep with severe mammary pathology unresponsive to medical treatment. The unanimous owner's willingness to repeat the procedure under similar circumstances further underscores the perceived therapeutic value of the intervention.

Keywords: inappropriate lactation, chronic mastitis, sheep, goat, udder

Bacterial Dissemination and Risk for Mammary Infection During the Milking Procedure in Sheep Flocks

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BACKGROUND and OBJECTIVE: The general objective of this work was the evaluation of the potential for bacterial dissemination within the milking system during the actual milking procedure in dairy sheep flocks. Specific objectives of the study were i) the evaluation of bacterial recovery from samples from ewes (teat duct material, milk) collected before and after milking and ii) the description of sources of potential bacterial dissemination to ewes during the milking procedure and iii) the identification of predictors related to the milking routine for increased dissemination of pathogens.

MATERIALS and METHODS: Four dairy sheep farms were enrolled into the study and visited thrice, 1, 3-4 and 5-6 months after start of milking. In the milking parlour, three different teatcups were selected at random and swab samples were obtained from these, as well as from the mammary glands milked into these teatcups before and after completion of milking. Dissemination sources (milk, teat duct material) were considered for potential bacterial dissemination to subsequent dissemination targets (teatcups, teat ducts). Dissemination was defined only when all isolates considered to be implicated in a chain (teat duct to teatcup to teat duct of subsequent ewes) were identified similarly. The rate of bacterial dissemination cases during the milking procedure was considered and predictors were evaluated.

RESULTS: The proportion of samples from which no bacteria were isolated after milking although bacteria had been isolated before that: 48.4%, was significantly higher than proportion of samples with a shift in status in the opposite direction: 27.5%. A significantly higher proportion of teatcups from which bacteria were recovered during the milking procedure, was recorded during the second visit to the farms (85.0%) ($p < 0.0001$). In total, 118 incidents of dissemination of staphylococci were recorded, of which a higher proportion was seen also during the second visit to the farms (41.6%) ($p < 0.0001$). In multivariable analysis, the decreasing ambient temperature within the parlour during the milking procedure emerged as a predictor for the rate of bacterial dissemination ($p = 0.041$).

CONCLUSIONS: The study highlighted a tendency for bacterial flushing from mammary glands during the milking procedure, which may contribute to reduction of potential intramammary infections. However, the bacterial flushing, occurring primarily from populations in the teat duct, can also contribute to the dissemination of pathogens to mammary glands that will be milked subsequently in the same teatcup. Low environmental temperatures appeared to increase the rate of such dissemination

Keywords: machine-milking, mastitis, milking routine, Staphylococcus, teatcup

Whole Blood Selenium Levels in New Zealand Sheep Flocks and the Observation When Comparing Results Alongside Selenium Supplementation Options

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BACKGROUND and OBJECTIVE: Traditionally in New Zealand, supplementation of ewes for trace elements would occur at two to three points in the productive season, using a short acting oral multimineral supplementation. But with the changing farming systems, production and genetics of flocks, is this form of supplementation still adequate?

MATERIALS and METHODS: Blood samples were collected from ewes over 14 different flocks within North Canterbury, New Zealand, between 2021 and 2025. Samples were collected before the ram was introduced and before the start of lambing.

RESULTS: From the data collected, on average, whole blood selenium (WBS) levels decreased between lambing and mating (by 537.7nmol/L, SD=401) and increased between mating and lambing (by 620.5nmol/L, SD= 266). Before lambing 2021 and mating 2022, all flocks average WBS levels were

within the adequate reference range (250-6500nmol/L), with 10 farms below 1000nmol/L. At this stage all farms routinely treated with oral selenium supplementation. 3 of these farms proceeded to drop below the minimum reference range (MRR) before lambing 2022 and a further 2 before mating 2023. By lambing 2025, 8 farms reviewed their supplementation plans based on results and all now had WBS levels above 1000nmol/L throughout the season. As a point of comparison a further 15 farms using oral selenium supplementation were tested in 2025, showing 50% had an average WBS level below 1000nmol/L, suggesting they risked dropping below the MRR as previously observed. WBS were also taken from 41 individually identified ewes over three different farms before ram introduction, with all samples within the adequate range (average 965.6nmol/L). These ewes were given no selenium supplementation. By pregnancy scanning 30/41 ewes WBS levels decreased by a median of 37.5%(-276nmol/L), with 2 animal's WBS levels now below the MRR. This suggests, without supplementation, levels can drop at a time previously observed to increase, meaning supplementation may be required for the mating period.

CONCLUSIONS: If WBS levels can decrease over the lambing to mating period by an average of 500nmol/L, this could help with supplementation decisions. WBS levels below 800nmol/L would warrant alternative supplementation for a typical decline in selenium levels, however levels below 1600nmol/L may warrant consideration to cover all observed declines. These results highlight the need for individual assessment and monitoring of farms to ascertain what is the most effective form of supplementation for individual flocks, accounting for risk management and season variation.

Keywords: Whole blood selenium, Selenium supplementation, Flock health monitoring, Ewe reproductive cycle, Seasonal variations, Trace element deficiencies

Case Study: Amputation of the Medial Claw of the Hindleg in an Adult Ram – Clinical Presentation, Surgical Technique, Postoperative Care

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BACKGROUND and OBJECTIVE: Lameness in sheep is a problem with great economical importance and animal welfare considerations. In Hungary, severe individual lameness usually results in culling for multiple reasons. Only select types of lameness can be succesfully treated, and most of them have a bad prognosis in the chronic stage. Cases in Hungary are rarely presented in time for effective treatment, and surgical interventions can be costly.

MATERIALS and METHODS: A 2 year old Landschaf Merino breeding ram was presented with severe (5/5) hind leg lameness. Physical examination did not reveal any other health problems. The affected foot showed marked swelling at the coronary band of the medial claw with widening of the interdigital space, and multiple eruption points. The lateral claw was unaffected. X-ray examination revealed advanced inflammation of the distal interphalangeal joint, with necrosis and absorption of soft and bony tissues up to the distal end of the third phalanx. Preoperative examination (physical examination, haematology and blood biochemistry) determined that the animal is fit for surgery. Following the sedation of the animal (0.1 mg/kg xylazine iv, BelaZin, bela-pharm GmbH Co.KG, Vechta, Germany), intravenous distal limb anaesthesia (10 ml 2% lidocaine, LidoBel, bela-pharm GmbH Co.KG, Vechta, Germany) was carried out on the affected leg. The claw was surgically prepared and isolated. An oblique incision was made through the soft tissues at the proximal third of the first phalanx. The bone was sawn through with saw wire. No skin flap was made. A compression bandage was placed on the surgical wound. Postoperative pharmaceutical treatment included three weeks of antibiotic therapy locally (oxytetracycline spray, Cyclo spray, Eurovet Animal Health B. V., Bladel, Netherlands) and systemically (0.5 ml/kg Shotapen, Virbac, Carros, France) and non-steroidal anti-inflamatory drugs (0.5 mg/kg meloxicam, Melovem 20 mg/ml, Dopharma Research B.V, Raamsdonksveer, Netherlands). Bandage change with debridement

and local disinfection was carried out every two days for 3 weeks.

RESULTS: Full weight bearing was seen after one week. Following granulation tissue formation, the wound healed with a scar, with no complications. The ram was culled for economical reasons one year later. Until then, it was used for breeding purposes. The owner was advised to avoid overconditioning the animal, and to be attentive to regular hoof trimming.

CONCLUSIONS: Amputation is a good treatment option in cases of distal interphalangeal joint inflammation in sheep, if the animal is otherwise healthy, and adequate aftercare is possible.

Keywords: distal interphalangeal joint inflammation, orthopedic surgery, sheep

Clinical Mastitis Incidence and Etiology in Dairy Goats

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BACKGROUND and OBJECTIVE: Mastitis is a commonly diagnosed disease in dairy goats which impacts health and welfare, and has financial implications for the farmer. Mastitis can be categorized in subclinical mastitis and clinical mastitis. Subclinical mastitis in dairy goats has been extensively researched, but relatively little is known about clinical mastitis. The incidence of clinical mastitis and the relationship between clinical presentation and causative bacterial agents has been poorly researched. This study aims to determine the incidence and etiology of clinical mastitis in dairy goats.

MATERIALS and METHODS: During the year 2025 milk samples from all cases of clinical mastitis on 26 dairy goat farms in the Netherlands were collected by the farmers. Farmers were asked to fill out a questionnaire about the signs, symptoms and characteristics of each clinical mastitis case. Based on these data and previous literature in dairy goats and cows, mastitis cases were classified as from less to more severe: grade 1) only milk abnormalities, grade 2) milk abnormalities and udder abnormalities, grade 3) milk and udder abnormalities, and the goat is systematically ill, and gangrenous mastitis) the color of the udder turns blue, milk abnormalities, and the goat is systematically ill. The farmers were reminded every two weeks to collect samples of all clinical mastitis cases. All samples were bacteriologically cultured, colony forming units were counted, and bacterial species were identified using MALDI-TOF.

RESULTS: During the study, 323 samples of clinical mastitis cases were collected on 26 farms with a total of 30,275 goats at risk, resulting in an overall incidence of 8.64 sampled cases/1000 goats per year. Farm level incidences differed between 0.0 and 88.71 sampled cases/1000 goats/year. *Staphylococcus spp.* were most frequently isolated. The proportion of *Staphylococcus aureus* increased with an increased severity score of the clinical mastitis.

CONCLUSIONS: "The most severe cases of clinical mastitis were mainly caused by *Staphylococcus aureus*. Less severe cases of clinical mastitis were caused by non-aureus *Staphylococcus spp.* These findings help to understand the incidence of clinical mastitis that occurs in dairy goat farms and which bacteria are related to which clinical signs. This knowledge could be helpful to develop preventive strategies against mastitis in dairy goats.

Keywords: clinical mastitis, goat, bacteriology

Staphylococcus Caprae is Associated with Udder Asymmetry in Dairy Goats

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BACKGROUND and OBJECTIVE: An asymmetry of the udder of dairy goats might be the result of a (sub)clinical mastitis, in one of the udder halves. To our knowledge, the association between intramammary bacterial infections, the somatic cell count, and udder asymmetry in dairy goats has not been studied. Previous research showed that primiparous dairy goats already can have an udder asymmetry. Staphylococcus aureus is seen as one of the most important pathogens causing mastitis in dairy goats. In previous research, some non-aureus Staphylococci have been described as pathogenic bacteria causing mild forms of subclinical mastitis. The aim of this study was to determine associations between the presence of intramammary infections and somatic cell counts or udder asymmetry in dairy goats during the first three months of lactation.

MATERIALS and METHODS: A longitudinal study was performed on 5 dairy goat farms. On each farm around 50 primiparous dairy goats were sampled at the following timepoints: during the 1st, 2nd and 3rd month of lactation, and after one year of lactation. Milk samples were collected for bacteriological culturing. An additional milk sample was taken to determine the somatic cell count, protein, fat, lactose and urea content. At the 3rd and 4th sampling, symmetry of the udder was scored. All udders were scored on all farms by the same assessor (MP).

RESULTS: After three months of lactation, more intramammary infections and higher somatic cell count were found in goats with an asymmetrical udder as compared to goats with a symmetrical udder. Surprisingly, the presence of *Staphylococcus caprae* was associated with goats having asymmetrical udders.

CONCLUSIONS: A higher number of intramammary infections with *Staphylococcus caprae*, and an increased somatic cell count are associated with asymmetrical udders in primiparous dairy goats, when scoring after the first three months of lactation. No differences were found in the prevalence of *Staphylococcus aureus* intramammary infections between symmetrical and asymmetrical udders. This shows that non-*aureus* Staphylococci play a role in udder asymmetry in dairy goats.

Keywords: intramammary infections, udder asymmetry, mastitis, goats

Efficacy of Fipronil Against amblyomma Hebraeum ticks on Boer Goats and Detection Of ehrlichia Ruminantium in Ticks in Limpopo Province, South Africa

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BACKGROUND and OBJECTIVE: Heartwater, caused by *Ehrlichia ruminantium*, causes economic losses to commercial boer goat farmers in the Limpopo Province of South Africa. Heartwater is endemic in this region where *Amblyomma hebraeum*, the vector for this disease occurs. Due to their long mouthparts and specific attachment sites on goats, *A. hebraeum* is associated with wounds, secondary abscess formation and lameness. Various methods are used to control *A. hebraeum* and the negative effects associated with this tick, including heartwater. To determine the efficacy of a 1.0% Fipronil pour-on solution against *Amblyomma hebraeum* ticks on boer goats. To isolate and cryo-preserve current strains of *Ehrlichia ruminantium* to facilitate the implementation of novel disease control methods.

MATERIALS and METHODS: A therapeutic- and persistent efficacy trial using a 1.0% fipronil pour-on solution against *A. hebraeum* on boer goats was performed by counting ticks on day 0, 4 and 7 post-treatment on animals in the treatment- and control group. Detection and cryopreservation of *E. ruminantium* in *A. hebraeum* ticks were performed

by collecting adult *A. hebraeum* ticks from boer goats and cattle, preparing ground-up-tick-supernatants in the laboratory and cryopreserving samples. DNA extraction and nested PCR was used to target the pCS20 gene of *E. ruminantium* and agarose gel electrophoresis was used to demonstrate amplified DNA of *E. ruminantium*.

RESULTS: A 1.0% fipronil pour-on solution is 100% effective and has a persistent efficacy of seven days against *A. hebraeum*, but only if a targeted treatment approach is followed whereby the pour-on solution is applied to the predilection sites of *A. hebraeum*. *E. ruminantium* was detected in the cryo-preserved ground-up-tick-supernatant samples.

CONCLUSIONS: A 1.0% fipronil pour-on formulation can be used by boer goat farmers as an effective acaricide to control *A. hebraeum*. *E. ruminantium* can be detected and cryo-preserved from *A. hebraeum* ticks collected from cattle and goats in a region where heartwater is endemic. DNA extraction, nested PCR analysis targeting the pCS20 region of the genome of *E. ruminantium* and visualizing the amplified DNA by agarose gel electrophoresis is an appropriate method to demonstrate successful detection of *E. ruminantium* from adult *A. hebraeum* ticks. These novel cryo-preserved samples can be used for further research including the isolation of *E. ruminantium*, identification of genetic diversity and comparing current strains with historical isolates to improve our understanding of the dynamics of heartwater. It may facilitate the implementation of novel disease control methods like virulence studies, cross protection studies and region-specific vaccination strategies.

Keywords: Amblyomma hebraeum, Fipronil, Efficacy, Ehrlichia ruminantium, Heartwater, Boer goats

Evaluation by Thermography of Changes in the Body Temperature of the Goat in Relation to the Proximity of Parturition

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BACKGROUND and OBJECTIVE: The use of thermography in animal production is to detect abnormalities in animals using non-invasive methods, which facilitates the detection of some problems without the need to introduce any device; also, allows for the real-time of an animal's body temperature. In goats it has been investigated to determine reproductive stages such as estrus. The main findings have confirmed that vulvar temperature is associated with heat. Also, vulvar temperature is correlated with fertility levels. The objective of this study was to evaluate body temperature, using thermography, in different areas of the body of goats to detect possible variations associated with the proximity of parturition.

MATERIALS and METHODS: Nineteen pregnant goats were used, nine of which had single births and nine had multiple births. Starting four days prior to the estimated parturition date, and continuing daily until the day of parturition, the temperature of the vulva, udder, and caruncle was measured using a thermal camera. Measurements were taken at 50 cm with the animal immobile and at the same time of day.

RESULTS: Caruncle temperature was found to be lower on the day of parturition than on day 2 prepartum (32.8 ± 1.0 vs. 31.3 ± 0.9 °C, P = 0.06). Vulvar temperature tended to be higher on day 4 prepartum than on day 2 prepartum (36.3 ± 0.4 vs. 34.1 ± 0.6 °C, P = 0.06). When comparing between zones, it was found that on day 4 prepartum, vulvar temperature was higher than udder and caruncle temperature (P = 0.04). On day 3 prepartum, vulvar temperature tended to be higher than udder temperature (P = 0.080). Again, on day 1 prepartum and on the day of calving, vulvar temperature was higher than udder and caruncle temperature (P < 0.011). Finally, a trend

was found that on day 3 prepartum, the caruncle temperature was higher in multiple-pregnant females than in single-pregnant females (34.7 +/- 1.1 vs. 31.8 +/- 0.4 °C, P = 0.07).

CONCLUSIONS: It is concluded that the temperature of the caruncle decreases as parturition approaches, while that of the udder tends to increase. Meanwhile, the temperature of the vulva remains consistently elevated relative to the other two body areas evaluated. Funded by UNAM-FESC-CI2458.

Keywords: goat, birth, reproduction, welfare

Circadian Changes in Body Temperature, Heart Rate and Heart Variability During Transhumance in Sheep, Measured by Biologgers

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BACKGROUND and OBJECTIVE: Subcutaneous bio-loggers allow assessing physiological changes in body temperature (T), heart rate (HR), and HR variability (HRV), as variables related to welfare. The objective of this study was to analyze how transhumance, carried out from high mountain areas to grazing valley areas, affects sheep physiology, and evaluate their ability to adapt to stress during the journey.

MATERIALS and METHODS: A Segureña breed flock was used. Subcutaneous devices were implanted in six adult sheep, which travelled around 120 km for 5 days in transhumance from high mountain pastures to the destination area, a grazing pasture area combined with oak trees ("dehesa"). The devices recorded body T, HR and HRV every 20 min, from 2 d before the start of the trip until 10 days after arrival. Phase effects (before, during and after the trip) were tested using repeated-measures ANOVA, and paired t tests. For circadian analysis, a 24 h single-component cosinor model was fitted separately for each ewe and phase. MESOR, amplitude and acrophase were derived from the fitted parameters, and compared across phases using repeated-measures ANOVA.

RESULTS: Body T (°C) (±SE) varied among the studied phase (37.68±0.33 before transhumance; 37.08±0.66 ºC during transhumance; 36.28±1.66 at the destination) ($p<0.05$). HR (bpm) was similar the days before the start of transhumance (73.31±11.31), and during the journey (70.51±17.13), but significantly dropped when arriving at the destination (64.21±12.31). Regarding HRV, both SDNN and RMSSD were significantly higher during transhumance than before or after. Body T showed a significant reduction in MESOR from before to during and after transhumance ($p=0.003$), together with a significant delay in acrophase ($p=0.032$). No significant differences were detected between during and after transhumance, indicating that the main shift occurred during the transition from baseline to movement. HR displayed a progressive decrease in MESOR from before to after transhumance, although this did not reach statistical significance. HRV indices (SDNN and RMSSD) showed a tendency toward higher MESOR and greater amplitude during transhumance, suggesting enhanced autonomic modulation, but inter-individual variability limited statistical power.

CONCLUSIONS: Overall, transhumance was associated with a clear alteration of circadian T organization, while autonomic changes followed a trend to a coherent adaptive pattern. These results indicate an adaptation of the physiological variables of the animals to the new environmental conditions during and after transhumance. The use of biologgers allows the study of the responses of animals to novel or stressful situations.

Keywords: sheep, transhumance, biologger, temperature, heart rate, heart rate variability

Assessment of the Efficacy of Various Protocols with Progestogen Sponges Plus Equine Chorionic Gonadotrophin for Natural Mating in Dairy Sheep

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BACKGROUND and OBJECTIVE: The objective was the assessment of the efficacy of two protocols for oestrus induction and synchronization by using two newly licenced pharmaceutical products (progestogen sponges and equine chorionic gonadotrophin (eCG)), followed by natural mating.

MATERIALS and METHODS: In total, 362 ewes in two breeds of dairy sheep (Lacaune and Chios) were enrolled into the study, in two equal groups. Intravaginal insertion of sponges containing medroxyprogesterone (60 mg per sponge) was carried out, for 10 (group A) or 14 (group B) days. At removal of intravaginal sponges, ewes were injected with 500 IU of equine chorionic gonadotrophin. Rams of known fertility were introduced into the ewes two days after sponge removal (1 ram per 7.5 ewes). Pregnancy was evaluated by ultrasonographic examination 40-50 days after introduction of rams. All lambings were recorded appropriately.

RESULTS: Losses of intravaginal sponges occurred significantly less frequently in group A than in group B: in 4.4% versus 10.5% of ewes, respectively. There was no significant difference between group A and group B in cycling rate: 93.6% versus 90.1% of ewes, respectively. There was no significant overall difference between group A and group B in pregnancy rate (as evaluated by ultrasonographic examination): 92.6% versus 91.2% of ewes, respectively. Pregnancy rate was higher among Lacaune ewes, specifically over 97% for both regimes applied. There was no significant difference between group A and group B in the overall proportion of ewes that lambed: 86.7% versus 90.7% of ewes, respectively.

CONCLUSIONS: The study has provided baseline findings about the use of the two newly licenced pharmaceutical products for reproductive control in sheep. The overall findings are within the expected range of reproductive performance of ewes and did not show any irregularities after using either of the two protocols evaluated. The findings have not indicated a difference in the results after intravaginal insertion of progestogen sponges to ewes for 10 days or for 14 days. Shortening (by 4 days) of the period of intravaginal insertion of progestogen sponges, followed by injection of equine chorionic gonadotrophin, did not have any adverse effects in the efficacy of a protocol with the aim for synchronization of oestrous cycles and 'out-of-season' breeding of ewes.

Keywords: health management, progestogen, reproductive control, sheep

Ovarian Dynamics After Different Synchronization Protocols Combining Progestogen Sponges Plus Equine Chorionic Gonadotrophin for Artificial Insemination in Dairy Sheep

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BACKGROUND and OBJECTIVE: The objective was the assessment of ovarian dynamics (follicular growth, ovulation and formation of corpus luteum) of three different protocols for oestrus synchronization, which included the combined use of progestogen sponges and equine chorionic gonadotrophin (as two newly licenced pharmaceutical products) and would be aimed for application in artificial insemination programs in dairy sheep

MATERIALS and METHODS: We included 24 ewes into the study, which were divided in three equal groups. Intravaginal insertion of sponges containing medroxyprogesterone (60 mg per sponge) was carried out for 7 (group A), 10 (group B) or 14 (group C) days. At removal of the sponges, ewes were injected with 250 IU of equine chorionic gonadotrophin; ewes in group received 0.075 mg of D-cloprostenol (prostaglandin F2α). Both ovaries and the uterus of ewes in the study were examined ultrasonographically by a micro-convex array at 12-hour intervals from sponge removal until ovulation or until 72 hours post-sponge removal. The transrectal technique was applied. Settings were as follows: frequency 7.5 MHz, scanning depth 4.0-5.0

cm, gain 55%. Follicles on ovaries of each ewe were mapped.

RESULTS: The median number of total follicles observed per ewe during the ultrasonographic examinations did not differ among the three groups: 2.5, 2.5 and 3.0 for ewes in group A, B and C, respectively. The median time-period for the last observation of follicles after the start of the ultrasonographic examinations was 60 hours, 36 hours and 72 hours for ewes in group A, B and C, respectively. Overall, the median maximum volume of follicles before ovulation was 0.052 mL, 0.023 mL and 0.040 mL for ewes in group A, B and C, respectively. The median volume of corpora lutea formed after ovulation was 0.078 mL, 0.117 mL and 0.137 mL for ewes in group A, B and C, respectively.

CONCLUSIONS: The findings have provided a schedule for the timing of ovulation in ewes after using hormonal control, for use in cases of artificial insemination. The results have indicated that a regime of 7-day long intravaginal insertion of progestogen sponge coupled with administration of equine chorionic gonadotrophin and prostaglandin led to earlier development of larger follicles; a regime of 14-day long intravaginal insertion of progestogen coupled with administration of equine chorionic gonadotrophin led to a higher number of ovulating follicles. These two regimes appear to be the most suitable for use for artificial insemination.

Keywords: progestogen, reproductive control, sheep, ultrasonography

The Effect of Selenitetriglycerides on the Health Status and Expression of Selected Genes Encoding Enzymes Involved in Antioxidant Processes in Goat Kids

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BACKGROUND and OBJECTIVE: The prevention of selenium deficiency is currently based on its supplementation with injectable or oral preparations, containing selenium in inorganic or organic form. Selenitetriglyceride are an organic form of selenium that can be administered to animals orally. They are characterized by low toxicity, high bioavailability, and absorption from the digestive tract.

MATERIALS and METHODS: The research was carried out on 12 kids of the Alpine goat, aged 10 days divided into 2 groups: control and experimental. For the first 7 days of the experiment, the experimental group was orally supplemented with a solution of selenitetriglycerides, at a dose of 0.5 mg selenitertriglicerydes, per kg of body weight. Then, blood was collected from all animals on the 0th, 1st, 7th, 14th, 21st and 30th day of the experiment. On the day 30. of the experiment, lambs and kids from both groups were euthanized, then the samples of the longisimus dorsi muscle, biceps femoris muscle, myocardium and liver was taken from them. In the serum of kids were estimated activity of AST (aspartate aminotranferase), GGTP (gamma glutamyl transpeptidase), LDH (lactate dehydrogenase) and Se concentrations. The activity of the glutathione peroxidase (GPX) and superoxide dismutase (SOD) in whole blood of all animals were determined also. In the tissue samples of biceps femoris muscle, myocardium and liver mRNA expression of genes encoding enzymes GPX1 ad SOD1 were determined.

RESULTS: Biochemical parameters tested in the blood serum of the animals of the experimental and control groups showed significant differences ($p<0.05$) of GGT in kids, which was significantly higher ($p<0.05$) in the control group on the days 7, 21 and 30. The results of serum Se concentration in kids showed that supplementation causes a significant ($p<0.05$) increase of this microelement during entire experiment. In addition, significantly higher ($p<0.05$) GPX activity was observed on the 1st, 7th, 14th, 21st and 30th days of the experiment. Similarly, SOD activity was significantly higher ($p<0.05$) in selenitetriglyceride-supplemented kids. After supplementation with selenitetriglycerides, an increase in the expression of the GPX1 gene in the biceps femoris muscle, liver in kids was observed. Moreover, an increase in the expression of the SOD1 gene in the analyzed fragments of the liver in kids was observed.

CONCLUSIONS: Selenitetriglycerides cause a significant increase in the selenium concentration in the body of goat kids and significantly influenced the expression of GPX1 and SOD1 genes. acknowledgements: „Funded by the Minister of

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Keywords: selenite triglycerides, antioxidants, gene expression, goat kids

Field Trial Evaluation of a Commercial Subunit Vaccine Against Bovine Cryptosporidiosis in Dairy Sheep

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BACKGROUND and OBJECTIVE: Cryptosporidium parvum is a zoonotic pathogen causing neonatal diarrhea in pre-weaned ruminants, often leading to severe illness. No small ruminant vaccine exists, but a recombinant Gp40 protein subunit vaccine for calves provides passive immunity through colostrum. This field trial is the first to evaluate the vaccine's safety and immunogenicity in newborn lambs.

MATERIALS and METHODS: The study was conducted on a commercial dairy flock in Spain with a previous history of cryptosporidiosis. The trial was blind controlled, randomized and stratified into sheep vaccinated with half the dose as registered for cattle (n=75; Group A) and sheep inoculated with a vaccine solvent (n=75; Group B). Pregnant dams were vaccinated twice prior to lambing. Vaccine safety was monitored daily for 14 days after primo vaccination and booster. Systemic adverse effects and local reactions were evaluated according to an established score based on size and severity of lesion. Immunogenicity was evaluated by measuring anti-Gp40 antibodies by ELISA in blood from both dams and lambs. Finally, lamb health was monitored according to an established score including Wisconsin score for diarrhea and fecal samples were collected three times per week for the first 21 days of life in 126 and 101 lambs born from group A and B, respectively, to detect oocyst shedding by Ziehl Neelsen staining.

RESULTS: The vaccine was safe supported by the absence of systemic clinical signs and the development of mild local reactions in group A that disappeared during the first two weeks post-vaccination. There was a significant increase of antibody levels in dams from group A between both vaccinations (P<0.0001) (14 days post first vaccination and 14 days after booster) and when compared to group B (P<0.0001). Anti-Gp40 antibodies were transmitted to the lambs via colostrum (P<0.0001). In terms of efficacy, the delayed exposure with C. parvum made it impossible to observe notable differences in clinical signs between the two groups, as oocyst shedding was only recorded at the final sampling (among 14–21-day-old lambs: three from group A compared to five from group B). Lambs were likely benefited from the vaccination and associated management measures that included the uptake of transition milk for the first five days of life.

CONCLUSIONS: Although the vaccine is not currently intended for use in sheep, it may be a safe intervention in sheep and could contribute to improved livestock welfare and productivity. However, there is no evidence at present to support its effectiveness in reducing oocyst shedding.

Keywords: Cryptosporidiosis, subunit vaccine, passive immunity, sheep

Assessment of Thoracic Ultrasound as a Diagnostic Tool for Lower Respiratory Tract Disorders in Sheep

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BACKGROUND and OBJECTIVE: Lower respiratory tract diseases (LRTDs) represent one of the leading causes of morbidity, mortality, and productivity losses in sheep worldwide. Many conditions, including ovine respiratory complex (ORC), small ruminant lentivirus (SRLV) infection, ovine pulmonary adenocarcinoma (OPA), gangrenous pneumonia (GP), and verminous pneumonia (VP), often present with subtle or overlapping clinical signs, making accurate field diagnosis difficult. Thoracic ultrasonography (TUS) has gained attention as a rapid, non-invasive diagnostic tool in ruminant medicine; however, comprehensive validation studies in sheep of different ages and production systems remain scarce. To compare the diagnostic performance of distance observation, comprehensive respiratory diagnostic assessment (CRDA), and thoracic ultrasonography in sheep with suspected LRTDs, using post-mortem examination as the reference standard.

MATERIALS and METHODS: A total of 103 sheep (51 adults and 52 lambs) from commercial flocks were prospectively examined. Each animal underwent sequential evaluation including remote visual assessment, detailed clinical respiratory examination, TUS performed by blinded operators, and complete necropsy with histopathology and microbiological/molecular pathogen detection. Diagnostic performance was assessed through sensitivity, specificity, predictive values, overall accuracy, and Cohen's kappa coefficient.

RESULTS: Remote observation showed extremely low sensitivity (8.6%) and negligible agreement with post-mortem findings (κ=0.057). CRDA improved sensitivity (72.4%) but demonstrated low specificity (44.4%) and limited agreement (κ=0.172), reflecting the difficulty of differentiating diseases with overlapping clinical presentations. TUS showed superior overall diagnostic performance, with 79.3% sensitivity, 86.7% specificity, and 82.5% accuracy (κ=0.65; p<0.001). Disease-specific agreement was substantial for ORC (κ=0.75), SRLV infection (κ=0.75), and GP (κ=0.78). Sensitivity was lower for VP (22.7%), likely due to its typical dorsocaudal distribution, which limits ultrasonographic accessibility. Importantly, TUS accurately identified healthy animals (κ=0.66), supporting its value in flock-level screening and decision-making.

CONCLUSIONS: TUS significantly outperforms clinical examination in the diagnosis of LRTDs in sheep and demonstrates substantial agreement with post-mortem findings. Its high specificity and ability to characterise lesion patterns make it a reliable, practical tool for field application. Integration of TUS into routine flock health programs may improve early detection, optimise targeted treatments, and contribute to more rational antimicrobial use in sheep production systems.

Keywords: Sheep, lung disorders, thoracic ultrasound

Pathological Characterization and Hereditary Pattern of An Extreme Wrinkle Phenotype in Neonatal Merino Lambs

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BACKGROUND and OBJECTIVE: Congenital and hereditary dermatological disorders have been described in sheep, although the clinical and histopathological features of some recently observed phenotypes remain poorly defined. A severe dermatological condition characterised by extreme skin wrinkling and early mortality has been identified in Spanish Merino lambs. This study aimed to characterise the pathological features of this condition and investigate its possible hereditary pattern.

MATERIALS and METHODS: Eight neonatal lambs from a pedigree Spanish Merino flock were referred between 2024 and 2025 for clinical and pathological evaluation. The flock included New Zealand and Dutch bloodlines, and the referring veterinarian reported several similarly affected lambs within related lineages. Lambs appeared clinically normal at birth, although the precise onset of dermatological alterations could not be determined. During the first weeks of life they developed marked skin folding and wrinkling, producing a characteristic “wrinkle lamb” phenotype, together with progressive wool loss. At approximately three to four weeks of age, some lambs developed proliferative oral lesions compatible with naturally acquired contagious ecthyma. All lambs died shortly after clinical deterioration. Serial biopsies of skin and oral mucosa were obtained for histopathological examination. Polymerase chain reaction (PCR) targeting the Orf virus B2L gene was performed on muzzle lesions to detect contagious ecthyma. Pedigree analysis was carried out to assess genealogical relationships and explore the possible mode of inheritance.

RESULTS: The main clinical finding was marked generalised skin wrinkling associated with diffuse wool loss. Histopathological examination revealed severe follicular dysplasia, marked follicular keratosis, degeneration of the follicular epithelium—mainly affecting the outer root sheath—and sebaceous gland atrophy, indicating a profound defect in follicular development and maintenance. PCR and histopathology confirmed that the oral lesions corresponded to contagious ecthyma (Orf virus infection). These lesions were interpreted as secondary infections, probably reflecting increased susceptibility to infection in affected lambs. Pedigree analysis showed clustering of affected animals within related lineages and supported a pattern consistent with autosomal recessive inheritance.

CONCLUSIONS: This study describes a previously unreported hereditary dermatological disorder in Merino lambs characterised by severe follicular dysplasia, extreme skin wrinkling, diffuse wool loss and early mortality. Pedigree analysis supports an autosomal recessive mode of inheritance. Contagious ecthyma lesions appear to represent secondary opportunistic infections rather than the primary cause of the condition. Early recognition of this phenotype is important for breeding management and genetic counselling to prevent dissemination within affected flocks. Further studies are needed to identify the underlying molecular mechanism.

Keywords: Sheep, Merino lambs, extreme wrinkle phenotype

Parasitic Resistance to Eprinomectin in Bavarian Goats

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BACKGROUND and OBJECTIVE: Small ruminants are often held on pasture and so ingest many endoparasites. These are acquiring multiples resistances, getting worse the last years. For minor species as goats very few medicaments are licenced. Formerly German veterinarians could use sheep medicaments. But since a few years in Germany there is only one eprinomectin product (Eprecis, Ceva) licensed for goats and has to be used exclusively. But there are many resistances to this drug in southern Germany. So the aim of this study was to describe the actual resistance patterns in Bavarian goat herds.

MATERIALS and METHODS: In 25 Bavarian goat Herds, mainly brown and white milking goats,10-15 animals were selected, preferably those with bad body condition. Individual fecal samples were taken and analyzed in a McMaster chamber. The animals were dewormed with injectable eprinomectin (Eprecis 20mg/ml, Ceva) dosed at 1ml/100kg as described by the producer. Only one farmer used the pour on version. After 10-14 repeated fecal samples were taken from the same animals and analyzed again.

RESULTS: The parasitic infestation varied widely between the farms, from an average of less than 500 to over 2,500 eggs per gram feces. For every individual animal the reduction of the egg count after deworming was counted. Only in 6 of the 25 herds the average reduction of the parasites was higher than 95%, the minimum to tell the product effective. The lowest reduction was 4% and the highest 100%. In one herd with a reduction rate of 27% in few months died 17% of the animals after deworming.

CONCLUSIONS: Especially in organic farms the milking goats have to go every day on the pasture. So, with ineffective drugs or bad pasture management, many animals get health problems. The good veterinarian practice implicates a change of deworming drugs, but actually this is not possible in German goats. Only if there is a proof of a reduction of less than 95% of the worm eggs, the drug is described as not effective in the herd and another medicament can be used by the veterinarian.

Keywords: Goat, eprinomectin, parasites, resistance

Effect of Maternal Age, Body Condition Score, and Lamb Sex on The Birth Weight of Kangal-Akkaraman Lambs

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BACKGROUND and OBJECTIVE: Sheep production is a fundamental component of global food security and sustainable livestock systems, particularly in arid regions. The Kangal-Akkaraman sheep is a regionally adapted genotype in Türkiye, known for its hardiness and enhanced growth rates. In sheep production, lamb birth weight is a primary determinant of survival rates, physiological adaptation, and economic profitability. This study aimed to investigate the effects of maternal factors, including maternal age, live weight, and body condition score (BCS), along with lamb sex, on the birth weight of Kangal-Akkaraman lambs.

MATERIALS and METHODS: The study was conducted on a commercial farm with 110 clinically healthy Kangal-Akkaraman ewes. Maternal age was determined by farm records and dentition. Ewe live weight, and BCS (1–5 scale) were recorded during late pregnancy. Withers' height was measured as a morphological indicator. Lamb birth weights were recorded within two hours postpartum using a precision scale (±0.05 kg) to minimize confounding factors. Data were analyzed using the General Linear Model (GLM) procedure, and linear regression was applied to evaluate the relationship between maternal withers height and birth weight. Statistical significance was set at $P<0.05$.

RESULTS: The flock exhibited high reproductive efficiency with a pregnancy rate of 93.6% and a lambing rate of 95.2%. Single births predominated (99.0%), and the pre-weaning survival rate was 98.1%. Analysis revealed that lamb sex and maternal age significantly influenced birth weight. Male lambs were significantly heavier (5.22±0.80 kg) than female lambs (4.21±0.53 kg) (P<0.001). Maternal age also had a significant effect (P<0.05), where 3-year-old ewes lambed heavier lambs (4.59±0.76 kg) compared to 2-year-old ewes (4.23±0.51 kg). A significant positive correlation was observed between maternal withers height and birth weight (P<0.05). However, maternal live weight and BCS did not significantly affect birth weight (P>0.05).

CONCLUSIONS: Maternal age and lamb sex are the primary determinants of birth weight in Kangal-Akkaraman sheep. While the breed demonstrates high reproductive capacity, the findings suggest that optimizing the age structure of the breeding flock, improving neonatal management, and minimizing environmental stress are essential to maximize production efficiency and lamb survival.

Keywords: Akkaraman, lamb, maternal age, sheep, weight

Hydroxy-Selenomethionine Supplementation Promotes Greater Selenium Deposition in the Muscle Tissue of Growing Lambs Compared with Sodium Selenite

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BACKGROUND and OBJECTIVE: Organic selenium (Se) sources, such as hydroxy-selenometionine (OH-SeMet), are known to have higher bioavailability than inorganic Se forms; however, their effects on Se deposition in lamb tissues have not been extensively investigated. This study aimed to determine the selenium concentration in the longissimus dorsi (LD) muscle of lambs fed diets supplemented with different levels of OH-SeMet or sodium selenite (SS).

MATERIALS and METHODS: Twenty-one Kıvırcık male lambs were randomly assigned to three dietary treatments in a randomized complete block design. Following a 10-day adaptation period, lambs were fed for 56 days with concentrate-based diets individually supplemented with either 0.2 mg Se/kg dry matter (DM) from SS, or OH-SeMet providing 0.2 or 0.4 mg Se/kg DM.

RESULTS: At the supplementation level of 0.2 mg Se/kg DM, OH-SeMet increased muscle Se concentration by 61.17% compared with SS. Moreover, Se deposition in muscle exhibited a linear dose-dependent response to increasing OH-SeMet levels.

CONCLUSIONS: The greater incorporation of OH-SeMet into muscle tissue may enhance the selenium content of lamb meat, potentially contributing to increased dietary Se intake for consumers of animal-derived foods.

Keywords: Hydroxy-selenomethionine, Lamb, Muscle Se concentration, Sodium selenite

Lentiviruses: Co-infection of A & B Genotypes in Flocks of Small Ruminants

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BACKGROUND and OBJECTIVE: Small ruminant lentiviruses (SRLV) affect ovine and caprine species. Currently, it is well known that there are 5 genotypes (A–E) and more than 30 subgroups. For establishing a diagnosis, the laboratory methods are essential. In Portugal, highly diverse production systems in different regions may contribute to the high profitability and social value of small ruminant production. The main aim of this work was to determine which SRLV genotypes are present in Portugal.

MATERIALS and METHODS: The SRLV genotyping research was conducted in sheep and goat flocks in Portugal that had previously participated in a seroepidemiological study. Two hundred and five [r1.1] positive samples to SRLV ELISA were randomly selected from 105 different farms for analysis (one or two samples of positive animals' sera by flock. With the primary objective of determining the circulating genotypes, each sample was subjected to a commercial ELISA genotyping test (In3diagnostics®).

RESULTS: Of the 205 samples analysed, 124 tested positive: 54 for genotype B, 32 for genotype A, and 38 were unclassified, indicating coinfection or cross-reactions. Upon further analysis by species, we found that 20 of the genotype A positive samples were in sheep and in 12 goats. As for genotype B, 24 samples were from sheep and 30 from goats. In five flocks, one animal was positive for genotype A while another was positive for genotype B; two of these were goat flocks, and three were sheep flocks.

CONCLUSIONS: The results indicate that both SRLV genotypes A and B circulate among ovine and caprine flocks in Portugal. Genotype A was predominantly detected in sheep, whereas genotype B was more frequently identified in goats. Nevertheless, these genotypes are not species-specific and may occur in both host species. Furthermore, coinfection with both genotypes was observed and may represent an important epidemiological concern at both the flock and individual animal levels.

Keywords: Small ruminant lentiviruses, Sheep, Goats, Genotype

Preliminary Findings on a Safe and Refined Orosophageal Probing Technique for Serial Rumen Fluid Sampling in Sheep

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BACKGROUND and OBJECTIVE: This protocol was implemented within a research project evaluating nutraceuticals on biological processes in sheep. A significant challenge was ensuring non-invasive rumen fluid extraction. Due to the scarce scientific information available on this topic, we developed and refined an oroesophageal probing technique adapted from bovine and experimental ovine protocols. The primary requirements were maintaining sufficient and safe aspiration pressure, ensuring consistent fluid progression without prior fasting or specific treatments, and avoiding adverse side effects. Therefore, the objective of this study

was to develop a safe, standardized technique for serial rumen fluid collection in sheep.

MATERIALS and METHODS: The sampling probe consisted of a 1.5 m flexible, transparent PVC tube (Spirocristal®; 12 mm internal diameter, 15 mm external diameter) featuring a rounded distal tip and eighteen 5 mm diameter apertures distributed across the distal 7 cm. External markers indicated the distances from the incisors to the glottis and the cardia. The system was connected to an airtight collection vessel and a regulated vacuum pump (Millipore WP61). Sampling was ideally performed 2–3 h postprandially with the animal standing and the head and neck extended. Following the insertion of a mouth gag, the probe was gently advanced to elicit a swallowing reflex. Intraruminal placement was confirmed by the presence of characteristic ruminal odor. A controlled vacuum (500–670 mbar absolute pressure) was subsequently applied. Obstructions to flow were resolved through minor probe manipulations or brief air insufflation. To ensure safe withdrawal and prevent aspiration, it was critical to terminate the vacuum, purge residual fluid via air insufflation, and manually occlude the probe before a swift, steady extraction.

RESULTS: Rumen fluid was extracted every 8 weeks from 11 Lacaune rams (mean age 13.4±0.8mo. mean weight 60±2kg) over three serial procedures (n = 33 extractions). Post-procedural respiratory pathology occurred in only one animal during the first session, after which the precautions of marking probe length and plugging the end during withdrawal were strictly implemented. The target volume (100 ml) was achieved in most cases. In 7/33 instances (21%), a lower volume was collected (mean 73 ±14ml), which remained sufficient for analysis. The mean procedure duration was 4±2 minutes per animal.

CONCLUSIONS: The oroesophageal probing technique described herein represents a safe, effective, and reliable method for serial rumen fluid collection in sheep. This standardized protocol ensures sample quality while minimizing animal distress and preventing potential complications.

Keywords: Ruminal fluid, intraruminal tube, sampling

Early Pregnancy Detection in Goats (Between Days 10 And 19 Post-Insemination) Using Ultrasonographic Measurements and Algorithm-Based Echotextural Analyses of Luteal Structures

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BACKGROUND and OBJECTIVE: Early, accurate, and relatively inexpensive pregnancy diagnosis enables timely re-breeding of non-pregnant females, thereby enhancing herd productivity, improving overall farm profitability, and supporting animal welfare. In goats, pregnancy is typically diagnosed after day 19 (D19) post-mating or artificial insemination (AI). Advancing pregnancy detection would allow prompt re-synchronization of non-pregnant does. Although the embryo proper is not visible before D19 of gestation in goats, changes in luteal size and ultrasonographic characteristics (echotexture) have been suggested as potential indicators of pregnancy. This study evaluated morphometric and algorithmically derived echotextural characteristics of luteal structures in goats as pregnancy indicators between D10 and D19 post-AI.

MATERIALS and METHODS: Nineteen primiparous Saanen goats (~9 months old; body weight 35-37 kg) from a commercial farm in Northwestern Sardinia, Italy, underwent laparoscopic AI (D0) in November (breeding season), following an estrus synchronization protocol using prostaglandin F2α/equine chorionic gonadotropin injections and progestin-soaked intravaginal sponges inserted for 6 days. Transrectal ovarian ultrasonography was performed daily on D10-D12 (late blastocyst/early tubular stage) and D17-D19 (maternal recognition of pregnancy), using a portable ultrasound scanner

connected to a 10-MHz linear-array transducer. Pregnancy was confirmed ultrasonographically on D40. Total luteal area (TLA) was measured with built-in electronic planimetry tool (area measurement) and analyzed using two-way repeated measures ANOVA with required assumption diagnostics and post-hoc tests. Digital images of luteal structures were analyzed using proprietary software (r-Algo) developed at the University of Guelph to identify pixel intensity ranges (PIR) within the 0-255 scale (0=absolute black to 255=absolute white) for which first-order echotextural variables (mean pixel intensity-MPI, pixel heterogeneity-MPH, and pixel concentration-MPC) of luteal structures differed significantly between pregnant (P) and non-pregnant (NP) does. Sensitivity and specificity values were calculated using the threshold-based discrimination method.

RESULTS: Mean ovulation rate (1.7±01) did not vary (P>0.05) between P and NP animals. TLA was 1.6-fold greater (P<0.05) in P (n=9) compared to NP (n=10) goats on D18, yielding 80% sensitivity (detection of P does) and 89% specificity (detection of NP does). Combination of the three echotextural parameters enabled accurate determination of pregnancy status in all animals on every examination day, with MPH offering the highest sensitivity/specificity (90-100%) on D10 (PIR 128-145), D12 (PIR 141-157), and D18 (PIR 12-27).

CONCLUSIONS: Luteal area measurements provide a simple yet reasonably accurate practical method of caprine pregnancy detection on D18 post-AI. However, r-Algo–derived echotextural parameters represent significantly more robust indicators of pregnancy in goats, as early as D10-D12 post-AI, with potential applicability across multiple animal species.

Keywords: Pregnancy, Goat, Image analysis, Corpus Luteum

Can Macroscopic Hydatid Cyst Characteristics Predict Hepatic Damage and Oxidative Stress in Sheep?

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BACKGROUND and OBJECTIVE: Cystic echinococcosis caused by Echinococcus granulosus is a common parasitic disease of sheep and frequently involves the liver. Hydatid cysts are easily recognized during slaughter inspection; however, their macroscopic features are rarely interpreted in relation to the severity of hepatic lesions. Understanding whether cyst burden reflects underlying tissue damage may help clinicians better evaluate the impact of infection. This study aimed to examine the relationship between animal characteristics (age and breed), hydatid cyst burden (number and diameter), hepatic histopathological lesions and oxidative stress responses in naturally infected sheep.

MATERIALS and METHODS: Twenty-five sheep livers obtained from slaughterhouses were examined, including 15 hydatid cyst–infected and 10 healthy control samples from Akkaraman and Morkaraman breeds aged between 1 and 5 years. Cyst diameter was classified macroscopically as score 1 (<1 cm), score 2 (1–5 cm) and score 3 (>5 cm). Cyst number was scored as 1 (1–3 cysts), 2 (4–10 cysts) and 3 (>10 cysts). Histopathological evaluation was performed on hematoxylin–eosin stained sections. Fibrosis, hepatocellular degeneration/necrosis, inflammatory cell infiltration and bile duct hyperplasia were assessed using a semi-quantitative scoring system (0–3). Immunohistochemistry was performed to evaluate oxidative stress responses using inducible nitric oxide synthase (iNOS) and superoxide dismutase (SOD).

RESULTS: Protoscoleces were detected in 6 of the 15 cyst-containing samples (40%). Although protoscolex positivity was observed only in cyst-containing samples, the association between cyst diameter and protoscolex presence was not statistically significant (Pearson x^2=3.30, p=0.069; Fisher's exact test p=0.051). Histopathological

lesions including fibrosis, hepatocellular degeneration/necrosis, inflammatory cell infiltration and bile duct hyperplasia were commonly observed in cyst-containing livers. However, histopathological lesion scores did not differ significantly according to protoscolex presence ($p>0.05$). Immunohistochemical analysis revealed strong inducible nitric oxide synthase expression in hepatocytes and inflammatory cells surrounding hydatid cysts. Superoxide dismutase expression was detected in both infected and control livers. Nevertheless, iNOS and SOD expression levels were not significantly associated with protoscolex presence ($p>0.05$). Older animals and those with higher cyst burden tended to exhibit more pronounced hepatic lesions.

CONCLUSIONS: Macroscopic hydatid cyst burden, particularly cyst number and diameter, appears to be more closely related to the severity of hepatic pathology than the presence of protoscoleces. Animals carrying multiple or larger cysts showed more pronounced inflammatory and degenerative liver lesions together with increased oxidative stress responses. These findings suggest that macroscopic characteristics of hydatid cysts may provide useful information for estimating the extent of liver damage in naturally infected sheep.

Keywords: Cystic echinococcosis, hydatid cyst, liver pathology, oxidative stress, sheep

Blood Biomarkers for Early Pregnancy Detection in Ewes: A Comparative Evaluation of Progesterone, PAG, PSPB, and IFN-tau

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BACKGROUND and OBJECTIVE: Early pregnancy diagnosis is essential for reproductive management in sheep, improving flock productivity and efficiency. Blood-based biomarkers have been proposed as alternatives or complements to ultrasonography, reflecting distinct physiological processes during early gestation. This study aimed to evaluate the diagnostic potential of peripheral interferon-tau (IFN-τ), progesterone (P4), pregnancy-associated glycoproteins (PAG), and pregnancy-specific protein B (PSPB) for early pregnancy detection in ewes, using ultrasonography as the reference method.

MATERIALS and METHODS: A total of 53 clinically healthy, multiparous Ile de France ewes underwent estrus synchronization followed by natural mating. Pregnancy status was determined by ultrasonography on day 30 post-mating and confirmed by lambing outcomes. From this population, 27 ewes (non-pregnant, single-pregnant, and multiple-pregnant; n=9 per group) were selected for biomarker evaluation. Blood samples were collected on day 17 for IFN-τ, day 18 for P4, and day 30 for PAG and PSPB.

RESULTS: Progesterone concentrations on day 18 were significantly higher in pregnant than non-pregnant ewes, yielding the highest diagnostic accuracy (AUC=0.93; sensitivity=83.33%, specificity=66.67%) and moderate agreement with ultrasonography (κ≈0.50). PAG levels differed significantly among groups and demonstrated good diagnostic performance (AUC=0.80) with fair agreement (κ≈0.33). PSPB concentrations showed a non-significant trend toward higher values in pregnant ewes and moderate diagnostic accuracy (AUC=0.70) with fair agreement (κ≈0.21). In contrast, peripheral IFN-τ concentrations did not differ significantly among groups and showed limited diagnostic value (AUC=0.62) with fair agreement (κ≈0.40), likely due to its predominantly paracrine mode of action and the use of a cross-species (bovine) ELISA kit.

CONCLUSIONS: In conclusion, progesterone and PAG are the most reliable peripheral biomarkers for early pregnancy diagnosis in sheep, whereas peripheral IFN-τ has limited utility under field conditions.

Keywords: Early pregnancy diagnosis, interferon-tau, progesterone, pregnancy-associated glycoproteins, sheep, ultrasonography

Identification and Molecular Characterization of a Novel Pestivirus Aydinense Isolate from Sheep in Türkiye: Further Evidence for a New Subgroup

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BACKGROUND and OBJECTIVE: Pestiviruses are significant pathogens causing substantial economic losses in the livestock industry worldwide. Pestivirus genus comprises 19 species, among these viruses, BDV (Pestivirus ovis) causes significant economic losses by infecting small ruminants (sheep and goats), leading to abortions, weak offspring, and persistently infected (PI) animals. BDV is quite heterogeneous, including eight identified genotypes (BDV-1-BDV-8) in addition to variants yet to be classified. The reference isolate Pestivirus aydinense, included as a new species in the latest ICTV classification, was first reported in Turkey as an Aydın-like group and has become endemic in some regions. This study aims to characterize a pestivirus isolate obtained from sheep in Türkiye and to determine its phylogenetic relationship within the Pestivirus aydinense species.

MATERIALS and METHODS: Blood serum samples were collected from lamb exhibiting symptoms of congenital anomaly in Türkiye. Cell culture studies for virus isolation were performed using Sheep Fetal Thymus (SFT) and Madin-Darby Bovine Kidney (MDBK) cell lines. The Indirect Immunoperoxidase Monolayer Assay (IIPMA) technique was used to confirm the growth of non-cp BDV in MDBK cells. The virus titer of the isolate was calculated in $TCID_{50}$/ml using the classical Spaerman-Karber method. To characterize the isolate, RT-PCR and sequenced amplified the 5' UTR of the viral genome. Phylogenetic analysis was performed using BioEdit and MEGA-X, comparing the sequence with global pestivirus reference strains available in GenBank.

RESULTS: Cell culture studies revealed that the sample was a pestivirus isolate with a non-cytopathogenic biotype, obtained from lamb blood serum. The molecular analysis confirmed the presence of a pestivirus closely related to Pestivirus aydinense. Sequence comparison based on the 5'UTR region revealed high homology with previously reported Turkish Pestivirus aydinense strains. However, phylogenetic tree construction studies have shown that this isolate forms a distinct cluster separate from the known reference lineage. The genetic distance and specific nucleotide substitutions within the 5'UTR region support the existence of a potential novel subgrouping within the Pestivirus aydinense species.

CONCLUSIONS: This study reports the identification of a genetically distinct Pestivirus aydinense isolate for the 5' UTR gene region in a Turkish sheep population. The identification of a potential new subgroup highlights the ongoing evolution and high genetic diversity of pestiviruses in the region. These findings contribute to the epidemiological surveillance of pestiviruses in small ruminants.

Keywords: Pestivirus aydinense, 5'UTR, Türkiye, Sheep, Molecular characterization

Knowledge, Attitude and Practices of Small Ruminants' Veterinarians from Portugal Towards Antimicrobial Resistance

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BACKGROUND and OBJECTIVE: Antimicrobial use (AMU) and antimicrobial resistance (AMR) are critical issues in veterinary medicine, affecting both animal and public health. Understanding veterinarians' knowledge, attitudes, and practices is essential to develop targeted interventions and stewardship programs. This study aimed to characterize veterinarian profiles regarding AMU and AMR by evaluating their knowledge, conscious attitudes, and clinical practices, and to identify professional profiles.

MATERIALS and METHODS: A structured questionnaire was administered to 176 veterinarians. The knowledge dimension included seven dichotomous and four multiple-choice questions; the attitude dimension included one dichotomous and three multiple-choice questions; and the practices dimension included five dichotomous and seven multiple-choice questions. All non-dichotomous items were recoded into binary variables (correct/incorrect) to standardize the data for analysis. Given the dichotomous nature of the items, exploratory factor analysis (EFA) based on tetrachoric correlation matrices was performed separately for each dimension. Internal consistency was assessed using Cronbach's alpha. Factor scores derived from the EFA were subsequently used as input variables for cluster analysis.

RESULTS: The initial knowledge EFA suggested a two-factor structure explaining 35.3% of the total variance. However, only the first factor showed acceptable psychometric properties (Cronbach's α= 0.54) and was retained. This factor included items related to AMU and was interpreted as "Knowledge on antimicrobial resistance." The initial four-item solution for attitude showed very low internal consistency (α= 0.19). After removing two weak items, the remaining two items formed a single factor explaining 34% of the variance. Despite limited reliability (α= 0.30), this factor captured perceptions regarding AMU and AMR and was interpreted as "Conscious attitude toward antibiotics." Practices EFA resulted in a final two-factor solution: "Rational use of antimicrobials", reflecting evidence-based prescribing practices and "Prescription without direct clinical assessment". Together, these factors explained approximately 66% of the total variance. Cluster analysis based on the four resulting factors identified three veterinarian profiles. Cluster 1 (n=9) showed low technical rigor and high levels of non-presential prescription. Cluster 2 (n= 117), displayed balanced scores across all dimensions, representing a generally consistent professional profile. Cluster 3 (n= 48) demonstrated relatively high technical rigor but lower conscious attitudes toward AMU. Silhouette analysis indicated moderate cluster separation (mean= 0.368).

CONCLUSIONS: The combined use of factor and cluster analysis allowed the identification of meaningful dimensions and professional profiles related to antimicrobial use among veterinarians. The results highlight heterogeneity in knowledge, attitudes, and prescribing practices, emphasizing the need for targeted educational and stewardship

interventions to promote responsible antimicrobial use in veterinary medicine.

Keywords: Antimicrobial, Resistance, Veterinary, Small, Ruminants

Antibiotic Prescription in Small Ruminants – Questionnaire Survey of Veterinarians

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BACKGROUND and OBJECTIVE: The widespread use of antibiotics in food-producing animals imposes selective pressure that contributes to the emergence, spread, and persistence of antibiotic-resistant bacteria. In addition to the impact on public health, the increase in antimicrobial resistance (AMR) can reduce therapeutic options, increase the burden of animal diseases, and production losses. The prescription of antibiotics is the exclusive responsibility of the veterinarian This work aimed to characterize the use of antibiotics (UAB) in small ruminants through the application of a questionnaire to veterinarians dedicated to field medical-surgical practice

MATERIALS and METHODS: A questionnaire was implemented, which was disseminated on social networks, sent by email to the sector's professional associations, and applied in person at scientific events, obtaining 176 responses.

RESULTS: The majority of the veterinarians who responded to the questionnaire were male (59.7%) and under 41 years old (50.6%). The main functions performed were medical-surgical clinic (63.1%), OPSA executor (64.2%) and consultancy (13.6%). Most veterinarians stated that they prescribed antibiotics for the treatment of respiratory (84.7%), mammary (68.8%) and foot (65.9%) pathologies. Regarding antibiotics used, the vast majority stated that they never use, rarely use, or occasionally use polymyxins (59.7%, 36.4%, and 2.8%, respectively), 3rd and 4th generation cephalosporins (21.0%, 38.1%, and 31.3%, respectively), and fluoroquinolones (8.0%, 36.9%, and 36.9%, respectively), preferring to frequently or very frequently use tetracyclines (28.4% and 60.8%, respectively), aminopenicillins without (34.7% and 30.1%, respectively) and with β-lactamase inhibitors (42.0% and 21.6%, respectively). The most frequently used antibiotics in respiratory pathology were macrolides (51.7%), fluoroquinolones (51.7%), and tetracyclines (49.4%). In mammary pathology, narrow-spectrum penicillins (46.0%), aminopenicillins with β-lactamase inhibitors (43.2%), and fluoroquinolones (34.7%) were used, while in foot pathology, tetracyclines (83.0%) and macrolides (60.2%) were used

CONCLUSIONS: The results obtained allow us to characterize the antibiotic prescribing practices of the veterinarians surveyed, highlighting trends that generally align with the recommendations of the European Medicines Agency. The preference for antibiotics classified in EMA categories B and C, namely tetracyclines and aminopenicillins, is aligned with European recommendations, which advocate their use as first-line therapy. On the other hand, the relatively frequent use of fluoroquinolones in respiratory and mammary pathologies is not fully aligned with guidelines, which recommend their use only in exceptional situations. This data reinforces the importance of continuing to invest in the ongoing training of veterinarians and the implementation of monitoring programs, in order to promote increasingly rational prescribing harmonized with the critically important European objectives for reducing adverse drug reactions

Keywords: Antibiotic, Prescription, Veterinarians, Small Ruminants

Comparison of Serum Protein Profile Between Seropositive and Seronegative Goats for Caprine Arthritis Encephalitis Virus

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BACKGROUND and OBJECTIVE: Caprine arthritis encephalitis (CAE) is an infectious, degenerative, and chronic disease responsible for considerable economic losses, including decreased milk production, premature culling of animals, increased mortality, and devaluation of the herd and its products. Research conducted in recent decades shows that the analysis of serum protein profiles in biological fluids can provide useful information in the diagnosis, prognosis, and monitoring of infectious/inflammatory diseases. Therefore, the objective of this study was to compare the values of total serum proteins, immunoglobulins, and acute phase proteins (APPs) in seropositive and seronegative animals for CAE, since information on serum proteins related to this disease is still scarce.

MATERIALS and METHODS: Twenty-two venous blood samples were collected from male and female goats, aged between 12 and 21 months, of Saanen and crossbred goat breeds, without clinical signs of CAE, raised in the State of São Paulo, Brazil. These samples were classified as positive (n = 6) and negative (n = 16) according to the results of antibody testing against the caprine arthritis encephalitis virus performed by Western blotting. Separation of the protein fractions of serum samples was performed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and the serum concentration of total proteins was determined by the biuret method.

RESULTS: Seropositive animals presented mean values of total proteins: 6.86 ± 0.43 g/dL; IgA: 71.15 ± 18.98 mg/dL; IgG: 1,450.26 ± 394.97 mg/dL; ceruloplasmin: 37.59 ± 16.43 mg/dL; transferrin: 375.76 ± 52.14 mg/dL; albumin: 4,472.44 ± 349.31 mg/dL; haptoglobin: 12.38 ± 3.72 mg/dL; α1-acid glycoprotein: 10.90 ± 5.06 mg/dL; molecular weight protein 23,000 Da: 201.62 ± 34.92 while negative animals presented mean values of total proteins of: 6.79 ± 0.43 g/dL; IgA: 68.78 ± 20.41 mg/dL; IgG: 1,157.17 ± 270.62 mg/dL; ceruloplasmin: 37.43 ± 10.36 mg/dL; transferrin: 366.75 ± 42.54 mg/dL; albumin: 4,719.34 ± 402.99 mg/dL; haptoglobin: 15.01 ± 5.14 mg/dL; α1-acid glycoprotein: 9.80 ± 2.41 mg/dL; molecular weight protein 23,000 Da: 200.49 ± 33.85, respectively. There was no significant difference (p>0.05) in the values of total proteins and protein fractions (immunoglobulins and APPs) between seropositive and seronegative animals.

CONCLUSIONS: No differences were observed in serum protein profile of goats that were seropositive or seronegative for the caprine arthritis encephalitis virus and that did not present clinical signs of the disease.

Keywords: Goat, CAE, acute phase proteins, immunoglobulins, SDS-PAGE

Presence of Anti-Leishmania Infantum Antibodies in Small Ruminants in Portugal

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BACKGROUND and OBJECTIVE: Leishmaniosis is a vector-borne disease caused by Leishmania parasites and transmitted by infected phlebotomine sand flies. *Leishmania infantum* is endemic throughout the Mediterranean region and the

Balkans. Domestic dogs are the main reservoir hosts, sustaining the parasite's domestic transmission cycle. As a zoonotic, multi-host disease, higher infection rates in animals or greater vector abundance are directly associated with increased human incidence. Currently, there is limited scientific evidence regarding the role of livestock, including domestic ruminants, in the maintenance and transmission of L. infantum in European countries, with only a small number of published studies available. This epidemiological study aimed to assess exposure to Leishmania infantum in small ruminants - sheep (*Ovis aries*) and goats (*Capra hircus*) - from mainland Portugal.

MATERIALS and METHODS: A seroprevalence study of *Leishmania infantum* was conducted in small ruminant herds in Portugal between 2020 and 2023. Herds were randomly selected, and farmers were invited to participate in the study. Samples were collected from 19 municipalities, including 12 in Trás-os-Montes, two in the Centre region, and five in the South. Across these municipalities, a total of 87 distinct localities were identified within the three geographical regions. All serum samples were analysed using an in-house ELISA (sensitivity: 99.37%; specificity: 97.50%).

RESULTS: A total of 2,124 animals were tested, including 1,820 sheep and 304 goats. The overall seroprevalence was 21.3% (453/2,124; 95% confidence interval [CI]: 19.6–23.1), with higher seroprevalence in sheep than in goats (22.4% vs 15.1%). Seropositive animals were detected in all years and regions analysed. Annual seroprevalence for 2020–2023 was 14.7% (95% CI: 11.7–18.0), 19.9% (95% CI: 17.2–22.7), 32.2% (95% CI: 28.1–36.4), and 17.9% (95% CI: 13.6–23.0), respectively. No significant differences were observed among 2020, 2021, and 2023, whereas 2022 differed significantly from the other years. No statistically significant differences in seroprevalence were observed among the different regions of the country (North, Centre, and South; p = 0.183).

CONCLUSIONS: The findings demonstrate widespread exposure of sheep and goat flocks to *L. infantum* in Portugal and highlight the importance of understanding the epidemiology of infection, including the role of management and biosecurity practices. Furthermore, additional studies are warranted to determine whether small ruminants develop clinical disease and to clarify the potential implications for animal and public health.

Keywords: Leishmania, sheep, goats, Portugal

Effects of Anastrozole on Libido, Sperm Quality, Testicular Morphology, and Testosterone in Kangal Rams During the Non-Breeding Season

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BACKGROUND and OBJECTIVE: Reproductive activity in rams and bucks decreases during the non-breeding season, characterized by reduced testosterone levels, deterioration in spermatological parameters, and decreased testicular size. Consequently, pregnancy rates achieved in synchronization programs applied outside the breeding season are generally lower. To improve male reproductive performance during this period, several stimulation methods such as exogenous hormone treatments or light–dark manipulations have been used. However, these approaches often require complex infrastructure, prolonged application periods, and may be difficult to implement under extensive field conditions. Therefore, practical alternative strategies are needed. Aromatase inhibitors have been investigated as a potential approach to enhance male reproductive activity during the non-breeding season in small ruminants. Although previous studies reported increased testosterone levels in bucks following aromatase inhibitor treatment, data regarding their effects in rams remain limited. Therefore, this study aimed to evaluate the effects of anastrozole on testicular morphology, spermatological parameters, libido, mating behavior, and serum testosterone levels in rams during the non-breeding season.

MATERIALS and METHODS: This randomized controlled clinical study was conducted on eight Kangal rams. The animals were divided into two groups: anastrozole (n = 4) and control (n = 4). Anastrozole was administered orally to the treatment group. Testicular measurements and semen collection were performed twice weekly starting 7 days before anastrozole administration (day −7) and continued for two months after treatment. Blood samples were collected every two

days starting 7 days before treatment and during the 15-day treatment period, and twice weekly thereafter to determine testosterone levels. Libido and mating behavior were evaluated once weekly.

RESULTS: Two-way ANOVA revealed a significant effect of time on libido ($p < 0.01$) and scrotum length ($p < 0.05$). Group effects were significant for scrotal circumference ($p < 0.05$), testicular length ($p < 0.01$), scrotum length ($p < 0.01$), and scrotum volume ($p < 0.01$). No significant group differences were detected for libido, testicular diameter, or testosterone levels. Additionally, no significant time × group interactions were observed for any parameter ($p > 0.05$).

CONCLUSIONS: In conclusion, aromatase inhibitor administration during the non-breeding season may affect certain testicular and scrotal morphometric parameters in rams; however, no significant effects were observed on libido, testicular diameter, or testosterone levels. These findings suggest that the reproductive impact of aromatase inhibition remains unclear, and further studies with larger sample sizes are required.

Keywords: Aromatase inhibitor, Ram, Spermatological parameters, Testicular morphometry, Libido, Testosterone

Comercial Global Positioning System as a Tool for Continuous Locomotion Monitoring in Lactating Sheep: A Case Report

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BACKGROUND and OBJECTIVE: The availability of precision livestock technologies for small ruminants remains limited. Commercial Global Positioning System (GPS) devices offer a viable alternative for monitoring movement patterns. This case report describes the use of a commercially available GPS device to quantify locomotor deviations associated with lameness in a lactating ewe.

MATERIALS and METHODS: A 3-year-old lactating crossbred ewe (Texel × Ile de France) with a body condition score of 3 was monitored starting three days postpartum. The study spanned 31 days, providing 19 days of effective GPS data collected during the daily grazing period, which averaged 6.84 ± 0.59 hours. Monitoring was divided into two functional periods: basal (asymptomatic = 8 days) and symptomatic/treatment phase (11 days). Parasitological monitoring confirmed low strongylid egg counts (< 500 eggs per gram). Locomotor data (global mean speed and percentage of time spent stationary during grazing) were compared between the basal and symptomatic phases using an independent t-test ($p < 0.05$).

RESULTS: On day 9, no abnormalities were detected during routine farm observations. On day 10, the shepherd observed marked lameness and requested veterinary evaluation. A clinical examination confirmed interdigital myiasis in the right hind limb, and treatment was initiated immediately. The mean speed decreased considerably ($p = 0.004$) from 0.140 ± 0.032 km/h during the basal period ($n = 8$) to 0.099 ± 0.019 km/h during the symptomatic period ($n = 11$). Conversely, inactivity increased ($p = 0.004$) from 92.57% ± 1.95% in the basal phase to 95.26% ± 1.37% during the symptomatic period. Complete clinical resolution was not established within the monitoring period, and the ewe remained under recovery during the symptomatic/treatment phase.

CONCLUSIONS: Commercial GPS technology has proven to be operationally feasible for the objective quantification of locomotor alterations in extensive sheep systems. The system objectively quantified the reduction in mean speed and increase in inactivity following the onset of lameness. Locomotion parameters remained persistently altered for 11 consecutive days, reinforcing the utility of continuous monitoring tools for quantifying the clinical impact and delayed functional recovery.

Keywords: lameness, sheep, monitoring tool, GPS

Optimization of Semen Collection Training in Young Rams: Use of Prostaglandin F2α and eCG as an Effective Alternative to Electroejaculation

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BACKGROUND and OBJECTIVE: Traditionally, training young rams to ejaculate in artificial vagina can be time-consuming, often leading to electroejaculation in non-responsive animals. However, electroejaculation is associated with animal welfare concerns and samples of inferior quality. This study describes the result after a stimulation protocol, previously described, with scarce published evidence of effectiveness, using prostaglandin F2α (PGF2α) and equine Chorionic Gonadotropin (eCG), aiming at inducing smooth muscle contraction in the epididymis and ductus deferens, while promoting a transient testosterone pulse. The combination of PGF2α+eCG directly stimulates Leydig cells activity.

MATERIALS and METHODS: The study involved 12 young Lacaune rams (breed with a minimal seasonality; age: 5.45±0.25 mo. old) beginning in March 2025 (out-of-season breeding). Training sessions used a "dummy" ewe synchronized to ensure a constant follicular phase. Sessions focused on sexual interest (flehmen response, perineal sniffing, pawing) and excitement (erection, mounting attempts). For rams not exhibiting mounting behavior after 16w of training, 2 ml d-cloprostenol was administered 10' prior to exposure. In refractory cases (no response after four consecutive PGF2α-administrations), a combined treatment of PGF2α+1000 IU of eCG was administered.

RESULTS: The transition from initial exposure to the first successful voluntary ejaculation was achieved in all individuals, after a mean interval of 104±47.13d, with a mean age of 7.18±0.25 mo. Notable individual variability was observed: the most precocious ram achieved ejaculation at 37d, while the most refractory required 158d. The pharmacological protocol proved to be highly effective in "triggering" the desired behavior. Seven rams required PGF2α administration to initiate mounting and ejaculation (after 16 weeks of training without success), being effective in 5/7 cases (71.42%) after the first PGF2α-administration. In the two most difficult cases, the combination of PGF2α+eCG (after 22.5 weeks of the beginning of the training and after four non-effective PGF2α-administrations), successfully induced the first ejaculation. The group of the five precocious rams ejaculated at first instance after 53±18.63d of training (7.37±0.69 mo. old), while the further seven rams ejaculated after 140±12.2d (9.91±0.26 mo. old). No adverse effects were recorded, and no rams required electroejaculation.

CONCLUSIONS: The application of synthetic Prostaglandin PGF2α, either alone or in combination with eCG, seems to be a highly effective tool for the sexual training of young rams where accelerating the training of young sires is necessary. This protocol significantly facilitated the use of the artificial vagina, ensuring voluntary semen collection and improving animal welfare by avoiding electroejaculation. Funding: FEDER (EU), Spanish MCIN&AEI; PID2022-136956OR-C32

Keywords: Ram, semen, collection, prostaglandin, eCG, PGF2α

Effects of Antepartum Melatonin Implantation on Blood Gas Parameters and Apgar Score in Newborn Lambs

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BACKGROUND and OBJECTIVE: Neonatal mortality is frequently associated with postpartum hypoxia and metabolic disturbances in lambs. Melatonin has been shown to modulate fetal development and facilitate perinatal adaptation. The aim of this study was to investigate the effects of prenatal melatonin implant on blood gas, acid-base balance and APGAR score in lambs.

MATERIALS and METHODS: Merino×Kivircik ewes were synchronized with intravaginal fluorogestone acetate sponges; pregnancy confirmed by ultrasonography on day 30. Eighty pregnant ewes were assigned to a 2×2 factorial design with treatment (melatonin vs control) and parturition type (elective cesarean vs normal parturition). Ewes received one melatonin implant on day 100 of pregnancy (18 mg, Regulin; MEL, n=40) or remained untreated (CON, n=40). Thirty-eight ewes (CON=18, MEL=20) underwent elective cesarean section at 146±1 days of gestation, whereas the remaining forty-two ewes lambed normal (CON=22, MEL=20). Within each treatment, ewes were additionally classified by litter size (single vs multiple). Venous blood gas analysis was performed at 0, 15, 60 and 180 minutes postpartum in cesarean-born lambs (n=59), and once within the first 3 hours in normal-born lambs (n=64). Apgar scores were evaluated in cesarean-born lambs at 0, 15 and 60 minutes. Data were analyzed using a mixed general linear model including treatment, birth type, litter size, time, and their interactions as fixed effects, with repeated measures for time-dependent variables ($p<0.05$).

RESULTS: In cesarean-born lambs, pH decreased after birth and gradually recovered until 180 min ($p<0.001$), but remained lower than in normal-born lambs ($p<0.01$). Oxygen pressure (PO_2) was higher in MEL groups at all time points ($p<0.05$). Apgar scores also showed a significant time ($p<0.001$) and time×group interaction ($p<0.05$), and MEL lambs showed significantly higher Apgar scores at 15 minutes ($p<0.01$). In normal-born lambs, carbondioxide pressure (PCO_2) was lower ($p<0.01$) and glucose concentration was higher ($p<0.05$) in MEL groups. The most favorable metabolic status was observed in MEL singleton normal-born lambs and the most severe acidosis occured in CON multiple cesarean lambs ($p<0.05$). Lactate was higher in CON, multiple and normal-born lambs ($p<0.05$), while creatinine was elevated in all cesarean subgroups compared to normal parturition ($p<0.001$).

CONCLUSIONS: Antepartum melatonin implantation was associated with improved oxygenation, Apgar score, and selected acid–base parameters in newborn lambs. Birth type and litter size also influenced neonatal metabolic status, with better adaptation observed in singleton and normal-born lambs, whereas multiple and cesarean-born lambs showed more pronounced metabolic alterations. These findings indicate that antepartum melatonin supplementation may contribute to improved neonatal adaptation.

Keywords: Apgar score, blood gas, lamb, melatonin, neonatal adaptation

Enucleation in Lacaune Sheep – Case Reports

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BACKGROUND and OBJECTIVE: The case describes the clinical symptoms, treatment failure, and subsequent enucleation in a goat. The patient, a 6-month-old Lacaune sheep weighing 50 kg, was kept on a small farm with year-round pasture access. This sheep was seriously injured, with a cut on its eyeball and eyelid.

MATERIALS and METHODS: The animal exhibited signs of ocular pain, tearing, conjunctivitis, and corneal ulceration, while its general health remained unaffected. An ophthalmological examination,

including a fluorescein test, confirmed the corneal ulcer. Initial treatment included topical ocular ofloxacin at a dose of 2 drops four times daily for 7 days, along with a single intramuscular injection of oxytetracycline at 1ml/10 kg. However, no improvement was observed after two days, leading to the decision to perform enucleation.

RESULTS: The surgery was carried out under general anesthesia (ketamine, xylazine, and butorphanol) combined with local anesthesia (lidocaine). We used methods Four-point retrobulbar nerve block, where four needles are inserted around the orbit, and a small amount of local anesthetic is injected at each site The entire eyeball was excised, and bleeding was controlled through pressure compression. After the bleeding has stopped, we fill the entire orbit with a special absorbable gelatinous sponge. The sponge will help to stop the bleeding, but most importantly, it will fill the empty space and the eye will look aesthetically better, and the skin will not fall inward. The orbit was sutured in two layers – subcutaneous – using absorbable material and skin - non-absorbable suture material.

CONCLUSIONS: Etamsylate was administered intravenously at a dose of 1ml/10 kg as a premedication to promote blood coagulation. Postoperative therapy included amoxicillin-clavulanate (1 ml/20 kg) administered for 7 days and ketoprofen (1 ml/30 kg) for 3 days. For prevention of myasis antiparasitics like ivermectin one time is used. The sutures were removed after 14 days, and the sheep was returned to the herd without complications. Small ruminants were very recovered with enucleation.

Keywords: sheep, enucleation, surgery

Investigation of the Source of İnfertility in Kangal Sheep

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BACKGROUND and OBJECTIVE: In recent years, infertility problems in sheep breeding have reached undeniable levels. Increasing yield characteristics have brought along infertility problems. For sheep, while the rate of non-pregnancy in mating during the breeding season was low, it is now seen at higher rates. In order to address this problem in all aspects, a sheep farm that had fertility problems (exhibiting consecutive cycles without getting pregnancy) in previous years was examined.

MATERIALS and METHODS: At the beginning of the season, a flock of 905 multiparous Kangal ewes between the ages of 3-6 years with proven fertility and 44 Kangal rams between the ages of 4-6 years with proven fertility were included in the study. All ewes in the flock were subjected to ultrasonographic examination and vaginal discharge evaluation prior to mating. After this screening, 880 ewes with normal vaginal discharge characteristics and no pathologic conditions in the ovaries and uterus were followed up during the breeding season. After the first mating, 171 ewes were mated for the second time. Pregnancy was achieved from 50 more ewes and 121 ewes that did not get pregnant were mated for the third time and 36 more pregnancies were achieved. Blood samples were taken from 85 ewes that did not get pregnant despite three matings. After DNA isolation from the swabs, PCR was used to investigate the existence of Brucella species (spp), Mycoplasma spp., Chlamydophila spp., Campylobacter spp., C. burnetii, Salmonella Abortusovis, Trueperella pyogenes and Klebsiella pneumonia agents.

RESULTS: 21 of them were positive for Coxiella burnetii and BDV and were slaughtered. Vaginal swabs were taken randomly from 25 of 64 ewes that

were negative for Brucella spp., Coxiella burnetii and BDV. Preputial swabs were also taken from rams used during the breeding season. The swab samples obtained were cultured and investigated by Polymerase Chain Reaction (PCR). In the study, it was determined that some pathogens obtained from swaps of rams and sheep were not obligant or opportunistic pathogens of the genital tract of sheep.

CONCLUSIONS: Mycoplasma spp., Truperalla pyogenes and Campylobacter spp. were predominantly isolated from the swap samples of ewes and rams in the flock suffering from infertility. Thus, the fact that these microorganisms are found in higher concentrations in ewes experiencing fertility problems led to the conclusion that they may be the cause of infertility.

Keywords: fertility, pregnancy, infertility, kangal, sheep

Microalgae- and Camelina-Derived Ω-3 Fat Improves Acrosomal Integrity and Sperm Morphology in Rams

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BACKGROUND and OBJECTIVE: Optimizing reproductive efficiency in livestock is related to semen quality. Nutritional supplementation with ω-3 polyunsaturated fatty acids (PUFAs) has been demonstrated to enhance sperm characteristics. Novel sources of PUFAs were explored as suitable additives. The present study focused on the impact of the addition of microalgae (Nanochloropsis gaditana; NG; rich in eicosapentaenoic acid, EPA) or Camelina sativa (CS, rich in alpha-linolenic acid, ALA) as oral sources for rams

MATERIALS and METHODS: Eleven males were assigned to three groups for ten weeks replacing 1% fat with N.gaditana-fat (NG; n=4; 25g/d), camelina-fat (CS; n=4; 50g/d), or saturated fat (palmitoleic acid; Control; n=3; 4g/d), all receiving isocaloric, isoproteic diets. A total of 110 ejaculates were evaluated for qualitative (color, consistency, mass motility) and quantitative parameters (membrane and acrosome integrity, abnormal forms, vitality). Sperm concentration and motility were analyzed by CASA. Flow cytometry assessed plasma membrane integrity, acrosomal status, mitochondrial activity, early apoptosis, membrane destabilization, oxidative stress, and enzymatic activity. Statistical analysis with IBM-SPSS-Statistics-v30 employed Mann–Whitney-U-tests, and Mixed-models.

RESULTS: Seminal volume and mass motility fluctuated over time regardless of diet (P<0.001). Acrosome integrity (NAR) was significantly affected by diet (P=0.003), time (P<0.001), and their interaction (P<0.001). Control group showed lower NAR than NG (P=0.034). By week-8, Control group showed higher acrosomal damage (DAR: 11.25±6.61%) than NG (4.00±2.25%) and CS (2.13±0.78%; P=0.027). In week-8, CS had more normal forms (93.75±4.72%) than NG (82.25±4.86%) and Control (81.17±3.92%). Total motility peaked at week-6 (92.06±2.36%), declining in weeks 1 and 5 (P<0.05). A marginal interaction (P=0.054) showed a trend toward higher progressive motility in CS at week-1 (46.50±5.54%). CS showed higher straightness in weeks-0 and 1 (P<0.05). The lateral head displacement and beat cross frequency were influenced by time (P<0.001), peaking mid experiment. Cytometry markers were time dependent (P<0.05), with week-3 showing peak quality and week-5 a generalized decline. Maximum viable sperms with intact acrosomes (77.57±4.61%), mitochondria active non oxidized sperms (78.30±4.82%), and non oxidized viable sperms (72.90±4.74%) occurred in weeks 2–3, followed by a decline in week-5 (P<0.05). Membrane stability peaked in week-3 (73.51±4.80%) and declined by week-9 (41.30±4.80%; P<0.001).

CONCLUSIONS: Dietary supplementation improved key sperm traits in rams compared with saturated fat. While kinetic and flow cytometry markers were mainly driven by time, possibly due to seasonality or maturity, ω 3 sources helped maintain superior acrosomal health. These findings support N.gaditana and C.sativa as effective, sustainable PUFA supplements to enhance semen quality in ruminants. Funding: FEDER (EU), Spanish MCIN&AEI; PID2022-136956OR-C32.

Keywords: fresh semen, sheep, PUFAs, semen quality

Microalgae- and Camelina-Derived Ω-3 Fat Improves Acrosomal Integrity and Sperm Viability During Cryopreservation in Rams

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BACKGROUND and OBJECTIVE: Nutritional ω-3-polyunsaturated fatty acids (PUFAs) supplementation lipid composition, though rumen biohydrogenation reduces its availability. While enriching the sperm membrane with PUFAs improves fluidity and cryotolerance, it simultaneously increases susceptibility to oxidative damage, making the net functional outcome unpredictable. The objective of the study was, therefore, to evaluate the effect on ram sperm cryopreservation resistance of the addition of oral microalgae (Nanochloropsis gaditana; NG; rich in eicosapentaenoic acid) or camelina (Camelina sativa; CS, rich in alpha-linolenic acid).

MATERIALS and METHODS: Eleven rams were divided into three experimental groups for a 6-week study, receiving NG (n=4; 25g/d), CS (n=4; 50g/d), and CON (control group, n=3; isocaloric control diet). Semen collected at 6 moments was evaluated fresh (0h) to establish a reference for quality and to evaluate the ejaculate's resistance to the cryopreservation process. Semen was analyzed at refrigeration (4h), post-thawing (0h-PT), and after a thermoresistance-test (2h-PT; 37ºC/120 min). Cryopreservation involved primary dilution (1: 1, 37ºC) and slow cooling (-0,25ºC/min) to 5ºC (2h). After a second dilution (400x106spz/ml; 100x106spz/straw) samples equilibrated at 5ºC for 2h (4h total) before packaging. Freezing was performed in LN2 vapor (10 min) followed by immersion (-196ºC). Thawing (37ºC, 30s) was performed within 3 weeks. Ejaculates were analyzed for quantitative parameters (membrane and acrosome integrity tests, and vitality). Linear mixed effects model (REML) was performed with ram as a random intercept, accounting for repeated measures (IBM_SPSS v30.0, USA).

RESULTS: The ram effect remained in the mixed model, being statistically significant in most variables. Regarding acrosome integrity at 4h, the NG group maintained more spermatozoa with normal apical ridges (NAR;87.57±14.18%) compared to CON (78.22±10.49%; P=0.003) and CS (75.79±18.50%; P=0.012). The proportion of damaged acrosomes (DAR) at 4h was lower in NG (5.00±3.02%; P=0.005) and CS (5.96±3.71%; P=0.017) than in CON (9.61±4.89%). Similarly, the NG group showed the lowest incidence of losing acrosomes (LAR; 6.78±14.56%), differing from CON (8.67±5.49%; P=0.020) and CS (15.96±16.55%; P=0.003) at 4h. For sperm vitality, NG outperformed CS (20.54±12.37%; P=0.057) and CON (19.89±14.52%; P=0.079) at 2h-PT (29.10±12.97%). However, vitality loss during equilibration (difference in viable spermatozoa between 0h and 4h) was lower in CS (7.00±7.73%) compared to NG (16.27±11.05%; adjusted P=0.005).

CONCLUSIONS: Dietary supplementation with microalgae was effective at preserving acrosome integrity and overall sperm viability during cryopreservation, although camelina provides a

targeted advantage in minimizing cell death during the initial equilibration phase. Therefore, oral ω-3 supplementation in ram diets seems a strategy to improve reproductive efficiency. Funding: FEDER (EU), Spanish MCIN&AEI; PID2022-136956OR-C32

Keywords: PUFAs, semen quality, sheep, freezing

A Novel Deletion Variant in an Ovine Immune Response Gene Is Associated with Increased Visna Maedi Susceptibility in Sheep

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BACKGROUND and OBJECTIVE: Visna/maedi (VM) is a chronic multisystemic lentiviral disease of sheep that causes important health and production losses. Although transmembrane protein 154 gene variants are known to influence VM resistance, protective haplotypes are rare or absent in some native breeds. Therefore, additional host genetic markers are needed. This study aimed to investigate whether variation in the ovine major histocompatibility complex class II *DRB1* gene is associated with VM susceptibility in Turkish sheep.

MATERIALS and METHODS: A total of 151 case-control matched pairs were constructed from 2266 serologically tested ewes representing native, improved, and composite Turkish sheep populations. Matching was based on age, breed type, and flock. VM serostatus was determined by enzyme-linked immunosorbent assay. Exon 2 and the adjacent intron 1 region of *DRB1* were amplified by polymerase chain reaction and analyzed by bidirectional Sanger sequencing. Haplotypes were reconstructed, linkage disequilibrium was assessed, and associations with VM serostatus were tested using McNemar's test. A secondary analysis was performed after excluding sheep carrying protective transmembrane protein 154 diplotypes.

RESULTS: Sixty-five *DRB1* exon 2 haplotypes were identified, indicating broad haplotype diversity across breeds. A novel 2 base pair deletion in *DRB1* intron 1, located 11 base pairs upstream of exon 2, was detected and showed strong linkage disequilibrium with haplogroup 13. In the dominant model, carriers of the deletion had significantly greater VM susceptibility than non-carriers. In the informative matched-pairs analysis, the deletion was associated with increased seropositivity with an odds ratio of 2.36. After excluding animals with protective transmembrane protein 154 diplotypes, the association remained significant and became stronger, with an odds ratio of 2.90 and an exact p value of 0.002. In silico analyses also suggested weaker viral epitope docking for the associated haplotype.

CONCLUSIONS: A novel *DRB1* intronic deletion was identified as a genetic risk factor for VM in Turkish sheep. This variant may serve as a complementary marker for selective breeding, particularly in native populations where protective transmembrane protein 154 haplotypes are uncommon. Its use could support more effective breeding strategies to reduce flock-level VM susceptibility.

Keywords: Ovar-DRB1, Visna/maedi, genetic susceptibility, sheep breeding, lentivirus, Turkish sheep

Microalgae-Derived Ω-3 Bypass Fat Modulates Blood Lipid Metabolism and Seminal Plasma Composition in Rams

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BACKGROUND and OBJECTIVE: Livestock reproductive efficiency relies partly on semen quality and systemic metabolic health, both influenced by dietary ω-3 polyunsaturated fatty acids (PUFAs). This study evaluated blood and seminal plasma metabolic effects of supplementing rams with rumen-protected PUFA sources—microalgae (Nannochloropsis gaditana; NS, rich in eicosapentaenoic acid) and camelina (Camelina sativa; CS, rich in α-linolenic acid)—designed to bypass microbial biohydrogenation and enhance absorption.

MATERIALS and METHODS: Eleven rams were assigned to three groups for twenty-one weeks, receiving isocaloric and isoproteic diets in which 1%fat was replaced by NG (n=4; 25 g/d), CS (n=4; 50 g/d), or CON (n=3; palmitic acid). Blood was collected from the jugular vein into EDTA-vacuum tubes at w0, w5, w9, w16 and w21; ejaculates at w0, w2, w6, w8, w11, w14 and w19. Samples were centrifuged at 35,000 rpm x15 min and supernatants analyzed for glucose (mg/dl), fructosamine (mg/dl), lactate (mg/dl), triglycerides (TG; mg/dl), cholesterol (CHOL; mg/dl), HDL and LDL (mg/dl), alanine aminotransferase (ALT/GPT), aspartate aminotransferase (AST/GOT), creatine kinase (CK-NAC) and lactate dehydrogenase (LDH; U/l), urea (mg/dl), β-hydroxybutyrate (BHB; mmol/l) and non-esterified fatty acids (NEFAs; mmol/l), using a bioanalyzer (Konelab 20). Mixed-effects models assessed effects of time, diet, and interactions (IBM SPSS v30.0).

RESULTS: Dietary PUFA supplementation altered systemic and seminal plasma lipid profiles, particularly in the NS group. NS notably increased blood TG and total CHOL by the end of the study (w21; 60.50 ± 8.27 and 280.25 ± 44.21 mg/dl, respectively; $P < 0.01$) compared to CON and CS groups, while reducing LDL levels (7.50 ± 1.29 vs. 40.50 ± 7.05 mg/dl; $P < 0.001$). Seminal plasma reflected these dietary shifts: NS rams tended to exhibit higher TG ($P = 0.068$) and showed significantly elevated NEFAs at M8 and M19 compared to CON and CS ($P < 0.05$). Independently of diet, blood and seminal plasma exhibited significant temporal metabolic fluctuations ($P < 0.05$) throughout the experiment, characterized by progressive decreases in fructosamine, total proteins, and albumin, alongside temporal variations in energy metabolites (glucose, lactate, blood NEFAs) and enzymes (CK NAC, GPT). Most seminal and blood plasma metabolite concentrations remained within physiologically acceptable limits for healthy young adult rams at every moment of analyses, not related to pathological alterations.

CONCLUSIONS: Dietary supplementation with microalgae modulates lipid metabolism in rams, driving lipid mobilization that successfully translated into the reproductive tract, demonstrating that NS derived lipids effectively bypassed rumen biohydrogenation and reached the reproductive system. These results highlight microalgae as an effective nutritional strategy to modify plasma seminal composition, whereas camelina exerts a comparatively milder metabolic impact under these experimental conditions. Funding: FEDER (EU), Spanish MCIN&AEI; PID2022 136956OR C32.

Keywords: lipidic metabolism, HDL, bypass PUFAs, sheep

Viral Causes of Abortion in Goats: A Historical and Regional Analyses of Published Data Over the Past 50 Years

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BACKGROUND and OBJECTIVE: Viral abortions remain a persistent constraint on reproductive efficiency and herd profitability in goats. Although infected does are often asymptomatic or exhibit only mild clinical signs, transplacental infection can lead to abortion, stillbirth, weak offspring, and congenital malformations. This review evaluates temporal trends (1975–2026) in four major viral abortifacient agents, Akabane virus (AKAV), Border disease virus (BDV), Caprine herpesvirus 1 (CpHV-1), and Bluetongue virus (BTV), focusing on changes in geographical distribution, diagnostic approaches, and prevention and control strategies.

MATERIALS and METHODS: A structured literature search was conducted using PubMed and Google Scholar. In PubMed, the keyword "Akabane virus goat" retrieved 50 records, of which 19 were included. "Border disease virus goat" identified147 records, with 65 meeting inclusion criteria. For "CpHV-1 goat," 190 records were identified and 70 were included. The term "Bluetongue virus goat" produced 372 records, of which 183 were deemed eligible. To improve coverage, Google Scholar was searched using the same keywords to identify additional sources not indexed or not readily retrievable in PubMed. This complementary search resulted in 24 additional references, including three books, two non-indexed publications, and 19 PubMed-indexed articles not captured in the initial search.

RESULTS: Early studies were predominantly descriptive and experimental, focusing on fetal pathology, abortion patterns, and host susceptibility. Over time, the literature documented expanded geographical distribution, including vector-borne transmission of AKAV and BTV, and herd-level persistence of BDV and CpHV-1. More recent studies demonsrate a transition toward molecular epidemiology, including genotype diversity, atypical strain detection, and risk factor analysis. PCR and RT-PCR have largely replaced reliance on serology alone, while ELISA, virus isolation, and histopathology remain important complementary tools. BDV gained prominence due to persistently infected offspring acting as reservoirs within herds. CpHV-1 was consistently associated with abortion outbreaks and latent infection, whereas AKAV and BTV remained strongly linked to transplacental fetal injury and vector ecology. Despite advances in diagnostics, no specific antiviral treatments are available. Control strategies therefore rely on vaccination, culling of persistently infected animals, quarantine of introduced stock, biosecurity, and vector control.

CONCLUSIONS: Over the last five decades, understanding of caprine viral abortion has evolved from descriptive characterization of reproductive loss to an integrated framework incorporating epidemiology, molecular diagnostics, and herd-level prevention strategies. The most consistent message across all four infections is that Early laboratory diagnosis and preventive management remain essential due to limited treatment options and the risk of fetal loss without evident maternal clinical signs.

Keywords: goat, viral abortion, review of 50 years

Clinical, Hematological, Immunocytochemical and Serological Response to Thrice Long-Acting Oxytetracycline Administration in Sheep Naturally Infected by *Anaplasma phagocytophilum*

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BACKGROUND and OBJECTIVE: *Anaplasma phagocytophilum* is a zoonotic, tick-transmitted,

intracellular bacterium and the causative agent of ovine granulocytic anaplasmosis, a disease associated with significant economic losses in livestock. Diagnosis is typically based on compatible clinical signs and hematological findings in tick-infested sheep, along with confirmatory laboratory testing. The present study aimed to evaluate the therapeutic response of sheep naturally infected by *A. phagocytophilum* to repeated administration of a long-acting oxytetracycline formulation.

MATERIALS and METHODS: Seventeen tick-infested sheep with a compatible clinical picture and a confirmed diagnosis of ovine granulocytic anaplasmosis, based on an immunocytochemical assay for morulae detection in buffy coat smears, were included in the study. All animals were further subjected to antibody testing and hematological evaluation, and were subsequently treated with a long-acting oxytetracycline formulation (Oxyvet® LA 20% inj., Provet SA, Greece), intramuscularly administered at a dose of 20 mg/kg body weight, three times at 56-hour intervals. To evaluate treatment efficacy, all the above clinical and laboratory examinations were repeated one month after the last oxytetracycline administration. Statistical significance of changes in hematological parameters was assessed using the Wilcoxon signed-rank test

RESULTS: One month after completion of treatment, clinical recovery was recorded in 16/17 sheep (94.1%). Sheep with immunocytochemically detected *A. phagocytophilum* morulae in buffy coat smears decreased from 17/17 (100%) before treatment to 5/17 (29.4%) after treatment. In contrast, seropositivity increased from 5/17 (29.4%) before treatment to 12/17 (70.6%) after treatment. Although anemia and thrombocytopenia persisted in two animals, post-treatment hematological evaluation revealed a significant increase in PCV and platelet count (PCV: [Median: 28.00; IQR: 25.75-30.00] vs [Median: 29.00; IQR: 27.00-31.00], P = [0.0002]; platelets: [Median: 158000.00; IQR: 132250.00- 212500.00] vs [Median: 189000.00; IQR: 166750.00-232500.00], P = [0.0008]). Total WBC count also increased significantly after treatment ([Median: 4300.00; IQR: 3800.00-4800.00] vs [Median: 4810.00; IQR: 4422.50-5832.50], P = [<0.0001]), mainly due to significant increases in lymphocyte, monocyte, and eosinophil counts.

CONCLUSIONS: Treatment with a long-acting oxytetracycline formulation administered thrice was effective, resulting in clinical remission, marked improvement of hematological parameters, and elimination of the pathogen. In contrast, antibodies against *A. phagocytophilum* persisted or even developped later in the course of infection despite successful elimination of the pathogen. Therefore, serology for antibody detection constitutes a rather inappropriate method for post-treatment monitoring.

Keywords: {Anaplasma phagocytophilum}, sheep, Long Acting Oxytetracycline, efficacy, immunocytochemistry, serology

FSH and GnRH-Induced Cyclicity and Fertility Outcomes in Anestrous Ewes During a Progesterone-Based Estrus Synchronization Protocol: Preliminary Results

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BACKGROUND and OBJECTIVE: In ewes during the anestrus period, progesterone-based synchronization protocols often result in insufficient estrus synchronization and low pregnancy rates, despite various modifications and hormone additions. This study aimed to stimulate follicular development and induce ovulation by applying FSH and GnRH during a 12-day progesterone-based estrus synchronization protocol in anestrous ewes, with the goal of initiating cyclicity within the protocol and increasing pregnancy rates.

MATERIALS and METHODS: A total of 147 Merino and Merino-cross ewes were included. On day 0, all animals received an intravaginal progesterone-releasing sponge. On day 5, ewes were randomly allocated into three groups: DFSH (double-dose FSH, n = 45), SFSH (single-dose FSH, n = 51), and control (n = 46). DFSH ewes received 40 mg FSH in two equal doses at 12h intervals, whereas SFSH ewes received a single 20 mg FSH dose. The control group received no FSH. Forty-eight hours after the last FSH dose,

FSH-treated ewes were injected with 0.008 mg buserelin (GnRH analogue). On day 12, at sponge removal, all ewes received 600 IU PMSG and 125 µg $PGF_2\alpha$. Rams were introduced 24 hours later for 5 days. Pregnancy was diagnosed on day 25 by transrectal ultrasonography.

RESULTS: Mating rates were similar among groups: DFSH 93.4% (46/49), SFSH 82.4% (42/51), control 86.7% (40/46). Pregnancy rates were 41.3% (19/46) in control, 43.1% (22/51) in SFSH, and 62.2% (28/45) in DFSH. DFSH had significantly higher pregnancy rates than control and SFSH (P = 0.04).

CONCLUSIONS: Adding FSH and GnRH to a progesterone-based protocol increased pregnancy rates, particularly with the split-dose regimen. The higher fertility in DFSH indicates that cyclicity can be induced within the protocol during the out-of-season period, improving pregnancy outcomes. Further studies with larger sample sizes are needed to confirm these effects.

Keywords: sheep, reproduction, synchronisation

Impact of Melatonin Administration on Pregnant Ewes and Fetal Hemodynamics by means of Doppler Ultrasonography

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BACKGROUND and OBJECTIVE: Doppler ultrasonography is a non-invasive, real-time imaging modality that has become an useful tool in veterinary obstetrics for assessing maternal and fetal well-being during pregnancy. The objective of this study was to evaluate the blood flow in the umbilical artery, ductus venosus and abdominal aorta (adjacent to the renal arteries) of fetuses of pregnant ewes treated with melatonin throughout their pregnancy

MATERIALS and METHODS: Twenty Lacunae ewes (8–9 months of age, 35–40 kg) were enrolled and randomly assigned into two equal groups, M (Melatonin) and C (Control), based on whether they received melatonin implants or not, respectively. Doppler examinations were performed using a micro-convex phased-array transducer (5–8 MHz, scanning depth 60–120 mm). Doppler examinations began in the 13th week of pregnancy and were performed at weekly intervals until parturition. A total of 8 examinations per ewe were performed for umbilical artery and 5 examinations per ewe for ductus venosus and abdominal aorta. The haemodynamic parameters calculated were the Pulsatility Index (PI), Resistive Index (RI), Peak Systolic Velocity (PSV), End Diastolic Velocity (EDV), Mean Velocity (Vmn), Systolic Acceleration Time (AT), Systolic/Diastolic Velocity Ratio (SV/DV), and Fetal Heart Rate (HR). Moreover, body weight of lambs was recorded at birth, and on days 15, 30 and 45 after birth.

RESULTS: The results showed that the PSV was higher and the EDV was lower in group M than in C ($P<0.05$), irrespectively of litter size. The birth weight was greater in single M-lambs than in C-ones and the growth rate of M-lambs was better than that of C-lambs ($P<0.05$).

CONCLUSIONS: This study shows that melatonin administration throughout pregnancy in ewes enhances the blood flow in fetuses which may have a positive impact on the growth rate of the born lambs.

Keywords: Doppler Ulreasonography, Aorta, Ductus venosus, Umbilical artery, Pregnant Ewes, Melatonin

Nasal Swabs Improve Herd-level Detection of Rotavirus a in Neonatal Lambs by RT-qPCR

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BACKGROUND and OBJECTIVE: Rotavirus is one of the most common primary pathogens implicated in neonatal diarrhea syndrome in lambs. However, limited data suggest low detection rates in fecal samples collected during diarrhea, raising concerns about reliable identification of the infection at the herd level. Moreover, fecal shedding can occur in non-diarrheic lambs, indicating asymptomatic carriage. While extra-intestinal detection of rotavirus in blood and nasopharyngeal swabs has been documented in piglets and human infants, such information for neonatal lambs is currently lacking. This study aimed to determine the optimal sampling time, the most appropriate biological samples, and the minimum number of animals required for accurate herd-level detection of rotavirus by RT-qPCR.

MATERIALS and METHODS: Two experiments were conducted. In Experiment 1, blood, nasal swabs, and fecal samples were collected every 48 h from eight healthy lambs between 2 and 10 days of age. In Experiment 2, the same specimen types were collected from 31 diarrheic lambs at 2 days of age and again on the first day of diarrhea. All samples were subjected to RT-qPCR testing for rotavirus A and bovine coronavirus, and fecal parasitology. Positivity rates were calculated for each specimen type and sampling time point. The minimum number of animals required for herd-level detection of rotavirus A was estimated using a binomial probability model implemented in R.

RESULTS: All samples tested negative for bovine coronavirus, and Cryptosporidium spp. In Experiment 1, fecal positivity for rotavirus A declined from 8/8 on day 2 to 1/8 on day 10, whereas nasal swabs showed higher and more sustained detection, remaining positive in 8/8 lambs on days 2 and 4. Rotavirus A was detected only sporadically in blood. In Experiment 2, rotavirus A was detected in at least one specimen from all 31 lambs. At 2 days of age, positivity rates were 16.13% in fecal samples, 54.8% in nasal swabs, and 64.5% when both specimen types were considered jointly. On the first day of diarrhea, corresponding rates were 25.8%, 32.3%, and 41.9%, respectively. The minimum number of animals required for ≥95% herd-level detection was 18 for feces, 4 for nasal swabs, and 3 for the combined approach at 2 days of age; on the first day of diarrhea, the corresponding numbers were 11, 8, and 6.

CONCLUSIONS: Nasal swabs comprise an effective, non-invasive specimen for rotavirus A detection in neonatal lambs. When combined with fecal sampling, they substantially improve herd-level detection efficiency and reduce the number of animals required for sampling.

Keywords: Rotavirus A, nasal swabs, RT-qPCR, lambs, neonatal diarrhea syndrome, herd-level detection

Age Differences in the Type and Anatomical Distribution of Skin Lesions in Hong Kong Local Goats

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BACKGROUND and OBJECTIVE: Skin disorders are a common reason for consultation among local goats in Hong Kong. Literature on caprine skin disorders in subtropical regions and in non-intensive production systems is scarce. This study aimed to compare the prevalence of skin lesions (types and

anatomical locations) across age groups in Hong Kong goats.

MATERIALS and METHODS: This cross-sectional study (Winter 2025) involved 324 randomly selected goats from 17 farms serviced by the City University of Hong Kong's ambulatory clinic. A trained assessor recorded sex, age, and skin lesions (types and anatomical locations), excluding mucosal lesions. For each anatomical site, age group differences in skin lesion types were analyzed using pairwise tests (estimated marginal means). To control for multiple comparisons, $p < 0.01$ was considered significant.

RESULTS: Most goats were female (71%, 230/324) and < 1 year old (63%, 204/324). 85% (275/324) of goats presented at least one skin lesion. Calluses were the most prevalent skin lesion (78%, 253/324) and more frequent in goats > 4 years old than in goats < 1 year old on carpi ($p < 0.001$) and tarsi ($p < 0.001$). Scales (27%, 87/324) were more common in goats > 4 years old than in goats < 1 year old on the thoracic ($p = 0.006$), lumbar ($p < 0.001$), and sternal backline ($p < 0.001$).

CONCLUSIONS: Skin lesions were highly prevalent in Hong Kong local goats, with environmental lesions (calluses on carpi and tarsi) being the most common, particularly in older goats.

Keywords: Capra hircus, Dermatology, Skin disorders

Quick Thoracic Ultrasonography for Early Detection of Pneumonia in Goat Kids: A Pilot Study

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BACKGROUND and OBJECTIVE: As the goat industry expands, respiratory disease in goat kids remains a leading cause of economic losses, impaired welfare, and antimicrobial use. Sudden death is a common first sign in goat kids, hence metaphylactic antimicrobial therapy is a frequent practice. On farm thoracic ultrasonography, as frequently used in calves, may offer opportunities to more rapidly detect pneumonia and better tailor antimicrobial use. Therefore, this study evaluated the feasibility of the quick thoracic ultrasonography (qTUS) point of care ultrasound application for detecting pneumonia in goat kids. Additionally, pneumonia prevalence estimates from Belgium are provided.

MATERIALS and METHODS: A total of 78 Saanen goat kids (3–6 weeks old) were randomly selected from four dairy goat farms in Eastern Flanders, Belgium. Farms were included based on willingness to participate, resulting in a convenience sample. On each farm, 18–20 kids were examined by means of qTUS using a portable ultrasound with a 7.5 MHz linear probe. Hair was not clipped; isopropyl alcohol was applied as a contact medium. The lung was visualized from diaphragmatic to cranial lobes in one smooth cranio-ventral movement on each side. Landmarks included caudally a start image containing "half liver – half lung," mid scan image "half heart – half lung" and "a. and v. thoracica" as the end-point. (right side). On the left side the caudal start image consisted of 'lung-spleen-rumen', whereas the other two landmarks were identical to the right side. All ultrasounds-findings were recorded. Pneumonia was defined as a lung consolidation ≥ 1cm.

RESULTS: Lung consolidations (0.5–3 cm) were observed in 41.0% (32/78) of scanned goat kids, with farm-level prevalence ranging from 0% to 85.0%. Cranial quadrants were most affected: right 30.8% (24/78), left 29.5% (23/78), while caudal quadrants were affected in 21.8% (right) and 14.1% (left) of the cases, respectively. These findings are consistent with general thoracic ultrasound patterns reported in calves. None of the herds reported severe clinical signs or mortality at the time of screening.

CONCLUSIONS: This pilot study showed that qTUS is feasible in goat kids, under practice conditions. Results suggest that pneumonia may be an underestimated problem on goat farms, with large differences in prevalence between farms. Thoracic ultrasound allows veterinarians to detect pneumonia in an earlier phase and has potential to rationalize antimicrobial use and timely identify the need for changes in control and preventative measures.

Keywords: Thoracic ultrasonography, qTUS, Pneumonia, Welfare, Rational antimicrobial use

Antimicrobial Residues in Small Ruminant's Sub-Products and Environment

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BACKGROUND and OBJECTIVE: When antimicrobials are administered to animals, their metabolism is not 100% effective, so active metabolites may be excreted through the urine and feces and potentially be present in meat and milk. The presence of residues in foods intended for human consumption may cause chronic, long-term exposure, thereby increasing the risk of antimicrobial resistance. This study aimed to determine the occurrence and concentration levels of antimicrobial residues in meat, milk, and environmental samples from Small Ruminants (SR) in Portugal's Central Region.

MATERIALS and METHODS: SR meat samples were collected from two slaughterhouses. Thirty-nine SR farms (27 sheep; 10 goats; 2 mixed) were visited to collect individual milk samples, pasture, feed, bedding and soil samples. The samples were placed in identified plastic bags or containers and stored at -80ºC until analysis. Residue detection was performed by ultra-high-performance liquid chromatography coupled to high-resolution mass spectrometry with a time-of-flight analyzer (UHPLC–TOF). Compound identification was carried out based on mass accuracy, retention time, and isotopic profile.

RESULTS: Overall, 526 samples were analyzed (314 meat, 85 milk, 39 soil, 37 bedding, 37 pasture, 14 feed samples). Antimicrobial residues were detected in 12 (6.2%) sheep meat samples and 18 (15.0%) goat meat samples. Overall, two anthelmintics (Albendazole Sulfoxide, Albendazole Sulfone) and five antibiotics (Tilmicosin, Ciprofloxacin, Tetracycline, Trimethoprim, Sulfadiazine) were detected in meat samples. Ten (11.8%) milk samples (2 from goat, 8 from sheep) contained antimicrobial residues, namely, two anthelmintics (Hydroxymebendazole, Albendazol) and six antibiotics (Ciprofloxacin, Cefazolin, Cephalexin, Cefquinome, Sulfamethoxypyridazine, Sulfamerazine). Antimicrobial residues were detected in 13 (14.4%) environmental samples (5 feed, 2 bedding, 6 soil), namely 4 anthelmintics (Albendazole, Albendazole Sulfoxide, Albendazole Sulfone, Aminoflubendazole) and 16 antibiotics (Cefazolin, Cefquinome, Tetracycline, Danofloxacin, Ofloxacin, Sarafloxacin, Nalidixic acid, Sulfadimethoxine, Sulfadimidine, Sulfadoxine, Sulfamethizole, Sulfamethoxazole, Sulfapyridine, Sulfaquinoxaline, Sulfatiazole, Tiamulin). All residues detected were within the maximum residue limit (MRL) stipulated by law.

CONCLUSIONS: The compounds identified were mainly antibiotics and anthelmintics, reflecting their prevalent use in animal production. Although residue levels were below MRL, ensuring the safety of food products and compliance with withdrawal periods, environmental matrices showed higher diversity of compounds, indicating the environment as a reservoir for these residues. The detection of critically antibiotic classes for human medicine, such as quinolones, fluoroquinolones, and fourth-generation cephalosporins, raises public health concerns, as their persistence in environment may exert selective pressure on bacteria, promoting antimicrobial resistance. Overall, these findings highlight the need for prudent antimicrobial use in SR and effective monitoring strategies within One Health.

Keywords: Antimicrobial, Residues, Small Ruminants

Effect of Strategic Injectable Supplementation with Vitamins and Minerals Involved in Antioxidant Defense on Dairy Goat Performance

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BACKGROUND and OBJECTIVE: The aim of this study was to evaluate the effect of a strategic injectable supplementation with vitamins and trace minerals involved in antioxidant defense on milk yield, milk composition, somatic cell count (SCC), and lactation length.

MATERIALS and METHODS: A completely randomized study was conducted on a dairy goat farm located in Suipacha, Argentina. Multiparous Saanen goats were randomly assigned to one of two groups. Supplemented group goats (SG; n=63) received subcutaneously three 1-mL doses of two injectable products: the first containing copper (10mg/mL), manganese (10mg/mL), zinc (40mg/mL), selenium (5mg/mL) (Adaptador® MIN, Biogénesis Bagó SA), and the second containing vitamins A (59500UI/mL) and E (50UI/mL) (Adaptador® VIT, Biogénesis Bagó SA). Treatments were administered at drying-off, one month pre-kidding, and within 48h postpartum. Control group (CG; n=65) received saline solution. All animals received the same housing and diet throughout the study. Milk yield was recorded every 30.5±5.9 days in milk (DIM), and samples from 108 goats were analyzed for fat, protein, lactose, total solids, and SCC. Lactation length was determined individually, with drying-off occurring when daily milk yield fell below 0.5 L/day. Milk yield, milk composition variables, SCC, and lactation length were analyzed using generalized linear mixed models, with repeated measures when appropriate, using R software (version 4.3.2). Fixed effects included treatment group, DIM (third-order orthogonal polynomial), parity, kidding season, body condition score, dry period length (DPL), and biologically relevant interactions. Goat was included as a random effect. Statistical significance was set at $p \leq 0.05$.

RESULTS: Milk yield was affected by treatment ($p<0.01$), DIM ($p<0.01$), DPL ($p<0.01$), interaction parity*DIM ($p<0.01$), and interaction treatment*DIM showed a tendency ($p=0.09$). Supplemented group showed higher average daily milk yield than CG (2.36 L/day, IC95%: 2.17–2.55 vs 1.90 L/day, IC95%: 1.70–2.10, respectively; $p<0.01$). The tendency for treatment*DIM interaction was attributed to a later and higher production peak in the SG lactation curve. Supplemented group had longer lactation length than CG (294 days, IC95%: 268-319 vs 264 days, IC95%: 238-291, respectively; $p=0.04$). Milk SCC, fat, protein, lactose, and total solids were affected by DIM ($p<0.01$) and milk yield ($p<0.01$). Treatment did not affect average SCC, fat, protein, lactose or total solids concentrations ($p>0.05$).

CONCLUSIONS: In conclusion, strategic injectable supplementation with vitamins and trace minerals involved in antioxidant defense improved milk yield and extended lactation length in dairy goats, without altering milk composition or SCC.

Keywords: Dairy goats, Micronutrient supplementation, Milk yield, Lactation length.

Keywords: Calf rearing, Herd size, Poland

Red Blood Cell Parameters and Their Associations with Parasitic Burden, Age and Reproductive Stage in Clinically Healthy Indigenous Greek Goats *Capra Prisca*

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BACKGROUND and OBJECTIVE: Red blood cell (RBC) parameters are essential indicators of health and physiological status in goats. However, the influence of parasitic infections, age and reproductive stage on RBC parameters remains insufficiently characterized in indigenous Greek goats. The objective of this study was to investigate RBC parameters and evaluate their associations with parasitic burden, age and reproductive stage in clinically healthy *Capra prisca* goats.

MATERIALS and METHODS: A total of 200 clinically healthy *Capra prisca* goats were classified according to parasitic status (gastrointestinal nematodes, *Eimeria* spp. and lungworms), age group (3–6 months kids, ≤3 years and >3 years adults) and reproductive stage (non-lactating pregnant and lactating non-pregnant does). Blood samples were analyzed using automated hematology analyzers to determine RBC indices. Statistical analyses were performed to assess associations between RBC parameters and parasitological, age-related and reproductive variables.

RESULTS: Heavy gastrointestinal nematode infections (>300 epg) were associated with significant reductions in RBC count and hematocrit, accompanied by increased mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, suggesting a compensatory hematological response. Lungworm infection was associated with lower RBC counts and reduced red cell distribution width. In contrast, *Eimeria* spp. infection did not significantly affect RBC parameters in growing kids. Age exerted a marked influence on RBC values, with younger animals exhibiting higher RBC counts and hematocrit compared to adults. Reproductive stage also affected hematological indices, as pregnant does demonstrated higher hemoglobin concentration and hematocrit than lactating non-pregnant does.

CONCLUSIONS: RBC parameters in *Capra prisca* goats are significantly influenced by parasitic burden, age and reproductive stage, even in the absence of clinical signs. These findings emphasize the need to interpret hematological results within the appropriate physiological and parasitological context. Incorporating RBC profiling into routine health monitoring may enhance the identification of subclinical alterations and support evidence-based management strategies for improving health and productivity in indigenous goat populations.

Keywords: Capra prisca, red blood cells, parasitic infection, age, reproductive stage, goat hematology

White Blood Cell Parameters and Their Associations with Parasitic Burden, Age and Reproductive Stage in Clinically Healthy Indigenous Greek Goats *Capra Prisca*

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BACKGROUND and OBJECTIVE: White blood cell (WBC) parameters are essential indicators of immune function and overall health status in goats. However, the influence of parasitic infections, age and reproductive stage on WBC profiles remains insufficiently characterized in indigenous Greek

goats. The objective of this study was to investigate WBC parameters and evaluate their associations with parasitic infections, age, and reproductive stage in clinically healthy *Capra prisca* goats.

MATERIALS and METHODS: A total of 200 clinically healthy *Capra Prisca* goats were categorized according to parasitic infection (gastrointestinal nematodes, *Eimeria* spp., and lungworms), age group (3–6 months kids, ≤3 years and >3 years adults) and reproductive stage (non-lactating pregnant and lactating non-pregnant does). WBC parameters, including total leukocyte counts and differential leukocyte profiles, were determined using automated hematology analyzers in conjunction with manual blood smear evaluation. Statistical analyses were performed to assess associations between WBC parameters and parasitological, age-related and reproductive variables.

RESULTS: Heavy gastrointestinal nematode infection (>300 epg) did not significantly affect total WBC counts but was associated with alterations in differential leukocyte distributions. Lungworm infection was associated with lower neutrophil counts and higher lymphocyte proportions. *Eimeria* spp. infection influenced basophil counts in growing kids only. Age exerted a significant effect on WBC parameters, with younger goats exhibiting higher total leukocyte counts, neutrophils and lymphocytes compared to adults. Reproductive stage also influenced leukocyte profiles, as non-lactating pregnant does demonstrated higher neutrophil and monocyte counts than lactating non-pregnant does, consistent with physiological immune modulation.

CONCLUSIONS: WBC parameters in clinically healthy *Capra prisca* goats are significantly influenced by parasitic burden, age and reproductive stage, even in the absence of overt clinical disease. These findings underscore the importance of interpreting leukocyte profiles within their physiological and parasitological context to avoid misclassification of normal adaptive responses as pathological alterations. Integrating WBC assessment into routine health monitoring may facilitate earlier recognition of subclinical parasitic impacts and support more precise, evidence-based management strategies in indigenous goat populations.

Keywords: Capra prisca, white blood cells, parasitic infection, age, reproductive stage, goat hematology

Induction of Maternal Behavior in Goats: Effects of Hormonal Treatment and Vaginocervical Stimulation

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BACKGROUND and OBJECTIVE: Maternal behavior is fundamental for the survival of the offspring. In precocial species, there is a known sensitive period that is controlled by hormonal and proprioceptive factors. It is known that vaginocervical stimulation triggered by fetal expulsion promotes maternal behavior in the females of several species. In sheep, this maternal behavior is associated with plasma concentrations of estradiol and progesterone, however, there are almost no studies on this subject in goats. Furthermore, there is evidence that in both species the maternal behavior in goats is affected by experience. The objective of this study was to determine the influence of hormonal treatment and vaginocervical stimulation on maternal behavior in ovariectomized nulliparous and multiparous goats.

MATERIALS and METHODS: Nulliparous (n=7) or multiparous (n=10) goats were ovariectomized and treated either with estradiol benzoate or with progesterone + estradiol benzoate; before or after vaginocervical stimulation. Maternal behaviors were assessed in a two-phase test (before and after vaginocervical stimulation), hormonal treatments were applied randomly. For 30 minutes in each phase, the treated goat was exposed to a non-goat kid between 6 and 24 hours old. Acceptance (low bleating, sniffing and cleaning the young, acceptance and allowing breastfeeding) and rejection (loud bleating, aggression, and refusal to nursing) behaviors toward the kid were measured.

RESULTS: Goats treated with estradiol benzoate showed a better maternal response after vaginocervical stimulation than before (P=0.002). Goats treated with progesterone and estradiol benzoate only exhibited positive behaviors before

and after stimulation, but there were no differences between the two groups (P=0.861). Nulliparous goats, regardless of treatment, showed negative responses before and after vaginocervical stimulation (P>0.05), while multiparous goats, regardless of treatment, showed more positive responses after vaginocervical stimulation (P=0.007).

CONCLUSIONS: It is concluded that estradiol and progesterone influence the establishment of maternal behavior, and in experienced goats, these treatments promote better maternal behavior. Funded by UNAM-DGAPA-PAPIIT IN224220.

Keywords: goats, maternal behavior, estradiol, progesterone.

Seroprevalence of Coxiella Burnetii in Small Ruminants in the Northeast of Portugal

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BACKGROUND and OBJECTIVE: Coxiella burnetii is the intracellular bacterium responsible for Q fever, a major zoonosis that causes significant reproductive disorders in domestic ruminants and poses a severe public health threat. The present study aimed to assess the seroprevalence of C. burnetii in sheep and goats at both the individual animal and farm levels, providing insight into the distribution of infection and informing strategies for monitoring and control in the northeast of Portugal.

MATERIALS and METHODS: A seroprevalence study was conducted in small ruminant herds in the northeast of Portugal between 2020 and 2023. Herds were randomly selected, and farmers were invited to participate. Samples were collected from 12 municipalities, encompassing 105 farms and 1832 animals, including 1621 sheep and 211 goats. Serum samples were screened using a commercial indirect Enzyme-Linked Immunosorbent Assay (ID Screen Q Fever Indirect Multi-species; Innovative Diagnostics) utilising a purified whole-cell bovine isolate antigen, following the manufacturer's instructions. Seroprevalence was calculated at the individual-animal and farm levels, separately for sheep and goats, and for farms specialised in sheep or goats. Statistical associations between species and sampling years were analysed using the chi-square test (x^2) to assess the relationships among species, years, and farm types, with significance set at $p < 0.05$.

RESULTS: The overall seroprevalence at the animal level was 40.39% (95% CI: 38.23–42.72). Seroprevalence in sheep was 38.72% (95% CI: 36.36–41.13), whereas in goats it was 52.81% (95% CI: 46.38–59.15). At the farm level, 87.61% (95% CI: 79.96–92.61) of farms were positive, including 87.2% of sheep farms and 91.67% of goat farms with at least one seropositive animal. Annual seroprevalence at the animal level was 25.34% in 2020, 40.12% in 2021, and 55.99% in 2022. Differences in seroprevalence between sheep and goats were statistically significant ($x^2 = 16.3$, $P < 0.001$). Furthermore, the data revealed a statistically significant temporal progression of the infection ($x^2 = 96.3$; $P < 0.001$).

CONCLUSIONS: This study provides updated seroprevalence data for C. burnetii in small ruminants in northeast of Portugal, displaying an ascending temporal trend. Sheep and goats were both affected, with seropositive animals distributed across multiple farms. These results emphasise the importance of monitoring Q fever in small ruminant populations under a One Health perspective, demanding the urgent implementation of integrated control strategies.

Keywords: {Coxiell burnetii}, Sheep, Goats, Seroprevalence

AI-Driven Prediction of Sheep Artificial Insemination Outcomes Based on Semen Quality Metrics

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BACKGROUND and OBJECTIVE: Artificial intelligence is increasingly being incorporated into livestock reproductive management to enhance decision making and improve efficiency. This study evaluated the feasibility of applying a machine learning model to predict the outcome of cervical artificial insemination in sheep based on detailed semen quality traits.

MATERIALS and METHODS: Semen was collected from four Lacaune rams (15–20 months old), extended, and used for inseminating 63 Lacaune ewes (14–15 months old) following a standardized estrus-synchronization protocol involving a 12-day CIDR treatment and eCG (500 IU) administration, with inseminations performed 36 and 60 hours after device removal. Semen assessment included computer-assisted sperm analysis (CASA) of motility and kinematic variables (total and progressive motility, VCL, VSL, VAP, ALH, BCF, STR, LIN, WOB), along with viability, morphology, and Hypo-Osmotic Swelling Test (HOST) response. Reproductive outcomes recorded were pregnancy status, lambing, and prolificacy. Data were analyzed using the XGBoost algorithm implemented on the Akkio AI platform.

RESULTS: The pregnancy-prediction model achieved an accuracy of 76.9%, with VAP, LIN, VSL, and progressive motility identified as the most influential predictors. Prediction of prolificacy showed lower performance with accuracy of 56.4%, RMSE 0.6, MAE 0.4, reflecting the multifactorial nature of this trait.

CONCLUSIONS: These results demonstrate the potential of machine learning tools to support reproductive decision-making in sheep production systems. Expanding the dataset to approximately 150–200 insemination records is expected to substantially improve model robustness and predictive accuracy.

Keywords: Sheep reproduction, Semen quality, CASA, Predictive modeling, Akkio, Artificial insemination

Effects of Melatonin Implantation on Metabolic Indicators and Offspring Growth in Late-Pregnant Ewes

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BACKGROUND and OBJECTIVE: BACKGROUND: Late gestation in ewes, particularly in cases of multiple pregnancies, is characterized by increased energy demands and an elevated risk of pregnancy toxemia. Although the effects of melatonin on energy metabolism have been well documented in monogastric species, its role in the regulation of energy metabolism and subsequent offspring performance in ruminants remains insufficiently elucidated. OBJECTIVE: This study aimed to determine the effects of melatonin implantation administered to late-pregnant ewes on metabolic indicators, as well as its effects on lamb birth weight and postnatal growth performance.

MATERIALS and METHODS: On day 100 of gestation, ewes with single and twin pregnancies (n=96) were allocated to melatonin (M: n=47; 18 mg subcutaneous ear implant) and control (C: n=49) groups. BHBA, glucose, and BCS were assessed on days 100, 114, 135, 142, and 149 of gestation, and lamb body weights were recorded from birth to day 75. Data were analyzed using repeated measures mixed models and general linear models including treatment, time, birth type, and their interactions.

RESULTS: Time had a strong effect on all maternal parameters (P<0.01). BHBA concentrations increased toward late gestation and were influenced by the

time × birth type interaction (P = 0.006), with a greater increase observed in twin-bearing ewes. Glucose levels remained stable, whereas BCS declined over time (P=0.002). Melatonin had no significant effect on maternal parameters. Similarly, melatonin did not affect lamb birth weight or postnatal growth. In contrast, birth type was the main determinant of lamb performance: single-born lambs were heavier at birth (P<0.0001) and maintained higher body weights throughout the growth period (P=0.009). Sex showed a tendency to influence birth weight (P=0.071).

CONCLUSIONS: Melatonin administered during late gestation had no effect on maternal metabolic or fetal parameters. Metabolic adaptation was dependent on pregnancy type, whereas lamb growth performance was associated with sex and birth type. Further studies are required to better elucidate the effects of melatonin.

Keywords: Ewe, Melatonin, Late gestation, Energy Metabolism, Lamb growth

SMART FARMING AND ARTIFICIAL INTELLIGENCE

Associations Between Milk Fat-to-Protein Ratio, Metabolic Biomarkers, and Behavior in Early-Lactation Dairy Cows

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BACKGROUND and OBJECTIVE: Early lactation is a critical period for dairy cows, during which metabolic disturbances such as subclinical ketosis and low-grade ruminal acidosis frequently occur without obvious clinical signs. The milk fat-to-protein ratio (FPR) is a practical indicator of energy balance and metabolic status. This study aimed to investigate the relationships between FPR, milk composition, blood biochemical parameters, and behavioral indicators in early-lactation dairy cows using continuous precision monitoring.

MATERIALS and METHODS: The study included 27 Holstein cows between 9 and 59 days in milk. Animals were classified into three groups based on FPR values: low-grade ruminal acidosis (LGRA; FPR < 1.2), healthy (H; FPR 1.2-1.5), and subclinical ketosis (SCK; FPR > 1.5). Milk fat, protein, and lactose concentrations were continuously recorded using an in-line milk analysis system. Rumination time, physical activity, water intake, and reticulorumen temperature were monitored using intraruminal sensors. Blood samples were collected weekly to determine metabolic and biochemical indicators, including non-esterified fatty acids (NEFA) and selected minerals and enzymes. Associations between FPR and monitored variables were evaluated using SPSS statistical analyses.

RESULTS: Cows classified with SCK exhibited higher milk fat content and lower milk protein and lactose concentrations compared with both H and LGRA cows, resulting in significantly elevated FPR values. Rumination time was markedly reduced in the SCK group, whereas activity levels were higher than in both LGRA and H cows, indicating altered feeding and locomotion behavior. Blood analysis revealed increased NEFA concentrations in SCK cows, consistent with intensified lipomobilization during negative energy balance. In contrast, serum iron concentrations were lowest in the SCK group, while LGRA and H cows showed higher and comparable values. Correlation analysis demonstrated positive associations between FPR and NEFA as well as cow activity, and negative associations between FPR and milk protein, lactose concentration, rumination time, water intake, and serum iron levels, confirming that increasing FPR was accompanied by progressive metabolic and behavioral disruption.

CONCLUSIONS: Elevated milk FPR is closely associated with metabolic, behavioral, and biochemical alterations characteristic of subclinical metabolic imbalance in early-lactation dairy cows. The integration of real-time milk composition data with continuous behavioral and physiological monitoring provides a non-invasive approach for early detection of subclinical metabolic disorders. This strategy has the potential to support proactive herd health management, improve animal welfare, and enhance productivity during early lactation.

Keywords: Innovations, Dairy cows, precision, biomarkers, milk composition

From Data to Decisions: A Qualitative Study on the Day-to-day Use of Youngstock Health Monitoring Sensors in Dairy Farming

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BACKGROUND and OBJECTIVE: Monitoring technologies represent a promising tool with significant potential to shape the future of dairy farming by enabling more proactive and data- driven management of young calves. The SenseHub Dairy Youngstock sensor (SH- YS; Merck & Co., Inc., Rahway, NJ, USA) provides real-time information to support these advancements. Despite its potential, there is currently no published field experience in the literature describing how this system is applied on dairy farms. The primary objective was to explore how farmers use the SH-YS technology in their daily management practices and to identify the factors influencing its integration.

MATERIALS and METHODS: This study used a qualitative exploratory design. Eleven semi-structured interviews were conducted with dairy farmers physically on farm (n = 9) or virtually (n = 3) in the UK, Spain, Italy, and Israel. Recruitment of participants was done based on the time of adoption, which ranged from 3 to 8 months. Interviews were audio- recorded, transcribed and analyzed. A thematic analysis was used to identify patterns and insights from the data, focusing on the experiences, challenges, and operational impacts of the SH-YS.

RESULTS: The analysis revealed five main themes: (1) acquisition and implementation, (2) sensor usage, (3) user experiences, (4) alternative use cases, and (5) future perspectives. Most farmers reported that implementation was straightforward; however, their approaches to managing alerts and responding to early warnings varied. For example, when a calf was identified by the system prior to exhibiting symptoms, some farmers increased monitoring, others conducted clinical examinations, and some initiated treatment protocols. Farmers also reported enhanced disease intervention capabilities, improved efficiency in calf management, and varying levels of trust in the technology, reflecting a shift in traditional management practices driven by sensor data. Finally, participants described additional use cases, including evaluating treatment response, supporting weaning decisions and herd health management. They also suggested improvements, such as integration with farm management software and treatment records, and features to locate lost tags.

CONCLUSION: The findings highlight the SH-YS potential for transforming youngstock management practices by enabling proactive health monitoring and decision-making. While benefits such as earlier disease detection and reduced labor requirements were noted, standardized protocols and user education programs must be developed or improved to maximize the technology's potential. Further research is recommended to explore additional use cases of the SH-YS technology in youngstock management.

Keywords: Sensor, Youngstock, Management, Health, Monitoring, Technology

Milk Yield Dynamics as Indicators for Disease in Dairy Cows

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BACKGROUND and OBJECTIVE: Dairy cows experience most diseases in early lactation. Deviations in milk yield have been shown to add value to health monitoring. This study aimed to investigate the relations between health status until 56 days in milk (DIM) and: 1) lactation curve characteristics, 2) dynamic indicators of milk yield.

MATERIALS and METHODS: Daily milk yield data and disease treatment records from 4 previous experiments were used. Cows were categorized in 3 ways based on disease treatments until 56 DIM: 1) in 3 groups based on the health status: no health problem (NHP), systemic or inflammatory health

problem (SHP), other health problem (OHP), 2) the number of disease treatments per cow (0, 1, or ≥ 2), 3) the presence or absence of disease treatments for either mastitis, endometritis, claw disorders, vaginal discharge or cystic ovaries. Six lactation curve characteristics, such as peak milk yield and persistency, were calculated. Four dynamic indicators of milk yield were calculated derived from daily deviations in milk yield during 10-56 and 10-305 DIM: log-transformed variance (LnVar), lag-1 autocorrelation (Rauto), skewness and mean negative residuals (MNR). Relations between disease treatments and milk yield dynamics were investigated using mixed models.

RESULTS: Peak yield and total milk yield until 56 DIM were lower in SHP cows than NHP and OHP cows. Cows of parity ≥3 in SHP had a later day of peak yield and lower slope to peak yield than NHP and OHP cows of parity ≥3. LnVar during 10-56 DIM was higher for SHP and OHP cows than NHP cows, and LnVar increased with increasing number of disease treatments. Cows treated for endometritis or cystic ovaries had a higher Rauto throughout lactation and cows treated for claw disorders during 10-56 DIM had a lower MNR than healthy cows.

CONCLUSIONS: This indicated that cows with health problems in early lactation had a different shape of the lactation curve and more perturbations in milk yield than healthy cows, but the exact response was disease-dependent. While lactation curve characteristics specifically indicated SHP cows, dynamic indicators of milk yield indicated both SHP and OHP cows. The impact of specific diseases on milk yield dynamics should be further investigated to allow for application in on-farm health monitoring tools.

Keywords: Milk yield dynamics, indicators of disease, early warning

Challenges and Opportunities for a Private Practitioner with a European Qualification in the United Kingdom

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BACKGROUND and OBJECTIVE: The European College of Bovine Health Management (ECBHM) was established in 2003. Initially a group of cattle veterinarians was admitted to the college on merit. Soon after an exam was set and a learning trajectory was devised. Even though content of residencies and alternative residencies varied per institution the examination sets a standard for qualified Diplomates of the ECBHM. Or as the College puts it: The primary objective of the College shall be to advance health oriented bovine production management in the herd context in Europe and increase the competency of those who practice in this field by establishing guidelines and standards of training for postgraduate education and experience prerequisite to become a specialist in the speciality of bovine health management. The aim of this study was to explore the challenges and opportunities of a European Board of Veterinary Specialisation (EBVS) qualification, in this case being a ECBHM Diplomate, when working in private practice in the United Kingdom (UK).

MATERIALS and METHODS: The ins and outs of a EBVS qualification was explored. This was then compared to national (Royal College Veterinary Surgeons) qualifications. Subsequently sociological educational literature concerning the relevance of postgraduate qualifications was reviewed in depth. Lastly all gathered information was applied to discuss the challenges and opportunities of a ECBHM Diplomate working in private practice in the UK.

RESULTS: EBVS and RCVS qualifications are partly interchangeable. Also, the relevance of further qualifications in society was shown. Nevertheless, it is questionable if farmers find the ECBHM diploma of much relevance in a daily setting.

CONCLUSIONS: By researching and discussing the relevance of further qualifications in livestock veterinary medicine, future opportunities for the ECBHM Diplomate that wants to work in practice were identified.

Keywords: ECBHM, RCVS, European qualification, sociology

Importance of Recording Prepubertal Estrus in the Reproductive Management of Heifers Monitored with Sensors from Birth

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BACKGROUND and OBJECTIVE: Reproductive management of dairy heifers is a key economic component of dairy herds. Automated Monitoring Systems (AMS) are widely used in adult cows, but their value for identifying puberty and cyclicity in heifers has not been fully explored. This study had two main OBJECTIVES: (1) to assess the agreement between AMS-detected estrus and serum progesterone concentrations ≥1 ng/mL; and (2) to describe age at first estrus, establishment of regular cyclicity, and their associations with reproductive performance in Holstein heifers monitored with sensors from birth.

MATERIALS and METHODS: Phase 1: Twenty-five Holstein heifers were tagged at approximately 4 months of age with SenseHub™ Dairy Youngstock (SHYS). Weekly blood samples were analyzed for progesterone level for 35 weeks (ELISA). AMS alerts were processed with Matlab and compared with physiological events. Peripubertal estrus was defined as two estrus events 16–26 days apart and progesterone >1 ng/mL. Sensitivity and specificity of AMS estrus detection were calculated. Phase 2: Data from 120 heifers ≥420 days old, monitored with SHYS from birth on a commercial farm (Zaragoza, Spain), were analyzed. Variables included age at onset of regular cyclicity (≥2 regular estrous with a 16–26 day interval), and reproductive status at 435 days (pregnant, under AI, open, culled). Heifers with regular cyclicity were classified as early (<280 days), normal (280–330 days), or late (>330 days). Associations between onset of cyclicity and reproductive status were assessed using a chi-square test.

RESULTS: Phase 1, the mean age at first progesterone >1 ng/mL was 272 ± 33 days (mean 2.8 ± 1.44 ng/mL). AMS sensitivity and specificity for peripubertal estrus detection were 95.7% and 100%, respectively. The mean interval between first progesterone positivity and first AMS-detected estrus was 6.5 days. Phase 2, 79/120 heifers (65.8%) achieved regular cyclicity at 324 ± 26 days; 3.8% were early, 62.0% normal, and 34.2% late. Forty-one heifers (34.2%) did not establish regular cyclicity, although most showed at least one estrus event. Age at onset of regular cyclicity significantly affected pregnancy rate at 430 days ($x^2=14.0$, $p<0.01$): early 66.7%, normal 42.9%, late 3.8%.

CONCLUSIONS: AMS detects peripubertal estrus with high reliability and provides useful information about puberty and cyclicity. Heifers that reach puberty and regular cycles earlier generally reflect better growth conditions and tend to achieve better reproductive outcomes, whereas later onset of cyclicity may indicate increased risk. Monitoring prepubertal estrus with AMS can support selection and management decisions in replacement heifers.

Keywords: reproductive management, automated monitoring systems, puberty detection, estrus, Holstein heifers

Automated Monitoring Systems in Dairy Youngstock: Using Rumination Time as an Indicator of Poor Transition from Milk Fed Calf to Ruminant

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BACKGROUND and OBJECTIVE: Rumination behavior increases rapidly during early calf development and has been consistently associated with solid feed intake, rumen maturation, health, and welfare. Automated monitoring systems (AMS) capable of recording rumination time (RT) may provide valuable information during the pre weaning period—a stage in which farms typically lack reliable indicators of starter intake or rumen development. The objectives of this observational study were: (1) to evaluate rumination behavior recorded by AMS; (2)

to assess correlations between average daily gain (ADG), weekly mean rumination time (WMRT), and milk consumption percentage (MCP); and (3) to determine practical WMRT thresholds for identifying calves at risk of reduced growth.

MATERIALS and METHODS: The study was conducted from September 2024 to January 2025 on a dairy farm in Huesca, Spain, monitoring 249 calves. After receiving two colostrum feedings, calves were group housed and weighed individually. They were placed in groups of 14 per automatic milk feeder (H&L CalfExpert) from 3–20 days of age, then transitioned to another feeder until 70 days. Calves were re weighed at weaning using a calibrated scale. SenseHub™ Dairy Youngstock tags were applied at disbudding (3–4 weeks of age). Individual data collected included colostrum quality score, morbidity, mortality, weights, MCP, and WMRT.

RESULTS: WMRT averaged 263 ± 2.24 min/day from weeks 5–10 and 371 ± 2.37 min/day from weeks 11–20, representing a significant increase ($p < 0.0001$). The correlation between WMRT and ADG during weeks 5–10 was low ($r = 0.21$). In contrast, MCP showed a strong correlation with ADG ($r = 0.67$). To better evaluate growth implications, WMRT was categorized as >200 min/day vs. ≤200 min/day. Calves with WMRT >200 min/day had significantly higher ADG (0.947 vs. 0.864 kg/day; $p < 0.0001$). Notably, 18% of calves did not reach 200 min/day, suggesting this threshold may serve as a useful indicator for on farm decision making. Calves with WMRT ≤200 min/day between days 48–62 were offered an extended milk period (three additional weeks). Compared with historical farm data, this strategy increased ADG in delayed weaning calves by 98 g/day (improvement of 5.4 kg total gain) and reduced post weaning culls by 3%.

CONCLUSIONS: Rumination data obtained through AMS may provide a valuable decision support tool during the pre weaning period, especially in environments where starter intake and rumen development are not routinely measured. WMRT appears useful for identifying calves at risk of reduced growth and guiding individualized weaning or feeding strategies adapted to farm conditions.

Keywords: rumination, automated monitoring, dairy calves, growth threshold, weaning strategy

Estrus Index and Subsequent Fertility After Artificial Insemination Using Automated Activity Monitoring in Lactating Dairy Cows

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BACKGROUND and OBJECTIVE: Automated monitoring systems (AMS) are widely used in dairy operations for estrus detection in Holstein cows. The heat index (HI) generated by these systems reflects the intensity of estrus-associated activity, rumination and interval between heats and may serve as a useful predictor of pregnancy in lactating dairy cows (Madureira et al., 2015a; Tippenhauer et al., 2021a, b). The objective of this observational field study was to evaluate the association between HI and insemination outcomes analyzing other possible factors.

MATERIALS and METHODS: A retrospective observational study was conducted across 29 Holstein dairy herds located in Spain using SenseHub™ Dairy (SH) integrated with Gando reproduction software. A total of 8,365 estrus alerts registered resulting in AI were analyzed. Animals were classified by HI into three categories: low (<69), moderate (70–89), and high (>90); and by parity. Additional covariates included estrus detection method (natural estrus vs. synchronization protocol) or AI number.

RESULTS: Overall P/AI was 35.6% (2,975/8,365). Pregnancy rates increased significantly with HI category: low: 24.4% P/AI (95% CI: 20.3–28.5%; n=426), moderate: 32.2% (95% CI: 29.8–34.6%; n=1,457), and high: 37.1% (95% CI: 35.9–38.2%; n=6,482; P<0.001). Binary analysis confirmed greater P/AI for cows with high HI (≥90) vs. moderate-low HI (<90) (37.1% vs. 30.4%; difference: +6.7 p.p.). Parity had a marked effect on fertility; however, within each parity group, high HI was consistently associated with superior P/AI, ranging from 43.4% in heifers to 29.5% in cows with ≥4 parities. Cows inseminated following a synchronization protocol (25.48% of AI and 82.18% after high-fertility synchronization protocols) with high HI showed greater P/AI (41.7%) than those detected in spontaneous estrus (36.8%), likely reflecting differences in underlying reproductive

status. AI number also influenced fertility: P/AI was highest at first AI (40.4%) and declined progressively with subsequent inseminations (37.0%; 36.5 and27.8% at 2ndo to fourth or beyond AI). Substantial between-herd variability was observed, with P/AI ranging from 47.8% to 96.3%, highlighting the impact of herd-level factors; however correlation between HI and P/AI is preserved throughout farms.

CONCLUSIONS: These findings demonstrate that HI is a reliable indicator of fertility potential under commercial Spanish dairy conditions. A HI ≥90 is consistently associated with higher pregnancy rates regardless of AI number. Incorporating estrus detection method and insemination number into the analysis enables a more precise interpretation of reproductive outcomes and supports the clinical utility of HI as a decision-support tool in dairy cattle reproductive management programs.

Keywords: estrus detection, heat index, pregnancy rate, artificial insemination, dairy cows

Early Rumination as a Functional Indicator of the Transition from Milk Fed Calf to Ruminant

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BACKGROUND and OBJECTIVE: The transition from liquid to solid feed is a critical stage for dairy calves, yet early detection of suboptimal adaptation remains difficult under commercial conditions. Rumination time (RT), measured through automated monitoring systems (AMS) may offer an objective indicator of rumen development. This study aimed to determine whether weekly mean rumination time (WMRT) between 43–50 days of age can predict subsequent body weight (BW) and health outcomes up to 210 days.

MATERIALS and METHODS: The study was conducted on a dairy farm in Huesca, Spain, using 384 group housed calves. All calves received two colostrum feedings, transition milk until day 3, and were then moved to an automatic milk feeder from days 3 to 75. They were kept in groups of 14 and fed a high nutrition milk plan with gradual weaning and ad libitum starter. SenseHub™ Dairy Youngstock tags were attached at ~30 days. Individual body weight was measured at 3,75, 147 and 210 days. Based on WMRT from days 43–50, calves were classified as high rumination (RumHIGH; ≥200 min/day, n = 278) or low rumination (RumLOW; <200 min/day, n = 106). RT, BW, milk intake, and health events were recorded until 210 days. Statistical analyses used SAS 9.4 (logistic regression for binomial outcomes; ANOVA for continuous variables).

RESULTS: From weeks 5 to 22, both groups showed increasing rumination, but RumHIGH calves maintained higher values (317 ± 10 vs. 279 ± 10 min/day; $p < 0.001$). RumHIGH calves exhibited a faster rise in RT between weeks 5 and 11, reaching peak levels by week 6, whereas RumLOW calves increased more gradually, achieving similar levels only around week 13. Rumination between weeks 5–13 was significantly higher in RumHIGH (264 ± 10 vs. 200 ± 10 min/day; $p < 0.001$). No group differences were observed in milk intake, weaning weight (~110 kg), or incidence of diarrhea and pneumonia. However, RumHIGH calves achieved greater body weights at 147 days (176 vs. 167 kg; $p = 0.001$) and 210 days (241 vs. 231 kg; $p = 0.004$).

CONCLUSIONS: Weekly rumination measured before weaning was associated with distinct rumination trajectories and post weaning growth. Calves with higher early rumination established rumination behavior earlier and more consistently, translating into superior post weaning body weight. Early rumination appears to be a promising functional indicator of the transition from milk fed calf to ruminant and may help identify animals undergoing suboptimal transition in intensive systems.

Keywords: rumination time, dairy calves, body weight, weaning, growth outcomes

Effect of Artificial Insemination Compliance Based on Automated Heat Detection on Conception Rate at 30 Days Post-Ai: A Case Study from a Dairy Farm in Turkey

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BACKGROUND and OBJECTIVE: In dairy cattle, fertility management is a key driver of production and profitability. On larger farms, automation is increasingly used to improve efficiency and reproductive performance. Activity monitoring systems enable continuous, individual-level detection of behavioral changes associated with estrus and health status and are becoming a new gold standard compared with visual heat detection. However, the insights provided by monitoring systems must be followed by timely human actions to achieve the desired reproductive outcomes. Conception rate (CR) is a key performance indicator of reproductive efficiency, directly affecting herd profitability and sustainability. The objective of this case study was to evaluate the effect of artificial insemination compliance with automated heat detection alerts (AI-AHD) on conception rate at 30 days post-AI.

MATERIALS and METHODS: During 2025, a large dairy farm in Turkey (around 1000 lactating dairy cows) equipped with an AHD and monitoring system (SenseHub Dairy™, MSD Animal Health) was followed. Monitoring collars were installed on virgin heifers (nulliparous), primiparous cows (first lactation), and multiparous cows (second or higher lactation). AIs performed based on AHD alerts were recorded every 10 days. Pregnancy diagnosis was performed systematically by transrectal ultrasonography 30 days after the recorded AI to determine CR. A 75% AI-AHD compliance threshold was defined for all animal categories. AI-AHD compliance was calculated as the proportion of AIs performed in response to AHD alerts. Conception rates were evaluated at ≥75% and <75% AI-AHD compliance for heifers, primiparous and multiparous cows.

RESULTS: A total of 5,178 AIs were recorded during 2025: 2,515 in heifers, 984 in primiparous cows, and 1,679 in multiparous cows. Overall, heifers had a higher CR (48.7%) than primiparous (45.0%) and multiparous cows (40.2%). AI-AHD compliance was highest in multiparous cows (84.7%), followed by primiparous cows (77.9%) and heifers (75.7%). Conception rates in animals with ≥75% AI-AHD compliance versus <75% were 47.4% vs 50.8% for heifers, 46.6% vs 46.6% for Primiparous cows and 41.0% vs 34.2% for Multiparous cows respectively. Higher AI-AHD compliance was associated with improved CR in multiparous cows, while no clear numerical difference was observed in heifers and primiparous cows.

CONCLUSIONS: Cow monitoring systems can support improved fertility management on dairy farms. In this case study, higher compliance with AHD-based AI guidance was associated with increased conception rate in multiparous cows, highlighting that the effectiveness of cow monitoring technologies depends not only on accurate estrus detection but also on consistent and timely action by farm management.

Keywords: Smart Farming, Dairy Farming, AI

Developing Data Driven Management Decision Support Tools for Dairy Stability and Sustainability Indices (SASI)

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BACKGROUND and OBJECTIVE: Animal well-being is central to sustainable dairy production and

maintaining social license to farm animals. However, objective welfare monitoring remains limited because current assessments rely on intermittent, subjective scoring. This project develops three data driven tools that provide objective time series indicators of cow health, resilience and welfare, collectively termed Sustainability and Stability Indices (SaSI).

MATERIALS and METHODS: Precision Livestock Farming (PLF) time series tools were integrated into a smartphone application using daily milk yield and sensor data. i. Transition The SaSI Transition Score (SaSI T) quantifies early lactation performance by comparing projected (P_{305}) and expected (E_{305}) 305 day yields. P_{305} was derived from latest test day records (TDR) using Interpolation with Standard Lactation Curves (ISLC). E_{305} was predicted using a multi output Random Forest trained on the first eight TDRs of the previous lactation across 41 commercial herds (n = 5,543 cows; mean herd size = 135). Positive SaSI T indicates successful transition; negative values reflect challenges. ii. Resilience Resilience was quantified using the log of milk yield residual variance from days in milk (DIM) 11–295 on 13 AMS farms (4,001 cows; mean herd size = 308). Lower variance implies higher resilience. Each cow received a ranking SaSI Resilience Score (SaSI R) per lactation. Early lactation SaSI-R_t at various DIM cut-offs were compared with full-lactation SaSI-R_{305}. iii. Welfare A pilot Welfare Score (SaSI W) was developed using PLF sensor data and validated against observational assessments on farm. Welfare audits were conducted by a trained veterinary assessor on a PLF equipped reference population (2 herds; n = 40 cows). Assessments included the Cow Positive Welfare Score (CPWS), based on facial expression (eyes, muzzle, ears) and head position. These manual scores were correlated with simplified PLF derived models, including milk only features.

RESULTS: Transition: P_{305} accuracy improved substantially from one to three TDRs (RMSE 1949.98 → 1230.02 kg; Spearman correlation 0.61 → 0.83), with minimal gains thereafter. SaSI T was therefore based on ≥2 TDRs. E_{305} prediction achieved per test day RMSE 5.71 kg and mean absolute percentage error (MAPE) 10.61%. Resilience: Early lactation ranking at 30-150DIM correlated 0.58–0.81 with RR_{305}. Rankings after ~90 DIM (r = 0.72) could potentially inform management decisions. Welfare: Median CPWS was 2.25. Correlation analyses evaluating how PLF indicators and milk-only features infer observational welfare scores are ongoing.

CONCLUSIONS: These tools offer potential indicators of transition success, resilience and welfare, supporting decision making and data driven authenticated overview of lifelong cow well being.

Keywords: Data-driven, decision-support, dairy, stability, sustainability, resilience

Early Life Behavior Assessed by an Automated Behavior Monitoring System and Health, Survival, and Subsequent Milk Production in Holstein Dairy Cattle

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BACKGROUND and OBJECTIVE: Automated behavior monitoring technologies assist producers to identify calves that require attention. Nonetheless, reports demonstrating the association between early life behavior captured by monitoring systems and long-term performance is limited in the literature. Therefore, the objective of this study was to determine whether early life behavior assessed through an automated monitoring system was associated with health and survival before calving, and milk production in the first lactation.

MATERIALS and METHODS: This observational retrospective study was conducted in one farm located in the USA. A total of 1,244 calves were fitted with a behavior monitoring system (SenseHub Dairy Youngstock, Merck Animal Health, Rahway, NJ, USA) after birth. Using behavioral data from the system, a score was established at 50 days of age for each calf. Scores 1, 2, and 3 corresponded to excellent, intermediate, and poor behavior, respectively. Information regarding respiratory treatments, mortality, sale, and milk production in the first 140 days in milk (DIM) were evaluated. Dichotomous variables were analyzed by logistic regression. Milk data was analyzed by ANOVA for

repeated measures. Time to culling was analyzed by Cox proportional hazard regression.

RESULTS: The distribution of calves scored as 1, 2, and 3 were 10%, 54%, and 36%, respectively. The proportion of respiratory treatments from birth to calving differed (P < 0.01) across scores (1: 66.1 ± 5.6%; 2: 87.5 ± 1.9%; 3: 97.2 ± 0.8%). Similarly, the proportion of animals that had ≥ 3 respiratory treatments was different across scores (1: 11.8 ± 3.2%; 2: 38.7 ± 2.7%; 3: 73.3 ± 2.4%). Score 1 calves were culled at a slower rate, score 3 at the fastest rate, and score 2 was intermediate between the other scores. The percentage of animals that were not culled and calved were 83.4 ± 3.9%, 76.6 ± 2.2%, 62.5 ± 2.7% for score 1, 2, and 3, respectively. Milk yield differed (P = 0.04) between score 1 and 3 (35.6 ± 0.5 vs. 34.4 ± 0.5 kg/d), whereas score 2 did not differ with the other groups (35.0 ± 0.2 kg/d).

CONCLUSIONS: Behavior before 50 days of age assessed through an automated monitoring system is associated with health, heifer survival, and milk yield in the first lactation. The scoring system successfully identified differences in short- and long-term performance of dairy calves. This study suggests that the scoring system has the potential to be used as a management tool at an individual and herd level.

Keywords: automated monitoring system, youngstock, culling, technology, dairy

Automated Classification of Lung Sounds Using Machine Learning in Pre-Weaned Dairy Calves: A Pilot Study

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BACKGROUND and OBJECTIVE: Thoracic auscultation is a common diagnostic test used in the field to detect bovine respiratory disease in calves; however, its interpretation is subjective and may lead to misdiagnosis, delayed treatment, and inappropriate antimicrobial use. In human medicine, artificial intelligence has been increasingly applied to support the automated classification of lung sounds, whereas its use in veterinary medicine remains limited. The aim of this study was to develop and evaluate a machine learning–based algorithm for the classification of lung sounds in pre-weaned dairy calves.

MATERIALS and METHODS: The dataset consisted of 296 lung sound recordings collected under field conditions and categorized using two classification schemes: a binary system ("healthy" vs. "not healthy") and a five-class system ("healthy", "crackles", "increased bronchial sounds", "increased breath sounds", and "wheeze"). The class distribution included 110 healthy, 43 crackles, 49 increased bronchial sounds, 62 increased breath sounds, and 32 wheeze recordings. Two classifiers from the scikit-learn library were evaluated: Gradient

Boosting and Random Forest. For both classification tasks, the dataset was split into training (80%) and testing (20%) subsets. Feature extraction and audio analysis were performed using the Librosa library in Python, comparing Mel-frequency cepstral coefficients and Mel-scaled spectrograms, converted to decibel (dB) scale prior to training. Model performance was assessed using stratified 10-fold cross-validation on the training set, with accuracy as the primary metric, and hyperparameters were optimized via grid search.

RESULTS: For binary classification, the best-performing model used Mel-scaled spectrograms with a Random Forest classifier, achieving an accuracy of 89.4% on the training set and 88.3% on the test set, with an F1 score of 0.82. For the five-class classification, the optimal model used Mel-frequency cepstral coefficients features with a Random Forest classifier, achieving an accuracy of 61.8% on the training set and 55.0% on the test set, with an F1 score of 0.55.

CONCLUSIONS: These results demonstrate that machine learning approaches have significant potential for the classification of lung sounds in calves, particularly for binary discrimination between healthy and diseased animals. This approach could support veterinarians and producers as a practical screening test under field conditions. Larger datasets and further validation are needed to improve multi-class classification performance.

Keywords: Bovine respiratory disease, lung sounds, Mel-frequency cepstral coefficients, Mel-scaled spectrograms, machine learning, artificial intelligence

Predicting Live Weight in Nomadic Guney Karaman Sheep Using Catboost: A Birth-Record-Only Approach with Meteorological Data

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BACKGROUND and OBJECTIVE: Nomadic sheep production systems present a particular challenge for precision livestock farming: breeders have no access to controlled environments, records are sparse, and the logistical burden of repeated weighing is high. For Güney Karaman sheep managed under nomadic conditions in Türkiye, timely estimation of live weight holds direct economic value, as income depends almost entirely on meat production at the point of sale. The objective of this study was to assess whether a gradient boosting model trained exclusively on birth records and meteorological data — with no intermediate weighing — could reliably estimate live weight.

MATERIALS and METHODS: Records from 21,085 Güney Karaman sheep, collected between 2023 and 2025 from flocks participating in the National Sheep Improvement Programme in Mersin, Türkiye, were used. Predictor variables included birth weight, sex, birth type, birth date, age of dam, and average meteorological measurements between birth and final weighing — comprising temperature-humidity index (THI), precipitation and wind speed. Notably, weaning weight was deliberately excluded to enable prediction at birth, without requiring an intermediate measurement. CatBoost, a gradient boosting algorithm with native handling of categorical features, was trained and evaluated using a stratified nested cross-validation framework with a 5-fold outer loop and 3-fold inner loop for hyperparameter optimisation. Model performance was assessed over 10 independent runs using R^2, root mean squared error (RMSE), mean absolute error (MAE), and mean absolute percentage error (MAPE).

RESULTS: CatBoost produced consistent predictions across runs, with mean R^2 = 0.787, RMSE = 2,856 g, and MAE = 2,024 g on the held-out external test set (n = 4,217). MAPE averaged 8.0%, reflecting reasonable percentage-based accuracy given the

absence of any post-birth weight measurements. SHAP analysis identified the day of weighing, age of dam, and meteorological variables — particularly THI and precipitation — as the most influential predictors, with birth weight contributing a comparatively modest effect.

CONCLUSIONS: A CatBoost model trained on birth-stage records and weather data can estimate live weight at a variable measurement age in nomadic Güney Karaman sheep, without any requirement for intermediate weighing. The prominence of meteorological variables alongside the day of weighing underlines the extent to which pasture-based environmental conditions shape growth trajectories in this system. These findings suggest that machine learning tools, when aligned with what breeders can realistically record at birth, may support practical weight estimation and decision-making even under nomadic management conditions.

Keywords: Güney Karaman sheep, CatBoost, Live weight prediction, Meteorological data

Integrating Biosensor Data and Machine Learning For Early Heat Stress Detection in Dairy Cows

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BACKGROUND and OBJECTIVE: Heat stress (HS) poses a significant challenge to dairy production systems, especially during early lactation, when cows are particularly vulnerable to metabolic and physiological disruptions. Traditional approaches for assessing HS, mostly reliant on the temperature-humidity index (THI), sometimes inadequately detect early or subclinical responses. This study sought to provide a comprehensive framework that integrates biosensor-derived physiological and behavioural data with milk quality metrics and machine learning (ML) algorithms to enhance early identification of HS in dairy cows.

MATERIALS and METHODS: Around 36,000 synchronised records were collected from 200 early-lactation Holstein cows on a Lithuanian commercial dairy farm for the first 60 days postpartum. Under license number G2-298, this research followed the Lithuanian Animal Welfare and Protection Law. Intraruminal boluses and in-line milk analysers recorded reticulorumen pH, body temperature, rumination time, cow activity, milk temperature, milk yield, fat, protein, and lactose levels. Environmental conditions were recorded every 10 minutes, and the THI was calculated. Six composite HS conditions were established by integrating THI thresholds with internal physiological, behavioural, and milk-related markers, based on biological rationale and empirical analysis (C1-C6). Five supervised ML algorithms— Random Forest (RF), Neural Network (NN), Partial Least Squares Discriminant Analysis, Support Vector Machine, and Ensemble model—characterized cows by condition. The model's performance was assessed using repeated cross-validation and standard measures, such as the area under the receiver operating characteristic curve (AUC) and the Matthews correlation coefficient.

RESULTS: Among the assessed ML algorithms, the RF and NN models demonstrated the most robust classification efficacy, especially under composite HS conditions C1 (THI > 73 in conjunction with milk temperature > 38.6 °C) and C6 (THI > 74 in conjunction with milk temperature > 38.7 °C), with area under the ROC curve (AUC) values consistently surpassing 0.90. SHAP examination of model interpretability revealed that milk temperature and THI were the primary predictors of HS, although rumination time and milk lactose significantly enhanced classification accuracy under various situations.

CONCLUSIONS: This work illustrates that the incorporation of biosensor-derived physiological, behavioural, and milk quality data with ML models facilitates effective early identification of HS in dairy cows. Composite, biologically informed categorisation criteria surpassed traditional single-indicator methods, especially when internal heat load and behavioural modifications were evaluated in conjunction with environmental stressors. RF and NN models demonstrated significant prediction accuracy, whereas explainable modelling identified milk temperature, rumination duration, and milk lactose as critical early indications.

Keywords: Heat stress, Dairy cattle, Biosensor data integration, Machine learning models, Explainable AI, Precision dairy monitoring.

Artificial Intelligence Models Integrating Biosensors, Blood Biomarkers, and Milk Traits for Lameness Detection in Dairy Cows

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BACKGROUND and OBJECTIVE: Lameness continues to be a predominant health issue in dairy cattle, severely affecting animal comfort and productivity, frequently going undiagnosed due to the constraints of subjective visual evaluation techniques. This study sought to assess the diagnostic efficacy of machine learning (ML) models developed using integrated physiological, behavioural, blood biochemical, and milk composition data for the objective detection of lameness in dairy cows during early lactation.

MATERIALS and METHODS: The study used 272 Holstein dairy cows maintained in commercial farm settings, consisting of clinically lame and healthy control animals matched by parity and lactation stage. This study was performed under licence number G2-298 in compliance with the Lithuanian Animal Welfare and Protection Law. Data were gathered at the point of clinical diagnosis utilising intraruminal biosensors, inline milk analysers, automated milking equipment, and laboratory blood analyses. The resultant dataset included physiological, behavioural, biochemical, and milk-related markers into a cohesive analytical framework. Six supervised ML algorithms—Random Forest (RF), Neural Network (NN), Logistic Regression (LR), Support Vector Machine, k-Nearest Neighbours, and an Ensemble model—were employed to categorise cows as either lame or healthy. The model's performance was evaluated by repeated cross-validation and conventional classification metrics to verify robustness and generalisability of the findings.

RESULTS: Among the assessed ML models, the RF algorithm exhibited superior classification efficacy in differentiating clinically lame from healthy dairy cows, attaining an accuracy of 97.04 ± 4.91%, flawless specificity (100%), and the highest normalised Matthews correlation coefficient (nMCC = 0.94). NN and Ensemble models exhibited robust performance (accuracy above 94%), while LR demonstrated significantly inferior results (accuracy 66.53 ± 6.09%), underscoring the superiority of nonlinear artificial intelligence methodologies. Clinically lame cows demonstrated substantial physiological and metabolic changes, including diminished water consumption ($p < 0.001$), increased body temperature ($p < 0.001$), and elevated amounts of non-esterified fatty acids ($p < 0.001$). The composition of milk was altered, exhibiting diminished protein levels ($p < 0.001$) and decreased lactose content ($p = 0.003$) in lame cows, although milk output, fat content, and temperature were statistically unchanged ($p > 0.05$).

CONCLUSIONS: In summary, the amalgamation of biosensor-derived data with blood biomarkers and milk characteristics using sophisticated ML algorithms offers a reliable, objective instrument for the detection of lameness. This methodology facilitates early, data-informed clinical decision-making and signifies a substantial progression in precision livestock husbandry and the control of dairy cow welfare.

Keywords: Lameness, Dairy cows, Artificial intelligence, Blood biomarkers, Milk composition

Use of Images for Assessing Body Condition Score in Breeding Cows

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BACKGROUND and OBJECTIVE: Body condition score (BCS) is a fundamental tool for assessing the nutritional status of cows and predicting their reproductive response, especially in the postpartum period. However, visual estimation of BCS is subjective, as it depends on the evaluator's experience and the correct application of technical criteria. Within the framework of the digitization of agriculture, the development of tools based on artificial intelligence represents an opportunity to standardize and automate this type of assessment. The overall objective of this study was to generate a database of images of breeding cows correctly classified by body condition, which would serve as input for the development of an application capable

of automatically estimating this indicator using artificial intelligence models. The specific objectives were to design an appropriate methodology for image capture and to build a database representative of the different body condition scores.

MATERIALS and METHODS: The study was carried out in October 2025 at the "Las Nazarenas" farm, located in the department of Flores, Uruguay. The study involved 140 multiparous cows of the Aberdeen Angus and Red Angus breeds, all with calves at foot. Each animal was photographed individually from a standardized rear view and classified by body condition through visual assessment at the time of fieldwork by three evaluators. Subsequently, the images were reclassified in an independent desktop instance. The data obtained were analyzed using descriptive statistics, analysis of variance (ANOVA), simple linear regression models, and Pearson correlation coefficients, using SAS software (V9.4).

RESULTS: The average body condition score determined in the field was 3.42 ± 0.66, with a range between 2 and 5.5, which showed adequate variability for the generation of the database. The regression models were highly significant ($p < 0.0001$) for the three evaluators, with coefficients of determination of 0.83, 0.72, and 0.67. The analysis of variance showed significant differences between observers ($F = 10.66$; $p < 0.0001$), although the evaluator effect explained only 4.9% of the total variability observed. Likewise, the correlations between the field assessment and the assessment based on images were high ($r = 0.82–0.91$), indicating a strong association between the two measurements. It is concluded that the estimation of body condition using images is a technically viable and statistically validated tool.

CONCLUSIONS: The database generated represents a significant contribution to the development of artificial intelligence-based applications, with the potential to improve objectivity, repeatability, and efficiency in the assessment of body condition in livestock breeding systems.

Keywords: body condition, beef cattle, artificial intelligence, image-based assessment

Early Detection and Characterization of Ketosis in Primiparous and Multiparous Dairy Cows Using a Reticuloruminal Bolus-Type Biosensor

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BACKGROUND and OBJECTIVE: Ketosis is a prevalent metabolic disorder in transition dairy cows. Early detection is critical; however, traditional blood sampling is typically delayed until clinical signs emerge. This study aimed to analyze distinct data patterns using a reticuloruminal bolus (equipped with a 3-axis accelerometer and temperature sensor) to enable early differential diagnosis of ketosis.

MATERIALS and METHODS: Twenty-one Holstein cows (5 primiparous, 16 multiparous) were monitored from 30 days pre- to 30 days post-calving using reticuloruminal biosensors. The sensors continuously recorded temperature (T) and 3-axis acceleration metrics (movement magnitude [V], temporal variability [V1], and high-activity frequency [COUNT]). Ketosis was diagnosed via bi-weekly blood sampling (b-HB > 1.4 mmol/l or NEFA > 0.7 mmol/l). Data were analyzed using two-way repeated-measures ANOVA and t-tests to evaluate the effects of parity and health status.

RESULTS: During the postpartum period, multiparous ketotic cows exhibited elevated body temperatures (+0.33 °C, $p < 0.05$), whereas primiparous cows showed a non-significant delayed rise. Behaviorally, ketotic cows displayed significantly higher high-activity frequency (COUNT; Primiparous: Control 12.5 ± 10.6 vs. Ketosis 17.6 ± 9.2, $p < 0.001$; Multiparous: 20.5 ± 14.3 vs. 23.8 ± 14.7, $p < 0.01$) and significantly lower individual

movement vigor (V; Primiparous: 1053.6 ± 136.3 vs. 1027.0 ± 99.6, p < 0.001; Multiparous: 1026.0 ± 97.0 vs. 1023.1 ± 87.8, p < 0.001). Movement variability (V1) numerically increased but lacked statistical significance. Clinical ketosis was diagnosed at 8.5 ± 3.5 and 18.4 ± 6.8 days postpartum for primiparous and multiparous cows, respectively, with sensor anomalies detectable 4–6 days prior. Excluding the calving window (day -1 to +1) to isolate metabolic effects, ketotic cows consistently exhibited higher activity frequency (COUNT; Primiparous: 11.6 ± 7.3 vs. 17.3 ± 2.3; Multiparous: 21.7 ± 5.4 vs. 25.2 ± 6.1) and altered temporal variability (V1; Primiparous: 1208.4 ± 96.1 vs. 1220.8 ± 69.3; Multiparous: 1274.2 ± 54.9 vs. 1261.9 ± 57.8). In contrast, individual movement vigor (V) was distinctly lower (Primiparous: 1061.3 ± 52.2 vs. 1021.5 ± 6.9; Multiparous: 1024.7 ± 14.1 vs. 1018.6 ± 7.4). While these pre-diagnostic trends clearly indicated a state of restlessness without physical vigor, statistical significance varied across specific days and metrics (p = 0.002–0.890).

CONCLUSIONS: This study reveals that ketosis manifests through divergent, parity-specific thermal and behavioral changes. To establish these findings as a universal early diagnostic algorithm, future research must validate these patterns using large-scale cohort data and apply advanced analytics, such as machine learning models.

Keywords: Ketosis, Transition dairy cows, Reticuloruminal biosensor, Early detection, Parity

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Zero-Shot Visual Assessment of Ovine Colostrum Quality by General-Purpose Multimodal Artificial Intelligence Models

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BACKGROUND and OBJECTIVE: Neonatal mortality in sheep is largely attributable to failure of passive transfer (FPT), which depends on timely ingestion of high-quality colostrum. Digital Brix refractometry (DBR) is a validated field tool for estimating colostrum immunoglobulin G (IgG); however, its adoption remains limited in resource-constrained settings. General-purpose multimodal large language models (MLLMs) capable of zero-shot visual reasoning from smartphone photographs represent a potentially accessible, training-free alternative. This study evaluated the zero-shot diagnostic performance of three commercially available MLLMs — ChatGPT (GPT-5; OpenAI), Gemini (Gemini 2.0 Pro; Google DeepMind), and Claude (Claude Sonnet 4.6; Anthropic) — for ovine colostrum quality classification, using DBR as the reference standard, in Kivircik-Merino crossbred ewes.

MATERIALS and METHODS: One hundred and twenty clinically healthy Kıvırcık-Merino ewes (parity 1–3) were enrolled. Colostrum samples were collected within the first hour postpartum and measured with a digital Brix refractometer. A threshold of 22% Brix classified samples as high quality (Brix ≥22%) or low quality (Brix <22%). Standardised photographs were captured under uniform conditions and independently submitted to each MLLM via a zero-shot prompt requesting qualitative classification and numerical Brix estimation. One veterinary practitioner with postgraduate experience in theriogenology assessed photographs without access to refractometer data. Diagnostic sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), overall accuracy, Cohen's kappa (κ), area under the ROC curve (AUC), and Bland–Altman bias were calculated for all evaluators.

RESULTS: Of the 120 samples, 69 (57.5%) were classified as high quality and 51 (42.5%) as low quality by DBR. Among MLLMs, Gemini demonstrated the highest accuracy (80.8%), sensitivity (66.7%), specificity (100%), PPV (100%), and κ = 0.630 (substantial). ChatGPT and Claude achieved accuracies of 70.0% and 62.5% with κ values of 0.438 and 0.312, respectively. The veterinary evaluator achieved 81.7% accuracy (sensitivity 68.1%; κ = 0.645; substantial). All evaluators achieved 100% specificity and PPV, with no false-positive classifications. ROC analysis showed excellent discriminatory performance (AUC: Gemini 0.976; veterinarian 0.975; Claude 0.933; ChatGPT 0.929). Bland–Altman analysis revealed systematic Brix underestimation (bias: +4.2 to +5.9 Brix).

CONCLUSIONS: Gemini demonstrated diagnostic performance comparable to a trained veterinary practitioner for zero-shot visual classification of ovine colostrum quality. Perfect specificity across all MLLMs suggests utility as reliable rule-in tools. General-purpose MLLMs may serve as accessible, minimal-cost supplementary screening aids for on-farm ovine colostrum assessment, particularly where refractometry is unavailable.

Keywords: artificial intelligence, brix, colostrum, quality

Developing Quality Assessment of Frozen Bull Semen Using Machine Learning

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BACKGROUND and OBJECTIVE: Reproductive performance in cattle farming depends on both female and male factors, with semen quality playing a potentially critical role. To support confidence in artificial insemination (AI) outcomes, farmers need reliable information on the quality of frozen–thawed bull semen (FTBS) before use. However, accurately assessing FTBS quality in the field is challenging due to its inherent variability and fragility. This study investigates the use of machine learning (ML) to support objective, field based FTBS quality assessment.

MATERIALS and METHODS: The SemenRate® system (RAFT Solutions Ltd, UK) integrates Computer Assisted Semen Analysis (CASA), flow cytometry and light microscopy to objectively measure semen parameters. This study analysed 216 semen batches from 163 bulls, used in 19,896 inseminations with known pregnancy outcomes. Parameters included straight line velocity (VSL), progressive motility (PM), total motility (TM), motile dose (MD), 2 hour thermo resistance testing for TM and PM (TM_Stress, PM_Stress), normal morphology, acrosomal membrane integrity (AMI) and mitochondrial membrane potential (MMP). A machine learning model was developed to characterise semen breeding potential. Eighty percent of the dataset (172 batches) was used for training and 20% (44 batches) for testing. Model validation employed 5 fold cross validation, with each fold using an 80: 20 division. K Means clustering, an unsupervised technique, identified three clusters based on motility and morphology parameters. Expert review proposed these clusters

represented three semen quality grades. Confounding factors such as cow reproductive physiology and farm management were not included as model features. Following clustering, a supervised random forest classification model was trained to categorise batches into the three quality groups. Cluster membership probabilities were used to generate a quality score for each batch, enabling ranking of semen according to their score. Feature importance values, calculated using the Gini impurity metric, quantified each feature's contribution to quality classification.

RESULTS: The random forest model achieved 95% accuracy, correctly classifying 42 of 44 test batches. Cross validation produced an average accuracy of 88%. Feature importance analysis identified TM, PM_Stress, TM_Stress and MMP as the most influential in the classification model.

CONCLUSIONS: Previous research has relied on correlations between individual semen parameters and fertility outcomes. This study presents novel approaches combining K Means clustering, random forest classification and weighted probability scoring. Preliminary findings imply that this model potentially provides objective tools for predicting and ranking FTBS quality prior to AI, independent of cow or farm level confounders. This offers farmers robust, actionable information prior to breeding to support more informed decision making.

Keywords: Semen, quality, assessment, machine-learning

Feasibility of Contactless Radar Sensing for Multispecies Monitoring of Heart and Respiratory Rates in Livestock

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BACKGROUND and OBJECTIVE: Physiological parameters such as heart and respiratory rates can be good indicators of animal health, stress, and disease. In farm and clinical settings, the two main methods to obtain these measurements are usually through manual examination or wearable sensors. However, these methods require close handling, specialised equipment, or animal restraint, which can limit their practicality for routine or repeated monitoring. This study evaluated the feasibility of a contactless sensing approach based on micro-Doppler radar to detect cardiac and respiratory activity in multiple animal species. The system measures subtle body surface movements generated by cardiopulmonary activity and converts them into physiological signals that can be analysed remotely.

MATERIALS and METHODS: Data were collected from four species under routine handling conditions. Included animals were a dairy cow restrained in a crush, a sheep and two pigs housed in a livestock facility at the University of Glasgow. Repeated radar recordings were obtained for each individual animal with simultaneous measurements from a heart rate monitoring device and manual clinical observations. These reference measurements were used to compare radar-derived physiological signals with established monitoring methods.

RESULTS: The initial signal processing demonstrated that periodic motion associated with cardiac activity could be detected in the radar recordings in all studied animal species. The estimated heart rate values which were obtained from the radar signals were broadly consistent with the results from reference monitoring devices. Respiratory patterns were also identifiable within the radar data; however, limited comparative

measurements were available for full validation of respiratory rate estimates.

CONCLUSIONS: These initial results suggest that radar-based sensing could provide a non-invasive method for monitoring animal vital signs. The system requires further validation, through testing with larger datasets and diverse farm conditions, to establish its potential for remote physiological monitoring and less invasive assessment of health and welfare in livestock management.

Keywords: Contactless sensing, Micro-Doppler radar, Livestock monitoring, Heart rate, Respiratory rate, Animal welfare

SURGERY AND LAMENESS

Surgical Management of Notomelia in a Male Korean Native Calf

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BACKGROUND and OBJECTIVE: Polymelia is a congenital anomaly characterized by the presence of one or more supernumerary limbs. When the accessory limb arises from the dorsal thoracic region, the condition is classified as notomelia. Reports of notomelia in cattle are limited, and occurrence in male calves appears uncommon. Surgical management may be complicated by vertebral-scapular fusion, which increases the risk of vertebrospinal injury during complete scapulectomy. Although advanced cross-sectional imaging can improve preoperative anatomical assessment, it is often unavailable or cost-prohibitive in farm animal practice. The objective of this report was to describe the surgical strategy and clinical outcome of notomelia management in a male Korean native calf when advanced imaging was unavailable.

MATERIALS and METHODS: A 24-day-old, 37 kg male Korean native calf was presented with a non-functional supernumerary limb originating from the right dorsal thoracic region. Physical examination revealed no additional abnormalities, and the calf demonstrated normal posture and ambulation. A single lateral thoracic radiograph identified an accessory scapula adjacent to the thoracic spinous processes. Under general anesthesia, the calf was positioned in sternal recumbency. Following sterile preparation with hanging limb draping, a semicircular skin incision was made over the accessory scapula. Blunt and sharp dissection exposed the scapular structure, and major vessels were double ligated using 3-0 polydioxanone. Intraoperative palpation suggested vertebral–scapular fusion with potential risk of vertebrospinal injury. Accordingly, complete scapulectomy was not pursued, and the distal scapula, including the acromion process, was transected. The limb was amputated using an oscillating saw. The exposed bone surface was smoothed, covered with bone wax, and overlaid with a muscle flap prior to routine closure.

RESULTS: The procedure was completed without intraoperative complications. The calf recovered uneventfully and stood unassisted within three hours after surgery. Postoperative antimicrobial therapy was administered for seven days. At 18 days postoperatively, the calf was ambulating normally without neurological deficits. The surgical site healed appropriately, with no evidence of dehiscence, infection, or clinically significant residual scapular prominence.

CONCLUSIONS: Advanced cross-sectional imaging remains the preferred modality for preoperative assessment. However, successful surgical management of notomelia in calves is achievable without it through integration of radiographic evaluation and careful intraoperative palpation. When vertebral–scapular fusion is suspected, distal scapular transection including the acromion may provide a practical and safe surgical landmark. This approach offers a clinically applicable and economically feasible strategy for bovine surgical practice.

Keywords: Cattle, Congenital Abnormalities, Notomelia, Surgical Amputation

The Effect of Meloxicam in Pasture-Grazing Dairy Cattle with Hoof-Horn Lameness on Time to Soundness and Reproductive Performance

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BACKGROUND and OBJECTIVE: Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly recommended in lameness treatment protocols, yet published evidence of benefit in hoof horn (HH) lesions that are non-acute is limited. This study evaluated whether administration of meloxicam at the time of treatment for HH lameness in dairy cattle reduced time to lameness cure and improved reproductive performance.

MATERIALS and METHODS: A randomized clinical interventional trial was conducted on five seasonal-calving, pasture-based dairy farms. Farmers identified lame animals, which were confirmed by trained technicians using a 0-3 locomotion score system (LS; 0 (no lameness; sound) to 3 (very lame)). Cows with LS ≥ 2 and HH lesions were examined by the same veterinarian who performed therapeutic claw trimming and applied wooden blocks as per established treatment protocols. After trimming, cows were randomized to receive either meloxicam or no NSAID (control), with blocking by farm and lesion type. Follow-up LS assessments occurred at a median interval of four days until LS = 0. Conception date and pregnancy status were determined by rectal ultrasound. Primary outcomes were days to lameness cure and days to conception. Time-to-event outcomes were analyzed using Cox proportional hazards models, and binary outcomes of conception within six weeks of breeding and final pregnancy status were analyzed using multivariable logistic regression.

RESULTS: A total of 241 cows were enrolled: 123 meloxicam treated and 118 control. There was no difference in the time taken to become sound (LS=0) between treatment group (p = 0.08). Among 229 cows with reproductive data (117 meloxicam, 112 control), meloxicam-treated cows conceived at a rate 1.42 times faster compared to controls (p = 0.02). Meloxicam-treated lame animals had 15% greater probabilities of conceiving within the first six weeks of the breeding period (63% vs 48%; p=0.04) and being pregnant at the end of the breeding period (85% vs 70%; p=0.02), respectively, compared with control lame animals.

CONCLUSIONS: Adding meloxicam to the treatment of dairy cows with HH lesions did not shorten time to soundness. Given the current body of evidence and application of wooden blocks as per established treatment protocols for lameness, the is not an unexpected finding. Adding meloxicam to the treatment was associated with significant improvements to reproductive outcomes including earlier conception, higher six week in calf rate, and final pregnancy rate. This work adds to the published evidence on the long-term benefits of pain management in dairy cattle with meloxicam.

Keywords: Hoof-horn, lameness, reproduction, NSAIDs

A Practical Field Approach to Severe Unilateral Renal Disease: Two Nephrectomies in Dairy Cows

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BACKGROUND and OBJECTIVE: Unilateral renal pathology, including renal rupture, can present as acute systemic illness in dairy cows. Under field conditions, rapid clinical deterioration and limited time for diagnostics may require immediate decision-making. This two-case report describes a field-based diagnostic and therapeutic approach to suspected right renal rupture, and illustrates how lessons learned from the first case enabled earlier recognition and decision-making in the second.

MATERIALS and METHODS: Two Holstein Friesian cows in early lactation (20 and 31 days in milk) from different farms were examined in the field after sudden milk drop, anorexia and depression. Clinical examination, rectal palpation and urinary catheterisation were performed, supported by hematology, serum biochemistry and urinalysis. In both cases, the cows deteriorated rapidly and severe unilateral right renal pathology with suspected rupture was considered likely. Treatment options

were discussed: conservative therapy, euthanasia, or exploratory right-flank laparotomy with possible nephrectomy. Surgery was elected; preoperative ultrasonography was not performed to avoid delay and because laparotomy was required for definitive diagnosis and surgical removal of the affected kidney.

RESULTS: Both cows had pyrexia and tachycardia, with dehydration and rumen atony. Rectal palpation identified a large, firm right-sided intra-abdominal mass, and catheterisation yielded turbid, hemorrhagic urine. Hematology supported inflammation, while serum creatinine remained within reference limits, consistent with preserved contralateral renal function. Exploratory laparotomy revealed severe perirenal inflammation, a markedly enlarged and dilated right kidney with a thickened ureter, and a ruptured renal lobe with release of red, turbid fluid. The kidney contained clear gelatinous material with fibrin strands. Right nephrectomy was performed after ligation of the renal vasculature and ureter. Cytology and culture indicated mixed infection with *Escherichia coli* and *Trueperella pyogenes*. Case 1 recovered and remained a productive herd member three years after surgery. Case 2 was normothermic within 48 h and produced 20 L/day by day 14.

CONCLUSIONS: Severe renal pathology should be considered in dairy cows with pyrexia, hematuria and a right-sided intra-abdominal mass, even when creatinine is normal. Under field conditions, exploratory laparotomy with the option of nephrectomy can provide both definitive diagnosis and effective treatment when severe unilateral renal pathology is suspected and contralateral function appears preserved.

Keywords: Nephrectomy, Field surgery, Unilateral renal disease, Dairy cow, Renal rupture

Total Duplication of the Femur and Polydactyly in the Same Limb of a Korean Native Calf

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BACKGROUND and OBJECTIVE: Congenital limb malformations are structural abnormalities present at birth and are relatively common in cattle, often arising from genetic or environmental factors. While various forms of limb malformations have been documented, complete femoral duplication—where two distinct femurs are present within a single limb—is exceptionally rare in both human and veterinary medicine. To date, no case of concurrent complete femoral duplication and distal polydactyly in the same limb has been reported in cattle. The objective of this report is to describe the clinical presentation, surgical management, and successful rehabilitation of this unique musculoskeletal anomaly in a neonatal calf.

MATERIALS and METHODS: A 2-day-old, 31 kg male Korean native calf was presented with an inability to stand and congenital polydactyly of the right hindlimb. Physical examination revealed abnormal bony protrusions in the femoral region and five fully developed hooves. Radiographic evaluation confirmed the presence of two distinct femoral shafts and heads within the right hindlimb, alongside multiple supernumerary metatarsal bones and phalanges. Under general anesthesia using sevoflurane, surgical intervention was performed on day 6 of life. The procedure involved the surgical excision of the duplicated femur and distal limb amputation while preserving the proximal metatarsal bones for stability.

RESULTS: Postoperative radiographs confirmed the successful removal of the duplicated structures. To facilitate recovery, a structured rehabilitation program was initiated using a large-breed dog wheelchair to promote weight-bearing. Therapy included forelimb-guided stimulation, hindlimb

massage, and passive range of motion exercises conducted twice daily. No postoperative wound infections or complications were observed. At the 1-month follow-up, the calf achieved the ability to stand independently, although a partial weight-bearing deficit remained.

CONCLUSIONS: This case represents the first documented evidence of concurrent complete femoral duplication and polydactyly in a calf. The findings demonstrate that early diagnosis through radiographic imaging combined with timely surgical intervention and structured rehabilitation can significantly enhance animal welfare and functional recovery. This report offers valuable clinical insights for managing complex congenital musculoskeletal anomalies in livestock.

Keywords: Cattle, Congenital Abnormalities, Femur, Polydactyly

Identification of Risk Factors for Lameness in All-Year Grazing Dairy Cows on Terceira Island

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BACKGROUND and OBJECTIVE: Lameness is a major contributor to economic losses in dairy production systems and represents a significant animal welfare concern. Contrary to widespread assumptions, lameness is also highly relevant in pasture-based dairy systems. In the Azores Islands, dairy cows are 24h on pasture, except during milking periods. Understanding the risk factors associated with lameness in these herds is essential for the development of effective prevention and control strategies.

MATERIALS and METHODS: An analytical cross-sectional observational study was conducted to estimate the prevalence of lameness in grazing dairy herds and to identify associated risk factors within this milk production system. Fifteen dairy herds from Terceira Island (Azores) were enrolled. The target population comprised adult lactating cows and pregnant heifers. Data collection included a structured questionnaire administered to farmers and standardized mobility scoring sessions performed on animals. Additionally, records from the routine hoof inspection and trimming program were analysed to determine the most prevalent hoof lesions in these herds.

RESULTS: Mobility scoring revealed a mean lameness prevalence of 21%. Hoof trimming records indicated that the most prevalent disorders were white line disease, thin sole, and subsolar abscess. Multivariable analysis identified thirteen principal risk factors associated with lameness: larger herd size ($p < 0.001$); inclusion of biotin in the concentrate ($p = 0.012$); reduced grazing time ($p = 0.006$); presence of a pre-milking waiting area ($p = 0.002$); concrete flooring in the waiting area ($p = 0.002$); longer waiting time before milking ($p = 0.039$); presence of a collecting yard preceding the milking parlour waiting area ($p < 0.001$); concrete flooring in the collecting yard ($p < 0.001$); milking system type ($p = 0.004$); mean duration of each milking session ($p = 0.019$); number of daily movements between pasture and the milking facility ($p = 0.002$); mean duration of daily movements ($p < 0.001$); and mean distance travelled during daily movements ($p < 0.001$).

CONCLUSIONS: Lameness prevalence in permanently pasture-based dairy cows can be substantial. Infrastructure associated with daily milking—particularly waiting areas, collecting yards, milking systems, and the quality and extent of walking routes—constitutes important risk factors. Targeted management and facility improvements should therefore be prioritised in pasture-based dairy systems to mitigate lameness risk.

Keywords: dairy cow welfare, pasture, lameness risk factor, Azores

Lumbar Epidural Anaesthesia for Standing Flank Surgery in Dairy Cattle

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BACKGROUND and OBJECTIVE: Standing flank laparotomy is a common surgical procedure in cattle and requires effective regional anaesthesia to ensure animal welfare and surgical success. Conventional local infiltration techniques, such as line blocks, are widely used but have limitations, including large anaesthetic volumes, inconsistent analgesia, and potential negative effects on wound healing. Lumbar epidural anaesthesia (LEA) has been used for more than forty years in Japanese cattle practice to provide effective anaesthesia for standing flank surgery. Reported advantages include low drug-volume requirements, limited interference with tissue handling and wound healing, and the ability to achieve circumferential trunk anaesthesia while maintaining hindlimb function. Despite this long history of use, LEA has not been evaluated in many other countries, where breed types, body size and management systems may differ. This study aimed to assess the clinical efficacy, safety, and practicality of a small fixed-volume LEA protocol in adult dairy cows undergoing standing rumen fistulation surgery in New Zealand, and to evaluate its potential as an alternative to conventional local anaesthetic techniques.

MATERIALS and METHODS: Fourteen healthy, non-pregnant, and non-lactating Kiwi-cross (Jersey x Holstein-Friesian) cows were enrolled. LEA was performed at the L1-L2 intervertebral space (or at T13-L1) using an 18-gauge, 130mm epidural needle. A mixture of 2% xylazine (0.4-0.6ml) and lidocaine (4.6-5ml) was injected slowly after confirming needle placement with the hanging-drop method. Analgesic onset was evaluated over 15 minutes. Assessed parameters included ease and success of epidural access, quality and duration of analgesia (pinprick and surgical response), motor function, physiological parameters (e.g. skin temperature changes), behavioural responses such as sedation and ataxia, and complications during or after surgery.

RESULTS: Epidural space access was successful in 11/ 14 cows (78.5 %); failures were associated with a narrow intervertebral space. Among cows with successful LEA, 10/11 (90.9 %) achieved excellent analgesia for surgery. Mild ataxia (swaying or shifting weight) occurred in 8/11 (72.7 %) cows, but none became recumbent or showed difficulty returning to their recovery pens. No postoperative injection-site complications were observed.

CONCLUSIONS: This study demonstrates that LEA is a safe, practical, and effective anaesthetic technique for standing flank surgery in New Zealand dairy cattle. The combination of low drug volume, reliable analgesia, and preservation of hindlimb function suggests that LEA is a viable alternative to traditional local anaesthetic methods. Further field-based evaluations in diverse production settings and countries are recommended to confirm consistency and broader applicability.

Keywords: Laparotomy, lumbar epidural anaesthesia, cattle, lidocaine

Intensive Antibiotic-Free Footbath as a Management of Digital Dermatitis in Dairy Cattle

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BACKGROUND and OBJECTIVE: Digital dermatitis (DD) is one of the main infectious and endemic hoof diseases, characterized by painful lesions with variable macroscopic appearance. Managing DD lesions remains a challenge and footbaths are the most common preventive measure. Although they are routinely used as a treatment, evidence regarding their isolated therapeutic effect is limited. This study aimed to evaluate the impact of an intensive footbath protocol using 5% HoofCare® on DD lesions without additional treatment and to determine whether individual treatment is necessary.

MATERIALS and METHODS: The study was conducted in the highest-producing lot of a commercial dairy farm, with approximately 360 Holstein cows housed in a free-stall system and averaging 48 kg/day of milk. The protocol consisted of once-daily footbath application after morning milking, five consecutive days per week, for eight weeks. Hind limbs were inspected during milking in a rotary parlor at four time points: D0 (before the first footbath passage), D15, D45, and D60 (final day of the protocol). D30 evaluation was not performed, and all assessments were conducted by the same trained veterinarian. Lesions were classified by M-stage as active (M1, M2, and M4.1), inactive (M3 and M4), or non-existent (M0). Only cows that presented at least one lesion at some point and were present for the full 60 days were included, regardless of whether they had lesions at D0.

RESULTS: Overall, 30 cows and 60 limbs were monitored for the entire period. The statistical unit was the limb. Lesion severity not differed over time (P=0.06) according to the ordinal logistic model in SAS, with lesion prevalence decreasing slightly over the 60 days, from 36.67% (22/60) at D0 to 35% (21/60) at D60, reflecting a 10% reduction in inactive and an 8.33% increase in active lesions. The final incidence of new lesions decreased by approximately 40% compared with initial incidence calculated between D0 and D15. Among limbs without lesions at D0, 78.95% remained lesion-free, while 21.05% (8/38) developed lesions. Active lesions persisted longer: 66.67% (8/12) remained active at D60, and only 16.67% (2/12) healed. In contrast, 70% (7/10) of inactive lesions healed. The cure, control, and prevention rate were respectively: 40.91% (9/22), 65% (39/60), and 78.95% (30/38).

CONCLUSIONS: These results indicate that intensive use of 5% HoofCare® footbaths is a promising preventive and control strategy; however, its therapeutic efficacy appears limited and mainly effective for inactive lesions. Therefore, identifying cows with active DD lesions and treating them individually remains essential.

Keywords: Prevention, Hoof lesions, Treatment, HoofCare®, M-stages

Economic and Environmental Impacts of Footbath with Cooper, Zinc, Tea-tree Oil and Citric Acid

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BACKGROUND and OBJECTIVE: Footbaths are the most common preventive measure for infectious hoof diseases in dairy cattle. However, despite their widespread use and economic relevance, concerns remain regarding their environmental impact, particularly related to residue discharge. This study aimed to describe economic and environmental impacts of a footbath protocol using a commercial product composed of copper citrate, zinc chlorate, tea tree oil, and citric acid (HoofCare®) in a free-stall dairy farm with more than 2,200 Holstein cows.

MATERIALS and METHODS: Footbath protocol was based on previous studies evaluating number of passages through pH analysis and intensive footbath use. A 5% solution was applied three times per week, once daily, for 15 weeks. Considering a pH threshold <5, footbath was replaced after a maximum of 800 cow passages, resulting in three changes per milking. Preparation consisted of adding 20 kg of product, diluting it with a small amount of cold water, and then filling the bath to a minimum depth of 10 cm. To evaluate treatment, prevention and control of digital dermatitis, lesions were classified by stage as non-existent, inactive, or active at eight time points over 15 weeks.

RESULTS: The protocol resulted in a cure rate of 70.12% and a prevention rate of 95.46%, with significant reductions in lesion prevalence and incidence. In 2022, HoofCare® cost per cow was US$0.63 per week (US$30.24/year). Implementing this protocol generated annual savings of US$4.32 per cow compared with copper sulfate (US$34.56/year), which required replacement every

350 cows. Additional indirect benefits were reduced labor linked to the footbath preparation and management. Regarding environmental impact, considering footbath capacity, density corrections, and an estimated 1 cm loss of solution depth every 100 cow passages, copper discharge ranged from 4,133.400 to 4,337.560 mg from the 5% copper sulfate footbath, while the 5% HoofCare® solution discharged 501.869 to 526.658 mg of copper. As this product contains five times less copper concentration than copper sulfate pentahydrate, its use results in lower environmental discharge, even when accounting for the zinc impact (1,003.739 - 1,053.316 mg). Additionally, copper citrate is formed from copper sulfate and citric acid, which is less toxic and more bioavailable, potentially reducing environmental impact.

CONCLUSIONS: HoofCare® represents a promising strategy for managing digital dermatitis, offering economic, practical, and environmental advantages. Further studies are needed to confirm these findings assess environmental impact of tea tree oil.

Keywords: Copper sulphate, Digital dermatitis, Hoof health, Prevention, HoofCare®

Diagnosis and Treatment of Pectoral Granulomatous Wound in a Bull

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BACKGROUND and OBJECTIVE: Pectoral wounds in cattle are frequent clinical findings and may be associated with traumatic or infectious aetiologies. The main differential diagnoses include stephanofilariasis (Stephanofilaria spp. infection), bacterial abscesses, complicated traumatic injuries, and granulomatous inflammatory processes. These conditions may present with swelling, fistula formation, and purulent discharge, requiring thorough clinical assessment and complementary laboratory investigations to establish an accurate diagnosis and appropriate therapeutic approach. The present case report aims to describe aetiologies, differential diagnoses and treatment of pectoral granulomatous wounds in cattle.

MATERIALS and METHODS: A five-year-old Bos indicus bull of the Gir breed from a bovine reproduction centre was evaluated, with a previous diagnosis of stephanofilariasis based on direct parasitological examination. The animal presented a proliferative granulomatous lesion of approximately 20 cm in size, infiltrated and poorly delimited in the pectoral region, with an evolution of approximately four months, with about a 10-cm diameter ulcerated surface and associated with fistula formation and purulent discharge. The lesion was cleaned and surgically excised, removing a fibrous mass associated with the affected area, followed by haemostasis through cauterization. During the procedure, abscess cavities containing purulent exudate were identified.

RESULTS: Collected samples were submitted for parasitological re-evaluation for stephanofilariasis, which yielded negative results. Microbiological culture identified Trueperella pyogenes, establishing the definitive diagnosis of a chronic bacterial granuloma associated with abscess formation. The antimicrobial susceptibility testing revealed sensitivity to enrofloxacin which was administered at a dose of 5 mg/kg SC SID for 30 consecutive days. Postoperative management consisted of local wound care, including periodic cleaning and topical insect repellent to promote tissue protection and support healing. Clinical follow-up was maintained until complete wound closure, which occurred approximately two months after surgical intervention.

CONCLUSIONS: This case highlights the importance of performing a thorough differential diagnosis when evaluating pectoral lesions in cattle. Reliance solely on initial clinical suspicion may lead to misdiagnosis and inappropriate treatment. Definitive etiological identification through complementary diagnostic methods is essential to guide therapeutic decision-making, improve prognosis, and reduce economic losses, particularly in high-value animals such as breeding bulls.

Keywords: cattle, stephanofilariasis, Trueperella pyogenes, granulomatous wound

Field Caesarean Section in Cattle: Contributing to Best Practice Evidence

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BACKGROUND and OBJECTIVE: Caesarean section in cattle is commonly performed yet has scant evidence base for best practice. There are some publications on success rates, however limited reporting on factors potentially influencing outcomes. This presentation will put the results of the authors' prospective cross-sectional observational study into context of evidence for best practice procedural aspects.

MATERIALS and METHODS: A convenience sample of veterinary surgeons across five UK practices was invited to report patient and procedure details every time they performed a cattle caesarean section over a 13-months period. Cases were followed up by telephone call to the farmer at 7 days post-operative regarding dam and calf survival, and at 60 days regarding dam survival, health and fertility.

RESULTS: A total of 280 cases were submitted (87.9% beef, remainder dairy). All but two dams and 84.2% of calves were alive at point of veterinarian leaving the farm. Of 246 cases with follow-up data, seven-day survival for calves was 80.1%, and for dams 97.6%. Key influencing factors for dam and calf survival were position during, and duration of, the surgery. Pre-operative calving assistance affected calf survival and oestrus expression at 60 days post-op. The use of clenbuterol also affected oestrus expression. Just over 10% of surgical wounds were reported as 'dirty' by the farmer at the 7-day post-op follow up. Preferred technique for the majority of surgeons was: left flank approach; clipping followed by a 2-step scrubbing protocol with either chlorhexidine or povidone-iodine then surgical spirit; catgut for both uterus and muscle closure; two layers of inverted uterine suture. Less than one-third of surgeons wore sterile gowns, and none wore sterile gloves. Almost all cases received systemic NSAIDS and antibiotics, administered pre-operatively in about three-quarters of patients. Clenbuterol pre-op and Oxytocin post-op were administered in a high proportion of cases.

CONCLUSIONS: Field caesarean section to address dystocia carries a good prognosis for dam and calf survival. The high dam survival rates in this study are particularly encouraging in light of free text comments describing problems encountered during the procedure in multiple cases. The pre-operative administration of NSAIDs and antibiotics follows good practice. Lack of wearing surgical gloves is of concern regarding risk of adhesion formation. Number of suture layers used suggests a desire for a 'safety net'.

Keywords: caesarean, survival, fertility, best practice

A Survey Exploring Hoof Health Practices on UK Dairy Farms

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BACKGROUND and OBJECTIVE: The UK Hoof Health Registry (UKHHR) collates electronic hoof health records into a national database to improve genetic evaluations for lameness and for epidemiological research. This study aimed to explore (i) farmers current management of hoof health, (ii) how hoof health data is being captured, stored and used (iii) use of genetic evaluations and pattern analysis tools to manage lameness and (iv) barriers to farmers sharing data with UKHHR.

MATERIALS and METHODS: A cross-sectional online survey (maximum 40 mixed-type questions) was designed by the research team, piloted on farmers and distributed non-randomly by multiple platforms. The target population was UK dairy farmers (n=7,000) who were key decision makers regarding lameness management.

RESULTS: A total of 167 responses covered the four UK nations. Routine hoof trimming was conducted on 77% (n=129) of respondent's herds, with the majority of these (47%, n=61) occurring biannually. Of those respondents who are routine trimming, 76% (n=127) of them would record animals as having had a hoof inspection. For hoof trimming events where a

problem was identified, 72% (n=121) would record all observed lesions and all treatments applied. Paper recording systems were utilised by 23% (n=38) of respondents, 32% (n= 52) used a combination of paper and electronic recording, while 45% (n=73) used electronic recording only. Time and cost were commonly cited as barriers to recording hoof health data, with usability of software and hardware capacity cited as barriers to utilising electronic recording systems. Genetic evaluations were being utilised by 42% (n=70) of respondents to support hoof health, 28% (n=46) of respondents were unsure if they were, and 31% (n=51) were not using them. When asked what genetic evaluations were being used, 24% (n=12) of respondents described the Lameness Advantage genetic evaluation, in comparison to 62% (n=31) who described conformation traits (e.g. leg conformation). Hoof health data analysis was utilised by 53% (n=89) of respondents, with 43% (n=11) of those analysing the data monitoring prevalence/incidences of lesions causing lameness. When asked how likely they were to share data with the UKHHR, 66% (n=109) of respondents replied "likely" or "very-likely", with 17% (n=29) replying "unlikely" or "very-unlikely". Further results will be reported at the conference.

CONCLUSIONS: Our study has provided insight into how UK dairy farmers are currently managing hoof heath, and the quality and quantity of data available to the UKHHR. It has identified barriers to improving the recording of hoof health data and the uptake of tools to manage lameness.

Keywords: Lameness, Hoof Health, Genetics, Survey, Dairy, Motivators

Diagnostic and Therapeutic Approaches in Cattle with Oral Cavity Trauma

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BACKGROUND and OBJECTIVE: Oral injuries in cattle are not uncommon and exhibit considerable variation in both extent and anatomical location. In ruminants, such injuries must be regarded as prognostically more critical than in monogastric species. In a substantial number of cases, these lesions result in impaired feed intake and reduced rumination activity due to mechanical trauma, inflammatory processes and pain. The clinical work-up of three patients suffering from oral trauma, with special emphasis on pain assessment and management will be presented.

MATERIALS and METHODS: Three clinical cases of cattle with injuries involving the oral cavity and associated structures were retrospectively evaluated. All animals underwent thorough physical examination, including detailed inspection and palpation of the oral cavity. Diagnostic imaging, particularly radiography and ultrasonography, was performed when deeper soft tissue or osseous involvement was suspected. Treatment strategies included surgical debridement and reconstruction when indicated, appropriate anaesthetic management, and implementation of multimodal analgesia. Postoperative monitoring focused on feed intake, rumination activity, wound healing, and overall clinical condition. Additionally, the Botucato pain scale was used for repeated pain assessment and evaluation. Supportive care included adapted feeding regimens and intensive nursing management.

RESULTS: Oral injuries in cattle varied considerably in severity and anatomical localization. Impairment of feed intake and rumination was common and represented a critical prognostic factor. Diagnostic

imaging findings enabled a reliable assessment of therapeutic feasibility. Clinical outcomes were frequently more favourable than initially anticipated, particularly if early surgical intervention was combined with effective and closely monitored pain management. Adequate analgesia was strongly associated with restoration of feed intake and rumination. Intensive postoperative care and dietary modification further improved recovery rates.

CONCLUSIONS: Clinical experience gained in recent years at the Clinical centre for Ruminants, Camelids and Herd Health Management, University of Veterinary Medicine Vienna, indicates that oral injuries in cattle frequently carry a more favourable prognosis for functional recovery than previously assumed. Close monitoring of analgesic efficacy, intensive nursing care, and appropriate dietary management appear to be the most critical factors contributing to successful recovery.

Keywords: surgery, pain assessment, pain management

Congenital Atypical Giant Cutaneous Neurofibroma in an Angus Calf: Surgical Management and Diagnosis

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BACKGROUND and OBJECTIVE: This case highlights the clinical presentation, diagnostic features, and surgical management of a cutaneous neurofibroma, emphasizing the importance of clinical decision making and the role of pathology in treatment planning and assessing the feasibility of full productive achievement. The objective of this case was to describe the advanced diagnostic imaging investigation (by X-ray and CT) and to describe the "customized" marginal resection, surgical skin reconstruction and the healing pattern of the hock region.

MATERIALS and METHODS: This study presents the case of a 1-week old Angus calf, admitted to the Surgery Clinic of the Faculty of Veterinary Medicine in Cluj-Napoca with an atypical non-painful mass, located on the medial side of the distal tibia and left hock. Clinical examination revealed a congenital, giant, pediculate, pendulous, mobile mass, attached to the deep facial plane. Radiographic and computed tomography investigations were subsequently performed, revealing a large, heterogeneous, very low-density mass (based on Hounsfield Unit values), with fluid accumulation, presence of cystic structures, and no invasion of osteoarticular compartment. The calf underwent general inhalatory anesthesia, followed by a customized marginal resection and skin reconstruction. Surgery was performed through a medial approach, and due to the high vascularization of the tumor, Ligasure was used for hemostasis. The wound was partially sutured over a drain in a routine pattern. A post-operative protective bandage was applied, with topical L-Mesitran gel.

RESULTS: The diagnostic in this case was based on the integration of clinical appearance, imaging findings, and pathology examination. Surgical management followed oncological principles, adapted to the specific characteristics of farm animals, and aimed to provide optimal wound healing after resection. Histopathological analysis revealed a myxoid stroma, spindle cells dispersed in a collagenous matrix; anisocytosis and anisokaryosis were mild, and the tumor showed marked vascularity. Postoperatively, the patient regains a complete range of motion, with no lameness and observable signs of pain, or gait alteration, ultimately achieving full productive performance.

CONCLUSIONS: The favorable postoperative outcome in this case of giant neurofibroma, together with the recovery of full productive performance, supports that surgical tumor resection in farm animals can be an effective, reliable, and curative therapeutic approach while maintaining overall animal welfare.

Keywords: neurofibroma, calf, Angus, surgery, diagnosis

Parity-Dependent Patterns in Hoof Lesion Recording Across Lactation: Insights from the UK Hoof Health Registry

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BACKGROUND and OBJECTIVE: Hoof trimming records are a routinely collected source of lesion data in dairy cattle; understanding when lesions occur and whether this varies by parity could support targeted prevention. This study described hoof lesion episode distribution across lactation by lesion type and parity using UK Hoof Health Registry data.

MATERIALS and METHODS: Data comprised 54,248 electronic hoof trimming records from 19,412 cows across 52 UK farms, collected between 2007–2025; trimming protocols were not recorded. Three lesion groups were classified: infectious lesions (IL; digital dermatitis, heel horn erosion, interdigital phlegmon, interdigital superficial dermatitis); claw horn disruption lesions (CHDL; sole ulcer, white line abscess, toe ulcer, bulb ulcer, sole haemorrhage, white line disease); and other lesions (OL; thin sole, interdigital hyperplasia). A new episode was defined as an initial recording or a repeat recording after a 60-day gap. Episode prevalence was calculated as new episodes per trimmer visits. Episodes were grouped by lactation stage: early (DIM 0–100), mid (DIM 101–200), and late (DIM >200). Analyses were stratified by parity group (1, 2, 3+). Trimmer visit distribution across stages were also calculated.

RESULTS: A total of 54,248 trimmer visits were recorded; evenly distributed across lactation stages (early 35.8%, mid 32.3%, late 32.0%). 14,920 trimmer visits (27.5%) recorded no lesions (>60 days from a recorded lesion). In total 37,913 new lesion episodes were identified: 8,814 IL; 26,576 CHDL; and 2,523 OL. IL episodes peaked in early lactation across all parity groups (prevalence 17.5–20.6%), with digital dermatitis the most common infectious lesion (13.0% in early lactation). CHDL episodes followed a parity-dependent pattern: parity 1 and parity 3+ cows both peaked in early lactation (38.8% and 57.6% respectively), whereas parity 2 cows showed a sustained and elevated recording level across lactation (~40% throughout lactation). Sole haemorrhage and sole ulcer were the most prevalent CHDLs, both peaking in mid-lactation (11.8% and 11.4% respectively). OL episodes were low in recording overall, peaking in late lactation across most parity groups. Overall, 39.2–43.3% of IL episodes and 34.3–44.1% of CHDL episodes occurred within early lactation.

CONCLUSIONS: Lesion type and parity may influence the timing of lesion recording. Infectious lesions may present an immediate post-calving challenge. Regarding CHDL, parity 2 cows show a distinctly different pattern of recording from other parities, with recording levels remaining elevated stable across the lactation. Our results support findings that preventive interventions should be timed according to lesion type and parity.

Keywords: Dairy cattle, Lameness, Hoof health, Hoof trimmer records

Phimosis in Tropical Cattle and Its Surgical Treatment: Case Study

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BACKGROUND and OBJECTIVE: Among the main problems affecting the reproductive health of zebu cattle, the high incidence of preputial lesions stands out, with phimosis being one of the most frequent. This pathology is closely related to extensive grazing conditions in tropical areas, as well as the anatomical characteristics of the prepuce in these breeds which facilitates exposure to trauma. These lesions have a high economic impact on production systems due to temporary or permanent loss of the stud's reproductive function and cost of pharmaceutical and surgical treatments This case study aims to describe a surgical alternative for the resolution of phimosis in cases where pharmacological options are insufficient, especially when mucosal damage is severe. In these scenarios, surgical intervention represents a viable solution to restore reproductive function and mitigate the economic losses resulting from this pathology.

MATERIALS and METHODS: Seven-year-old beefmaster bull. It began with preputial laceration that was treated with anti-inflammatories. However there was no clinical response and the lesion worsened, leading to phimosis (inability to exteriorize the penis) leading to a surgical solution. For the technique, the animal is sedated. Through palpation and observation, the boundary between healthy tissue and fibrous tissue is identified, at which point the incision begins. This is performed delicately and in a segmented manner, avoiding penetrating the prepuce lumen. Once this dissection is complete, the affected portion is completely removed, and both parts are joined with U-shaped sutures. In the postoperative period, sexual rest, semi-stabilization, cold showers, broad-spectrum antibiotics, non-steroidal anti-inflammatory drugs, and high-dose proteolytic enzymes are recommended.

RESULTS: A follow-up evaluation was conducted after 15 days and the result was satisfactory. It showed a clear favorable evolution, proceeding to remove the stitches after 30 days.

CONCLUSIONS: As this is a surgery with a significant economic impact due to the cost of the stallion and both medical and surgical treatments, preventive measures are suggested, such as strict weed control, timely diagnosis of the inflammatory process of the prepuce, and pharmaceutical treatment. As it is a delicate operation due to the handling of soft tissues, the postoperative period is the most fundamental since fibrosis can recur.

Keywords: phimosis, cattle, economic impact, surgical treatment

Ovarian Ligation to Enhance Beef Quality in Hanwoo Heifers: Ultrasonographic Regression, Progesterone Suppression, and Minimal Inflammatory Response

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BACKGROUND and OBJECTIVE: Ovarian ligation is a surgical technique designed to inactivate ovarian function without removing the ovaries. This procedure may offer a practical strategy for suppressing estrus and potentially improving beef production efficiency in heifers. However, objective evaluation of ovarian regression, hormonal suppression, and procedure-related inflammation after ovarian ligation remains limited. The present study aimed to evaluate ultrasonographic ovarian regression, progesterone suppression, and inflammatory response following ovarian ligation in Hanwoo heifers.

MATERIALS and METHODS: Forty Hanwoo heifers were enrolled in this study. Twenty-five heifers underwent bilateral ovarian ligation using a large-animal ligator, and fifteen heifers served as non-ligated controls. Ovarian size and morphology were monitored by transrectal ultrasonography by measuring the longest ovarian axis. Blood samples were collected at predefined time points to measure serum progesterone (P4) and haptoglobin concentrations using enzyme-linked immunosorbent assay (ELISA). Clinical recovery and estrous behavior were also monitored during the observation period.

RESULTS: All ligated heifers recovered uneventfully, and no estrous behavior was observed after the procedure. Transient ovarian edema or hematoma occurred in 15 of the 25 ligated heifers. Ovarian regression was confirmed in 23 of the 25 heifers by day 45 and in the remaining two by day 59. In the ligated group, the median serum progesterone concentration was 1.923 ng/mL on day 1 and remained at or below this level thereafter. Two heifers with edema or hematoma persisting to day 45 had progesterone concentrations (1.703 and 1.367 ng/mL) comparable to the day 45 median value (1.367 ng/mL; IQR, 1.012–1.810), indicating effective luteal

suppression despite transient ovarian enlargement. Haptoglobin concentrations did not differ significantly between groups; however, a transient increase was observed on day 4 in the ligated group and on day 7 in the control group.

CONCLUSIONS: These findings demonstrate that ovarian ligation effectively induces ovarian regression and suppresses estrous activity in Hanwoo heifers while causing minimal systemic inflammatory response. The procedure appears to be safe and functionally effective, supporting its potential application as a reproductive management strategy in beef cattle.

Keywords: heifer, meat quality, ovarian ligation, ultrasound, progesterone, haptoglobin

Lateral Patellar Luxation in an Anglo-Nubian Goat - a Case Report

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BACKGROUND and OBJECTIVE: Lateral patellar luxation is a relatively rare condition in goats and is most commonly congenital. Goats of the Anglo-Nubian breed are more susceptible to this disorder due to the more upright conformation of the hind limbs. The goat was surgically treated for congenital lateral patellar luxation to restore the stifle motion and tibio-patellar alignment. The objective of this case was to describe the advanced diagnostic imaging investigation (by X-ray and CT) and to describe the „ modified wedge recession" sulcoplasty performed for surgical repair.

MATERIALS and METHODS: A 2-year-old Anglo-Nubian doe was presented to the Surgery Clinic of the Faculty of Veterinary Medicine in Cluj-Napoca for orthopedic evaluation, following the owner's observation of intermittent lameness that recurred immediately after discontinuation of anti-inflammatory treatment. Clinical examination revealed abnormal bilateral patellar lateral displacement, and further diagnostic imaging was requested. Radiographic and computed tomography investigations were subsequently performed, and confirmed the clinical diagnosis. Surgical repair was performed by „modified wedge recession" sulcoplasty, by three osteotomies, the first two for removing a V-shaped wedge, and the thirds cuts to deepen the sulcus defect. The bone and cartilage wedge was replaced without fixation, and the patella was repositioned on the new created sulcus.

RESULTS: Imaging findings confirmed bilateral grade III patellar lateral luxation, with intra-articular degenerative changes. Surgical management was performed in two stages, with a one-month interval between procedures. The selected technique was wedge recession sulcoplasty, adapted from small animal orthopedics procedures. Postoperatively, the patient regained complete stifle range of motion, resolution of lameness, and no observable signs of pain or gait alteration.

CONCLUSIONS: In conclusion, lateral patellar luxation represents a significant orthopedic condition in goats, being already reported as common in certain populations such as Hejazi goats in Libya, where a possible association with inbreeding has been suggested. The favorable postoperative outcome, characterized by complete resolution of lameness and absence of pain, supports surgical correction as an effective and reliable therapeutic approach for severe lateral patellar luxation in goats, contributing significantly to improved limb function and overall animal welfare.

Keywords: Goat, Patellar Luxation, Sulcoplasty

Early Detection and Adhesive Gel Bandaging Enable Effective, Rapid and Antibiotic-Free Management of Digital Dermatitis-Associated Lameness in Dairy Cows

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BACKGROUND and OBJECTIVE: Digital dermatitis (DD) is a leading cause of infectious lameness in dairy cattle. Treatment commonly involves topical antibiotics; however, increasing emphasis on antimicrobial stewardship has encouraged investigation of alternative, non-therapeutic approaches. Adhesive gel bandages (AGB) are now commercially available and these repel the ingress or adsorption of water, detritus and bacteria. They also self-degrade, sustainably, over time without the requirement for removal. Previous work has shown that the use of AGB over topically-applied antibiotic improves outcomes in DD compared with antibiotic treatment alone. It remains unclear whether the barrier properties of this hydrophobic AGB can support natural healing without prior antibiotic application. This study aimed to investigate this question, with particular emphasis on DD-associated lameness.

MATERIALS and METHODS: A 280-cow extended autumn block calving dairy herd, was followed over seven months. Baseline lameness prevalence was 21.8% with DD the predominant cause. Lameness was identified both ad-hoc (by trained farm staff) and via fortnightly mobility scoring of the herd using the AHDB scoring system (0–3). Lame cows were examined to determine causality, and any DD lesions were classified by the M-stage scoring system. AGB was applied to all hooves carrying DD lesions at days 0 and 7 and only thereafter if lameness had not resolved. Primary outcome was mobility score at circa four weeks and categorised dichotomously as sound (score 0–1) or lame (score 2–3). Analysis considered application reason (routine mobility scoring or ad-hoc detection), M-stage at application, and number of AGB applications per lesion. Resolution rates were compared using a two-proportion Z test (α=0.05).

RESULTS: 92 AGB applications were included in the analysis, of which 81 (88%) were classified as sound at final follow up (day 21-35). 68 out of 92 were a cow's first intervention in the study period; the remaining 24 were a separate intervention (over a month from the previous) or occurred on a different foot. Lameness resolution was similar regardless of initial M-score stage, albeit M3 and M4 lesions were of low prevalence (12% combined). Importantly, 100% of cows receiving AGB that had DD lesions without lameness were sound at outcome (n=34), vs 79.3% with lameness (n=58) at t=0 (p=0.004).

CONCLUSIONS: AGB in dairy cows appears to be an effective management option for isolation of the foot from the environment, allowing rapid remission of DD-associated lameness. The earlier in the process this is applied the higher the likelihood of resolution and subsequent reduction in the use of antibiotics.

Keywords: Digital Dermatitis, Bovine, Lameness

Diprosopus Dirrhinus (Nasal Duplication) in a Female Holstein Friesian Calf: Clinical Findings, Diagnostic Imaging and Surgical Management

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BACKGROUND and OBJECTIVE: Congenital craniofacial abnormalities are rarely reported in cattle, with nasal duplication (diprosopus dirrhinus) representing an exceptionally uncommon condition. The aim of this case report was to add to the limited veterinary literature on congenital nasal anomalies in cattle, emphasises the diagnostic value of advanced imaging techniques such as computed tomography (CT), and describe the surgical management of diprosopus dirrhinus with favourable outcome.

MATERIALS and METHODS: Case Presentation: An 11-week-old Holstein Friesian heifer calf was referred for evaluation of a congenital nasal deformity present since birth. Clinical examination revealed a dorsal, turbinate-like structure on the nasal bridge, associated with blind-ended cavities producing mucoid discharge but no respiratory compromise. Advanced imaging using CT identified

abnormal duplicated nasal turbinates with incomplete bony enclosure, consistent with diprosopus dirrhinus.

RESULTS: Intervention and Outcome: Surgical excision of the accessory nasal structure was performed under general anaesthesia. Intraoperatively, the abnormal tissue was found to be separated from the normal nasal cavities by a soft tissue bridge. Histopathology confirmed the presence of respiratory epithelium, cartilage and bone, consistent with ectopic nasal tissue. Postoperative recovery was uneventful, and the calf remained clinically normal with appropriate growth at follow-up 6 months later.

CONCLUSIONS: Diprosopus dirrhinus, although rare, should be considered in cases of congenital nasal abnormalities in calves. This case highlights the diagnostic value of computed tomography in characterising complex craniofacial malformations in cattle and guiding surgical decision-making. This report contributes to the limited evidence base and demonstrates that surgical management can result in a favourable long-term outcome.

Keywords: Craniofacial duplication, CT, Diprosopus dirrhinus

Evaluation of Ozonated Cream Therapy in the Treatment of Foot Lesions in Dairy Cattle

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BACKGROUND and OBJECTIVE: Foot diseases in the daily cattle industry represent a major health and welfare concern, contributing to live weight loss, reduced milk yield, decreased feed intake, shortened productive lifespan, and impaired reproductive performance. The implementation of effective preventive measures, early diagnosis, and timely treatment is essential to minimize economic losses, enhance recovery, and improve animal welfare.

MATERIALS and METHODS: The study material consisted of 145 Holstein cows with foot disorders from two different farms. Animals presenting with lameness were clinically examined, and lesions located at the heel, interdigital skin, sole, and hoof wall were evaluated for structural changesBased on clinical findings, the animals were classified into three groups: digital and interdigital dermatitis, heel horn erosion, and sole ulcer. Following mechanical cleaning of the hoof, excessive horn tissue was removed through routine hoof trimming procedures. Subsequently, ozone cream was applied topically to the affected areas as part of the treatment protocol. Foot lesions were treated with a commercially available Vetozon cream, applied before each bandaging. This protocol was maintained until complete healing was achieved. During the treatment period, several parameters were recorded, including the number of bandages applied, healing time, duration of lameness, and milk yield.

RESULTS: Lameness duration and healing time were significantly longer in cows with sole ulcers compared to those with digital/interdigital dermatitis and heel horn erosion. No significant difference was observed between digital/interdigital dermatitis and heel horn erosion in terms of these parameters. Milk yield decreased during the treatment period in all groups and increased following treatment. The most pronounced reduction was observed in the sole ulcer group, indicating a greater negative impact of this condition on production performance. Locomotor activity, as assessed by step count, increased in the post-treatment period across all groups. While step counts remained relatively stable between baseline and treatment periods in digital/interdigital dermatitis and heel horn erosion, a significant decrease during treatment followed by a marked increase after treatment was observed in the sole ulcer group.

CONCLUSIONS: The application of ozone cream contributed to improved healing outcomes in common foot diseases, including digital dermatitis, interdigital dermatitis, heel horn erosion, and sole ulcers. Treatment was associated with reduced healing time, recovery of milk yield, and improvement in locomotor activity. These findings suggest that ozone cream may serve as an effective adjunctive treatment option in the management of bovine foot diseases. However, further controlled

and large-scale studies are required to better elucidate its therapeutic efficacy and underlying mechanisms.

Keywords: Cattle, Ozone, foot diseases

Association Between Dorsal Wall Length and Foot Lesions in Finishing French Young Bulls: A Slaughterhouse Cross-sectional Study

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BACKGROUND and OBJECTIVE: Young bulls (YB) reared indoor in France exhibit a high prevalence of several foot lesions by the end of the fattening period. However, determinants of foot disorders in this population remain poorly understood. As YB are typically housed on straw yards without routine trimming, limited access to abrasive surfaces may promote excessive horn growth, increasing the risk of claw horn lesions (CHL), including heel horn erosion (HHE). Digital dermatitis (DD), often affecting the dorsal aspect of the coronary band in YB, may further exacerbate horn overgrowth, through inflammation or weight-bearing avoidance. This study aimed to (1) describe the distribution of dorsal wall length (DWL) in finishing French YB and (2) investigate its association with the occurrence of different foot lesions.

MATERIALS and METHODS: In this cross-sectional survey, the four feet of 2,102 YB (363 farms) were inspected at slaughterhouses. DWL was measured using a caliper (coronary band to dorsal wall tip). Feet were then trimmed, and lesions were recorded using the French nomenclature, aligned with ICAR Claw Health recommendations, including severity levels. Mixed-effects linear regression models identified factors associated with DWL, while multivariable mixed-effects logistic regression assessed associations between DWL and CHL.

RESULTS: DWL ranged from 60 to 152 mm, with the highest median value in the front medial claw (88 mm), followed by the lateral claws (85 mm for both front and hind), and the hind medial claw (83 mm). DWL was associated with claw location (front medial > front lateral > hind lateral > hind medial), presence of dorsal DD (+ 2 mm), breed, carcass weight (increase of 0 to + 0.5 mm per 100 kg, depending on claw location), and slaughter season (higher DWL in winter). Most of CHL were associated with increased DWL in at least some claws and/or breeds. Additional factors influencing CHL risk included claw location (risk varied by CHL type), breed (Limousine and rustic beef breeds at lower risk), weight, age (for HHE only), as well as region of fattening and slaughter season.

CONCLUSIONS: While this cross-sectional design precludes causal inference, results suggest that claw overgrowth may contribute to CHL, and may be consecutive to previous dorsal DD lesion. Providing abrasive surfaces or preventive trimming could potentially reduce CHL in indoor-housed YB.

Keywords: cattle, lameness, conformation, claw disorder, beef, dairy

The Effect of Functional Hoof Trimming on Milk Yield, Step Count, and Lying Time in Cows

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BACKGROUND and OBJECTIVE: Lameness is a major problem in dairy cattle, negatively affecting animal welfare and productivity. Functional hoof trimming (FCT) improves locomotion by balancing weight distribution on the hooves. Consequently, early changes may occur in behavioral and production parameters. These changes can be objectively evaluated using pedometric measurements such as step count and lying time. This study aimed to determine changes in milk yield, step count, and lying time in cows before and after FCT.

MATERIALS and METHODS: The study included 231 clinically healthy, high-yielding Holstein cows aged 2–8 years. Data were collected at four time points: one week before FCT (T0), one day after FCT (T1), one week after FCT (T2), and four weeks after FCT (T3). Step count (steps/day), lying time (min/day), and milk yield (kg/day) were automatically recorded using a herd management system. Cows were grouped by age (2, 3, and ≥4 years) and parity (low: 1–

2, medium: 3–4, high: ≥5). Data were analyzed using linear mixed models with cow ID as a random effect, and time, age, and time × age interaction as fixed effects. Bonferroni adjustment was used, and significance was set at $p < 0.05$.

RESULTS: Time significantly affected step count ($p = 0.016$), lying time ($p < 0.001$), and milk yield ($p < 0.001$). Step count decreased from 139.46±3.84 at T0 to 131.95±3.51 at T2 and 131.47±3.84 at T3, with no difference between T0 and T1 ($p > 0.05$). Lying time increased from 627.66±11.18 at T0 to 668.13±11.79 at T1, remaining elevated thereafter ($p < 0.001$). Milk yield decreased from 35.90±0.63 kg at T0 to 34.22±0.61 at T2 ($p < 0.001$) and 34.36±0.69 at T3 ($p = 0.010$), with no difference at T1 ($p > 0.05$). The time × age interaction was significant ($p = 0.007$), with a marked decline in cows aged ≥4 years, while 2-year-old cows remained stable. Parity also affected milk yield ($p = 0.002$), and a significant time × age × parity interaction was observed ($p = 0.008$).

CONCLUSIONS: FCT reduced step activity and increased lying time, indicating decreased mobility in the short term. Milk yield also declined, particularly at one and four weeks after trimming. These effects varied according to age and parity, suggesting that responses to FCT depend on the physiological status of the animal

Keywords: functional claw trimming, dairy cow, lying time, steps sount, milk yield

Longitudinal Assessment of Intensively Reared Dairy Heifer Foot Growth and Corkscrew Shape Development

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BACKGROUND and OBJECTIVE: As the future of the dairy herd, heifers represent a large investment, but lameness poses a significant risk to their productivity and longevity. Lameness development can be influenced by foot growth and shape, with corkscrew claw conformations now reported in heifers. This study aimed to assess foot growth of pre-calving heifers, along with claw shape observations for identification of when corkscrew claws become visible.

MATERIALS and METHODS: A convenience sample of 250 pre-conception heifers aged between 8-14 months from a single fully housed dairy herd in the UK milking 2,500 cows were recruited. They were observed every three months and scored for foot shape following a visual guide based around dorsal and axial wall locations, over a total of five visits. In addition, 20 heifers had their feet measured, with the horn marked at the dorsal wall and 1cm from the lateral heel wall to allow growth and wear measurements to be taken every 3 months.

RESULTS: Mean claw growth was 0.22 mm/day prior to calving, but this reduced to 0.17 mm/day along with increased wear (0.25 mm/day) and reduced total claw length around the time of calving and heifers entering the milking herd. The heel wall tended to grow faster and have more wear than the dorsal wall, but there was no difference found between any of the claws. With regard to observed foot shape, the odds of higher scores increased over time ($P<0.001$), with the odds of heifers having more severe shape scores increasing as the number of claws affected by the change in foot shape increased (OR=11.77, 95% 8.81-15.73, $P<0.001$). Shape score 1 claw prevalence ranged from 12.7-39.6%, but may be considered as long toes given these young heifers had dorsal wall lengths of 79.2mm, which is very similar to that described for adult Holstein cows despite these heifers only having a mean age of only 529 days. Shape score 2 claws were seen in 13.9% of heifers, which was a similar level seen in the adult milking herd. The most commonly affected claw with both shape 1 and 2 scores was the medial claw across all limbs.

CONCLUSIONS: Given the high rates of growth and the early observations of claw shape change in intensively reared Holstein heifers, use of post-conception foot inspections may be of benefit in maintaining good claw conformation.

Keywords: Heifer, Corkscrew, claw

A Cross-Sectional Study of Dairy Cattle Lameness Prevalence in Farms in Central and Northern Greece

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BACKGROUND and OBJECTIVE: The dairy cattle population of Greece is currently estimated at around 90,000 animals, with 79% of them found in its central and northern regions. Despite lameness being recognised as one of the most prevalent problems for the dairy industry worldwide, the only available data for Greek herds were collected approximately 20 years ago. The aim of this study was to update lameness prevalence estimates by surveying dairy farms across Central and Northern Greece.

MATERIALS and METHODS: A convenient sample of twenty-five dairy farms across the regions of Thessaly (n=11), Macedonia (n=8), and Thrace (n=6) were visited during summer and autumn 2021. During the visit, all cows comprising the milking herd were scored for lameness upon exiting the milking parlour, by one of two trained assessors, both veterinarians. Cows were scored on a 0-3 scale; score 0 described normal gait, score 1 was for cows presenting imperfect mobility, score 2 corresponded to impaired mobility, and score 3 was assigned to cows presenting severe lameness. The method used is commonly known in the United Kingdom (UK) as the Agriculture and Horticulture Development Board (AHDB) mobility scoring system. Animals were classed as lame if they had a mobility score of 2 or 3. Statistical analysis of recorded data was performed in Rstudio.

RESULTS: A total of 3,571 milking cows were assessed for their mobility: 1,327 from farms in Thessaly, 1,133 from farms in Macedonia, and 1,111 from farms in Thrace. The median milking herd size per farm was 126 (interquartile range: 115, range: 41-490). Of all the cows assessed, 56.3% were found to be lame (scores 2 and 3); of those that were lame, 29% had a mobility score 3. The mean within farm lameness prevalence was 58%, with a standard deviation of 20% and a range of 18.8%-92.3%. The median mobility score observed was 2. One-way ANOVA revealed a significant effect of region on lameness prevalence. Post-hoc Tukey HSD comparisons indicated that mean prevalence for Thrace (38.2%) was significantly lower when compared to Thessaly (62.2%, $p < 0.05$) and Macedonia (67.1%, $p < 0.05$).

CONCLUSIONS: Lameness prevalence within farms across the main dairy producing regions of Greece was found to be concerningly high. Given the severe impact of lameness on animal welfare and production, these findings highlight the significance of the issue on a nation-wide level and a need for concerted efforts and targeted strategies to lower its prevalence.

Keywords: Dairy cattle, Lameness, Lameness prevalence, Greece

UDDER HEALTH AND MASTITIS

The Role of Mastitis in Elevated Aerobic Plate Counts of Sheep Milk

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BACKGROUND and OBJECTIVE: Aerobic plate count (APC) is a common measure of bulk milk (BM) quality. Elevated BM APC may result in reduced milk prices or penalties and can be a challenge to troubleshoot. Anecdotally, some producers have lowered their BM APC by removing ewes with subclinical mastitis. The objectives of this study were to describe the prevalence of elevated APC at the ewe and BM levels, assess the association between APC and somatic cell count (SCC) in BM, and determine if mastitis is a risk factor for elevated APC.

MATERIALS and METHODS: Gland-level milk samples were collected from approximately 15 randomly selected ewes on each of 20 New Zealand dairy sheep farms at early, mid, and late lactation. California mastitis test (CMT, measured on a scale of 0, trace, 1, 2, and 3) and aerobic bacterial culture were performed at the gland level, and SCC and APC at the ewe level. Subclinical mastitis was defined as the ewe having one or two bacteriologically positive glands and SCC >500,000 cells/mL and/or CMT ≥1. BM APC and SCC data were retrospectively collated from 907 milk consignments from 16 of these farms that supplied BM to a processor. Associations between ewe-level APC and risk factors were determined with a mixed Bayesian ordinal regression model. The relationship between BM SCC and APC was explored with correlation analyses and by constructing a mixed logistic regression model of the odds of APC exceeding >100,000 CFU/mL.

RESULTS: For individual ewe milk samples, APC was below the limit of quantification (1,000 CFU/mL) in 78% of ewes, <100,000 CFU/mL in 97% of ewes, and varied between visits and farms. Elevated CMT score and SCC, positive milk culture, and subclinical mastitis were confirmed as risk factors for elevated APC. Ewes with CMT scores 2 or 3 had 6.7 times (95% credible interval = 4.36–10.22) higher odds of being in a higher APC category than ewes with lower CMT scores. BM APC exceeded 100,000 CFU/mL in 200/896 (22%) consignments, peaked in August, and the farm-level proportion exceeding this threshold varied from 3% to 100%. A doubling in SCC was associated with approximately double the odds of APC exceeding 100,000 CFU/mL (OR = 2.09, 95% CI = 1.38-3.28), but high APC was not well predicted by SCC.

CONCLUSIONS: Udder health should be considered when troubleshooting high BM APC. The CMT can be used as a screening tool. BM SCC is not a useful tool for predicting elevated BM APC.

Keywords: sheep, aerobic plate count, mastitis, risk factors

Mastitis in Grazing Dairy Ewes: Epidemiology and Risk Factors

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BACKGROUND and OBJECTIVE: There is limited published research on milk quality and mastitis in grazing dairy ewes, leaving producers and their advisors in pasture-based systems reliant on extrapolation from bovine mastitis or overseas dairy sheep research. The objectives were to describe clinical and subclinical mastitis, and their risk factors, among grazing dairy ewes on New Zealand farms.

MATERIALS and METHODS: Two studies were run on 20 commercial New Zealand sheep milking farms in the 2022-2023 season. Clinical mastitis: a prospective cohort study was conducted using producer-diagnosed clinical mastitis (defined as a change in the appearance of milk and/or signs of inflammation in the gland). Case history data and milk samples were collected. Subclinical mastitis: a repeated cross-sectional study was conducted, where visible udder morphology and pathology was assessed and gland-level milk samples were collected from approximately 15 randomly selected ewes on each farm at early, mid, and late lactation. California mastitis test (CMT, measured on a scale of 0, trace, 1,2 and 3) was performed at the gland level and somatic cell count (SCC) at the ewe level. Subclinical mastitis was defined at the ewe level as one or two bacteriologically positive glands and SCC >500,000 cells/mL and/or CMT ≥1.

RESULTS: The season incidence of clinical mastitis was 2.3 (95% CI: 1.6–3.3) %. Cases occurred at a median of 7 days postpartum and most had clots in the milk (59%), swelling (55%), and unevenness (71%) of the glands. Pyrexia was diagnosed in 25% and depression in 26% of cases. Treatment was given to 46% of cases. The most common outcome was immediate drying off to be culled without treatment (32%). *Streptococcus uberis* (14%), non-aureus staphylococci (12%), and *Staphylococcus aureus* (11%) were the most common isolates, with 49% being culture-negative. The prevalence of subclinical mastitis was 6.4% (95% CI: 4.6-8.7%). The most common bacteria were non-aureus staphylococci (4.0% of glands) and *Staphylococcus aureus* (0.6% of glands). Ewes with moderate or

severe teat end hyperkeratosis had 6.4 times higher odds of subclinical mastitis and ewes with asymmetric udders had 2.3 times higher odds.

CONCLUSIONS: Compared to studies from more intensive systems, the incidence of clinical mastitis was low and comparable, *Streptococcus uberis* was relatively dominant among clinical mastitis cases, while the prevalence of subclinical mastitis was relatively low but the bacterial causes were consistent. The frequency of systemic signs suggests a role for supportive treatment. Teat end hyperkeratosis and udder asymmetry may be useful visual indicators for mastitis risk.

Keywords: sheep, milk quality, mastitis, risk factors

Effect of Oral Nutraceutical Administration at Dry-Off on Udder Pressure

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BACKGROUND and OBJECTIVE: Dry-off can be particularly challenging for modern high-producing cows, as it often leads to rapid milk accumulation in the udder during the first days after dry-off. This issue is exacerbated when dry-off is performed abruptly resulting in udder engorgement within hours. Udder engorgement and the resulting high udder pressure delay keratin plug formation, which is often visible as milk leakage from the teats. This condition increases the risk of new intramammary infections. Cows with high milk yields at dry-off face a greater risk than lower-producing cows. In the present experimental study, we evaluated the effect of a nutraceutical product on udder pressure at dry-off in high-yielding cows and during the days following its administration.The nutraceutical is designed to reduce milk accumulation at dry-off, thereby potentially limiting udder engorgement and its associated consequences.

MATERIALS and METHODS: A total of 20 cows were enrolled in the trial: 8 in the control group and 12 in the nutraceutical group. The nutraceutical was administered at dry-off and again the following day, as recommended for high-producing cows (those yielding more than 20 kg of milk per day during the week before dry-off). The last milking averaged 27.71 kg in the control group and 25.27 kg in the nutraceutical group (no significant difference; t-test, P = 0.19). Udder pressure was measured from dry-off (Day 0) to Day 3 on several points using an algometer, following the protocol described in a previous study that used the same device.

RESULTS: Both groups showed an increase in udder pressure on the day after dry-off, consistent with milk accumulation, followed by a gradual decrease. In the nutraceutical group, pressure values observed on Day 2 were similar to those observed on Day 3 in the control group (t-test, P = 0.97), suggesting a comparable pressure level one day earlier. In addition, udder pressure decreased significantly between Day 1 and Day 3 in the nutraceutical group (t-test, P = 0.0005), whereas no significant change was observed over the same period in the control group (t-test, P = 0.37).

CONCLUSIONS: Nutraceutical supplementation at dry-off was associated with an earlier reduction in udder pressure compared with non-supplemented cows, suggesting a potential positive effect on the reduction of milk accumulation. Lower udder pressure may help limit engorgement and its associated risks, contributing to improved udder health and potentially better cow comfort during the dry-off period. Further studies are warranted to investigate animal welfare implications.

Keywords: High-yielding cows, Dry-off, Udder pressure, Udder engorgement, Nutraceutical,

Nanoparticles in Textile Wet Wipes for Bovine Subclinical Mastitis Control: A Preventive Tool Against Antimicrobial Resistance

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BACKGROUND and OBJECTIVE: Bovine subclinical mastitis (SM) represents one of the most costly pathologies in the dairy industry, compromising the compositional and hygienic quality of milk. Current management often relies on the indiscriminate use of antibiotics, which exacerbates the critical challenge of bacterial resistance, negatively affecting both animal and public health. The main objective was to develop and measure the preventive efficacy of a wet wipe impregnated with nanoparticles (NPs) for udder antibiotic-free disinfection during pre-milking procedures.

MATERIALS and METHODS: Phase I focused on the product development, including the synthesis, characterization, and impregnation of NPs onto a non-woven textile material. Additionally, an in vitro evaluation was conducted to determine the minimum inhibitory concentration of the NPs against key SM pathogens (Staphylococcus aureus and E. coli). Phase II was a field trial involving lactating cows from the Universidad de Antioquia farm. Four cows were randomly divided into control groups (traditional management and wipes without NPs use) and two experimental groups (NPs at 2 mL/L and 5mL/L) for 21 days. Milk sampling was performed twice to monitor the SM prevalence using the CMT test and somatic cell count (SCC), and the compositional milk quality (fat, protein, total solids-TS, lactose, MUN)

RESULTS: This preliminary study resulted in a significant reduction in microbial load detected when using wipes with NPs, with a direct relationship observed between the biocide concentration and antimicrobial efficacy. Treatment with 2 mL/kg reduced the bacterial load by 84.7%, while the 5 mL/kg treatment achieved a 96.0% reduction compared to the control without NP´s. Milk quality was maintained after the NPs treatments compared to control groups with general means ± SD: fat 4.84±0.45 %, protein 3.74±0.24%, TS 13.62±0.72%, lactose 4.34±0.32, MUN 16.94±4.92. The NPs at 5mL/L treatment resulted in a persistent CMT negative cow and at 2mL/L improve CMT from 2 to 3 cruces before wipe use to 1-2 cruces and negative CMT after 20 days of treatments. The SCC do not vary significantly in the 2 cows NPs treated but cows in control groups showed increased SCC.

CONCLUSIONS: The routine application of the NPs wipe demonstrated a reduction in SM indicators (SCC and CMT) and a maintained milk hygienic quality, compared to the control groups. The main impact of this pilot study lies in validating a low-cost, easy-to-apply, and highly effective preventive tool that could substitute antibiotic treatments, aligned with the principles of the global fight against antimicrobial resistance.

Keywords: Prevention, udder health, nanobiotechnology, dairy cows, milk quality, veterinary medical supplies

Evaluation Of Three Methods for Determining Whether an Antibiotic Medicine Is Needed for The Treatment of Mild or Moderate Clinical Mastitis

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BACKGROUND and OBJECTIVE: In Denmark, it is mandatory to collect a quarter milk sample when a cow has clinical mastitis (CM) and is treated with antibiotics. Most analyses are performed by the veterinary clinics that oversee the health of the dairy farms. For mild or moderate cases of CM, antibiotic treatment (AB) is only beneficial for intramammary infections (IMIs) caused by organisms belonging to staphylococci and streptococci genus. Finding a way

to diagnose on the farm would make the whole process easier, save time on decisions, and cut down on unnecessary antibiotic use.

MATERIALS and METHODS: Producers were instructed to collect two CM milk samples: one fresh sample, which was refrigerated until use, and another frozen sample (+10% glycerol). The fresh sample was tested on-farm using two already marketed rapid tests, prepared according to the manufacturers' instructions and placed in an egg incubator for 24 hours, at which point the results were read. Tests yielded three possible

RESULTS: no growth (0), Gram-negative (-), or Gram-positive (+), or contaminated (X). Samples were then shipped to the veterinary clinic and cultured on selective media. Frozen samples were shipped to a professional testing laboratory for culturing (on blood agar and in broth) and identification (MALDI-TOF). Cultures with 3+ colony types were declared contaminated (CONT). Other results were grouped as STErile, MIXed, *S. aureus* (SAU), *S. uberis* (SUB), Coagulase-Negative Staphylococci (CNS), Other Gram-Positive (OGP), ENTerobactiaceae, and Other Gram-Negative (OGN).

RESULTS: Out of 177 samples collected by producers, 139 were MIX, SAU, SUB, CNS, OGP or CONT, and deserve antimicrobial treatment (78.5%). Routine in-clinic labs were able to correctly justify the AB in 74.1% of these cases. However, veterinary clinics and the reference lab agreed in the identification of the causative species in only 23.0% of cases. Rapid tests, performed either at the veterinary clinic or on the farm, both yielded poor results, being able to identify CM cases likely to benefit from AB in 39.6–55.5% of cases.

CONCLUSIONS: Even with pathogen identification disagreements between the reference laboratory and in-clinic labs, veterinarians were still able to satisfactorily identify animals requiring treatment. However, the Predictive Negative Value is low for the three methods due to numerous false negative results. Consequently, using these rapid tests to exclude negative-testing animals from AB would be hazardous, as it may significantly reduce their chance of recovery. Improving daily on-farm antibiotic stewardship through more precise diagnosis may require exploring milk culturing performed by better-trained veterinary practitioners.

Keywords: mastitis, diagnostic, antibiotic, predictive values

Udder Cleft Dermatitis: Current Understanding and a Uk Practitioners' Approach to Engaging with Clients, Raising Awareness of This Condition and How to Manage It

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BACKGROUND and OBJECTIVE: Udder cleft dermatitis (UCD) is an ulcerative skin condition affecting dairy cattle, typically located at the anterior udder junction. It poses significant welfare and productivity concerns, and prevalence varies widely with recent UK studies reporting within-herd prevalence of up to 26 %. Across Europe published studies undertaken with herds in Germany, Sweden and the Netherlands have shown prevalence ranging from 0 % to 38 %. UCD is thought to have significant implications for the animal health, welfare and productivity due to the severity of some of these lesions which can persist for months. Lesions can be overlooked due to their anatomical location making them difficult to detect, often until the characteristic pungent odour develops. Motivation for this project largely came about from the author's own lack of awareness of the prevalence of UCD within frequently visited dairy herds, and a concern that veterinary advice is often not sought for this condition leading to a number of unproven, extra-label products being used for treatment.

MATERIALS and METHODS: 10 dairy farms in the southwest of England were enrolled in the project, with herds ranging from 115 – 300 milking cows. Herds enrolled included housed herds on deep sand cubicles, mattresses (with sand or shavings) and straw, and outdoor grazing herds. One veterinary technician from the practice carried out whole herd UCD scoring on all farms between March and June 2025. Scoring primarily took place in the milking parlour or with self-locking yokes using an inspection mirror with a light source to inspect the udder cleft of each cow. A score was allocated to each cow, using a standardized scoring system developed by a UK-based UCD steering group.

RESULTS: In total 2087 lactating dairy cows were enrolled in the study and 168 udder cleft dermatitis lesions of varying grades were identified. The prevalence of lesions within herds ranged from 2 % to 18 %, with an average of 7.65 %.

CONCLUSIONS: Awareness of udder cleft dermatitis is growing, yet further work is needed to fully understand its impact on animal welfare and productivity, and how best to manage it. Veterinary team (vets and veterinary technicians) engagement raised awareness of UCD across the farms involved, with further work needed to further improve knowledge as to the prevalence, prevention and management of UCD amongst farmers.

Keywords: udder, cleft, dermatitis

Analysis of the Effectiveness of Danofloxacin in Controlling Somatic Cell Counts (scc) in Dairy Cows During the Dry Period

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BACKGROUND and OBJECTIVE: Mastitis poses the largest health challenges in the dairy industry. A retrospective analysis was conducted on the effectiveness of danofloxacin(Advocin, Zoetis) administered subcutaneously to dairy cows on the dry-off day. The objective of the study was to assess effectiveness of danofloxacin in reducing SCC in dairy cows treated during the dry period.

MATERIALS and METHODS: The study included data from 98 cows given danofloxacin at dry-off. Somatic cell counts (SCC) were monitored at six time points: before dry-off (T-4, T-2 months), at dry-off (T0), and three times postpartum (T+2, T+4, T+6 months). Descriptive statistics at each time point were performed evaluating SCC, and non-parametric Wilcoxon Rank Sum test was used to assess statistical significance ($P < 0.05$) of differences between median values of SCC.

RESULTS: The analysis showed a significant decrease in median SCC from 287 thousand/ml before dry-off to 111.5 thousand/ml at T+2 ($p = 0.0115$). The full therapeutic success rate (SCC <200 thousand/ml at T+2) was 56.1%.

CONCLUSIONS: While considered successful, several limitations were identified: 27.6% therapeutic failures, 7.1% relapses, and a concerning 42.9% deterioration among cows with initially normal SCC. Nevertheless, the findings in this study suggest selective dry cow therapy instead of routine antibiotic prophylaxis is warranted for use in dairy operations for treatment of mastitis.

Keywords: mastitis, danofloxacin, dry period, SCC

Cluster of Mastitis Cases Caused by Genetically Highly Diverse " "mycobaterium Smegmatis "" Strains

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BACKGROUND and OBJECTIVE: Fast-growing non-tuberculous mycobacteria (NTM) are uncommon cause of bovine mastitis. "*"Mycobacterium smegmatis"*" is a saprophyte in the soil and has been considered as an opportunistic causative agent of mastitis in individual animals. Recently, a possible link has been observed between "*"M. smegmatis"*" mastitis and the use of recycled manure solids (RMS) and composted bedding. We describe here a cluster of mastitis cases caused by genetically highly diverse "*"M. smegmatis"*" strains in research dairy herd utilizing RMS bedding.

MATERIALS and METHODS: In a research dairy herd, one clinical mastitis case yielded no pathogen findings in mastitis qPCR (PathoProof Mastitis Complete-16). The affected quarter was resampled for bacterial cultivation, resulting in the isolation of "*"M. smegmatis"*", identified by MALDI-TOF. Subsequently, duplicate quarter milk samples were taken in every mastitis case and PCR-negative samples were cultivated for M. smegmatis. A total of 10 isolates from 6 cows were characterized by whole

genome sequencing (WGS). The genome sequences were subjected to local ad hoc cgMLST analysis using Ridom SeqSphere+ software.

RESULTS: A total of 10 M. smegmatis mastitis cases occurred in seven cows. The clinical appearance included a high somatic cell count, swollen and indurated infected udder quarters, either yellowish mucous flakes in the foremilk secretions or no changes in milk, and no general symptoms. These cases occurred within 9 months after barn renovation, including implementation of an automatic milking system (AMS) and the replacement of rubber mattress stalls with deep-bedded stalls maintained with RMS. cgMLST demonstrated high genetic diversity among 10 "*"M. smegmatis"*" strain analyzed. Allele differences between 1766 and 3156 were seen between the majority of the strains. All cows and all quarters had different cgMLST profiles. In two cows, the same quarters were sampled twice, with 1-month and over 1-year intervals. In these cases, chronic persistent infection caused by a single strain within a quarter was shown.

CONCLUSIONS: Mastitis cases in a dairy herd were caused by genetically highly diverse "*"M. smegmatis"*" strains. However, chronic infections caused by persistent strains were observed in the quarter level. The cgMLST method proved to be an effective tool for molecular epidemiological investigation of bovine NTM mastitis strains.

Keywords: mastitis, Mycobacterium smegmatis, cgMLST, WGS

Advancing Bovine Mastitis Management and Reducing Amr: Insights from Over 200,000 Mastitis Milk Samples in New Zealand

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AgriHealth NZ Ltd

BACKGROUND and OBJECTIVE: Mastitis poses a significant global challenge in dairy production and judicious use of antibiotics is critical to reduce the risk of antimicrobial resistance (AMR). This paper analyses over 200,000 mastitis milk samples from New Zealand dairy farms, processed using the novel Mastatest diagnostic system (2018-2024).

MATERIALS and METHODS: Mastatest, a rapid, on-farm colour-change test, provides bacterial identification and, for clinical cases, minimum inhibitory concentration (MIC) data for common antibiotics within 24 hours. Our analysis of 198,737 clinical mastitis and 47,221 subclinical mastitis samples revealed critical trends that will assist mastitis treatment decisions and reduce antibiotic use on farms.

RESULTS: 'No growth' results were significantly lower at 10% compared to conventional laboratory cultures (20-25%). Strep. uberis was the most common isolate (23%) from clinical mastitis cases year-round in New Zealand, contrary to traditional views in seasonal farming systems. Gram-negative bacteria (E. coli, Klebsiella, Serratia) constituted 12% of isolates and demonstrated limited sensitivity to common mastitis antibiotics (99% had MIC >4.0), making antibiotic treatment inappropriate. Furthermore, Strep. uberis showed reduced cloxacillin sensitivity. While penicillin was broadly recommended, it was deemed inappropriate in 40% of clinical mastitis presentations, highlighting the need for diagnostics and targeted treatment. For subclinical mastitis, 47,221 samples identified Staph. aureus in 24% of cases, enabling strategic management to curb infection spread. These findings demonstrate that integration of cow side mastitis diagnostic testing substantially reduces antibiotic use. By identifying 'no growth' and Gram-negative cases best managed with non-steroidal anti-inflammatory drugs (NSAIDs), 45,351 cases (23%) avoided antibiotics, leading to an estimated saving exceeding $9 million NZD. This approach enhances animal welfare through pain relief based on diagnostic testing, and directly supports New Zealand's dairy industry antimicrobial resistance (AMR) goals via tailored treatment programs.

CONCLUSIONS: This research offers compelling evidence for rapid, on-farm diagnostics in optimising mastitis treatment, reducing antibiotic reliance, and supporting global efforts against AMR, providing a valuable model for other dairy industries.

Keywords: Mastitis, mastitis detection, mastitis bacteria, AMR, dairy cows, New Zealand

Managing Coliform Mastitis in a Large New Zealand Dairy Herd: Investigation and Control of E. Coli Mastitis Grades in Bulk Tank Milk

Steve Cranefield, Richard Emslie

AgriHealth NZ Ltd

BACKGROUND and OBJECTIVE: In early 2025, a large New Zealand dairy herd milking 700 cows experienced recurrent detection of coliform bacteria in bulk tank milk despite satisfactory milking machine hygiene and milk cooling systems. This paper details the complexity of the investigation and learnings applicable to veterinarians investigating coliform grades or managing Escherichia coli mastitis.

MATERIALS and METHODS: The investigation identified E.coli mastitis as the primary source of elevated bacterial counts. Individual cow cultures revealed sporadic, high-level E. coli shedding events associated with transient spikes (>6000 cfu/mL) in bulk milk grades. Most affected cows self-cured without antibiotic treatment, consistent with the high self-resolution rate of E. coli mastitis reported globally. Milking management, teat condition, and environmental factors were assessed. Environmental bacteria contamination of teats through milking routines and feeding pad, suboptimal teat-spray efficacy and inconsistent feed management were identified as risk factors. Milking practices and feeding pad management were changed to improve teat cleanliness and hygiene practices. Reinstating in-line mastitis detection allowed earlier identification of clinical and subclinical infections, leading to earlier detection of mastitis cases and reduced grading frequency. Parallel dietary evaluation revealed evidence of ruminal acidosis (variable dung scores, feed sorting), prompting ration reformulation with increased fibre inclusion and use of a mixer wagon. This intervention was informed by literature suggesting a potential link between subacute ruminal acidosis (SARA) and increased E. coli mastitis susceptibility through altered immune and gut barrier function.

RESULTS: Following implementation of environmental, nutritional, and detection-based interventions, bulk milk coliform counts stabilised, and clinical mastitis incidence declined. DNA sequencing of milk isolates was undertaken to determine whether specific E. coli strains, including curli-producing variants, contributed to herd-level infection dynamics.

CONCLUSIONS: This paper highlights the steps that veterinary practitioners should take when investigating E. coli mastitis causing coliform grades in bulk tank milk.

Keywords: E. coli mastitis, coliform grades, dairy cows, ruminal acidosis, mastitis detection, New Zealand

Descriptive Results on the Quality of the Bulk Tank Milk in Dairy Cattle Farms in Greece

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BACKGROUND and OBJECTIVE: The objective of the study was the presentation of data regarding the quality of the bulk tank milk in dairy cattle farms, based on a large-scale cross-sectional study conducted throughout Greece.

MATERIALS and METHODS: The study involved 49 dairy cattle farms, with on-site visits performed to all the farms. Samples of bulk tank milk were collected and were processed for cytological, bacteriological and chemical examinations.

RESULTS: Median somatic cell counts in the bulk tank milk collected from the 49 farms was 0.248×106 cells per mL (interquartile range (IQR): 0.060×10^6 cells per mL). In eight farms (16.3%, 95% confidence interval (CI): 8.5%–29.0%) somatic cell counts exceeded 0.400×10^6, while in one farm (2.0%, 95% CI: 3.6%–10.7%) cell counts exceeded 0.500×10^6 cells per mL. Median total bacterial counts in the bulk tank milk from the 49 farms was 42×10^3 colony-forming-units (cfu) per mL (IQR: 62×10^3 cfu per mL). In three farms (6.1%, 95% CI: 2.1%–16.5%), total bacterial counts exceeded 1500×10^3 colony-forming-units per mL. Staphylococci were isolated from the bulk tank milk collected from 32 farms (65.3%, 95% CI: 51.3%–77.1%), streptococci from 16 farms (32.7%, 95% CI: 21.2%–46.6%) and coliforms from 28 farms (57.1%, 95% CI: 43.3%–70.0%). Median values of fat and protein

content in the bulk tank milk were 3.99% (IQR: 0.68%) and 3.29% (IQR: 0.14%), respectively.

CONCLUSIONS: This study provides, for the first time in Greece, data regarding bulk tank milk quality in dairy cattle farms, based on an extensive investigation across many regions of the country.

Keywords: bulk-tank milk, cattle, Greece, mastitis, milk quality

Innovative Intramammary Oxygen-Ozone Therapy for Clinical Mastitis in Dairy Cows

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BACKGROUND and OBJECTIVE: The evaluation of innovative and complementary therapeutic strategies for the clinical management of bovine mastitis represents a highly relevant challenge for veterinary medicine. This study aims to describe the effects of two oxygen-ozone (O_2O_3)-based therapeutic protocols, used either as integrative therapy in association with antibiotics (Ab) or as monotherapy, on udder health in dairy cows affected by clinical mastitis.

MATERIALS and METHODS: A prospective, randomized, and comparative clinical trial was conducted on 45 mammary quarters with clinical mastitis (grades M1–M2) in multiparous Holstein cows (70–120 days in lactation), raised in a dairy farm. The quarters were randomly assigned to three groups (n=15): Ab-conventional antibiotic therapy (ampicillin + dicloxacillin, intramammary, 2 administrations); Ab+O_2O_3-the same antibiotic regimen followed by 4 intramammary infusions of O_2O_3; O_2O_3 – monotherapy with 4 intramammary infusions of O_2O_3. The O_2O_3 mixture was produced using a certified medical ozone generator, immediately prior to administration, to ensure stability and concentration accuracy. For the insufflation procedure per quarter, a total volume of 300 mL was administered at a concentration of 35 µg/NmL, with an infusion time of 100 seconds. Diagnosis was carried out through clinical examination, somatic cell count (SCC), and bacteriological culture (BC). Evaluations were performed at T0, T1 (7 d), and T2 (14 d). The considered outcomes included: etiological cure (negative BC), clinical cure (absence of clinical signs), variation of SCC, and milk yield. Statistical analyses were performed using x^2/Fisher's exact test and mixed ANOVA ($p<0.05$).

RESULTS: A total of 135 clinical evaluations, 135 BC, and 270 SCC were collected. At T2, etiological cure rates were: Ab=50.0%; Ab+O_2O_3=78.6%; O_2O_3=60.0% (all groups vs T0, $p<0.01$). Clinical cure rates at T2 were: Ab=71.4% ($p<0.001$), Ab+O_2O_3=92.9%, and O_2O_3=100.0% ($p<0.00001$). SCC significantly decreased in all groups ($p<0.00001$). Milk yield increased by +31.4% (Ab, $p<0.05$), +58.5% (Ab+O_2O_3), and +36.7% (O_2O_3) ($p<0.01$ for both).

CONCLUSIONS: Intramammary ozone therapy, either in combination with antibiotics or as monotherapy, demonstrated clinical and etiological efficacy comparable or superior to conventional therapy, with positive effects also on milk yield. Considering the absence of withdrawal times, the O_2O_3 approach may represent a promising and sustainable therapeutic option for the management of bovine clinical mastitis, applicable in both conventional and organic dairy farms, where antimicrobial use is strictly regulated and reduction of antibiotic treatments is a primary objective. However, these findings should be interpreted with caution and require further confirmation through larger-scale randomized studies in order to consolidate their clinical effectiveness and reproducibility.

Keywords: Mastitis, Ozone therapy, Antimicrobial reduction, Udder health, Welfare

Bibliometric and Scientometric Study in Mastitis and Animal Welfare

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BACKGROUND and OBJECTIVE: The objectives of the study were: (a) the evaluation of published papers on the association of welfare in mastitis and (b) the presentation of quantitative characteristics regarding the scientific content and bibliometric details of these papers.

MATERIALS and METHODS: The Web of Science platform was used with search terms: [mastitis] AND [welfare]; only articles or reviews published until the end of year 2023 were included. In total, 669 papers remained for individual assessment, after which a total of 150 published papers (102 original articles and 48 reviews) were found to fulfil completely the terms of the search.

RESULTS: The first paper on mastitis and welfare of animals was published in 1996. Thereafter, the number of articles published throughout the years increased progressively (slope: 1.477); moreover, the proportion of articles on mastitis and welfare among all papers on mastitis also increased progressively from 0.1% before 2001 to 1.3% during 2021-2023. Published papers originated from 43 countries; most papers originated from the United Kingdom and the Unites States of America (n=20) and Italy (n=15). Published papers originated from 114 different scientific establishments; most papers originated from the University of Aarhus (n=6) and the University of Minnesota (n=5). The majority of original articles dealt with mastitis and welfare in cattle (n=81); other species on which relevant studies were performed, were sheep (n=12), goats (n=4), buffaloes (n=2), pigs (n=2), mink (n=1) and yak (n=1). Most original articles described field studies (n=84), whilst fewer ones described experimental studies (n=16) or meta-analyses (n=2). All original articles described animal-based work, whilst fewer ones also described management (n=56) or resources (n=11) work. The papers were published in a total of 57 journals, most often in Journal of Dairy Science (n=24) or in Animals (n=11). Overall, there were 605 individual authors of the papers; the median number of authors per paper was 4. The number of authors per paper progressively increased with time (slope: 1.366). Most of the papers were published under subscription only access (n=88), whilst fewer were published as open-access (n=62); nevertheless, a trend emerged for progressive increase of the latter-type papers.

CONCLUSIONS: The work provided a mapping and scientometric analysis of the research output related to welfare research on mastitis internationally. The findings can be used to identify potential collaborators, as well as themes for future research work.

Keywords: bibliometric, cattle, literature, meta-analysis, One Health

Multilocus Sequence Typing of Staphylococcus Aureus Isolates from Clinical and Subclinical Mastitis Cases in South Korea

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BACKGROUND and OBJECTIVE: Staphylococcus aureus is one of the major etiological agents of bovine mastitis and remains difficult to eradicate due to its antimicrobial resistance and ability to evade leukocyte phagocytosis. Moreover, S. aureus shows the highest isolation rate among bacterial species associated with bovine mastitis in Korea. Therefore, the present study aimed to elucidate the molecular characteristics of S. aureus isolates obtained from clinical and subclinical cases and to provide insights for improving management strategies against S. aureus mastitis.

MATERIALS and METHODS: Clinical mastitis samples were obtained from cows that presented clinical signs, while subclinical mastitis samples were collected from asymptomatic cows at six dairy farms. The collected samples were immediately transported under refrigeration, and 10 μL of each milk sample was cultured on blood agar plates. One pure colony of culture-positive samples was sub-cultured for species identification by MALDI-TOF assay, and antimicrobial susceptibility testing based

on minimum inhibitory concentration (MIC) determination. Additionally, bacterial strains identified as S. aureus (5 clinical and 30 subclinical samples) were used for multilocus sequence typing (MLST) PCR analysis. The MLST allelic profiles of S. aureus strains (36 from this study and 91 international references) were uploaded to the PHYLOViZ online platform.

RESULTS: Among 162 culture-positive samples from the subclinical samples, the most prominent isolate was S. chromogenes (38.0%), followed by S. aureus (20.0%), S. simulans (7.33%) and S. epidermidis (6.67%). Antimicrobial susceptibility test revealed that 5 clinical S. aureus strains showed resistance against benzylpenicillin (2 strains out of 5), clindamycin (1/5 strains), erythromycin (1/5 strains), gentamicin (1/5 strains), and minocycline (5/5 strains), and intermediate resistance against cefpodoxime (5/5 strains). In contrast, 30 subclinical S. aureus strains showed antimicrobial resistance against trimethoprim/sulfamethoxazole (5/30 strains) and intermediate resistance against cefpodoxime (2/30 strains). MLST allelic profile analysis revealed that the 36 S. aureus isolates from Korea formed distinct farm-specific and clinical type-specific clusters. When compared with 91 international reference strains, all Korean isolates, except one clinical strain, were clearly separated from foreign strains.

CONCLUSIONS: In this study, S. aureus molecular characteristics varied across farms, clinical vs. subclinical cases, and domestic vs. international strains, forming distinct clusters. Clinical S. aureus mastitis strains demonstrated higher resistance rates to a greater number of antibiotics than subclinical strains. Our findings highlight the need to establish biosecurity systems adaptable to diverse local farming environments, which may influence the characteristics of S. aureus.

Keywords: Bovine mastitis, Staphylococcus aureus, clinical and subclinical, antimicrobial resistance, MLST

Adenosine Triphosphate (atp) Bioluminescence as An On-farm Tool for Assessing Pre-milking Teat Preparation

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BACKGROUND and OBJECTIVE: The adenosine triphosphate (ATP) bioluminescence detector has been advocated as a simple and useful tool for the evaluation of the degree of contamination of surfaces and liquids. The aim of the present study was to examine whether an ATP bioluminescence meter can be used as a cow-side tool to assess the degree of contamination of bovine teats, to objectively score the pre-milking teat preparation protocol.

MATERIALS and METHODS: Teat swab samples from 52 cows on two different dairy farms in The Netherlands were collected. Swabs were taken of each teat immediately after performing pre-milking routine teat preparation. The total bacterial cell count (TBCC) was determined at 22°C using an ISO 6222 standard method (Royal GD, Deventer, The Netherlands). The ATP bioluminescence, expressed as the number of relative light units (RLU) for each teat swab sample, was obtained. Simple linear regression and correlation was used to compare TBCC and RLU. Optimal cut-off points for the ATP bioluminescence method to classify the teat preparation protocol as 'microbiologically appropriate' (TBCC < 4.000 cfu/ml) were determined. Statistical analysis was performed in R (R Core Team 2022).

RESULTS: The median TBCC of teat swab samples was 9.3 Log10 cfu/ml and ranged from 4.7 to 17.9 log10 cfu/ml. The median ATP bioluminescence of the samples was 718 RLU (range 107 – 3566 RLU). A strong significant correlation was found between TBCC and RLU ($r = 0.69$; $p < 0.001$). The optimal cut-point for the ATP bioluminescence method to classify the teat prep protocol as 'microbiologically appropriate' or not was 500 RLU (Se 0.95, Sp 0.73).

CONCLUSIONS: The results of this study indicate that ATP bioluminescence measurements can be used as an on-farm screening tool to evaluate the pre-milking teat preparation. Teat swab samples with an RLU of less than 500 can be considered as 'microbiologically acceptable' and are indicative for an adequate pre-milking udder hygiene protocol.

Keywords: udder health, Microbiology, Adenosine triphosphate (ATP) bioluminescence, RLU

A Retrospective Analysis of the Pathogens Isolated from Mastitis Milk Samples from Uk Dairy Herds 2022-2025

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BACKGROUND and OBJECTIVE: An understanding of the aetiology and pathogens involved in mastitis is critical for developing targeted treatment and prevention plans. Environmental pathogens are thought to be the predominant cause of mastitis in UK dairy herds, however there is a paucity of evidence confirming this over the past decade. This study aimed to describe the frequency of bacterial pathogens isolated from individual cow mastitis samples from UK dairy herds cultured between 2022 and 2025.

MATERIALS and METHODS: Data were analysed from 1,355 samples submitted to a Gloucester-based laboratory by UK veterinary practices via a Boehringer Ingelheim subsidised sampling scheme between August 2022 and December 2025. Sample date and reason for submission were extracted from the submission forms. Samples were grown on Columbia, Modified Edwards, MacConkey and Sabouraud Dextrose agars using the streak plate method. Antimicrobial sensitivity of S.aureus isolates was determined using the Kirkby-Bauer disc diffusion method. Where more than three isolates were recovered, the sample was considered contaminated.

RESULTS: The reason for sampling was not recorded in 61.6% (n=835) of submissions, while 22.9% (n=310) of submissions were from clinical mastitis cases and 15.5% of submissions (n=210) were from high somatic cell count (SCC) cows. There was no bacterial growth in 315 samples (23.2%); a monoculture growth in 714 samples (52.7%); mixed growth in 303 samples (22.4%) and contamination of 23 samples (1.7%). Across all samples the most commonly isolated organisms were S.uberis (29.7%), E.coli (25.9%) and coagulase-negative staphylococci (CNS) (14.5%). For clinical submissions, E.coli (37.7%), S.uberis (29.0%) and CNS (13.5%) were most commonly isolated. For high SCC submissions, S.uberis (31.9%), CNS (21.0%) and S.aureus (14.3%) were most commonly isolated. Antibiograms were performed on 93 submissions where S.aureus was isolated. The majority (n=85; 91.4%) of isolates were sensitive to all tested antimicrobials. Resistance was most often found to Cloxacillin (n=4; 4.3%) and Penicillin (n=3; 3.2%). Only one S.aureus isolate was resistant to multiple (>3) antibiotics.

CONCLUSIONS: In agreement with previous UK studies, major environmental bacteria (E.coli and S.uberis) were most commonly isolated. The frequent isolation of CNS is considered significant as though traditionally considered to be a minor infectious pathogen, recent research identifies CNS species may contribute to both clinical mastitis and increase SCC. While the retrospective nature and inconsistency in submission details are unavoidable limitations, these findings characterise the key pathogens associated with bovine mastitis in the UK and contribute to the evidence-based foundation for informing targeted therapeutic approaches and effective prevention strategies.

Keywords: Udder health, bovine mastitis, milk, bacterial culture, somatic cell count

From Parlour to Ration: A Field Toolbox to Control Mastitis and High Somatic Cell Count in a Small Closed Dairy Herd

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BACKGROUND and OBJECTIVE: A closed dairy herd of 46 lactating cows presented with recurrent clinical mastitis and persistently elevated bulk milk somatic cell count (SCC). With limited replacement stock, extensive culling of chronic high-SCC cows

was not feasible. This case aimed to demonstrate a practical, field-applicable workup to identify dominant pathogens and modifiable drivers and to implement control measures that improve cure probability while reducing new intramammary infections.

MATERIALS and METHODS: Farm records were reviewed for mastitis events and SCC patterns. An on-farm wet milking assessment evaluated cow and teat hygiene, milking settings, milking duration and cluster removal. Pathogen testing used multiplex polymerase chain reaction (PCR) on bulk tank milk and pooled milk from five cows, and bacteriological culture from clinical cases. Selected high-SCC quarters underwent leukocyte profiling. Ration and forage management were assessed, including silage conservation and aerobic spoilage, and manure was sieved in high- and low-producing groups.

RESULTS: Over 12 months, 30 clinical cases occurred and 65% of cows were affected, mainly in cows in third or higher lactation, with clustering during summer. Marked manure contamination of udders and teat ends indicated a heavily contaminated housing environment. Milking observations showed prolonged milking and frequent overmilking due to delayed cluster removal. An iodine-based barrier post-dip was used, but teat-end coverage was inconsistent, and hygiene and handling of reusable items increased cross-contamination risk. Multiplex PCR detected predominantly Streptococcus uberis, with additional detection of Escherichia coli, Staphylococcus aureus and other staphylococci. Culture from clinical cases confirmed S. uberis. Maize silage showed mould spots and a hot area and was fed before full stabilisation. Together with manure consistency and sieve findings, this supported suboptimal starch digestion with increased hindgut fermentation pressure. Loose manure contributed to dirty cows and cubicles, reinforcing environmental contamination pressure.

CONCLUSIONS: Findings supported a multifactorial mastitis problem driven by high environmental contamination and milking-time transmission risk. Control required coordinated changes in digestion and manure consistency, cow cleanliness and cubicle hygiene, alongside improved milking hygiene, effective post-dip coverage and elimination of overmilking. Cow-level management with targeted diagnostics, evidence-based treatment protocols and structured follow-up was essential to maximise cure and limit progression to chronic infection. Vaccination against *S. uberis* was initiated as an adjunct measure. Where culling is constrained, persistently infected quarters may undergo permanent dry-off, with cows subsequently milked on three quarters and segregated when indicated.

Keywords: Mastitis, Somatic cell count, wet milking assessment, milking routine, Streptococcus uberis

Udder Health in Sub-Saharan Africa: Multi-Country Insights, Challenges, Solutions, and A Regional Roadmap

Bitsu Kiflu

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BACKGROUND and OBJECTIVE: Bovine mastitis is a major constraint to dairy productivity and milk quality across Sub-Saharan Africa (SSA). Regional progress is often hindered by fragmented data, limited diagnostic access, and variability in on-farm protocols. We sought to synthesize field insights from multiple SSA countries, describe current situations, highlight shared challenges, and review pathogens and antimicrobial resistance (AMR) patterns. Furthermore, we assessed the impact of a multi-national stakeholder forum on regional capacity and presented a feasible roadmap to improve udder health across the region.

MATERIALS and METHODS: A regional forum named "Udder Health makes a Difference" convened stakeholders including veterinarians, paraveterinarians, milk quality officials, final year veterinary students and dairy advisors from 11 African countries. The program included country insight presentations and discussions from representatives of Zambia, Uganda, Tanzania, Nigeria, Zimbabwe, Ethiopia, and Kenya, alongside sessions on diagnostics and learning. Scientific sessions provided updates on farm-level protocols, dry period management, economics, and therapy, plus a regional review of mastitis epidemiology and AMR in SSA. Thematic breakout sessions focused on designing udder health campaigns, developing modular training, mapping public private partnerships, and strengthening diagnostics. The program included a session on antibacterial residues and contaminants in milk, reflecting food safety concerns.

RESULTS: Cross-country reports identified shared barriers to improving udder health, including

inconsistent milking hygiene, limited routine use of diagnostic tools (CMT/SCC), and a lack of standardized treatment protocols. Participants highlighted the risk of antibacterial residues in urban milk supplies as a critical public health concern. Breakout sessions produced a stakeholder map for regional collaboration, a diagnostics integration plan, and a short-term training roadmap for dairy farmers and animal health practitioners. Post-congress survey results demonstrated high impact: 92% of respondents rated the forum as "very relevant" and 100% reported increased confidence in addressing udder health challenges, particularly when working with multiple stakeholders (vets/paravets, farm owners/attendants, and others involved in dairying). Furthermore, 93% of participants intended to implement a training session in their respective countries on simple hygiene and diagnostic protocols within six months.

CONCLUSIONS: Improving udder health in Sub-Saharan Africa requires shifting from isolated initiatives to a coordinated, sector-wide approach that actively involves veterinarians, paraveterinarians, farm owners, and farm attendants. The forum demonstrated that multi-stakeholder engagement can successfully bridge knowledge gaps and strengthen professional confidence across the dairy value chain. The proposed roadmap highlights practical detection strategies, hygiene bundles, and strong public private partnerships together offering a scalable framework to enhance dairy sustainability, milk quality and safety across the region.

Keywords: Udder health, Mastitis, Diagnostics, Antimicrobial stewardship, Public Private Partnership, Sub-Saharan Africa.

in vitro Antibacterial of Oregano Essential Oil Against Mastitis-associated Bacteria, Including a Methicillin-resistant Strain

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BACKGROUND and OBJECTIVE: Bovine mastitis remains one of the most significant diseases affecting dairy herds worldwide, compromising milk quality, animal welfare, and farm profitability. The extensive use of antimicrobials in mastitis control has contributed to the emergence of resistant pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), highlighting the need for alternative control strategies. Plant-derived compounds such as oregano essential oil (*Origanum vulgare*), rich in phenolic constituents including carvacrol and thymol, have demonstrated broad-spectrum antibacterial activity and have been proposed as potential alternatives in the control of bacterial infections.

MATERIALS and METHODS: This study evaluated the in vitro antibacterial activity of a commercial oregano essential oil against bacterial reference strains associated with bovine mastitis. Antimicrobial activity was determined using the broth microdilution method to establish the Minimum Inhibitory Concentration (MIC), followed by subculture to determine the Minimum Bactericidal Concentration (MBC). Bacterial suspensions were standardized to 0.5 McFarland and incubated at 35 ± 1 °C for 24 hours, with resazurin used as a metabolic viability indicator. The tested strains included Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923, Staphylococcus aureus ATCC 43300 (MRSA), and Klebsiella pneumoniae ATCC 13883.

RESULTS: Oregano essential oil inhibited all tested strains. MIC and MBC values for *E. coli* were 0.625 mg/mL. For *S. aureus* ATCC 25923, MIC and MBC were 5 mg/mL and 25 mg/mL, respectively, while for the MRSA strain they were 5 mg/mL and 15 mg/mL. For *K. pneumoniae*, both MIC and MBC were 5

mg/mL. Notably, methicillin resistance did not correspond to higher inhibitory concentrations.

CONCLUSIONS: These findings demonstrate consistent *in vitro* antibacterial activity of oregano essential oil against mastitis-associated pathogens, including a multidrug-resistant strain, and support further investigation into its potential role within integrated mastitis control approaches.

Keywords: Minimum inhibitory concentration, antimicrobial resistance, {Staphylococcus aureus}, phytotherapy

Enlarged Teat Morphology in Polish Red Cattle – a Hereditary Trait or a Sporadic Anomaly?

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BACKGROUND and OBJECTIVE: Udder conformation is an important trait in the selection of dairy cattle, particularly in herds using mechanical milking. In extensive production systems, however, this trait is assessed less frequently. Nevertheless, teat morphology may influence calf rearing efficiency and predispose animals to injuries or functional disorders of the mammary gland. This study describes a case of unusual teat enlargement accompanied by moderate udder enlargement in Polish Red cows raised under extensive conditions.

MATERIALS and METHODS: Unusual teat enlargement was observed during the periparturient period in two multiparous Polish Red (PR) cows. Clinical examinations were conducted one day postpartum (summer) and subsequently in autumn and winter. The examinations included visual inspection, palpation, assessment of teat canal patency, and detailed morphometric measurements of the teats. Additionally, the California Mastitis Test (CMT; Mastirapid) was performed and somatic cell count (SCC) was determined using a Fossomatic analyzer. The milk microbiome in a pooled sample was analyzed using MALDI/TOF/MS.

RESULTS: Pronounced teat enlargement was observed in both cows. In the most enlarged quarters, increased udder temperature and impaired milk flow were detected. Teat dimensions exceeded reference values reported for other cattle breeds. Immediately after calving, teat length in the first cow ranged from 8 to 16 cm, with diameters from 5.5 to 8.0 cm. In the second cow, teat length ranged from 12 to 13 cm and diameter from 4.9 to 6.9 cm. Over time, the teats gradually decreased in size. After 150 days, teat length ranged from 7 to 13.2 cm in the first cow and from 6.2 to 11 cm in the second. Elevated CMT scores were detected in some quarters until the fifth month of lactation. The SCC in pooled milk reached up to 800,000 cells/mL; however, no microorganisms were detected. Calves remained healthy and developed normally, and both cows are currently pregnant.

CONCLUSIONS: Marked teat enlargement in extensively managed cows may cause difficulties for calves during suckling and represent a management challenge. However, appropriate herd management and early colostrum intake may help prevent potential problems. The high SCC in the absence of detectable pathogens was notable and warrants further investigation. Additional studies, including cytokine analysis, are planned to clarify the underlying mechanisms.

Keywords: Teats, Udder, Cows

Laboratory-tested Efficacy of Numerous Teat Disinfectants for Dairy Cows Against Common Bovine Mastitis Pathogens from Czechia

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BACKGROUND and OBJECTIVE: To conduct a comprehensive laboratory screening of the disinfectant efficacy of the commonly used teat disinfectants ("dips") applied before and/or after milking against mastitis pathogens prevalent in Czechia.

MATERIALS and METHODS: The efficacy of 98 dips was tested against five species and one genus of mastitis pathogens, 5 isolates from each species/genus, thus against 30 isolates from mastitis cases in Czechia. These were Streptococcus uberis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Serratia marcescens and Prototheca spp. The dips were tested by the modified agar dilution test method, in dilution 1: 10 and were combined into four groups according to the disinfectant: iodine (n=25), chlorhexidine digluconate (n=20), chlorine dioxide (n=23) and lactic acid (n=30).

RESULTS: The highest proportion of inhibitions was observed in the dip groups containing chlorhexidine and chlorine dioxide (both in 93.8% of tests; P<0.05). The commonly used iodine products showed the lowest proportion of inhibitions (72.0%; P<0.001), due to a low proportion of inhibitions in tests with Gram-negative (G-) bacteria (53.1%; P<0.001; 40% of these dips did not inhibit any G- isolate), whereas their efficacy against Gram-positive (G+) isolates did not differ from that of the chlorine dioxide and lactic acid dips. Tests with G- bacteria showed the highest proportion of inhibitions for chlorine dioxide dips (93.9%; P<0.05), whereas tests with G+ isolates for chlorhexidine dips (99.3%; P<0.001). Against Prototheca spp. algae, the lactic acid group showed the lowest efficacy (inhibited all five isolates in only 73.3% of products). Across all dip groups, the proportion of dips that showed only partial efficacy (inhibited only some of the five isolates of a given species/genus) was small (2.5-6.0% dip–species/genus interactions for each dip group; n.s.). Thus, for a given species/genus, the test results depended primarily on the choice of dip (beginning with the supplier and the main disinfectant agent) and very little on differences in the characteristics of its isolates.

CONCLUSIONS: In a situation dominated by a specific main pathogen, such as a herd problem with Prototheca sp., selecting a lactic acid-based dip would likely carry a higher risk of choosing a product with insufficient efficacy against Prototheca spp. However, our results are based solely on laboratory testing and cannot be directly applied under practical conditions. Follow-up field testing would be required. Supported by the Ministry of Agriculture of the Czech Republic and the Technology Agency of the Czech Republic by Grants QL24010350 and TN02000017/21.

Keywords: Milking hygiene, teat dip class, germicide, screening, teat disinfectant products, mastitis isolate

Mastitis Vaccination as Key Tool in Modern Herd Management: Expectations, Protocols, and Results

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BACKGROUND and OBJECTIVE: The growing global concern about AMR has prompted initiatives to reduce antimicrobial use in food-producing animals through both regulatory measures and voluntary stewardship programs. Despite decades of research into alternative strategies, the dairy sector continues to rely heavily on antibiotics for mastitis treatment and control. Various non-antibiotic approaches to prevention have been explored including improved milking hygiene, a focus on cow environment management. Among these alternatives, vaccination stands out as the most extensively investigated and scientifically validated approach. Although the intramammary immune response is complex and not yet fully understood, vaccines targeting key pathogens such as Staphylococcus aureus, Streptococcus uberis, and Escherichia coli have shown potential to reduce the incidence, duration, and/or severity of mastitis cases. While no vaccine currently provides complete protection, vaccination remains a promising and sustainable strategy to reduce antibiotic.

MATERIALS and METHODS: Vaccination against whatever disease, including mastitis, has three specific characteristics: it is specific, time-limited, and it is a preventative and not a curative solution. The latter implies that basically every dairy farm should start vaccinating against mastitis as a kind of insurance. This is especially true for vaccination against environmental mastitis pathogens. After all, there is no farm in the world where dairy cows are not exposed to environmental mastitis pathogens including Streptococcus uberis and Escherichia coli. Of course, it is almost impossible to convince every dairy farm to start vaccinating cows against mastitis. The reality is that most farms only start vaccinating when they have already (huge) problems with mastitis. The farm's udder health status is always determined by the balance between the infection pressure (i.e. bacterial load to which the cow is exposed) and the cow's immunity.

RESULTS: This communication consolidates current evidence and expert insights to clarify the role of vaccination against mastitis in dairy herds. It outlines where vaccination can add value, how it can be effectively integrated into herd health programs, and what outcomes farms and veterinarians can realistically expect.

CONCLUSIONS: Vaccination should not be considered as a substitute for good management practices such as proper cow housing hygiene, optimized milking routines, or strong biosecurity. However, when vaccines are targeted at the appropriate pathogens and administered at the right time, they can substantially reduce the severity of clinical cases, lower risk of new infections, and decrease the overall need for antibiotics. Vaccination offers a scientifically supported, practical, and sustainable strategy to help reduce antibiotic use in dairy cows by preventing and mitigating bovine mastitis.

Keywords: Vaccination, mastitis, cow

A Comparison of Routine Treatment and Diagnostic-guided Targeted Selective Treatment for Clinical Mastitis on Northern German Dairy Farms

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BACKGROUND and OBJECTIVE: Antibiotic stewardship in dairy production is driven by regulatory frameworks and antimicrobial resistance concerns. In Germany, mastitis therapy is subject to national requirements (Antibiotika-Leitlinie, TÄHAV) emphasizing prudent and selective antimicrobial use. Targeted selective treatment (TST), supported by on-farm diagnostics, provides a framework for informing antimicrobial treatment decisions. This study assessed routine treatments for mild to moderate clinical mastitis on Northern German dairy farms and compared them with a diagnostic-guided TST protocol using Mastatest, focusing on antimicrobial selection, regulatory compliance (EMA classification, SPC), and antimicrobial use reduction.

MATERIALS and METHODS: The study was conducted on seven dairy farms in Northern Germany and included 67 mild to moderate clinical mastitis cases treated with antibiotics. Animals were treated according to farm-specific protocols without bacteriological diagnostics guiding treatment decisions. Aseptic quarter milk samples

were collected at mastitis detection and analyzed using Mastatest. The test provided pathogen differentiation and MIC-based susceptibility testing for six antimicrobial agents within 24 hours. Farm-level treatment decisions were subsequently compared with Mastatest results to evaluate antimicrobial use and compliance with German antibiotic guidelines.

RESULTS: Routine treatments relied mainly on broad spectrum antimicrobials in 91% (61/67) of cases, while narrow spectrum products were used in only 9% (6/67). At least one EMA category C antimicrobial was used in 88% (59/67) of cases and a category B agent in 2% (1/67). Products not approved for mastitis were administered in 25% (17/67) of cases, including one not authorised in Germany. All cows received intramammary therapy, with 37% (25/67) also receiving systemic antibiotics. Multiple antimicrobial use was common, with 66% (44/67) of cows receiving two agents and 22% (15/67) receiving three or more. SPC compliance was poor, particularly for treatment duration, which was off label in 84% (56/67) of cases. Based on on farm diagnostics, 25% (17/67) of cases would not have required antibiotic treatment, as no pathogens or Gram negative pathogens with high spontaneous cure rates were detected. All Gram positive isolates were penicillin susceptible; however, this narrow spectrum agent was used in only 6% (4/67) of cases.

CONCLUSIONS: The study demonstrates a discrepancy between pathogen susceptibility patterns and routine mastitis antimicrobial treatment on Northern German dairy farms, with substantial non-alignment to national guidelines and pharmacological principles. Diagnostic-guided TST bridges the gap between pathogen susceptibility and routine mastitis treatment, enabling rational, guideline-compliant antimicrobial use. Integration of on-farm diagnostic testing into routine practice supports reduced antimicrobial use, increased suitability of narrow-spectrum therapy, and improved antimicrobial stewardship in dairy production.

Keywords: targeted, selective, treatment, clinical, mastitis, antibiotic

Quarter-Level Field Epidemiology of Bovine Mastitis in a Modern Mediterranean Island Dairy District: Results from 12 Sardinian Herds

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BACKGROUND and OBJECTIVE: The Arborea district in Sardinia (Italy) is a geographically isolated dairy area with modern management and long-standing mastitis control efforts. All farms operate under continuous veterinary supervision within structured risk-based self-monitoring schemes (e.g., Classyfarm), and diagnostic-supported decision-making to minimize antimicrobial use in calves. The district benefited from the IZS-led long-term Milk Quality initiatives aimed at training farmers and reducing contagious udder pathogens in herds. In this real-world setting, we aimed to describe quarter-level clinical and etiological patterns of bovine mastitis, linking udder and milk signs to culture results.

MATERIALS and METHODS: Twelve farms with mastitis history were monitored February–August 2025. Quarter milk (n=622) was sampled following NMC guidelines, with standardized clinical records. Bacterial colonies were identified by MALDI TOF MS, providing quarter level linkage between clinical findings and bacterial species. Basic management information (herd size and structure, housing, milking routine, mastitis treatment choices, colostrum management and calf rearing practices) was collected through standardized questionnaires.

RESULTS: Of 622 quarter samples, 195 (31%) yielded one or two bacterial species, 67 (11%) were classified as mixed flora and 360 (58%) were culture negative. Overall, 37 bacterial species were identified (no mycoplasmas). Escherichia coli was the most frequent (46 isolates), followed by Streptococcus uberis (24 isolates) and non-aureus staphylococci such as Staphylococcus chromogenes (17 isolates); Staphylococcus aureus was confined to a single herd. Mastitis treatments mainly relied on

penetamate, amoxicillin, cloxacillin, and first-generation cephalosporins; calves with diarrhea received aminopenicillins or sulfonamide trimethoprim combinations, while some herds successfully managed calf diarrhea without antimicrobials. For some species (E. coli, Klebsiella pneumoniae, Mammaliicoccus sciuri, Staph. chromogenes, Staph. equorum, Staph. haemolyticus, Staph. microti and Streptococcus lutetiensis), clinical data could be robustly associated with bacteriological findings. Gram-negative infections were more frequently linked to visibly altered milk with flakes/clots and severe changes in secretion, whereas Gram-positive infections more often presented with serous milk and udder inflammation (edema, calor, rubor, dolor). Other species, frequently detected in quarters sampled because of elevated somatic cell counts, showed no consistent association with specific macroscopic patterns.

CONCLUSIONS: In this modern Mediterranean dairy district, mastitis shows a heterogeneous etiological profile dominated by E. coli, Streptococcus uberis and non-aureus staphylococci, with a substantial proportion of culture-negative and mixed-flora samples illustrating field diagnostic complexity. The low circulation of contagious pathogens is consistent with the sustained impact of structured control and farmer-training initiatives in the district. Quarter-level linkage between clinical signs and culture results provides a practical reference for refining decision-making in intensive settings.

Keywords: Mastitis, epidemiology, island

Bovine Mastitis Pathogens in Irish Dairy Herds: Incidence, Patterns and Laboratory Reported Antimicrobial Susceptibility from Practice Submitted Milk Samples in 2024 and 2025

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BACKGROUND and OBJECTIVE: Mastitis remains one of the main drivers of antibiotic use in Irish dairy herds and is a key cause for culling. As part of ongoing support to veterinary practices, Boehringer Ingelheim has funded milk sample culture and sensitivity testing through an external laboratory. This project aimed to describe the main mastitis pathogens identified in 2024 and 2025, look for seasonal patterns, and summarise the pathogens sensitivity results, ranking them as Sensitive, Limited Sensitivity or Resistant. All bulk tank sample submissions were excluded.

MATERIALS and METHODS: Two years of practice submitted quarter milk sample results (2024: 2,280; 2025: 2,371) were reviewed after removing bulk tank samples and standardising data fields. Samples were assigned into "Organism identified", "No growth", or "Contaminated", as reported in the laboratory reports. When mixed infections were reported, each organism was counted individually in order to assess incidence rates. "Limited sensitivity" was retained as its own category, as no zone diameter data was available. All figures are descriptive of this dataset only.

RESULTS: Across both years, just under 90% of submissions yielded a bacterial isolate. Streptococcus uberis, Staphylococcus aureus, and Escherichia coli were consistently the most commonly identified pathogens. This pattern was stable between years, although S. uberis showed a slight rise in 2025, while S. Aureus had a slight decline. A seasonal lift in isolate numbers was seen from roughly April to July in both years, and again from September to December. Susceptibility patterns varied by organism. For example, Staphylococcus aureus isolates were frequently categorised as resistant to penicillin (~75%), while remaining predominantly sensitive to cephalexin (~99%). In contrast, Streptococcus uberis showed low levels of penicillin resistance (~5.6%) and was largely sensitive to amoxicillin (>99%). These findings reflect the lab's categorical interpretations only as no quantitative susceptibility data was available.

CONCLUSIONS: The dominant pathogens in Irish practice submissions remained relatively consistent across the two-year period, with a clear Spring/Summer rise in case numbers, followed by another peak in Autumn/Winter, in line with peak risk periods in a predominantly Spring calving population. The susceptibility distributions reflected well established patterns, including frequent penicillin resistance among Staphylococcus aureus, while Streptococcus uberis showed low levels of penicillin resistance, remaining largely sensitive to amoxicillin. Presenting the laboratory's categorical interpretations as simple proportions offers a

practical view of treatment expectations while avoiding over interpretation of "Limited sensitivity", which cannot be quantified further without disk diameter data. These findings may help guide treatment discussions within herds.

Keywords: Bovine Mastitis, Milk Samples, Culture and Sensitivity, Dairy Production

Cytobacteriological Status of Bovine Udder Quarters at Dry-Off: A Descriptive Study in Germany (2022–2025)

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BACKGROUND and OBJECTIVE: Assessment of udder health at dry-off (DO) is essential for evidence-based dry-cow therapy. Knowledge of pathogens and antimicrobial susceptibility supports the decision. This descriptive epidemiological study evaluated bacteriological findings, somatic cell count (SCC), and antimicrobial susceptibility in quarter foremilk samples collected before dry-off in German dairy herds.

MATERIALS and METHODS: Between January 2022 and December 2025, 72,002 quarter foremilk samples (QFS) from approximately 18,000 cows in 216 dairy herds (predominantly Holstein Friesian) were collected 1–7 days before dry-off. The annual distribution of samples was 30.2% (2022), 25.9% (2023), 24.0% (2024), and 19.9% (2025). Samples were aseptically collected by farmers; parity and clinical history were not recorded. All QFS were analyzed at bovicare GmbH (Bernau, Germany) for bacteriology and SCC (FOSS Analytical, Denmark) following NMC and DVG guidelines. Intramammary infection (IMI) was defined as pathogen detection plus SCC above thresholds using either the DVG definition (>100,000 cells/mL) or the NMC definition (>200,000 cells/mL). Antimicrobial susceptibility testing was performed by agar diffusion; inhibition zones were interpreted as susceptible (S), intermediate (I), or resistant (R) based on published standards (e.g., CLSI). Ffollowing antimicrobials were tested: penicillin (P), oxacillin (OXA), cephapirin (CEPA), and cefquinome (CFQ). Chi-square tests were used; $p < 0.05$ was considered significant (IBM SPSS v29.0).

RESULTS: Of all QFS, 52,726 (73.2%) were bacteriologically negative, 1,557 (2.2%) were contaminated, and 1,292 (1.8%) were not collected. Pathogens were detected in 16,427 (22.8%) samples. Using the DVG IMI definition, 11,610 (16.1%) samples met IMI criteria; using the NMC definition, 8,960 (12.4%) samples. Among NMC-defined IMI samples, pathogen distribution was: Non-aureus staphylococci (NaS) 2,986 (33.3%), Streptococcus uberis 2,222 (24.8%), Corynebacterium bovis 1,995 (22.3%), Enterococcus spp. 489 (5.5%), Staphylococcus aureus 394 (4.4%), Streptococcus dysgalactiae 395 (4.4%), coliforms 352 (3.9%), and others 127 (1.4%). NaS proportions differed by year ($p < 0.001$; 2024: 29.7% vs 2023: 35.8%), S. uberis increased ($p < 0.001$; 2023: 20.5% vs 2025: 27.6%), and S. aureus increased ($p = 0.001$; 2022: 3.3% vs 2025: 5.6%). Antimicrobial susceptibility of NaS was OXA 96.5%, CEPA 94.9%, CFQ 88.3% (I: 8.2%), and P 56.8%. For S. aureus, susceptibility was CEPA 98.5%, OXA 97.0%, CFQ 96.0%, and P 76.3%.

CONCLUSIONS: The substantial number of QFS without IMI before DO supports implementation of selective dry-cow therapy. NaS is the predominant pathogen across the years. The using first- and fourth-generation cephalosporins offers no advantage over oxacillin, which is classified by the EMA as a group D antibiotic (recommended as first-line treatment whenever possible).

Keywords: udder health, selective dry-cow therapy, antimicrobials, susceptibility,

Field Evaluation of Long-acting Cloxacillin Intramammary Treatment for Clinical Mastitis in Dairy Herds: Bacteriological Results, Somatic Cell Counts and Satisfaction Survey

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BACKGROUND and OBJECTIVE: This study summarizes experiences with the use of a long-acting cloxacillin (LAC) intramammary preparation for treatment of clinical mastitis (CM) in dairy cows. Our aim was to explore the experiences of the users and the effect of LAC on the somatic cell count (SCC) and the quarter bacteriological status.

MATERIALS and METHODS: A questionnaire was conducted to evaluate the opinions of treating veterinarians and farmers regarding the practicality of LAC. 57 CM cases from three German dairy herds were evaluated. Bacteriological analysis (BA) was performed upon diagnosis (D0) and repeated after 14 days (D14). Somatic cell count (SCC, Fossomatic, FOSS Analitical, DK) of 55 milk samples was determined on D0 and D14. All cases were treated with LAC 200mg administered in three doses every 48 hours according to manufacturer's instructions. The % relative change of SCC (ΔSCC) was computed, and descriptive measures (mean±SD, median, range) were reported for SCC (D0, D14) and ΔSCC. (R language for statistical computing v. 4.5.2).

RESULTS: No adverse reactions were observed. Questionnaire responses indicated that 72% of farmers and 74% of veterinarians were satisfied with treatment outcomes (scoring >5 on a 1–10 scale). The SCC was 5,57±5,54 ×10³ cells/ml (median 2,50 ×10³ cells/ml, range 116–20,53 ×10³ cells/ml) and 2,66±3,96 ×10³ cells/ml (median 417 ×10³ cells/ml, range 27–14,04 ×10³ cells/ml) on D0 and D14, respectively. The SCC dropped by 79.6% (median) between D0 and D14; however, the disperse of ΔSCC (42.5%±484.5%, range 99.1% to 3,286% indicated the variable response to the treatment and the existence of strong outliers. On D14, 18 out of 57 samples with initial SCC >150 ×10³ cells/ml (D0) improved to ≤150 ×10³ cells/ml (D14). The BA at D0 was negative (germ-free) in 43.9% (25/57) of cases. BA

RESULTS: Staphylococcus aureus (24.6%, n=14), Streptococcus uberis (8.8%, n=5), coagulase-negative staphylococci (CNS; 7%, n=4), and Escherichia coli (5.3%, n=3). Bacteriological cure rates on D14: 50% (7/14) for S. aureus, 80% (4/5) for S. uberis, 75% (3/4) for CNS, and 100% (3/3) for Escherichia coli. Similar bacteriological cure can be expected treating clinical mastitis in Germany (Leon et al 2024).

CONCLUSIONS: Treatment of clinical CM with a LAC intramammary preparation was well perceived by clinicians and farmers. Pronounced reductions in somatic cell count and favorable bacteriological cure rates for key pathogens suggest effectiveness in managing clinical mastitis in dairy herds.

Keywords: long-acting cloxacillin, clinical mastitis, dairy herds, bacteriology, Somatic cell counts

in vitro Susceptibility of Clinical Mastitis-Associated escherichia Coli Isolates to the Most Relevant Intramammary Antibiotics Used in Germany

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BACKGROUND and OBJECTIVE: The efficacy of intramammary (IMM) antibiotics for treatment of clinical mastitis (CM) caused by Escherichia coli remains controversial. Recent evidence demonstrated benefit of IMM ceftiofur in non-severe gram-negative CM; however, use of third- and fourth-generation cephalosporins is restricted in parts of Europe. This study assessed the in vitro susceptibility of bovine CM–associated E. coli isolates to the most frequently used IMM antibiotics in Germany.

MATERIALS and METHODS: Quarter milk samples from dairy cows with recent CM were collected across Germany (2020–2023). Samples were analyzed for somatic cell count (SCC), bacteriological culture, and antimicrobial susceptibility by agar disc diffusion at a milk quality laboratory (bovicare GmbH, Germany), following NMC and DVG guidance with minor modifications. Susceptibility was determined for amoxicillin/clavulanic acid (AMC) and a cephalexin–kanamycin combination (CNK). Inhibition zone diameters were classified as susceptible (S), intermediate (I), or resistant (R) using the following breakpoints: AMC—S >17 mm, I 14–17 mm, R <14 mm; CNK—S >19 mm, I 18–19 mm, R <18 mm. Statistical comparisons were performed using Friedman tests (IBM SPSS v26.0); significance was set at P <0.05.

RESULTS: E. coli was isolated from 2,002 samples. Susceptibility differed significantly between antimicrobials (P <0.05). For AMC, S/I/R proportions were 80%/3%/17%. For CNK, S/I/R proportions were 31%/33%/36%. These findings align with results reported for mastitis-associated E. coli isolates in Austria using disc diffusion method and comparable thresholds.

CONCLUSIONS: The commercially available fixed combination AMC, consisting of a β-lactam antibiotic (amoxicillin) and a β-lactamase inhibitor (clavulanic acid) demonstrated high in vitro activity against E. coli in agar diffusion tests. In contrast, the cephalexin-kanamycin combination (CNK) showed low in vitro activity against mastitis-associated E. coli, with limited susceptibility and a high proportion of non-susceptible isolates. Similar findings have been reported in Europe.

Keywords: Dairy cow, mastitis, Escherichia coli, Intrammamary antibiotics, Agar diffusion test, antimicrobial susceptibility

in vitro susceptibility of Staphylococci to Antibiotics Used Against Bovine Mastitis Pathogens

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BACKGROUND and OBJECTIVE: In-vitro antimicrobial susceptibility data are essential to support veterinary prescribing. This epidemiological study evaluated antimicrobial susceptibility of staphylococci—non-aureus staphylococci (NaS) and Staphylococcus aureus—isolated from quarter foremilk samples (QFS) collected in German dairy herds.

MATERIALS and METHODS: From 2023 to 2026, 1,711 QFS were collected from: clinical mastitis (CM; 754), dry-off (DO; 625), high bulk tank SCC (286), and post-calving (46). Samples were aseptically collected by farmers; clinical histories were not recorded. All QFS were analyzed at bovicare GmbH (Bernau, Germany) for bacteriology and SCC (Fossomatic; FOSS Analytical, Denmark) following NMC and DVG guidelines. Antimicrobial susceptibility testing was performed by agar diffusion; inhibition zones were interpreted as susceptible (S), intermediate (I), or resistant (R) using published standards (e.g., CLSI). Tested antimicrobials were: penicillin (P), lincomycin (LIN), oxacillin (OXA), cephapirin (CEPA), amoxicillin/clavulanic acid (AMC), ampicillin (AMP), tylosin (TYL), erythromycin (ETM), the fixed combination penethamate/penicillin/framycetin (PPF), and cefquinome (CFQ). Friedmann tests were used; $p < 0.05$ was considered significant and statistically significant differences between antibiotics were represented using different letters (a, b, c, d, e, f) (IBM SPSS v29.0).

RESULTS: Of all 1,711 QFS, 1,006 (59%) were NaS and 705 (41%) were S. aureus. For NaS the susceptibility S/I/R (%) was: TYL(a) 99/1/0; AMC(a) 99/0/1; CEPA(a) 99/0/1; CFQ(b) 95/4/1; OXA(b) 95/1/4; ETM(c) 88/8/4; PPF(c) 87/9/4; AMP(d) 56/8/36; P(d) 57/5/38; LIN(e)

46/25/29. For S. aureus the distribution for S/I/R (%) was for the different antibiotics: AMC(a) 99/0/1; CEPA(a) 99/0/1; TYL(a) 97/3/0; OXA(b) 97/0/3; CFQ(c) 92/6/2; ETM(d) 77/22/1; P(d) 79/4/17; AMP(d) 76/6/18; PPF(e) 67/22/11; LIN(f) 41/46/13.

CONCLUSIONS: Staphylococci are important pathogens causing mastitis in dairy cows. Using agar diffusion method, Staphylococci were susceptible to most tested antimicrobials. TYL, AMC, and CEPA showed the highest in-vitro activity, while P, LIN, AMP and PPF showed the lowest in-vitro activity against both NaS and S. aureus.

Keywords: {In vitro} susceptibility, Staphylococci, bovine mastitis

Temporal Dynamics and Seasonal Patterns of Bovine Mastitis Pathogens in a Commercial Dairy Herd in the Comarca Lagunera Region (Torreón, Coahuila & Durango, México): A Two-Year Bacteriological Surveillance Study (2024-2025)

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BACKGROUND and OBJECTIVE: Bovine mastitis continues to be one of the diseases with the greatest economic impact on dairy production worldwide. This disease causes significant production losses due to a decrease in milk quality and quantity and an increase in production costs. The objective of this study was to evaluate the temporal dynamics, seasonal patterns, and monthly prevalence of pathogens associated with bovine mastitis in a commercial dairy barn over a two-year period (2024-2025). Monthly bacteriological culture data obtained from milk samples from cows positive to the California test (CMT) were analysed to determine the prevalence, interannual variation and seasonal distribution of pathogens within this production system.

MATERIALS and METHODS: This study consisted of a retrospective observational epidemiological analysis of bacteriological culture data collected between January 2024 and December 2025 on a commercial dairy farm. Monthly laboratory results were used to construct a database of mastitis pathogen detection frequencies. Milk samples were taken from California test-positive cows following standard aseptic sampling protocols to minimise contamination during sampling. Bacteriological diagnosis was performed using blood agar culture methods, including incubation at standard temperature, evaluation of colony morphology, and confirmatory testing. The pathogens evaluated in this study included Staphylococcus aureus, Streptococcus agalactiae, coagulase-negative Staphylococcus spp., Streptococcus spp., Escherichia coli, Klebsiella spp., Proteus spp., Arcanobacterium spp., and Pseudomonas spp. The monthly prevalence of each pathogen was calculated as the proportion of positive isolates relative to the total number of milk samples analysed during that month. Data visualisation included epidemiological heat maps, seasonal distribution graphs, and Pareto ranking analysis of pathogen prevalence to identify the dominant mastitis pathogens in the herd.

RESULTS: Throughout the two-year evaluation period, environmental pathogens accounted for the majority of bacteriological isolates detected in mastitis cases. Escherichia coli was the dominant pathogen in both years, with an annual prevalence of over 50% of isolates. Coagulase-negative Staphylococcus species represented the second most prevalent group of pathogens, followed by Staphylococcus aureus and Streptococcus spp., which remained present at lower but constant prevalence levels throughout the months. Visualisation of the heat map revealed a constant dominance of Escherichia coli throughout the study period, with seasonal peaks observed during the spring and summer months.

CONCLUSIONS: The predominance of environmental pathogens observed in this study reflects the effectiveness of control programmes targeting contagious pathogens such as Staphylococcus aureus and Streptococcus agalactiae. Their low prevalence suggests improvements in milking routines and herd health management.

Keywords: mastitis, epidemiology, dairy herd health, bacteriology, seasonal dynamics

Presence of Bacteria on Intramammary Tubes After Their Application Under Field Conditions

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BACKGROUND and OBJECTIVE: Application of intramammary tubes should be performed under aseptic conditions to minimize the risk of iatrogenic intramammary infections (IMI). This study assessed bacterial contamination on cannula tips collected immediately after routine on-farm use.

MATERIALS and METHODS: After routine use on a 600 cow dairy farm with highly hygienic milking and treatment practices, cannula tips from 10 intramammary tubes and from 10 internal teat sealants were collected immediately and submitted to the laboratory for bacteriological examination. Microbiological procedures followed GVA guidelines (GVA 2009). The tips were placed in Ringer's solution, which was then further examined using quantitative microbiological methods on esculin blood agar (Oxoid, Germany). Plates were incubated aerobically at 37°C and examined after 24 h and 48 h. Colonies were identified by MALDI-TOF MS (Bruker Daltonics; microflex LT/SH smart; MBT Compass Library v8). Bacterial load was reported as CFU per cannula tip.

RESULTS: Among the 10 internal teat sealants, 7 cannula tips (70%) showed no bacterial growth. One cannula tip yielded Pseudomonas putida (480 CFU). One cannula tip yielded a mixed culture of Clostridium botulinum, Lactococcus garvieae, and Pseudomonas putida (total 480 CFU). One cannula tip showed nonspecific growth (30 CFU). Among the 10 intramammary tubes, only one cannula tip (10%) showed no growth. Eight samples showed nonspecific growth (average 45 CFU, min 5 CFU, max 340 CFU). One cannula tip yielded Micrococcus luteus and Staphylococcus cohnii (3000 CFU).

CONCLUSIONS: Low-level contamination was observed, mainly with environmental organisms. Pseudomonas putida is commonly found in soil, water, and plants, and Micrococcus luteus is common in soil, dust, water, and human skin flora. Lactococcus garvieae has the potential to cause IMI and mastitis.

Keywords: Mastitis, IMI, Treatment, Hygienic Application

Determination of MIC (Minimal Inhibitory Concentration) for Cloxacillin Against Non-Aureus Staphylococci (NAS) Isolated from Quarters at Dry-Off

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BACKGROUND and OBJECTIVE: Cloxacillin (CLOX), a time-dependent isoxazolyl penicillin, has been used for decades for the intramammary treatment of bovine mastitis, both during lactation and at dry-off (DO). Testing pathogen susceptibility to antibacterial agents is an essential prerequisite for the targeted and responsible use of antibiotics. Non-aureus staphylococci (NAS) are the most frequently identified pathogens at DO (Klawitter et al. 2025). NAS are a species group with large variability, they have different pathogenicity, and they can develop easily resistance. In this study, we analysed the MIC of CLOX for NAS isolated from DO milk samples (MS).

MATERIALS and METHODS: The collection of MS and microbiological identification were performed in accordance with DVG guidelines (2009). Ten microliters (10 µL) of MS were plated on esculin blood agar (Oxoid, Germany). After 24 and 48 h of aerobic incubation at 37°C, microbiological investigations were conducted. Colonies were differentiated by MALDI-TOF analysis (Bruker Daltonics, microflex LT/SH smart, MBT Compass Library, V8, Germany). For MIC testing, the broth microdilution method (CLSI VET 01-A4, 2013) was applied. Bacterial growth was determined by the appearance of a visible turbidity; the well without turbidity corresponded to the MIC of the respective isolate.

RESULTS: The isolates are a representative cross-section of NAS from MS obtained immediately before DO in northern Germany. Seventy-two isolates were analysed. The distribution was as follows: 23 isolates of S. chromogenes; 17 of S. haemolyticus; 11 of S. epidermidis; six of S. xylosus, six of S. saprophyticus; four of S. succinus, three of S. simulans; and one each of S. equorum and S. hyicus. MIC values ranged from 0.125 to 4 µg/mL. For all the isolates, MIC50 was 0.25 µg/mL and MIC90 was 1 µg/mL. Minor variations were observed between groups: for S. chromogenes MIC50 was 0.5 µg/mL and the MIC90 1 µg/mL. For S. haemolyticus we found a MIC50 of 0.25 µg/mL and a MIC90 of 0.5 µg/mL. For S. epidermidis: MIC50 0.125 µg/mL and MIC90 0.25 µg/mL. For S. succinus, both MIC50 and MIC90 were 1 µg/mL.

CONCLUSIONS: The MICs described here can be considered low. Among NAS isolates, only small differences were observed. Determined MICs were lower compared with those from CM (1 µg/mL for MIC50 and 2 µg/mL for MIC50; Bolte et al. 2020). A high efficacy of high concentrated, long acting CLOX applications at dry-off can be expected for all NAS analyzed.

Keywords: antimicrobial resistance, dry off, staphylococci, cloxacillin

Determination of MIC (Minimum Inhibitory Concentration) for Cloxacillin Against Gram-Negative Bacteria Isolated from Bovine Mastitis Milk Samples

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BACKGROUND and OBJECTIVE: Cloxacillin (CLOX), a time-dependent, semisynthetic penicillin, is commonly used in the dairy industry at dry-off (DO). The intramammary tubes contain between 500 mg and 1 g CLOX. Gram-negative bacteria such Escherichia coli and Klebsiella spp. are not frequently identified pathogens at DO in Germany (Klawitter et al. 2025). Nevertheless, intramammary infections can occur after dry-off and may involve Gram-negative bacteria. This study evaluated CLOX MICs for Gram-negative isolates obtained from bovine milk samples (MS).

MATERIALS and METHODS: Milk sampling and microbiological identification were performed according to GVA guidelines (GVA 2009). Ten microliters (10 µL) of MS were plated on esculin blood agar (Oxoid, Germany). After 24 and 48 h of aerobic incubation at 37°C, microbiological investigations were conducted. Colonies were identified by MALDI-TOF MS (Bruker Daltonics; microflex LT/SH smart; MBT Compass Library v8). MIC testing was performed using broth microdilution (CLSI VET01-A4, 2013). Bacterial growth was determined by visible turbidity; the lowest concentration without turbidity was recorded as the MIC.

RESULTS: A total of 54 isolates (41 E. coli, 7 Klebsiella pneumoniae and 6 Klebsiella oxytoca) were analysed. The isolates were obtained from commercial dairies in northern Germany. MIC values ranged from 128 to >512 µg/mL. For E. coli, MIC50 was 256 µg/mL and MIC90 was 384 µg/mL (median 256 µg/mL; IQR 128 µg/mL; range 128 to >512 µg/mL). For K. oxytoca, MIC50 and MIC90 were 384 µg/mL (median 384 µg/mL; range 200 to 384 µg/mL). For K. pneumoniae, MIC50 was 384 µg/mL and MIC90 was >512 µg/mL (median 384 µg/mL; range 256 to >512 µg/mL).

CONCLUSIONS: CLOX-MICs against the isolates tested were high, which is consistent with limited intrinsic activity against Gram-negative bacteria. However, concentrations above this MIC can be temporarily achieved, especially in the teat area, in the first few hours after intramammary application, depending on the residual milk volume and the distribution of the drug. Consequently, high CLOX concentrations can reduce the growth of Gram-negative pathogens in residual milk after DO.

Keywords: antimicrobial susceptibility, resistance, Escherichia coli, Klebsiella, cloxacillin, mastitis

Monitoring the Association Between Virulence Factors, Somatic Cell Counts, and Antibiotic Susceptibility in Mastitis Pathogens Isolated from Korean Dairy Farms

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BACKGROUND and OBJECTIVE: Bovine mastitis causes significant economic losses in dairy farms due to treatment costs and milk disposal. Pathogen diversity leads to varied clinical symptoms and prognoses. Even within the same species, therapeutic responses differ based on virulence factors (VFs). This study investigates the associations between VFs, antibiotic susceptibility, and somatic cell counts (SCC) in major mastitis pathogens from Korean dairy farms.

MATERIALS and METHODS: A total of 290 bacterial isolates — Escherichia coli (ECO, n = 23), Enterococcus spp. (ENT, n = 80), Staphylococcus aureus (SA, n = 109), and coagulase-negative Staphylococci (CNS, n = 78)—were recovered from mastitis samples (2015-2019). PCR assays were performed to detect VFs associated with biofilms, enterotoxins, adhesion, and iron acquisition. SCC was measured, and antibiotic susceptibility against nine antimicrobials was evaluated via disk diffusion. Statistical analyses, including t-tests and Spearman's correlation, were performed using SigmaPlot.

RESULTS: VF analysis revealed that SA exhibited significantly higher detection rates of enterotoxins sea and seb compared to CNS (13.16% vs. 3.85% and 7.37% vs. 0.00%, respectively). Conversely, the biofilm-related factor icaA was not detected in SA (0%) but showed a 25.64% detection rate in CNS. Notably, over 80% of Staphylococcus spp. harboring enterotoxin-associated VFs were associated with SCC levels exceeding 1×10^6 cells/mL. In ENT, the presence of the enterococcal surface protein (esp) showed a significant negative correlation with the number of susceptible antibiotics ($r = -0.36$, $p < 0.01$). For ECO, an increase in the total number of VFs was significantly correlated with reduced antibiotic susceptibility ($r = -0.498$, $p < 0.05$). Specifically, iron acquisition-related VFs, fyuA and irp2, demonstrated strong negative correlations with antibiotic susceptibility (both $r = -0.529$, $p < 0.01$). Notably, ECO isolates positive for Shiga toxin-related VFs from specific farms exhibited significantly higher SCC ($3.65\times10^6 \pm 1.79\times10^6$ cells/mL) and lower antibiotic susceptibility (1.50 ± 0.76) compared to others.

CONCLUSIONS: A significant negative correlation exists between VF accumulation and antibiotic susceptibility across all pathogens, indicating that higher pathogenicity leads to poorer treatment responses. Shiga toxins in ECO and enterotoxins in SA were key drivers of elevated SCC. Individual farms showed variations in multidrug resistance rates. VF profiling serves as a valuable tool for predicting prognosis and establishing effective, farm-specific therapeutic strategies.
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Keywords: Antibiotic susceptibility, Enterococcus, Escherichia coli, Mastitis, Staphylococcus, Virulence factor

Milk Metabolome Characteristics in Dairy Cows Affected by Different Severity of Intramammary Infection

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BACKGROUND and OBJECTIVE: Mastitis is a main concern in dairy cows, as it negatively affects health and welfare. When the pathogen crosses teat duct and infects the mammary tissue, it triggers an innate immune and inflammatory responses characterized by an increase in SCC in milk above 200.000cells/mL. However, some intramammary infections are not associated with increase in milk SCC. Several studies were conducted to study milk metabolomic profiles. However, knowledge about the mechanisms by which milk metabolites are altered, and the influence of only intramammary infection on milk metabolome are still lacking. The aim of this study was to investigated the quarter-milk metabolomic profile of dairy cows affected by intramammary infection, subclinical mastitis, and clinical mastitis.

MATERIALS and METHODS: 80 quarters were enrolled following a cross-sectional study from multiparous Holstein-Friesian dairy cows between 30 and 100 days in milk, not under antimicrobial treatment, and without an history of treatment in the previous 6 months. Quarters were equally divided in four groups: healthy (H) quarters with SCC<200.000cells/mL and negative bacteriological milk cultures (BMC); intramammary infection (IMI) with SCC<200.000cells/mL and positive BMC; subclinical mastitis (SCM) with SCC<200.000cells/mL, positive BMC, and absence of clinical signs; and clinical mastitis (CM) with SCC<200.000cells/mL, positive BMC, and presence of clinical signs. Milk metabolomics analysis were conducted by 1H-NMR. Differences in metabolites were assessed with a linear mixed-effects model. A p-value<0.05 was accepted, whereas a 0.05≤p-value≤0.10 was considered as trend to significance.

RESULTS: Main pathogens were Staphylococcus aureus (24.4%) and Escherichia coli (23.3%). The SCC were 49.200, 66.100, 1.982.800, and 11.834.700 cells/mL for H, IMI, SCM, and CM, respectively. 45 metabolites were identified of which 34 differed significantly among groups. 18 metabolites changed from H to CM, with a progressive increase of 2-aminoadipate, alanine, dimethylamine, n-acetyl-glycine, and lactate, and decrease of creatine-phosphate, o-phosphocholine, glucose, lactose, maltose, cis-aconitate, carnitine, fumarate, phenylacetate, 2-ketobutyrate, acetoacetate, citicoline, and orotate. Other 12 metabolites differed only in CM compared to all other groups with greater levels in leucine, taurine, valine, acetate, formate, pyruvate, 5-dodecenoic acid, and 3-hydroxybutyrate, and lower levels in arabinose, galactose, ribose, and ascorbate.

CONCLUSIONS: In conclusion, milk metabolome suggested a progressive increase in inflammation, blood-milk barrier damage, oxidative stress, and microorganism metabolism. Moreover, increased cell proliferation was suspected during IMI and SCM, with progressive substrate consumption in CM. Furthermore, both SCM and CM showed impaired energy and lipid metabolisms, and altered systemic energy status with worse conditions during CM.

Keywords: Metabolomics, Mastitis, Dairy cows,

Using Quantitative Polymerase Chain Reaction Threshold Cycles for Evaluating Cow Survival After Coliform Mastitis

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BACKGROUND and OBJECTIVE: Rare but severe coliform mastitis caused by *Escherichia coli* and *Klebsiella spp.* is an urgent and economically significant disease of dairy cattle worldwide. Rapid and strong host immune response is responsible for the severe and often fatal symptoms. Choosing treatment options and setting a prognosis are at the moment based on limited

knowledge. The aim of our observational study was to examine the association between *E. coli* and *Klebsiella spp.* DNA amount in quarter-milk samples determined by quantitative polymerase chain reaction (qPCR) cycle threshold (Ct) values and 7-day survival after mastitis onset in dairy cattle.

MATERIALS and METHODS: The data included positive *E. coli* and *Klebsiella spp.* quarter-milk sample results from the Centralized Health Care Register for Finnish Cattle Herds, which were combined with animal data from the national milk recording database. A generalized linear mixed model with a logit link function and herd as a random effect was used to evaluate the effect of pathogen-specific Ct values with other cow-level covariates to predict the odds of 7-day survival after coliform mastitis. Samples excluded were Ct values within the minimum limit values (*E. coli* 34.1–37, *Klebsiella spp.* 32.1–37) and contaminated samples with more than two pathogens.

RESULTS: A total of 6.7% (261/3876) of cows with *E. coli* mastitis and 12.9% (75/581) of cows with *Klebsiella spp.* mastitis died or were euthanized within seven days after milk sampling. The odds of survival after mastitis increased per one-unit increase in Ct value (*E. coli*: odds ratio, [OR] 1.35, 95% CI 1.30–1.40, $p < 0.0001$; *Klebsiella spp.*: OR 1.36, 95% CI 1.24–1.48, $p < 0.0001$). The odds of survival decreased with each 10-day increase in days in milk (*E. coli*: OR 0.96, 95% CI 0.95–0.98, $p < 0.0001$; *Klebsiella spp.*: OR 0.96, 95% CI 0.93–0.98, $p = 0.001$). In the *E. coli* group odds of survival decreased when parity increased. In the *Klebsiella spp.* group, the association between parity and survival was weaker than in the *E. coli* group. Breed, number of infected quarters or medical treatment had no significant association with survival.

CONCLUSIONS: Based on these preliminary results, the amount of *E. coli* and *Klebsiella spp.* DNA in milk samples is associated with survival in the first week after disease onset. This information could help in evaluating severity of the disease and guiding treatment decisions. Quantitative PCR offers a diagnostic way for predicting an animal's survival from severe coliform mastitis by estimating pathogen DNA through Ct values.

Keywords: mastitis diagnostics, qPCR, coliform mastitis, E. coli mastitis, Klebsiella spp. mastitis

Impact of Clinical Mastitis at Different Stages of Lactation on Milk Revenue

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BACKGROUND and OBJECTIVE: Clinical mastitis (CM) has a substantial impact on milk yield and farm profitability. Although the negative association of CM on milk production over the entire lactation is well documented, less is known about how the timing of CM within lactation influences economic outcomes. We assess the impact of CM at different stages of lactation on milk revenues.

MATERIALS and METHODS: Data from four dairy farms comprising 1,102 Holstein cows (387 primiparous and 715 multiparous) that calved between February 13 and September 30, 2018, were analyzed. Monthly test-day records were used to obtain individual milk yield, and fat and protein contents. Trained farm personnel performed CM diagnosis. Cows were categorized into four lactation-stage groups according to days-in-milk (DIM) at the first recorded CM CASE: first-month (1–30 DIM), early lactation (31–100 DIM), mid lactation (101–200 DIM) and late lactation (201–305 DIM). Milk revenue was calculated as the area under the lactation curve of daily milk income, estimated by linearly interpolating between consecutive test-days, adjusted for fat and protein, and valued using component-specific prices. Milk revenue was modeled by lactation stage at first CM occurrence using linear mixed models (SAS Studio), with farm, parity, calving season, and uterine disease included as covariates. For mid and late lactation stages, models also included production level as a covariate. Milk revenue is reported as USD/cow/year (mean and 95 % CI). For each period-specific model, healthy cows were CM-free up to the end of that period and had lactation length ≥ that end-day (first-month: CM-free in the first 30 DIM, no minimum length).

RESULTS: Out of the 1,102 cows included in the analysis, 42 % (n=465) experienced at least one CM CASE: 190 in the first-month, 133 in early lactation, 95 in mid lactation, and 47 in late lactation. Compared with healthy cows, CM occurring within the first month of lactation was associated with a lower milk revenue of -195 USD/cow/year (-305 to -85; P = 0.0005). In contrast, estimated revenue differences were -63 (-155 – 28; P = 0.18) for early lactation, -7 (-105 to 90; P = 0.88) for mid lactation, and 75 (-52 to 204; P = 0.25) USD/cow/year for late lactation.

CONCLUSIONS: The impact of CM on milk revenue depended on the lactation stage at first occurrence. A first case during the first month was associated with substantial milk revenue losses, whereas revenue differences were small when the first CM case occurred in later stages.

Keywords: udder health, economics of animal health, Milk losses

Udder Health Indicators on Five Commercial Czech Dairy Farms After the Introduction of Selective Dry Cow Treatment in Accordance with European Union Legislation

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BACKGROUND and OBJECTIVE: To verify the implementation and sustainability of selective dry cow treatment (SDCT) in accordance with European Union legislation, under Czech conditions.

MATERIALS and METHODS: On farms (A, B, C, D, E), the introduction of SDCT began in 07-12/2023. It was supported through training and continuous consultancy until 12/2024. The farms/herds comprised an average of 241, 122, 522, 332, and 679 Czech Fleckvieh (A, B, C) and Holstein (D, E) cows, with a milk yield of 29.5-34.4 l/day. The multi-component algorithms used for SDCT differed according to farm-specific conditions. On-farm culture results were utilized on farms B, C, and E. First, milk production, average somatic cell count (SCC), average linear score (LS) of SCC, new SCC>200,000 and chronic SCC>200,000 cases were evaluated at the farm level using milk recording (MR) data. The period from 01/2022 until the start of SDCT was compared with the period from its implementation until 12/2024. Secondly, during the SDCT application period, the first MR SCC results after parturition were compared between cows dried off with antibiotics (DO+ATB) and those without (DO−ATB). This dataset included 190, 124, 188, 241, and 76 cows, respectively.

RESULTS: No DO−ATB cows on these farms died during the first days after drying off. The proportion of DO−ATB cows was 27%, 41%, 21%, 63%, and 37%, respectively. At the first comparison, results differed between farms. SCC-based indicators slightly worsened after the start of SDCT on farms A, B and C (average LS increased by 0.5, 0.5, and 0.1), whereas on farms D and E, LS decreased by 0.3 and 0.1. Similarly, the proportion of new SCC>200,000 increased by 1.1% on Czech Fleckvieh farms, while on Holstein farms, it decreased by 0.3% and 1.0%. On farm B, production indicators also deteriorated. In the second comparison, DO−ATB cows on all farms showed lower LS by 0.5, 0.8, 0.4, 0.9, and 0.2, respectively, and also the proportion of SCC>200,000 was lower in these cows by 9.2%, 3.1%, 4.2%, 15.3%, and 4.5%, respectively.

CONCLUSIONS: Although udder health indicators slightly deteriorated in the whole herds on three farms after the start of SDCT, cows dried off without ATB showed better SCC-based indicators at the first MR compared with cows dried off with ATB on all farms. Carefully performed drying off, particularly without dry cow (DC) ATB, and the resulting reduction in DC-ATB use remained sustainable on all farms. Supported by Grants NAZV-QL25020050 and TN02000017/02.

Keywords: Dry off, selective dry cow therapy, mastitis, somatic cell count, linear score, antimicrobial use

Sensitivity and specificity of the California Mastitis Test (CMT) in the Diagnosis of Subclinical Mastitis

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BACKGROUND and OBJECTIVE: The CMT is a test that estimates the somatic cell content in milk and is interpreted subjectively through the intensity of the reaction, which in most cases is classified as negative (–), mild (+), moderate (++), and intense (+++), indicating increasing degrees of inflammatory response in the udder and usually considered indicative of subclinical mastitis. Its advantages include low cost, ease of execution, immediate results at the collection site, and the ability to evaluate individual mammary quarters. The objective of this study was to evaluate the sensitivity and specificity of the CMT in relation to somatic cell count (SCC) for the diagnosis of subclinical mastitis in a dairy herd.

MATERIALS and METHODS: Milk samples from 76 mammary quarters of 19 lactating Holstein cows belonging to a dairy herd at an educational institution in the State of São Paulo, Brazil, were evaluated. The cows were raised under a semi-intensive system and mechanically milked twice a day, producing an average of 16 kg of milk/cow/day. Prior to morning milking and after teat cleaning, the strip cup test and CMT were performed. Subsequently, 50 mL milk samples were collected manually from each mammary quarter in sterile plastic bottles containing bronopol preservative for SCC determination using flow cytometry in an automated counter. The sensitivity of the CMT in relation to SCC was determined according to the proportion between positive test results and the presence of the disease (subclinical mastitis), while specificity was calculated according to the proportion between negative test results and the absence of the disease.

RESULTS: Of the 76 milk samples collected, no sample was positive in the strip cup test, nine samples were positive in the CMT, and 45 samples presented SCC > 200,000 cells/mL (ranging from 203,000 to 8,802,000 cells/mL). Taking into account the reference value of SCC for cows (up to 200,000 cells/mL), high specificity and low sensitivity were obtained for the CMT (100% and 20.5%, respectively).

CONCLUSIONS: While SCC provides a precise numerical measure of somatic cells per milliliter of milk, allowing the detection of subtle elevations indicative of early inflammatory responses, the CMT relies on a qualitative gel-forming reaction between a detergent reagent and the DNA of somatic cells. Despite these limitations, the CMT remains an indispensable tool in the health management of dairy herds, especially in small-scale production systems and in regions where laboratory infrastructure for SCC analysis is limited or economically inaccessible.

Keywords: cow, CMT, mammary quarter, milk, SCC, subclinical mastitis

Alternating Intramammary Benzylpenicillin and Intramuscular Penethamate Improves Bacteriological Cure of Chronic *Streptococcus uberis* Mastitis in Dairy Cows: A Randomized Field Trial

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BACKGROUND and OBJECTIVE: Streptococcus uberis is a major etiologic agent of bovine mastitis. In France, penicillin G remains the drug of choice for the treatment of Streptococcus uberis

intramammary infections (IMI). Field practitioners regularly report suboptimal cure rates with standard registered regimens. Daily intramuscular (IM) penethamate achieves high milk penicillin G concentrations for about 12 h; however, given the mechanism of accumulation of penethamate in milk (ion trapping), it is legitimate to doubt the maintenance of effective concentrations 12 to 24 hours after IM injection. Similarly, wash-out of intramammary (IMM) doses of penicillin G at subsequent milking may create subtherapeutic periods.

OBJECTIVE: To evaluate whether alternating IMM benzylpenicillin with IM penethamate improves bacteriological cure of clinical mastitis caused by Streptococcus uberis compared to IMM benzylpenicillin alone.

MATERIALS and METHODS: Design: Non-GCP randomized field study involving four veterinary practices in France. Animals: Lactating cows with non-severe clinical or subclinical mastitis yielding Streptococcus uberis in pure culture. Cows carrying infections with no history of any clinical case or high somatic cell counts (SCC) at least in the 2 months before the clinical case were defined as having recent infections. On the contrary, any clinical case or high SCC in the 2 previous months were used as definition of a chronic infection. Interventions: Protocol A (Control): IMM benzylpenicillin once daily (3 d for recent; 5 d for chronic). Protocol B (Test): same IMM therapy alternated with IM penethamate (15,000 IU/kg) 10–12 h after each IMM dose (3 d recent; 5 d chronic). Outcomes: Bacteriological cure on days ~14 and ~21 post-therapy; MIC distributions.

RESULTS: Among chronic cases with complete bacteriological follow-up (n=55), cure was 77.4% with Protocol B versus 45.8% with Protocol A (95% CI for difference: 6.3 to 56.9 percentage points). No difference was observed between the two protocols for recent infection in a partial dataset (64,7% vs. 63,6%; n =28) Logistic regression showed no significant association between MIC and bacteriological cure (p=0.591).

CONCLUSIONS: Alternating IMM benzylpenicillin with IM penethamate improves bacteriological cure in chronic Streptococcus uberis clinical mastitis under field conditions whatever MIC.

Keywords: streptococcus uberis, mastitis, penicillin, penethamate.

Efficacy of *Vitex pseudo-negundo* in a Bovine Mastitis Model Experimentally Induced by *Staphylococcus aureus* in Rats

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BACKGROUND and OBJECTIVE: Mastitis is one of the most prevalent and economically significant diseases in dairy cattle, causing to inflammation of the mammary gland and reduced milk yield. This study investigated the therapeutic efficacy of *Vitex pseudo-negundo* (VPN) extracts in an experimentally induced rat mastitis model.

MATERIALS and METHODS: The study was approved by the Local Ethics Committee for Animal Experiments of Karadeniz Technical University (protocol no. 2024/10, decision no. 53488718-352). Sixty female Wistar rats were randomly assigned into six groups of 10. Group I served as the control group. Mastitis was induced by intramammary injection of *Staphylococcus aureus* (1.5×10^8 CFU/mL; 10 µL per gland) into the L4, L5, R4, and R5 mammary glands. Group III received intramammary gentamicin (0.25 mg/10 µL/day) for five days. Groups IV–VI received *Vitex pseudo-negundo* extracts—water (0.2603 µg/kg/day), ethanol (0.0803 µg/kg/day), and petroleum ether (0.1042 µg/kg/day), administered at 10 µL per gland for the same duration. Rats were clinically monitored three times daily from the onset of clinical signs until euthanasia. Statistical analysis was performed using ANOVA with SPSS 23.0 (SPSS Inc., Chicago, IL). Data

were tested for normality using the Shapiro–Wilk test and were found to be normally distributed ($p > 0.05$). One-Way ANOVA followed by Tukey's post-hoc test was used to evaluate intergroup differences. Statistical significance was defined as $p < 0.05$.

RESULTS: *Staphylococcus aureus* infection significantly increased mammary gland weight, total oxidative status (TOS), bacterial load, and induced tissue pathology, while decreasing total antioxidant status (TAS). C-reactive protein levels remained unchanged. VPN significantly improved mammary gland weight, TOS, TAS, bacterial load, and histological parameters ($p < 0.001$). Mammary gland weight, oxidative stress, bacterial load, and histopathological lesion severity all decreased markedly following treatment. Histological examination showed near-normal restoration of mammary gland architecture in all treatment groups.

CONCLUSIONS: The result indicate that mastitis markedly increases mammary gland weight and that the tested herbal extracts exhibit anti-inflammatory effects comparable to gentamicin. Preparations of *Vitex pseudo-negundo*, particularly the water extract, demonstrated strong potential for restoring altered microbiological, biochemical, and histological parameters in this rat mastitis model. These findings suggest that *Vitex pseudo-negundo* extracts may serve as effective alternative or adjunct therapies for mastitis.

Keywords: Bacterial load, Experimental model, Mastitis, Oxidative stress, Phytotherapy, Vitex pseudo-negundo

fimA mediates Adhesion, Colonization and Inflammatory Injury in Bovine Mastitis Caused by *Klebsiella pneumoniae*

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BACKGROUND and OBJECTIVE: Bovine mastitis caused by *Klebsiella pneumoniae* is an important infectious disease associated with severe inflammation, poor therapeutic outcomes, and significant economic losses in the dairy industry. Type 1 fimbriae, with FimA as the major structural subunit, are recognized virulence factors in Enterobacteriaceae, but their role in bovine mastitis remains unclear. This study aimed to elucidate the contribution of fimA to bacterial adhesion, virulence, and host inflammatory responses during *K. pneumoniae* intramammary infection.

MATERIALS and METHODS: A *fimA* deletion mutant (Δ*fimA*) was constructed from a clinical mastitis isolate of *K. pneumoniae* using a CRISPR/Cas9-based genome editing system. The effects of *fimA* deletion were evaluated using in vitro adhesion assays, bacterial transcriptome analysis, and a murine intramammary infection model. Bacterial burden, histopathology, cytokine production, inflammasome related protein expression, and host transcriptomic responses were assessed to determine virulence and inflammation.

RESULTS: Deletion of *fimA* resulted in defective type 1 fimbrial assembly and significantly reduced adhesion to bovine mammary epithelial cells, confirming the essential role of FimA in fimbrial formation and epithelial attachment. At the bacterial level, transcriptomic analysis revealed downregulation of type 1 fimbrial genes, iron acquisition systems, outer membrane proteins, and stress-response genes in the Δ*fimA* mutant. In contrast, type 3 fimbrial genes and lysozyme inhibitors were upregulated, suggesting compensatory regulation of adhesion and stress resistance following *fimA* deletion. In the murine

intramammary infection model, Δ*fimA* infection led to significantly lower bacterial loads and attenuated mammary gland inflammation compared with WT infection. Histological analysis further demonstrated reduced inflammatory infiltration and tissue damage in Δ*fimA* infected mammary glands. Consistent with these observations, Δ*fimA* infection induced significantly lower levels of TNF-α and IL-6 and reduced cleavage of gasdermin D, indicating weakened proinflammatory and pyroptotic responses. Although IL-1β and NLRP3 expression showed downward trends, these differences were not statistically significant, suggesting partial attenuation of inflammasome activation. Collectively, these findings indicate that *fimA* promotes *K. pneumoniae* colonization and amplifies host inflammatory responses, thereby exacerbating mammary gland injury during mastitis.

CONCLUSIONS: *fimA* is a key determinant of type 1 fimbrial assembly, epithelial adhesion, and virulence in *K. pneumoniae*. Deletion of *fimA* attenuates bacterial colonization and inflammatory damage in the mammary gland, highlighting its importance in the pathogenesis of *K. pneumoniae* induced mastitis.

Keywords: bovine mastitis, klebsiella pneumoniae, type 1 fimbriae, virulence

Non-*aureus* staphylococci and Mammaliicocci in Large-scale Chinese Dairy Herds: Prevalence, Antimicrobial Resistance, and Genomic Characterization Of staphylococcus Chromogenes

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BACKGROUND and OBJECTIVE: Non-*aureus* staphylococci and mammaliicocci (NASM) are currently the most frequently isolated bacteria from subclinical mastitis (SCM) in dairy cows worldwide. The objectives of this study were to determine 1) the prevalence of NASM in SCM and clinical mastitis (CM) in large Chinese dairy herds, 2) the prevalence of antimicrobial resistance (AMR) of different NASM species, 3) genomic features of *Staphylococcus chromogenes*, the predominant NASM species.

MATERIALS and METHODS: A total of 1,916 samples, including 973 SCM and 943 CM, were collected on 28 large (>500 cows) dairy farms. NASM were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry combined with *cpn60* sequencing. AMR was determined by disk diffusion. Distribution and AMR of NASM were analyzed by beta regression modeling combined with the severity of disease, parity, and days in milk. *Galleria mellonella* was used to assess virulence of 78 strains of *S. chromogenes*, a dominant species; subsequently, 32 strains, representing high- and low-virulence phenotypes, were selected for whole-genome sequencing and comparative genomics.

RESULTS: A total of 19 species and 999 isolates of NASM were identified in this study. Isolation rates of NASM in SCM and CM were 60% and 27%, respectively. The predominant NASM species in SCM and CM were *S. chromogenes* (48 and

47%), *Mammaliicoccus sciuri* (29 and 16%), and *Staphylococcus haemolyticus* (7 and 11%), respectively. *S. chromogenes* and *M. sciuri* were more prevalent in SCM, whereas *S. haemolyticus* and *Staphylococcus simulans* were more common in CM. In addition, *S. chromogenes* and *M. sciuri* were more likely to cause moderate than severe CM. Overall, distribution of NASM was not different in a particular parity. NASM had the highest resistance to penicillin (CM: 29%; SCM: 30%). Additionally, 10% and 8% of NASM isolates from SCM and CM, respectively, were methicillin-resistant. *S. chromogenes* isolates from CM had higher virulence ($p < 0.05$) than those from SCM. The 32 genomes comprised 20 sequence types, indicating high genetic diversity. Resistance genes (n = 21) were detected, including a first report of *fusC* in *S. chromogenes*. Capsular polysaccharide biosynthesis genes were more frequent in high-virulence isolates.

CONCLUSIONS: NASM was prevalent in both SCM and CM on Chinese dairy farms. *S. chromogenes* and *M. sciuri* were dominant in SCM, whereas *S. haemolyticus* and *S. simulans* were more common in CM. Penicillin resistance was most common and methicillin-resistant isolates were detected in NASM. Genomic analyses of *S. chromogenes* revealed high diversity, including the first detection of *fusC* in this species.

Keywords: non-aureus staphylococci and mammaliicocci, Staphylococcus chromogenes, subclinical mastitis, clinical mastitis, antimicrobial resistance, China

Development of Chitosan Nanoparticles Loaded with Tea Tree Oil and Thymol as An Antibacterial Alternative for Bovine Mastitis

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BACKGROUND and OBJECTIVE: Bovine mastitis is one of the most costly diseases in dairy production and is associated with intensive antibiotic use. The development of sustainable, non-antibiotic alternatives has promoted the use of nanostructured systems based on biopolymers and natural antimicrobial compounds. To develop and characterize chitosan nanoparticles loaded with tea tree essential oil and thymol, and to evaluate their cytotoxicity, in vitro release, and antibacterial activity against mastitis-associated bacteria.

MATERIALS and METHODS: Chitosan nanoparticles were prepared using the ionic gelation method, encapsulating tea tree essential oil and thymol. Physicochemical properties, including particle size, polydispersity index, and zeta potential, were evaluated. Morphology was analyzed by scanning electron microscopy. Encapsulation efficiency was determined by high-performance liquid chromatography. Cytotoxicity was assessed in the MARC-145 cell line using a mitochondrial viability assay. In vitro release was evaluated using Franz diffusion cells at different pH values. The antibacterial activity of the essential oils was evaluated against strains isolated from clinical cases using agar diffusion and broth microdilution assays. In vitro efficacy was further assessed against Staphylococcus aureus and Escherichia coli through bacterial growth kinetics.

RESULTS: The nanoparticles showed submicron particle size, homogeneous distribution, and positive zeta potential, indicating good colloidal stability. Encapsulation efficiency exceeded 75%. Nanoencapsulated systems exhibited higher cell viability compared with free essential oils. Active compound release was pH-dependent and sustained in nanoparticle formulations. A reduction in bacterial load of 92% against S. aureus and 72% against E. coli was observed, with greater efficacy against Gram-positive bacteria.

CONCLUSIONS: Chitosan nanoparticles loaded with tea tree oil and thymol represent a promising non-antibiotic antibacterial alternative for bovine mastitis. Their controlled release, reduced cytotoxicity, and strong in vitro efficacy support their potential application in veterinary medicine, although further in vivo studies are required.

Keywords: Bovine mastitis, Chitosan nanoparticles, Essential oils, Antimicrobial alternatives, Dairy cattle

Streptococcus Agalactiae: Practical Eradication Experience in Italian Dairy Herd

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BACKGROUND and OBJECTIVE: Streptococcus agalactiae is one of the main contagious pathogens responsible for mastitis in dairy cattle. The infection primarily affects the mammary gland and is mainly transmitted during milking through contaminated equipment or contact between infected and healthy animals. The presence of this pathogen causes increased somatic cell count, reduced milk production and deterioration of milk quality, resulting in significant economic losses. Control programs based on microbiological diagnosis, herd health management, and improved milking hygiene practices have proven effective in limiting the spread of the pathogen. The aim of this study was to describe a practical eradication program for Streptococcus agalactiae and to evaluate the effectiveness of the diagnostic and management strategies adopted to control the infection.

MATERIALS and METHODS: This study was conducted in the North of Italy on a dairy farm counting approximately 110 lactating cows producing milk for Parmigiano Reggiano. Veterinary intervention was needed after a persistent increase in bulk tank somatic cell count and the occurrence of mastitis cases poorly responsive to antibiotic therapy. Real-time PCR analysis of bulk tank milk confirmed the presence of Streptococcus agalactiae. An eradication program was implemented, including microbiological testing of individual milk samples from all lactating cows to identify infected animals. Based on udder health status, the herd was subsequently divided into separate milking groups. The control protocol involved targeted antibiotic treatment of infected cows, periodic microbiological monitoring of bulk tank milk, and improvements in milking hygiene practices.

RESULTS: The first microbiological screening confirmed the presence of the infection and identified 48 positive animals (39.6% prevalence): • 8 animals culled • 23 animals treated during lactation (cure-rate: 91.3%) • 17 animals treated at dry-off (cure-rate: 94.12%) • a second therapeutic intervention resulted in recovery in all remaining cases The implementation of control measures helped limiting

pathogen transmission through the separation of milking groups, treatment of infected animals and improved hygiene procedures during milking. Monitoring over time showed a progressive reduction in pathogen presence and the eradication was obtained in 8 months. Consequently, a decrease in the somatic cell count in bulk tank milk was observed (from 430 cells/ml to 257 cells/ml in one year).

CONCLUSIONS: This experience demonstrates that an eradication program based on early diagnosis, proper herd health management and correct milking hygiene practices can be effective in controlling Streptococcus agalactiae infections in dairy cattle herds. Continuous monitoring and appropriate biosecurity measures are essential to prevent reintroduction of the infection and to ensure milk quality.

Keywords: Mastitis, Streptocuccus Agalactiae, contagious diseases, eradication program.

Association Between Automated Herd Monitoring System Data and the Number of Bacterial Pathogens in Subclinical Mastitis of Dairy Cows

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BACKGROUND and OBJECTIVE: Subclinical mastitis (SCM) is common in dairy cows, affecting physiological and milk composition parameters before clinical signs appear. Automated herd monitoring systems may provide early indicators of udder health disturbances. This study evaluated associations between data from SmaXtec (Austria, 2010; SXT) and Brolis HerdLine (Lithuania, 2015; BHL) and bacterial pathogen numbers in SCM.

MATERIALS and METHODS: Twenty-four dairy cows were included. Cows were selected based on DeLaval OCC (Sweden, 2006), with SCM defined as somatic cell count ≥200,000 cells/mL. Day 0 was the first diagnosis day. Affected quarters were identified using the California Mastitis Test (CMT), and milk was aseptically sampled. Cows were classified by bacteriology: Group 0 – no pathogen (G0), Group 1 – one pathogen (G1), Group 2 – two pathogens (G2). When multiple quarters were affected, classification followed the highest pathogen count. SXT-recorded body temperature (BT), activity (ACT), and rumination (RUM), and BHL-recorded milk yield (MY), milk temperature (MT), fat (MF), protein (MP), and lactose (ML) were analyzed for 30–15 and 15–0 days pre-diagnosis. ANOVA with LSD post hoc tests was used in IBM SPSS Statistics 31.0.2.0.

RESULTS: SXT BT differed between groups. At 30–15 days: 38.65 ± 0.11 °C (G0), 38.59 ± 0.15 °C (G1), 38.44 ± 0.14 °C (G2, $p = 0.042$). At 15–0 days: 38.56 ± 0.03, 38.52 ± 0.14, 38.38 ± 0.14 °C ($p = 0.029$). BHL MP was higher in G1 and G2 (30–15 days: 3.89 ± 0.58, 3.29 ± 0.18, 3.47 ± 0.28 g/dL, $p = 0.003$; 15–0 days: 3.49 ± 0.18, 3.28 ± 0.18, 3.43 ± 0.29 g/dL, $p = 0.035$). MT also differed (30–15 days: 34.88 ± 0.66, 36.18 ± 0.75, 35.58 ± 0.60 °C, $p = 0.008$; 15–0 days: 35.07 ± 0.73, 36.09 ± 0.56, 35.71 ± 0.54 °C, $p = 0.012$). No differences were observed for ACT, RUM, MY, MF, or ML. These results indicate early physiological and milk composition changes correlate with pathogen load and may signal SCM before clinical signs.

CONCLUSIONS: BT, MT, and MP were associated with bacterial pathogen numbers in SCM. Automated monitoring systems (SXT, BHL) help detect early physiological changes and support earlier identification of cows at risk. Findings highlight these systems' potential to guide timely interventions and improve herd management.

Keywords: Subclinical mastitis (SCM), Dairy cows, Automated monitoring, Body temperature, Milk composition, Udder health

Effect of *S. uberis* Infections on Subsequent Lactation in Dairy Cows

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BACKGROUND and OBJECTIVE: Streptococcus uberis is one of the most important pathogens in dairy cows, causing both clinical mastitis and persistent subclinical infections. While the impact of S. uberis during the affected lactation is well known, its potential consequences on subsequent lactations are less understood. The aim of this study was to evaluate whether exposure to S. uberis in a previous lactation influences udder health and survival during the following lactation.

MATERIALS and METHODS: 284 cows were enrolled in the studio. Quarter milk samples were collected at dry off and after calving to determine infection status. Animals were classified into three groups: (G1) 62 cows with S. uberis infection detected either as clinical mastitis during the previous lactation or in quarter milk samples collected at dry off; (G2) 37 cows infected with other major mastitis pathogens (*S. aureus*, other streptococci and coliforms) during the previous lactation or at dry off; and (G3) 185 cows without evidence of infection with major pathogens or clinical mastitis during lactation and at dry off. To avoid confounding due to ongoing infection, all positive cows for *S. uberis* in post calving quarter samples were excluded from the analysis. In the subsequent lactation, udder health indicators were evaluated, including average linear score (LS) and the proportion of test day records with LS ≥ 4. Differences among groups were assessed using ANOVA. Survival analysis was performed using restricted mean survival time (RMST), considering the first of these composite events occurring during the lactation: clinical mastitis, LS ≥ 4 at test-day, or cow removal from the herd.

RESULTS: Cows previously exposed to *S. uberis* showed poorer udder health in the subsequent lactation. Average LS was higher in G1 compared with G3 (4.3 vs 3.5; $p < 0.001$), and the proportion of test-day records with LS ≥ 4 was significantly greater (56% vs 38% $p<0.001$). Within the first 305 days in milk, RMST was 124.0 days in G3, 108.0 days in G2, and 81.4 days in G1. Compared with G3, cows in G1 experienced the composite exit event on average 42.9 days earlier ($p < 0.01$).

CONCLUSIONS: Exposure to *S. uberis* before dry-off was associated with persistently poorer udder health and earlier exit from a healthy state during the subsequent lactation, suggesting that the impact of this pathogen may extend beyond the lactation in which the infection occurs. Research funded by the Italian Ministry of Health, project number RC IZSVE 01/22

Keywords: mastitis, Streptococcus uberis, udder health, survival

Differential Somatic Cell Count (DSCC) as an Indicator of Treatment Response to Intramammary Therapy in Lactating Dairy Cows

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BACKGROUND and OBJECTIVE: Clinical mastitis remains one of the most economically important diseases in dairy production, affecting both milk yield and quality. Although antimicrobial therapy is widely used, objective indicators for evaluating treatment response and confirming cure are still limited in field conditions. Somatic cell count (SCC) is commonly used to assess udder inflammation, but it mainly reflects the magnitude of the inflammatory response and may not capture qualitative changes in immune cell populations during recovery. Differential somatic cell count (DSCC), which represents the proportion of polymorphonuclear leukocytes and lymphocytes in milk, has recently become available through automated milk analyzers and may provide additional information on inflammatory dynamics. The objective of this study was to investigate whether DSCC could serve as a practical indicator for evaluating treatment

response following intramammary therapy during lactation.

MATERIALS and METHODS: Fourteen quarters from 13 Holstein cows diagnosed with clinical mastitis on a commercial dairy farm in Hokkaido, Japan, were monitored between January and September 2025. Milk samples were collected before treatment, during treatment, and during the withdrawal period. Intramammary antimicrobial therapy was administered for 3–7 days depending on clinical progression. SCC and DSCC were measured using Fossomatic™7 DC. Cure was defined as an average SCC below 200,000 cells/mL during the first 7 days after milk shipment resumed. Quarters were classified as cured or recurrent, and temporal changes in SCC, DSCC, and the combined index SCC×DSCC were compared.

RESULTS: Nine quarters were classified as cured and five as recurrent. Pre-treatment SCC and DSCC did not differ significantly between groups. In cured quarters, SCC and SCC×DSCC gradually decreased during treatment and remained low after the withdrawal period. DSCC also showed a clear decline toward the end of the withdrawal period and at the start of milk shipment. In contrast, recurrent quarters showed only transient reductions in SCC and DSCC followed by re-elevation after treatment. Evaluation using udder health group classification demonstrated that most cured quarters shifted from infected categories to the healthy category during recovery, whereas recurrent quarters frequently remained in infected groups.

CONCLUSIONS: These findings suggest that DSCC may capture qualitative changes in inflammatory cell populations during the recovery phase of mastitis that are not fully reflected by SCC alone. Monitoring DSCC, particularly during the withdrawal period, may help distinguish cured from recurrent cases and provide a practical indicator for evaluating treatment response after intramammary therapy in lactating dairy cows.

Keywords: mastitis, differential somatic cell count, intramammary therapy, treatment response, dairy cows

Validation of an *Escherichia coli* Mastitis Model

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BACKGROUND and OBJECTIVE: *Escherichia coli* (ECO) infection is one of the most frequent causes of severe clinical mastitis in cattle and causes acute inflammation and reduced animal welfare. Animal models are beneficial in search for better treatment interventions and more knowledge on intramammary infections. Therefore, the objective of this study was to achieve a robust and reproducible bovine ECO mastitis model with use of a standardized inoculum and subsequent monitoring of clinical and laboratory parameters.

MATERIALS and METHODS: Four healthy dairy cows were included in this study, with low somatic cell count (SCC) and negative bacteriological culture in each quarter. From each cow two quarters were inoculated with an in-vitro standardized ECO-strain: two cows were inoculated in the hind and two in front quarters. Inoculation was performed with 100 cfu of a well-known low virulent ECO strain. After inoculation clinical variables and laboratory parameters were scored every three hours for day 0 and 1 post inoculation, every six hours for day 2 and 3 and every twelve hours for day 4, 5 and 6. General health and rectal temperature of each cow were monitored, as well as numbers of live ECO per ml milk, SCC, clinical score of quarter and milk, and quarter milk production. Quality of this study was guaranteed by a GCP system and ISO 9001 certification and milk samples were analyzed in a RvA ISO 17025-accredited laboratory.

RESULTS: Isolated numbers of ECO were far higher than 100 cfu for at least 2 to 3,5 days post inoculation. All inoculated quarters showed signs of clinical mastitis and all four cows showed mild signs of general illness with a short peak in body temperature and sharp increase in SCC. Milk production dropped in the first day in the inoculated and control quarters, which was restored afterwards. Onset of clinical signs started uniform in the inoculated quarters and in different cows. Hence, induction and progression of ECO mastitis was

similar and reproducible in all cows. Moreover, measures of symptoms and secretion of ECO were homogeneous in all cows and inoculated quarters.

CONCLUSIONS: We were able to induce a robust and reproducible ECO mastitis infection. This mastitis model can, therefore, be used for determination of efficacy of treatments that aim to reduce ECO mastitis signs and/or shedding of the bacterium.

Keywords: Mastitis infection model, Escherichia coli, intramammary

Ecological Diversity and Dominance of Bovine Mastitis Pathogens in a Dairy Farm in the Comarca Lagunera, (Coahuila and Durango, Mexico) Using Shannon Index and Pareto Analysis

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BACKGROUND and OBJECTIVE: Bovine mastitis is a multifactorial disease caused by various microorganisms that interact within the microbial ecosystem of the herd. The ecological analysis of the bacterial community allows us to understand: pathogen diversity, species dominance, structure of the mastitic microbiome, temporary changes in the bacterial community. Tools such as the Shannon diversity index allow bacterial diversity to be quantified, while Pareto analysis identifies the pathogens responsible for the highest proportion of infections. OBJECTIVE: Evaluate the diversity and dominance of pathogens causing bovine mastitis through ecological analyzes based on the Shannon index and the Pareto principle in a dairy system in the Comarca Lagunera during 2024–2025.

MATERIALS and METHODS: A retrospective observational study was conducted using bacteriological culture results from milk samples collected from clinical and subclinical mastitis cases between January 2024 and December 2025. Isolates were classified into nine main pathogen groups: Escherichia coli, coagulase-negative staphylococci (CNS), Streptococcus spp., Staphylococcus aureus, Klebsiella spp., Pseudomonas spp., Proteus spp., Streptococcus agalactiae, and Arcanobacterium spp. Monthly prevalence patterns were visualized using a heatmap approach. Bacterial diversity was evaluated using the Shannon diversity index (H'), and a Pareto analysis was performed to identify the most relevant etiological agents.

RESULTS: The results showed a clear predominance of environmental pathogens, with Escherichia coli accounting for approximately 70–75% of total isolates (≈1730 cases). Coagulase-negative staphylococci were the second most frequent group (≈240 cases), followed by Streptococcus spp. (≈120 cases), Staphylococcus aureus (≈110 cases), and Klebsiella spp. (≈60 cases). Other pathogens were detected sporadically. Monthly heatmap analysis revealed persistent dominance of E. coli throughout the study period. The Shannon diversity index ranged from 0.47 to 1.59, indicating moderate temporal fluctuations in bacterial diversity, with lower diversity associated with periods of strong E. coli predominance.

CONCLUSIONS: These findings highlight the epidemiological importance of environmental mastitis pathogens and emphasize the need to strengthen hygiene and environmental management strategies in dairy production systems. These results demonstrate that there is a predominance of environmental pathogens, related to seasonality, at the same time that infectious agents are in acceptable ranges, demonstrating that there is good management of the control of infectious pathogens, regardless of seasonality.

Keywords: Bovine mastitis, Dairy cattle, Mastitis epidemiology, Environmental pathogens, Shannon diversity index

Lipoteichoic Acid from *Staphylococcus aureus* Alters Filamentous Actin Organization and Induces Arp2 Protein Expression in Bovine Mammary Epithelial Cells

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BACKGROUND and OBJECTIVE: Bovine mastitis is an inflammatory disease of the mammary gland that causes tissue damage and alterations in milk composition, leading to significant economic losses in the dairy industry worldwide. Lipoteichoic acid (LTA), a cell wall component of *Staphylococcus aureus*, one of the main etiological agents of subclinical mastitis, plays a key role in disease pathogenesis. Previous studies suggest that LTA may contribute to disruption of the blood–milk barrier (BMB); however, its effects on actin cytoskeleton reorganization mediated by the Arp2/3 complex and on cellular bioenergetics in bovine mammary epithelial cells (bMECs) remain poorly understood. In this study, we evaluated the effects of S. aureus LTA on Arp2/3 complex activity and cellular bioenergetics in bMECs, *in vitro*. Cells were isolated from heifer udders, cultured, and treated with different LTA concentrations. We assessed the gene and protein expression of Arp2 and Arp3 subunits, actin cytoskeleton organization, and cellular bioenergetics.

MATERIALS and METHODS: bMECs were isolated from udders of 6 heifers and cultivated in DMEM/F12 culture medium with Penicillin, Streptomycin, Gentamicin and Amphotericin B, 10% fetal bovine serum, and 10 µL/mL of ITS (0.5 mg/mL bovine insulin, 0.5 mg/mL transferrin, 0.5 µg/mL sodium selenite). The cells were seeded on a culture plate at 37 ºC, 5% CO_2. The expression of the ARP2/3 complex was evaluated by PCR and western blot and the F-actin filaments were observed with confocal microscopy. The coherence of the distribution of actin filaments was quantified using ImageJ Fiji OrientationJ software. Finally the LTA-induced metabolic changes were measured using the Seahorse XF Pro instrument.

RESULTS: We observed that 20 µg/ml LTA increased ARP2 expression after 7 hours of treatment in bMECs. Analysis of the actin cytoskeleton revealed that 20 µg/ml LTA reduced filament organization in bMECs. Metabolic analysis showed no significant changes in oxygen consumption rate (OCR) or glycolytic activity, although a slight increase in mitochondrial respiration was observed.

CONCLUSIONS: These findings suggest that LTA from *Staphylococcus aureus* modulates Arp2/3 complex expression and actin cytoskeleton organization in bMECs, potentially contributing to the disruption of the epithelial barrier. Although no significant increase in ATP consumption was detected, cytoskeletal remodeling may be driven by Arp2/3 complex activation and the associated ATP hydrolysis.

Keywords: bovine mammary epithelial cells, lipoteichoic acid, Staphylococcus aureus, Arp2/3 complex, actin cytoskeleton, cellular bioenergetics

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