BOLOGNA

11-12-13 GIUGNO 2026

79° CONVEGNO SISVET

ALMA MATER STUDIORUM

Università di Bologna, Plesso Belmeloro

79° CONVEGNO SISVET
Programma

Giovedì 11 Giugno

08.00 – 10.00

REGISTRAZIONE

10.00 – 13.00

ORAL SOCIETÀ

- **AIPVET** Aula C
- **RNIV** Aula I
- **SICLIMVET** Aula F
- **SICV** Aula E
- **SIMMIV** Aula M
- **SIRA** Aula D
- **SOFIVET** Aula H
- **SOIPA** Aula L

13.00 – 14.00

LIGHT LUNCH

14.00 – 17.00

WORKSHOP 1 Aula A

RISK ASSESSMENT E COMUNICAZIONE DEL RISCHIO QUALI STRUMENTI DI PREVENZIONE IN OTTICA ONE HEALTH

Moderatori:
Prof. Aniello Anastasio, Dipartimento di Medicina Veterinaria e Produzione Animale - Università degli Studi di Napoli Federico II
Prof. Orlando Paciello, Dipartimento di Medicina Veterinaria e Produzione Animale - Università degli Studi di Napoli Federico II

14.00 – 14.15
Introduzione ed apertura dei lavori
Prof. Andrea Serraino, Presidente Associazione Italiana Veterinari Igienisti - Dipartimento di Scienze Mediche Veterinarie - Alma Mater Studiorum Università di Bologna

14.15 – 15.00
Governare la complessità in ottica One Health: il ruolo del risk assessment e la leadership del Ministero della Salute
Dr. Giovanni Filippini, Direttore Generale della Direzione Generale della Salute Animale (DGSAF)
Dr. Ugo Della Marta, Direttore Generale della Direzione Generale dell'Igiene e della Sicurezza Alimentare (DGISAN)

15.00 – 15.30
Valutazione del rischio sanitario legato al commercio di animali e prodotti di origine animale: una metodologia fondamentale per la prevenzione dell'introduzione di malattie infettive sul territorio nazionale
Prof. Marco De Nardi, Dipartimento di Scienze Mediche Veterinarie - Alma Mater Studiorum Università di Bologna

14.00 – 17.00

ORAL SOCIETÀ

- **AMV** Aula I
- **ARNA** Aula H
- **SICLIMVET** Aula F
- **SICV** Aula E
- **SIRA** Aula D

15:30 – 16.00
Il ruolo degli animali sentinella nella valutazione del rischio sanitario e ambientale in patologia veterinaria
Prof. Sandro Mazzariol, Dipartimento di Biomedicina Comparata e Alimentazione - Università degli Studi di Padova

16.00 – 16.30
Il ruolo delle scienze farmaco-tossicologiche veterinarie nella valutazione del rischio di contaminanti e additivi zootecnici da parte dell'Autorità Europea per la Sicurezza Alimentare (EFSA)
Prof. Carlo Nebbia, Esperto scientifico - Autorità Europea per la Sicurezza Alimentare - Università degli Studi di Torino

16.30 – 17.00
Chiusura lavori
Prof. Andrea Serraino, Presidente Associazione Italiana Veterinari Igienisti (AIVI)

17.00 – 19.00

ORAL SOCIETÀ

- **AIPVET** Aula C
- **AIVI** Aula L
- **AMV** Aula I
- **RNIV** Aula A
- **SICLIMVET** Aula F
- **SICV** Aula E
- **SIFTVET** Aula H
- **SIMMIV** Aula M
- **SIRA** Aula D

19.00

WELCOME PARTY

Cortile D'Ercole di Palazzo Poggi, Via Zamboni 33

Venerdì 12 Giugno

08.30 – 10.30

WORKSHOP 2 Aula B

LE TECNOLOGIE INNOVATIVE A SUPPORTO DELLA PROFESSIONE VETERINARIA
Moderatori:
Luciano Pinotti, Università di Milano
Erminio Trevisi, Università del Sacro Cuore-Piacenza

08.30 – 08.45
Introduzione e saluti dei moderatori

08.45 – 09.15
AI and Big Data: from data collection to decision support
Miel Hostens, Professor of Digital Dairy Management and Data Analytics - Cornell University (USA)

09.15 – 09.35
Nutrizione di precisione e salute della mandria: il ruolo dei dati aziendali
Cavallini Damiano, Dipartimento di Scienze Mediche Veterinarie - Università di Bologna

09.35 – 09.55
Dati epidemiologici e gestione della "One health": tecnologie innovative e AI
Alborali Loris, Istituto Zooprofilattico Sperimentale Emilia e Lombardia, Brescia
Bontempi Giorgio, Istituto Zooprofilattico Sperimentale Emilia e Lombardia, Brescia

09.55 – 10.15
Intelligenza Artificiale per sistemi di allerta precoce dei rischi climatici nell'acquacoltura mediterranea
Bonaldo Alessio, Dipartimento di Scienze Mediche Veterinarie - Università di Bologna

10.15 – 10.30
Chiusura dei lavori

10.30 – 11.00

PAUSA CAFFÈ

11.00 – 13.00

INAUGURAZIONE 79° CONVEGNO SISVET Aula B

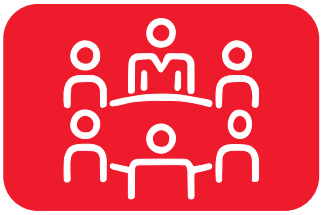
13.00 – 14.00

LIGHT LUNCH

14.00 – 16.00

ASSEMBLEA FEDERAZIONE

Aula B



14.00 - 15.00

ORAL SOCIETÀ

- **AIVI** Aula L
- **ARNA** Aula H
- **SICLIMVET** Aula F
- **SIRA** Aula D

15.00 - 16.00

ORAL SOCIETÀ

- **AMV** Aula I
- **SICLIMVET** Aula F
- **SICV** Aula C
- **SIFTVET** Aula H
- **SIMMIV** Aula M
- **SIPAOC** Aula E
- **SOFIVET** Aula D
- **SOIPA** Aula L

14.00 - 15.00

POSTER SESSION

Aule P/Q

- **AISMEVEM**
- **AMV**
- **RNIV**
- **SICV**
- **SIFTVET**
- **SIMMIV (ANIV)**
- **SIPAOC**
- **SOFIVET**

15.00 - 16.00

POSTER SESSION

Aule P/Q

- **AIPVET**
- **AIVI**
- **ARNA**
- **SICLIMVET**
- **SIRA**
- **SOIPA**

16.00 – 16.30

PAUSA CAFFÈ

16.30 – 18.00

SESSIONE CONGIUNTA DI ONCOLOGIA

Aula C

MAIN LECTURE
Cancer Diagnostics Beyond the Needle
Dr. Pachi Clemente

ORAL SOCIETÀ ▪ **AIPVET** ▪ **SICLIMVET** ▪ **SICV**

ORAL SOCIETÀ

- **AMV** Aula I
- **ARNA** Aula H
- **SIMMIV** Aula M
- **SIPAOC** Aula E
- **SOFIVET** Aula D
- **SOIPA** Aula L

18.00 – 19.00

PREMIAZIONE POSTER E RELAZIONE VINCITORI BANDO SANITÀ PUBBLICA	RIUNIONE DIRETTORI SANITARI	ORAL SOCIETÀ
Aula B	Aula F	▪ **SIMMIV** Aula M

20.30

CENA SOCIALE

Salone delle Feste di Palazzo Re Enzo, Piazza del Nettuno 1/C

Sabato 13 Giugno

08.30 – 11.00

SIMPOSIO FEDERALE Aule C/D

FARM, RICERCA TRASLAZIONALE, FAMILY PET: L'EVOLUZIONE DEI SUIDI NELLA MEDICINA VETERINARIA

08.30 – 08.55
Anatomia porci di Cofone
Annamaria Grandis, AISMeVeM /AMV

08.55 – 09.20
Il sistema immunitario del suino: interazione con l'ambiente
Elisabetta Razzuoli, RNIV

09.20 – 09.45
Il quadro normativo del suino nella medicina veterinaria: identificazione, tracciabilità e gestione del farmaco
Matteo Gianesella, SICLIM-VET

09.45 – 10.10
Il dolore nel maiale: insegnamenti tratti dal traslazionale per la clinica
Daniela Casoni, SICV

10.10 – 10.35
Il suino per la medicina dei trapianti e la medicina di precisione: la storia dello xenotrapianto
Maria Laura Bacci, SOFIVET

10.35 – 11.00
Passato presente e futuro della suinicoltura in Italia
Giovanna Martelli, ARNA

11.00 – 11.30

PAUSA CAFFÈ

11.30 – 13.30

TAVOLA ROTONDA Aule C/D

CONFERENZA DEI DIRETTORI
Dallo Stress nella Formazione Universitaria al BurnOut nella Professione

13.30 – 14.30

LIGHT LUNCH

14.30 – 18.30

ORAL SOCIETÀ

- **SICLIMVET**
  Aula F

Oral Communication(s)

POST-MORTEM INTERVAL DETERMINATION USING GROSS ORGAN ASSESSMENT IN THE CAT (*FELIS CATUS*)

Luca Botta[1], Paola Pregel[1], Isabella Di Nunno[1], Neva Cormio[1], Emanuele Ricci[2], Lorenzo Ressel[2], Frine Eleonora Scaglione[1]

[1]Dip. di Scienze Veterinarie, Università degli Studi di Torino; [2]Department of Veterinary Anatomy Physiology and Pathology, Institute of Infection Veterinary and Ecological Sciences, University of Liverpool

In forensic veterinary pathology, alongside classical techniques (i.e. the evaluation of the degree of rigor mortis) and more recently studied parameters (i.e. nucleic acid and protein degradation), body macroscopic evaluation remains a valuable resource to estimate the Post-Mortem Interval (PMI) [1]. This approach represents a simple but effective method, as previously demonstrated in studies on different animal species, especially pigs, often considered proxies for humans [2]. The sequential succession of time-dependent macroscopic changes occurring to deceased bodies is strongly influenced by environmental temperature and its variations. Therefore, Accumulated Degree Days (ADDs), obtained by summing daily average environmental temperature, represent a useful tool to improve the interpretation of post-mortem (PM) changes [3].

This study aims to describe PM gross changes occurring in organs of cats kept in different preservation conditions, to further improve PMI estimation. Seventy-two deceased cats, collected from veterinary clinics, underwent forensic autopsy. Six cats served as controls and were necropsied within one-hour PM. The remaining animals were divided into three groups (24 cats each) and exposed to either an outdoor environment (“field”), stored at +4oC (“refrigerated”) or frozen at -20°C (“frozen”). The cats remained in the assigned preservation condition for 1, 3, 14 and 28 days (6 cats each). ADDs associated with controls, “field” and “refrigerated” cats were then calculated. During the necropsy, pictures of each organ were taken (in situ, ex situ and cut section). A new scoring system was applied to evaluate PM gross changes in the organs (decomposition score, DS). Differences among groups were assessed by Kruskal-Wallis test, followed by Dunn’s post hoc tests. The “field” cats showed statistically significant increases in DS for liver, spleen, kidney, brain, lungs, subcutis and skinned body ($p<0.001$) in cats exceeding 200 ADDs compared to controls. In the “refrigerated” cats, the liver showed the most pronounced increases, particularly at later time points (14--days $p<0.001$; days $p<0.05$). Furthermore, significantly increased DS was observed in spleen (3-/28-days $p<0.05$), brain ($p<0.01$-0.05), lungs ($p<0.01$), and subcutis ($p<0.05$–0.01) after 14-28 days. Significant differences were also detected in the same organs ($p<0.05$-0.01) in the “frozen” cats (14-/28-days) compared with controls. Gross organ PM changes in cats preserved in different conditions showed increased DS, along with ADD increase, thus crucially describing organ decomposition and PMI, when combined. Compared to “field” cats, DS was much inferior in refrigerated and frozen cats. The liver, spleen, and brain were the most sensitive organs to decomposition and most useful to distinguish more advanced decomposition stages, in all experimental. These findings suggest that systematic gross evaluation may represent a useful tool for improving PMI estimation in forensic veterinary investigations, especially when integrated with either histological or biomolecular assessments.

[1] Ruiz López et al., Estimation of the post-mortem interval: a review, Forensic Science International, vol. 369, p. 112412, Apr. 2025.

[2] Indra et al., Evaluation of porcine decomposition and total body score (TBS) in a central European temperate forest, Journal of Forensic Sciences, vol. 69, no. 3, pp. 784–797, May 2024.

[3] Megyesi et al., Using Accumulated Degree-Days to Estimate the Postmortem Interval from Decomposed Human Remains, Journal of Forensic Science, vol. 50, no. 3, 2005.

Oral Communication(s)

FATAL ANIMAL ATTACKS IN HUMANS: AN INTEGRATED FORENSIC APPROACH

Giuseppe Piegari[1], Angela Serino[2], Antonietta Porzio[2], Ciro Di Nunzio[3], Vittoria Romano[4], Davide De Biase[5], Carlo Campobasso[6], Maurizio Saliva[2] e Orlando Paciello[4|7]

[1]Università degli Studi di Roma "Link Campus University"; [2]Azienda Sanitaria Locale ASL Napoli 3 Sud, Torre del Greco (NA); [3]Laboratorio di Genetica Forense, CEINGE – Università Federico II di Napoli; [4]Dipartimento di Medicina Veterinaria e Produzioni Animali, Università degli Studi di Napoli "Federico II"; [5]Dipartimento di Farmacia, Università degli Studi di Salerno, Fisciano (SA); [6]Dipartimento di Medicina Sperimentale, Università della Campania 'Luigi Vanvitelli', Napoli; [7]Istituto Zoprofilattico Sperimentale del Mezzogiorno, Portici (NA)

Animal attacks in humans represent a relevant public health and forensic issue. In Italy, however, the lack of a centralized registry limits a comprehensive assessment of fatal cases, and shared forensic protocols for the post-mortem investigation of suspected animal attacks are still lacking [1]. This study aimed to describe an integrated human–veterinary forensic approach for investigating suspected bite-related fatalities. To this aim, four suspected animal-related deaths were prospectively investigated through a multidisciplinary approach involving a human forensic pathologist and a veterinary pathologist. Gross post-mortem findings, scene evidence, and genetic analyses were jointly evaluated. Human–animal interaction occurred indoors (2/4), in the house garden (1/4), and in a regional park (1/4). Crime scene analysis revealed that, among the two cases occurring at home, one involved a child left alone with a dog, whereas the other involved an adult who died during dinner in the presence of his dog. Moreover, in the case occurring in the house garden, the man was pruning a tree and was found near a ladder. Finally, the adult who died in a regional park was discovered in a wooded area. Dog attacks were suspected in three cases, and wild boar attacks were suspected in one. In two cases, post-mortem tissue loss was documented. In one adult case, superficial linear excoriations associated with lacerated-contused wounds and filamentous biological material morphologically consistent with wild boar hair supported animal interaction. In the child, autopsy revealed circular to linear lacerated-contused, ecchymotic, and excoriated lesions, predominantly involving the head and neck. In this case, human hair recovered from suspected dog feces was confirmed by DNA analysis. Overall, the integrated evaluation indicated that lesions were predominantly post- or perimortem in three cases, whereas one case was consistent with a fatal animal attack. These findings highlight the value of a standardized multidisciplinary forensic workflow in distinguishing lethal animal attacks from post-mortem animal activity.

[1] Iarussi F et al. Fatal Dog Attacks in Italy (2009-2025): The Urgent Need for a National Risk Registry, Animals (Basel), Dec 6;15(24):3523, 2025

Oral Communication(s)

BODIES RECOVERED FROM WATER: EVALUATION OF THE POST-MORTEM INTERVAL AND PRELIMINARY AI-BASED DIATOM TESTING IN VETERINARY FORENSIC PATHOLOGY

Ilaria d'Aquino[1], Vittoria Romano[2], Giuseppe Piegari[3], Lorenzo Riccio[2], Valeria Russo[2], Davide De Biase[4], Evaristo Di Napoli[2], Orlando Paciello[2|5]

[1]Azienda Sanitaria Locale ASL Benevento; [2]Dipartimento di Medicina Veterinaria e Produzioni Animali, Università degli Studi di Napoli "Federico II"; [3]Università degli Studi di Roma "Link Campus University"; [4]Dipartimento di Farmacia, Università degli Studi di Salerno, Fisciano (SA); [5]Istituto Zoprofilattico Sperimentale del Mezzogiorno, Portici (NA)

Diagnosis of drowning is a complex challenge in veterinary forensic pathology, due to the highly nonspecific nature of macroscopic lesions [1]. The diatom test is considered the gold standard for confirming drowning; however, the risk of post-mortem contamination has been commonly reported in human literature. Conversely, few studies investigated this aspect in animals. Furthermore, manual microscopic analysis is slow and prone to human error. Although modern diagnostics increasingly rely on Artificial Intelligence (AI), the development of algorithms for diatom detection and quantification has so far been restricted to human forensic practice [2]. The aims of our study were to: 1) evaluate the gross and histological post-mortem cadaveric changes in cat cadavers experimentally submerged in water; 2) evaluate the post-mortem penetration of diatoms in different organs; 3) assess an AI-based approach using pre-trained backbones and Grad-CAM for model explainability. To these aims, 12 cats were divided into 2 groups based on their age range (group A: 5-7 years, group B: < 1 year). Animals were submerged in water under standardized diatom concentration (160-178 in 10 ml of sample) and environmental temperature. For animals in both groups a complete necropsy, histological examination and diatom test were performed at 7, 14 and 21 days. Concurrently, 100 µL aliquots of the same water were processed after centrifugation, and the recovered pellet was smeared onto 25 slides and digitized at 40× magnification. Whole-slide images were tiled into 256 × 256 pixel patches to build the training and validation datasets. Six pre-trained backbones were evaluated as frozen feature extractors, with additional in silico noise testing and Grad-CAM analysis. Cadavers showed progressive phases of decomposition, ranging from chromatic to skeletonization. Loss of cellular detail and nuclear fragmentation were the most detected histological changes. Diatom contamination was detected; however, the diatom test resulted negative in bone matrix and bone marrow. Among the evaluated models, ResNet-18 achieved the highest discriminative performance (inter/intra ratio: 1.142; silhouette score: 0.322). Latent space analysis (t-SNE) showed appreciable, although incomplete, separation between positive and negative samples. Preliminary in silico testing demonstrated the system's ability to localize diatoms, although occasional activation on background features was observed. Grad-CAM visualizations further confirmed that model decisions were mainly driven by biologically relevant morphological structures rather than visual artifacts. These findings could provide a valuable tool for the investigation of drowning in Veterinary Forensic Pathology and support the feasibility of a deep learning approach for diatom detection.

[1] McEwen BJ et al. "Veterinary Forensic Pathology: Drowning and Bodies Recovered From Water". Vet Pathol. (2016). doi: 10.1177/0300985815625757.

[2] Zhou, Y. et al. "Digital whole-slide image analysis for automated diatom test in forensic cases of drowning using a convolutional neural network algorithm." Forensic science international vol. 302 (2019): 109922. doi:10.1016/j.forsciint.2019.109922

Oral Communication(s)

UNEXPECTED PREDATION: ANATOMO-PATHOLOGICAL DESCRIPTION OF GOLDEN JACKAL (*CANIS AUREUS MOREOTICUS*) ATTACKS ON DONKEYS

Stefano Pesaro[1], Rita Lorenzini[2], Marco Galeotti[1]

[1]Università degli Studi di Udine, Dip. di Scienze Agro-alimentari, Ambientali e Animali; [2]Istituto Zoprofilattico Sperimentale del Lazio e della Toscana M. Aleandri, Grosseto

The presence and distribution of meso- and large carnivores in Europe, particularly within the Italian territory, have markedly expanded over the last decades. The increase in carnivore populations has led to a rise in conflicts with human activities. To promote greater acceptance of these predators, authorities have introduced financial compensation schemes for damages caused by predation animals. However, access to such measures requires a professional and integrated approach.T he golden jackal (Canis aureus moreoticus) is an opportunistic species, predominantly scavenging but also capable of active predation. However, knowledge of its predatory strategies, particularly toward larger prey, remains limited. The present study describes the lesions observed in cases of predation on donkeys attributed to the golden jackal in Friuli Venezia Giulia between 2016 and 2025. All eight individuals were females; four were found dead, while four were alive at the time of examination. Regarding body condition score, five animals were in good condition, two showed a moderate condition, and one was in poor condition. The main lesions observed were characterized by extensive and irregular lacerations involving the cutis and underlying musculature, predominantly localized in the perineal region. In particular, severe tissue disruption was evident at the level of the anus and external genitalia, with partial loss of soft tissues. The wound margins appeared uneven and frayed, with areas of hemorrhage and necrotic tissue. In some cases, lesions extended to the proximal tail region. The attribution of the predatory event was confirmed in two cases: in one instance through biomolecular investigations and in another through camera trapping. These findings highlight the importance of adopting an integrated diagnostic approach, which is essential not only for accurate ethological interpretation of predatory behavior but also, and above all, for supporting the competent authorities involved in the management of carnivores in Italy.

Oral Communication(s)

THE WOLF IN CAMPANIA: A FORENSIC APPROACH TO DEATH DYNAMICS, HEALTH STATUS AND POPULATION GENETICS

Nicola D'Alessio[1], Stefano Scarcelli[2], Giovanni Sgroi[1], Marco Paone[1], Emanuela Sannino[1], Valerio Toscano[3], Adriano Minichino[4], Vincenzo Veneziano[2], Marita Georgia Riccardi[1], Orlando Paciello[1]

[1]Dip. Cordinamento di Sanità Animale, Istituto Zoprofilattico Sperimentale del Mezzogiorno (IZSM), Portici (NA); [2]Dip. di Medicina Veterinaria, Università degli Studi di Napoli Federico II; [3]Centro di Riferimento di Igiene Urbana Veterinaria (CRIUV), Napoli; [4]Centro di Recupero Animali Selvatici (CRAS), Napoli

In the last decades, the wolf population (*Canis lupus italicus*) has deeply increased in Italy [1], especially in southern parts of the peninsula, such as Campania region, requiring a focus on health status and causes of death of this species. In the field of forensic medicine, postmortem examinations also allow to reconstruct the mechanism of injury, determination of cause of death, and identification of any human-related liability. Furthermore, postmortem examinations are a key component of health monitoring and data collection aimed to manage and conserve the wolf population. In particular, hybridization between dogs and wolves is a critical issue for wolf conservation, making genetic analysis a necessary component of autopsy.

Between 2015 and 2026, wolf carcasses in Campania were collected and delivered to the Istituto Zooprofilattico Sperimentale del Mezzogiorno (Portici, Italy). All carcasses underwent a complete necropsy run in accordance with forensic medicine guidelines, with particular attention to the description of lesions, their nature and chronology. For each animal, sex, estimated age, nutritional status, and geographic origin were recorded, along with photographic reports to ensure the data custody chain.

A total of 75 wolves was examined, including 23 from the province of Avellino, 14 from Benevento, 12 from Caserta, and 26 from Salerno. Based on year, the trend of traumatic events was characterized by an initial increase (from 2 cases in 2015 to 6 in 2017), followed by a stabilization between 2018 and 2023 (5-7 cases per year). In 2024, a decrease was observed (3 cases), while in 2025 there was a marked increase to 21 cases (increase of 280%). Although to date the trend of 2026 (6 cases) is in accordance with data up to 2023, the number is already high and seems to overlap with the trend of 2025. From a spatial perspective, Salerno and Avellino show a constant presence over time, while Benevento exhibits a more stable and limited trend. The Caserta province showed a significant increase in recent years, suggesting a possible expansion of the species into more heavily populated areas. The peak of 2025 may due to the increased anthropogenic pressure and wolf population growth confirming the need for effective mitigation strategies against anthropic factors other than continuous monitoring of wolf population. The main human-related causes of death included roadkill and injuries by gunshot wounds. Poisoning by anticoagulants and pesticides accounted for a significant proportion of cases. Our findings suggested a link between compromised health and increased susceptibility to traumatic events. The data show that wolf mortality in Campania is strongly influenced by human pressure, as previously established in other regions of Italy [2]. In a minor fraction of cases, pathological conditions such as Sarcoptic mange and Aujeszky's disease, were identified as cause of death. Integrating necropsy with genetic analyses is crucial to understand hybridization phenomena. The results highlight the need to strengthen monitoring activities, combat illegal practices such as the use of poisoned bait, and further investigate the phenomenon of hybridization. An integrated approach is essential for the wolf species management and conservation in Campania.

[1] Aragno et al. La popolazione di lupo nelle regioni dell'Italia peninsulare 2020/2021, ISPRA, 2022.

[2] Musto et al. Men and wolves: Anthropogenic causes are an important driver of wolf mortality in human-dominated landscapes in Italy, Global Ecology and Conservation, 32:e01892, 2021.

Oral Communication(s)

IL-1B DRIVES INFLAMMATORY PATHWAYS IN EQUINE TENOCYTES: INTEGRATED PROTEOMIC AND LIPIDOMIC INSIGHTS

Matteo Seccaroni[1], Alessia Tognoloni[1], Sandra Buratta[2], Mandy Peffers[3], Lorena Urbanelli[2], Roberto Maria Pellegrino[2], Antonio Di Meo[1], Elisabetta Chiaradia[1]

[1]Dip. di Medicina Veterinaria, Università degli Studi di Perugia; [2]Dip. di Chimica, Biologia e Biotecnologie, Università degli Studi di Perugia; [3]Dept. of Musculoskeletal and Ageing Science, University of Liverpool (UK)

Over the past decade, interleukin-1 beta (IL-1β) has been recognised as a factor in the pathophysiology of tendinopathy. Elevated IL-1β levels have been detected in degenerated tendons and are associated with increased expression of matrix metalloproteinases (MMPs), which promote extracellular matrix (ECM) degradation. In addition, IL-1β modulates tendon healing stimulating cell response. Despite these findings, the molecular mechanisms underlying these effects remain poorly understood [1,2].

This study investigated the effects of 48 hours of exposure to IL-1β (10 ng/mL) on equine tenocytes isolated from the superficial digital flexor tendon (SDFT). Untreated tenocytes cultured under the same conditions were used as control. Both targeted and untargeted approaches were employed, consisting in zymography, western blotting, ELISA, PCR as well as label-free quantitative proteomics (LFQ) and lipidomic analyses. For this study, 10 primary cultures of tenocytes obtained from were 10 horses aged between 5-10 years, were used. Each experiment was performed in triplicate and repeated at least three times.

IL-1β reduced tenocyte viability and increased MMP-2 and MMP-9 activities in the culture medium. Consistent with ECM structural and remodelling impairment, proteomic analysis revealed increased levels of MMP-2, MMP-13, and MMP-7, as well as changes in the abundance of collagen type Iα1, Iα2, Vα1/2, XII, XVI, COMP, and fibromodulin in IL-1β-treated cells compared with control cells. In addition, proteomic data highlighted that IL-1β increases the levels of proinflammatory proteins, including IL-6, IL-4, and COX-2,

Lipidomic analysis revealed reductions in arachidonic acid and sphingomyelin levels, indicating possible alterations in lipid-mediated inflammatory signalling. Notably, IL-1β–treated cells showed elevated levels of sphingomyelinase (SMPD3) and NF-κB activation. Together with the increase in COX-2, the decrease in arachidonic acid and sphingomyelin, and the rise in pro-inflammatory cytokines, these findings indicate that IL-1β may act through multiple inflammatory signalling pathways.

Overall, these results, combined with the observed alterations in ECM remodelling, confirm that IL-1β contributes to tendon homeostasis disruption by driving inflammation, matrix degradation, and impaired tissue regeneration.

In conclusion, the study provides a more detailed understanding of the molecular mechanisms by which IL-1β contributes to tendon disorders and highlights potential molecular targets for therapeutic intervention to mitigate inflammation and preserve ECM structure, thereby improving tendon healing.

[1] Beaumont et al. Mol Cell Biochem. 2024 May;479(5):1059-1078. doi: 10.1007/s11010-023-04779-

[2] Zhang et al. Front. Immunol. 16, 1657285. https://doi.org/10.3389/fimmu.2025.1657285

Oral Communication(s)

HISTOPATHOLOGICAL AND HISTOCHEMICAL CHARACTERIZATION OF DESTRUCTURED SKELETAL MUSCLE IN HEAVY PIGS: EVIDENCE OF AN UNDERLYING MYOPATHIC CONDITION

Davide De Biase[1], Giuseppe Piegari[2], Valentina Bonfatti[3], Sara Faggion[3], Paolo Carnier[3], Valeria Russo[4], Orlando Paciello[4|5]

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Destructured skeletal muscle represents an increasingly recognized defect in heavy pigs, with significant implications for meat quality and industrial processing [1]. Although commonly interpreted as a post-mortem alteration associated with metabolic imbalance [1], the underlying biological mechanisms remain poorly defined. The present study aimed to characterize the morphological, histoenzymatic, and immunohistochemical features of destructured muscle in heavy pigs, with particular focus on identifying evidence of intrinsic muscle pathology. A total of 144 samples of the *Semimembranosus muscle* from heavy pigs (*Sus scrofa domesticus*) were examined, including 77 samples displaying macroscopic destructured areas and 67 unaffected controls. Cryosections were stained with hematoxylin and eosin (H&E) and modified Gomori trichrome for structural evaluation. Histoenzymatic reactions, including succinate dehydrogenase (SDH), nicotinamide adenine dinucleotide–tetrazolium reductase (NADH-TR), and cytochrome c oxidase (COX), were performed to assess mitochondrial distribution and oxidative metabolism. Desmin immunohistochemistry was used to evaluate cytoskeletal organization. Affected muscles consistently exhibited widespread myofibrillar alterations, characterized by fiber atrophy, loss of structural integrity, and multifocal areas of moderate-to-severe disorganization. Trichrome staining revealed the presence of subsarcolemmal red-stained vacuolar structures. Histoenzymatic analyses demonstrated marked disruption of mitochondrial architecture and oxidative metabolism, with frequent core-like lesions showing central loss of enzymatic activity. Desmin immunoreactivity highlighted cytoskeletal disarray and abnormal protein aggregation within affected fibers. Overall, these findings indicate that destructured muscle is not merely the result of post-mortem metabolic events but rather reflects a condition of profound structural and metabolic impairment [2]. The coexistence of myofibrillar degeneration, mitochondrial dysfunction, and cytoskeletal disruption supports the hypothesis of an underlying myopathic process, likely exacerbated during the early post-mortem phase [3]. These results provide new histopathological evidence that destructured pork originates from a pre-existing muscle condition, with direct implications for meat quality and processing strategies. Furthermore, we highlight the critical role of detailed histopathological evaluation in identifying subclinical myopathies affecting food-producing animals.

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Oral Communication(s)

A DIAGNOSTIC ALGORITHM FOR CANINE AND FELINE MAMMARY TUMORS: SECOND RING TEST STUDY

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This study is the result of the ring test performed by the Group of Study of Mammary tumors that was organized within AIPVet. It is based on the application of a diagnostic algorithm and 96 authors totally have participated. Histological classification of mammary proliferative lesions in dogs and cats is essential for prognostic and predictive studies, as well as for comparative oncology; standardization of diagnosis is however highly affected by substantial interobserver variability. In this study, a structured diagnostic algorithm based on the Davis–Thompson Foundation classification was assessed for the ability to improve interobserver agreement in the morphological diagnosis of mammary gland lesions in dogs and cats. A consensus diagnosis of 114 selected mammary lesions (91 canine, 23 feline) was used as gold standard and 79 veterinary pathologists from 21 countries evaluated digitized whole-slides in two rounds: a first reading without the algorithm and a second reading with the algorithm. Interobserver concordance was measured using unweighted kappa statistics for lesion category (hyperplastic/dysplastic, benign, malignant) and histological subtype. Across all lesions, agreement was higher for lesion category than for histological subtype. A slight improvement of concordance was observed applying the algorithm, both for category. Also, by using the algorithm, more than 90% of raters (73/79, 92.4%) modified at least one diagnosis and, among these, approximately two-thirds reached agreement with the gold standard in at least one case. Overall, the algorithm provided a reproducible diagnostic framework that may support training and improve standardization of canine and feline mammary tumor classification. Additionally, this study showed how relevant is to perform a large team-work on complicated topic as tumor classification.

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Oral Communication(s)

STANDARDIZATION AND CHARACTERIZATION OF PRIMARY THREE-DIMENSIONAL CELL CULTURE PROTOCOLS FOR SPONTANEOUS CANINE TUMORS

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In medicine, three-dimensional (3D) tumor models have become essential for overcoming the limitations of two-dimensional (2D) cultures, which fail to fully reproduce the heterogeneity of cellular subpopulations and the tumor microenvironment [1,2]. Furthermore, obtaining physiologically relevant and reproducible data from patient-derived 3D cell culture systems remains a significant challenge, despite their crucial role in improving the predictive value of preclinical studies.

This study aimed to standardize the generation of primary 3D cell cultures derived from spontaneous canine tumors and to compare a ready-to-use bioreactor (VITVO®) with a miniaturized plate-based system (MPBS), both based on the same scaffold material.

A total of 23 tumors were collected, including hemangiosarcomas (n = 10), oral melanomas (n = 6), urothelial carcinomas (n = 2), and osteosarcomas (n = 5), from surgical procedures performed at three Italian veterinary hospitals. Fresh samples were used for routine diagnostic procedures (formalin-fixed, paraffin-embedded sections stained with hematoxylin and eosin) and to establish primary 3D cell cultures, which were subsequently analyzed through histological evaluation. Primary tumors (PTs) and primary 3D cell cultures were compared to achieve phenotypic characterization by an immunohistochemical panel of antibodies specific for each tumor type. The extent of necrosis and cell proliferation (anti-Ki-67 positivity) in PTs were evaluated to correlate these parameters with the cellularity of the primary 3D cell cultures.

Tissue dissociation (TD) proved to be a critical issue: mechanical dissociation with an automated device showed poor performance in preserving cell viability, whereas switching to manual protocol of TD significantly improved both cell viability and cellularity. Primary 3D cell cultures were successfully established in both the VITVO® bioreactor and the MPBS. The MPBS allowed the culture of small biopsy samples without compromising cellularity. Necrosis in PTs was inversely correlated with primary 3D cell culture cellularity ($r = -0.46$, $p = 0.026$).

Immunohistochemical staining confirmed that primary 3D cell cultures retained the phenotypic characteristics of the PTs: marker concordance was observed in 8/10 hemangiosarcomas, 5/6 melanomas, and 2/2 urothelial carcinomas. Osteosarcoma cultures exhibited limited growth, consistent with stromal predominance and low cellularity.

This study demonstrates that optimization of dissociation protocols, scaffold selection, and culture conditions is essential for preserving tumor heterogeneity and cellularity in primary 3D cell cultures. The MPBS provides flexibility for small biopsy samples, whereas VITVO® is more suitable for larger specimens. These 3D models retain morphological, histopathological, and immunophenotypic features, supporting the study of tumor biology in a physiologically relevant microenvironment.

Overall, this workflow establishes a standardized and reproducible platform for generating representative primary 3D cell cultures from spontaneous canine tumors, highlighting their potential as robust tools for comparative oncology research.

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Oral Communication(s)

A DEEP LEARNING FRAMEWORK FOR HISTOPATHOLOGICAL IMAGE ANALYSIS IN CANINE TESTICULAR TUMOURS

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Canine testicular tumours are common and can have significant implications for fertility, hormonal balance, and overall health [1]. Their histopathological classification may be affected by inter-observer variability, potentially leading to misdiagnosis or under-recognition of concurrent alterations. In this study, we developed a reproducible deep learning (DL) framework for automated histological classification of the three major canine testicular tumours and assessed model interpretability using Grad-CAM [2]. To this end, a dataset of 200 WSIs of canine testicular tumours (seminoma n = 75, Leydig cell tumour n = 75, Sertoli cell tumour n = 50) was collected from the archives of the DIPSA Laboratory, University of Naples Federico II, and digitized at 20× magnification following standardized staining procedures. From each WSI, up to 2,500 non-overlapping tiles (380 × 380 px) were extracted and subjected to multi-step quality control. After quality control, 91.6% of tiles were retained, yielding approximately 458.000 informative tiles for model development and validation. We fine-tuned an EfficientNet-B4 convolutional neural network end-to-end on the tile-level dataset and evaluated performance using 5-fold cross-validation stratified at the WSI level. Grad-CAM was then applied to the fine-tuned EfficientNet-B4 network to identify the regions of histological tiles most influential in classification decisions. Our results showed that the fine-tuned model achieved a mean tile-level accuracy of 0.82 ± 0.03, with F1-macro = 0.81, AUC-macro = 0.95, and AP-macro = 0.91, outperforming all feature-based classifiers trained on deep embeddings (Random Forest: accuracy = 0.74). Grad-CAM heatmaps revealed strong overlap between network attention and regions of diagnostic relevance defined by pathologists, confirming morphological consistency. Interpretability analysis quantitatively confirmed this finding, with Pointing Game Accuracy of 0.86 ± 0.05 and Top 20% Energy inside ROI of 0.73 ± 0.07. However, classification errors were mainly derived from morphologically overlapping areas or slide artefacts, highlighting the need for more accurate annotation and expansion of the dataset. Overall, our findings suggest that the automated system has the potential to assist pathologists in the diagnostic process and to reduce inter-observer variability. Nevertheless, the proposed system should be regarded as a decision-support tool for the veterinary pathologist rather than a standalone diagnostic system. These DL-assisted workflows can improve standardisation, reproducibility, and knowledge sharing. Future studies should focus on expanding multi-institutional datasets to reduce bias and improve generalisation and should include a blinded ring test involving veterinary pathologists with varying levels of experience.

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Oral Communication(s)

ASSESSMENT OF HEALTH CERTIFICATES ISSUED FOR ON-FARM EMERGENCY SLAUGHTERED BOVINE ANIMALS: A RECORDS-BASED INVESTIGATION FROM FARM TO THE SLAUGHTERHOUSE

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On-farm emergency slaughter (OFES), as provided for under Regulation (EC) No 853/2004 and subject to official controls under Regulation (EU) 2017/625, allows otherwise healthy animals that are acutely injured and unfit for transport to enter the food chain, reducing food waste and economic losses. Eligibility requires ante-mortem inspection (AMI) by an Official Veterinarian (OV) and completion of a health certificate compliant with national operational guidelines issued by the Italian Ministry of Health and the Emilia-Romagna region.

This records-based investigation analysed health certificates for bovine animals undergoing OFES within the Modena Local Health Authority (LHA) between 1 January and 31 December 2023, assessing compliance with EU and national requirements. The health certificate items under review included clinical history, detailed diagnosis, justification for OFES, exclusion of systemic disease or welfare compromise, and OV declaration. Compliance with mandatory fields and coherence with OFES eligibility criteria were evaluated in line with national and regional operational guidelines. An economic analysis was performed from both the farmer and LHA perspectives. Farmers' net income was estimated from carcass value minus transport, slaughter, and veterinary costs, while LHA costs and revenues were calculated using national tariffs, full labour cost estimates for OV, and travel-related expenses, allowing the net economic balance of the OFES service to be determined.

A total of 926 certificates were reviewed; 110 (11.9%) showed non-compliance. The most frequent issue (87/110; 79%) was the absence of a sufficiently specific diagnosis or clinical description (e.g. generic terms such as "possible trauma" or "walking difficulties" or "downer-cow syndrome" without further details on acute or chronic process or if animals were unfit to travel or if the clinical signs were due to trauma or metabolic conditions), limiting justification for eligibility for OFES. In 13 cases (12%), animals presented conditions incompatible with OFES eligibility (e.g. systemic illness, chronic conditions, or body temperature above 40.5°C) that should have led to rejection at AMI. These findings suggest variability in OV interpretation and highlight the need for harmonised training and clearer guidance as described in other studies (Fusi et al., 2025; Thomsen et al., 2026).

The economic evaluation estimated a net loss of €86,779 for the LHA, primarily driven by personnel time and operational costs. Similar economic and operational constraints have been reported in other European studies reporting variability in clinical decision-making and economic burden, where OFES is recognised as beneficial but resource-intensive if not optimised (Kautto et al., 2023). Overall, while OFES remains an important tool to safeguard animal welfare and reduce food waste, improvements in certification accuracy, standardisation of AMI practices, and optimisation of service delivery models are needed to ensure both regulatory compliance and long-term sustainability.

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Oral Communication(s)

VALORIZATION OF UNDERUTILIZED MEDITERRANEAN FISH SPECIES THROUGH A SUSTAINABLE AND MULTIDISCIPLINARY APPROACH

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The sustainable exploitation of marine biological resources is a key objective of the European Union's Common Fisheries Policy (CFP). Europe imports approximately 65% of its fishery products, while Italy reaches nearly 80%, indicating that domestic production does not satisfy consumer demand.

Although Ministerial Decree No. 175216 (16 April 2025) lists around one thousand commercially relevant fish species, consumption remains concentrated on a limited number of products (e.g., seabass, seabream). In contrast, several abundant native Mediterranean species remain poorly recognized by consumers. This represents both a waste of local biological resources and increased pressure on a few overexploited stocks, highlighting the need for strategies supporting a more sustainable and diversified seafood market.

This study aimed to enhance the value of five underutilized Mediterranean fish species through an innovative multidisciplinary food-feed approach, providing robust scientific evidence to strengthen consumer confidence in their consumption. The novelty of the study lies in the 360-degree evaluation of these species, combining nutritional, physicochemical, rheological, and molecular characterization to comprehensively assess their suitability for both large-scale industrial food processing and the valorization of processing side streams as feed-formulation ingredients.

Species were selected based on regional abundance and current low economic value: Atlantic horse mackerel (*Trachurus trachurus*), Bogue (*Boops boops*), Common dolphinfish (*Coryphaena hippurus*), Round sardinella (*Sardinella aurita*), and Silver scabbardfish (*Lepidopus caudatus*).

Nutritional (moisture, fat, protein, ash), physicochemical (TVB-N, histamine, pH), and rheological (color, texture, viscosity) properties were evaluated in both edible portions (fillets) and side streams.

Furthermore, because complete mitochondrial reference genomes for these species were scarce or fragmented in public databases, complete mitochondrial DNA (*mtDNA*) sequencing was performed using Illumina NGS technology. The obtained *mtDNA* sequences were aligned with homologous sequences from species within the same family, or when appropriate, the same order, to develop species-specific molecular markers. Phylogenetic analysis was carried out for each species to evaluate the relationships with better-known species to consumers.

Nutritional analyses revealed fillets with fat content below 3% and protein levels ranging from 22–23%, confirming profiles competitive with heavily exploited commercial species. Processing by-products showed elevated lipid (2.7–5%) and substantial protein (18–19%) levels. Combined with favorable rheological properties, including increased viscosity in saline solutions, these results demonstrate strong potential both for innovative food processing applications and for the formulation of high-value feed ingredients.

High-quality complete *mtDNA* was successfully obtained for all species, fully sequenced, annotated, and deposited in GenBank (PRJNA1035303). Innovative species-specific primers were designed and validated, supporting seafood authentication in prepared and processed products.

By integrating product valorization, by-product recovery, and molecular traceability within a single multidisciplinary framework, this approach offers an innovative model for sustainable fisheries exploitation. Promoting underutilized fish species can reduce pressure on overexploited stocks, contribute to marine biodiversity conservation, and support local economies, in line with CFP objectives.

Oral Communication(s)

SURVIVAL OF *STAPHYLOCOCCUS AUREUS* BELONGING TO CC5 IN RAW MILK CHEESE FROM THE FARM TO THE TABLE

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Staphylococcus aureus can produce enterotoxins, causing foodborne outbreaks. Raw milk cheese has been identified as one of the food matrices causing staphylococcal food poisoning in the European Union [1, 2, 3].

In this study, we investigated the concentration of *S. aureus* along the longitudinal sampling of one batch of raw milk cheese, testing raw milk (day 1) (n=3) and raw milk cheese during ripening (i.e., 7, 45, and 90 days) (n=3 for each sampling time) and at the end of 90 days of shelf-life after storage under vacuum and refrigeration temperature (i.e., 4±1) (TRV) (n=3), under air and refrigeration temperature (n=3) (TRA), under vacuum and abuse temperature (i.e., 7±1°C) (TAV) (n=3), under air and abuse temperature (TAA) (n=3).

The cheese was prepared from raw milk heated at 42.5 °C for 30 s and supplemented with starter. It underwent 3 stewing steps at 50 °C, was drained overnight at room temperature, brined at 8 °C for 5 h, treated with an antifungal coating, and then stored at 12 °C for 90 days.

Up to three *S. aureus* isolates were purified, subjected to DNA extraction using the MagAttract HMW DNA Kit and library preparation with the Illumina DNA Prep Kit, and whole-genome sequenced on the Illumina NextSeq 1000 platform. Genome assembly was performed using MetaSPAdes, followed by MLST-based strain characterization and identification of enterotoxin, virulence, and antimicrobial resistance genes using reference databases.

The counts of *S. aureus* were 1.14±0.14 log CFU/g in raw milk with a pH of 6.66 and increased to 2.73±0.16 and 3.02±0.23 after 7 and 45 days of ripening, when pH was 5.35 and 5.24, and aw 0.982 and 0.966. At the end of ripening and shelf life, the counts dropped to 1.20±0.55 and < 1 log CFU/g, respectively; pH to 5.28 and between 5.02 (TRV) and 5.94 (TRA); aw to 0.976 and between 0.9523 (TAV) and 0.950 (TRV).

Eight *S. aureus* strains were collected after 7 (n=3), 45 (n=3), and 90 days (n=2) of ripening. Moreover, 3 strains were collected at shelf life after storage under TAV, TRV, and TRA conditions (n=1 each).

The tested strains belonged to the clonal complexes CC5 (n=6) and CC97 (n=5). All strains carried virulence-associated genes, including *selX*. However, the *sea* gene, encoding enterotoxin A, was detected exclusively in CC5 isolates. Strains belonging to CC97 harbored genes associated with tetracycline resistance and efflux mechanisms, whereas CC5 strains carried beta-lactam resistance genes (*blaI, blaR1, blaZ*). Variability in adhesion-related genes (*clfB, fnbA, sdrD*) suggested differences in colonization and survival potential.

Although *S. aureus* counts remained below 4 log CFU/g, the lower limit of the process hygiene criterion for coagulase-positive staphylococci in raw milk cheese stated in Commission Regulation (EC) No 2073/2005, the results of this study show that CC5 strains were detected along the 90 days of ripening and survived in the final product after 90 days of storage at both packaging and temperature conditions. Analyzing up to 3 colonies in each positive sample increased the likelihood of detecting CC5 within the *S. aureus* population, because strains of different CC coexist in the same sample.

This study has been funded by the Italian Ministry of Agriculture, Food Sovereignty and Forests under the Contratto di Filiera, Stalla modello, DMI n. 0673777 del 22/12/2021, V Avviso n. 0182458 del 22/04/2022.

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Oral Communication(s)

BACTERIOCINS AND ANTIMICROBIAL PEPTIDES: IMPLICATIONS IN FOOD SAFETY

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Food safety remains a major global challenge due to the persistence of foodborne pathogens, the increasing spread of antimicrobial resistance, and the urgent need for sustainable alternatives to conventional chemical preservatives. In this context, bacteriocins, lantibiotics, and other antimicrobial peptides have attracted growing interest as natural molecules capable of controlling undesirable microorganisms in food systems. Produced by several bacterial groups, including lactic acid bacteria, these compounds display potent antimicrobial activity against bacteria of major relevance to the dairy and food-processing sectors. Among them, nisin is the best-characterized lantibiotic and, to date, remains the only bacteriocin authorized in Europe as a food preservative, making it a benchmark for current and future biopreservation strategies.

Within this framework, our work explored antimicrobial peptide production in food-associated competitive environments, focusing on both established and emerging sources of bioactive compounds. In an experimental model based on the interaction between *Lactococcus lactis* and *Listeria monocytogenes*, we investigated how bacteriocin production may be modulated under microbial competition. Under co-culture conditions, *L. lactis* did not simply maintain constitutive nisin production, but instead showed a shift in the represented lantibiotic forms, from a predominance of Nisin A under non-competitive conditions to a stronger expression of Nisin Z in the presence of *L. monocytogenes*. Since Nisin A and Nisin Z differ by a single amino acid substitution, yet Nisin Z is characterized by improved solubility and diffusion properties, this shift may be particularly relevant for antimicrobial performance in complex food matrices.

In parallel, and with a focus on *Staphylococcus aureus* as an important food-associated pathogen, we undertook a preliminary screening of 63 *Staphylococcus* strains isolated from raw milk samples collected from 21 Holstein cows in Poland. One isolate showed inhibitory activity against multiple indicator strains, suggesting the production of an extracellular antimicrobial compound. Early characterization indicates that this substance is likely proteinaceous or peptidic in nature. Although these findings are still preliminary, they support the possibility that staphylococci associated with raw milk may also represent a source of bioactive compounds of potential interest for food safety applications. Further analyses are currently ongoing to better define the identity of the compound, its antimicrobial spectrum, and its possible technological relevance.

Overall, these observations reinforce the view that antagonistic interactions in food ecosystems are dynamic processes that shape both pathogen behavior and the expression of protective antimicrobial compounds. At the same time, they highlight the potential of bacteriocins, lantibiotics, and related peptides as promising tools for the development of innovative and sustainable strategies to improve food safety.

Oral Communication(s)

PROPOSAL FOR A STANDARDIZED SAMPLING METHOD AND PROCESS HYGIENE CRITERIA TO ENHANCE FOOD SAFETY IN RABBIT SLAUGHTERHOUSES

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Rabbits, as lagomorphs, are classified as meat consisting of edible parts, including blood under Regulation 853/2004 that sets requirements for their inspection, slaughter hygiene, and ante- and post-mortem controls. Food Business Operators must also ensure compliance with standards for slaughterhouses, cutting plants, and hygiene during and after processing (Regulation 853/2004). However, Regulation 2073/2005 on microbiological criteria for foodstuffs does not specifically consider rabbit carcasses. The omission of this food category implies not only that no specific microbiological criteria, including target microorganisms and corresponding limit values are provided, but also that no sampling method is explicitly defined, representing a significant gap for assessing slaughter hygiene for both FBOs and Competent Authority.

This study aimed to evaluate a sampling method suitable for rabbit carcasses, and to select a calculation approach for the assessment of rabbit carcass process hygiene criteria through the analysis of different regulatory and normative sources.

Regarding the sampling method, after reviewing the scientific literature and BS EN ISO 17604:2015 “Microbiology of the food chain — Carcass sampling for microbiological analysis”, the Whole Carcass Rinse (WCR) method was selected, as it is non-destructive, exhibits lower variability than swabbing in terms of repeatability and reproducibility, provides greater representativeness of different carcass areas, and ensures higher microorganism recovery from small carcasses than swabbing and, in some cases, even than the excision method.

By means of WCR method a total of 203 carcasses were collected from 3 different slaughterhouses, specifically 5 carcasses were sampled every sampling day with a minimum of 12 sampling days conducted. Analyses were performed for Enterobacteriaceae enumeration and aerobic colony count. The aggregated data showing a normal distribution for both hygiene criteria classes were analyzed by considering the three regulatory alternatives represented by AFSSA (2008) [1], the Guidelines for the Application of Regulation (EC) No 2073/2005 [2], and AVIS 21-2013 [3]. Among the evaluated regulatory approaches, the AFSCA percentile-based approach was the most suitable for rabbit slaughters: the 75 and 95 th percentiles of the results obtained is respectively the “m” and “M” values. It also presents an improvement-oriented approach supporting progressive improvement and benchmarking of slaughter hygiene through adjustable percentiles (by increasing or decreasing the reference percentile used to calculate “m” and “M” values). A CFU/mL threshold based on experimental data percentiles may be defined either as slaughterhouse-specific, using historical data, or as a common limit for all plants, subject to validation with a larger and more diverse dataset.

This proposal represents a preliminary contribution that may facilitate the future harmonization of process hygiene criteria for rabbit carcasses in Italian and European legislation. Further studies with wider sampling and additional plants are needed to validate the approach and suggest applicable process hygiene criteria limits to be potentially integrated into national and EU regulatory frameworks.

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Oral Communication(s)

INTEGRATED ABATTOIR AND MOLECULAR INVESTIGATION OF CYSTIC LESIONS IN CATTLE FROM NORTHERN ITALY

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Post-mortem inspection at slaughterhouses represents a key surveillance tool for zoonotic parasites within the food chain. Cystic echinococcosis (CE), caused by *Echinococcus granulosus sensu lato*, is traditionally monitored through routine inspection of offal, although the diagnostic reliability of macroscopic evaluation alone may be limited, particularly in hypoendemic areas. This study aimed to assess the occurrence of cystic lesions compatible with CE detected during official meat inspection in cattle in Northern Italy and to verify their etiological origin through laboratory confirmation.

A retrospective observational study was conducted on official slaughterhouse records from January 2021 to September 2024, including 129,957 cattle originating from Emilia-Romagna and Lombardy regions. Post-mortem inspections were performed by official veterinarians in accordance with the European legislation, and lesions were classified as hydatid cysts based on macroscopic criteria only. Data on age, sex, origin and lesion localization were analysed using descriptive statistics. In parallel, a prospective investigation was carried out between October 2024 and March 2025 on 17,541 cattle slaughtered in the same facility. During routine inspection, organs presenting cystic lesions suspected of CE were collected and subjected to detailed anatomopathological and molecular analyses. Morphological evaluation included the assessment of cyst structure and microscopic examination of cyst fluid. DNA was extracted from cyst tissues and fluids. A validated multiplex PCR described by Trachsel D. et al. (2007) was used to discriminate *Taenia* spp., *Echinococcus granulosus*, and Echinococcus multilocularis followed by sequencing of positive amplicons. Positive and negative controls were included in each PCR run to ensure assay reliability.

The retrospective analysis showed a low mean prevalence of lesions compatible with CE (0.32%), with hepatic localization more frequently reported and a higher occurrence in older animals. In the prospective phase, 16 hepatic lesions (0.09%) were initially classified as compatible with CE; however, two were excluded after anatomopathological examination, reducing the suspected prevalence to 0.08%. Molecular analyses did not detect *Echinococcus* spp. DNA in any sample, while 50% of lesions were identified as due to *Taenia hydatigena*. Consequently, the confirmed prevalence of bovine CE in the investigated population was 0.0% (IC95%: 0-23.2%).

These findings demonstrate that cystic lesions detected during routine meat inspection are not always attributable to *Echinococcus* spp., particularly in low-prevalence settings, and the risk of misclassification when relying solely on macroscopic evaluation have to be evaluated. The integration of laboratory diagnostics with slaughterhouse surveillance improves etiological confirmation and provides more reliable epidemiological data, supporting risk-based management approaches and avoiding unnecessary investigations at farm level within a One Health framework.

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Oral Communication(s)

EVALUATION OF *SHEWANELLA SPP*. CONTAMINATION IN SEAFOOD PROCESSING: FOCUS ON ENVIRONMENTAL AND EQUIPMENT SURFACES

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Shewanella spp. are Gram-negative bacteria increasingly detected in food industries. Their remarkable adaptability combined with their biofilm-forming ability, raise concerns about their persistence in processing environments, potentially leading to food contamination. In recent years, this genus has been frequently isolated from several food matrices, particularly seafood (crustaceans and bivalve mollusks), where some species are associated with spoilage processes, off-odors and quality deterioration, fresh meat and occasionally vegetables. These findings suggest the surrounding environment, water and raw materials as primary contamination sources, possibly combined with insufficient hygiene practices. In addition, several species are also emerging as opportunistic pathogens associated with a wide range of human infections, further highlighting their relevance in food safety and public health [1]. Therefore, monitoring their distribution in processing facilities is essential to identify risks and improve preventive strategies. The current study aimed to investigate the presence of Shewanella spp. in five seafood processing plants, each including both bivalve mollusks depuration and finfish processing, located in the Campania region (southern Italy) during the year 2025. The objectives were to assess their spatial distribution within the facilities, identify operational areas with the highest contamination levels and evaluate the effectiveness of sanitation procedures as well as the biofilm-forming ability of the isolated strains. In this regard, microbiological sampling targeted environmental surfaces, tools and processing equipment using pre-moistened sterile cellulose sponges according to ISO 18593:2018. After collection, samples were serially diluted and plated on Baird-Parker agar for presumptive isolation. To ensure accurate taxonomic assignment, suspected colonies were further identified by MALDI-TOF MS. Only species-level identified isolates (n=11) were tested for biofilm production using the crystal violet method. During the monitoring period, a total of 100 sponge samples (20 per plant) were collected from seafood processing facilities, covering four operational areas: processing (n=20), storage (n=20), dispatch (n=20) and bivalve mollusks depuration (n=20) areas. Shewanella spp. were detected in 21 out of 100 samples (21%). The highest positivity rates were observed in both storage areas (7/20; 35%) and depuration-related surfaces (7/20; 35%), followed by processing areas (4/20; 20%) and dispatch areas (3/20; 15%). As far as biofilm activity assessed only in species-level identified isolates (n=11), the analysis showed that all strains were biofilm producers yet after 24 h (6 weak, 4 moderate, 1 strong), supporting their potential persistence in processing environments. These results suggest that environmental and equipment surfaces of both storage and depuration areas may represent potential reservoirs for Shewanella spp. contamination, highlighting water as a possible vehicle for these bacteria and indicating deficiencies in sanitation practices. In conclusion, the study underscores the persistence and distribution of Shewanella spp. in seafood processing environments and highlights the essential role of food-contact surfaces in sustaining contamination. The results stress the importance of enhancing sanitation protocols through targeted, stringent, and regularly validated cleaning strategies to reduce cross-contamination, safeguard product quality, and ensure the microbiological safety of final seafood products.

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Oral Communication(s)

MOLECULAR SCREENING OF HEPATITIS E VIRUS IN WASTEWATER SAMPLES FROM A SWINE ABATTOIR

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This study aimed to provide novel environmental data on the circulation of hepatitis E virus (HEV) RNA in effluent samples collected from a domestic pig slaughterhouse in Central Italy. Between January and June 2025, a total of 36 samples were collected over 18 days. More specifically, two 1.0 Lt aliquots were taken from two different sites: the first one was at the pre-treatment level (V_1 - stocking yard) and the second one at the post-treatment one (V_2 - after the depuration process at the wastewater treatment plant (WWTP)), following the protocol described by dos Santos et al. [1] in Brazil. Both of 18 V_1 and V_2 samples were centrifuged twice at 180,000 X g for 2 hours; the resulting pellets were used for the RNA extraction procedure using the Trizol LS method. Real time RT-qPCR and nested RT-PCR assays were performed to quantify and amplify the targeting ORF-1 and ORF-2 genetic regions of the HEV genome. Sequencing was followed by the amplicon analysis, publication on the GenBank platform, and phylogenetic tree construction according to the Neighbor-joining method [2]. The IBM SPSS Software (SPSS, Chicago, IL, USA) was used, and the two-tailed *t*-test was performed to assess possible significant statistical differences in HEV viral loads between V_1 and V_2 samples, expressed as genomic equivalent per millilitre (GE/mL). Results showed that 13.89% (5/36) of the screened effluent samples detected HEV RNA, and more specifically, 8.33% (3/36) were V_1 samples and 5.55% (2/36) V_2 ones. The highest viral load (10^5 GE/mL) was observed in a V_1 sample; the average load over the six months was 10^3 GE/mL. Overall, the *t*-test revealed statistically significant differences between positive V_1 and V_2 samples (*p*-value= 0.040). The amplified ORF-1 and ORF-2 sequences were deposited and published on the GenBank platform with the following accession numbers: PX925942, PX925944, PX925945, PX925946, PX925947, and PX925948. The observed prevalence (13.89%) was higher than 0.4% reported by Rusiñol et al. [3] from effluents collected from a swine abattoir in Spain and lower than 50.0% reported by dos Santos et al. [1] from slaughterhouse wastewaters samples in Brazil. This study represents the first Italian virological and molecular screening of HEV RNA circulation from an environmental perspective. It also confirms the limited efficacy of current WWTPs in removing viral contaminants and highlights the necessity of the inclusion of specific virological parameters in the legislation. These findings want to underline the crucial importance of molecular surveillance of zoonotic viruses among ecosystems.

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Oral Communication(s)

EVALUATING THE LEVELS OF OCHRATOXIN A IN DRY-CURED HAM BY MEANS OF A VALIDATED HIGH SENSITIVITY HPLC-FLD METHOD AND CONTRIBUTION TO RISK ASSESSMENT

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Ochratoxin A (OTA) is a mycotoxin produced by several fungal species including *Aspergillus ochraceus*, *A. carbonarius*, *A. niger* and *Penicillium verrucosum*. This mycotoxin causes nephrotoxicity and renal tumors in humans and animals. In particular, OTA exposure has been linked with the human diseases Balkan endemic nephropathy and chronic interstitial nephropathy, as well as other renal diseases. Cereals, coffee, dried fruits, wine, and spices are recognized as the most contaminated types of food which can be contaminated by OTA. However, recent studies and investigations have reported about the possible not negligible levels of contamination of OTA in dry-cured ham [1]. In this regard, it is important to underline that the Regulation (EU) No. 2023/915 does not establish so far specific legal limits for the OTA contamination in dry-cured ham. This is due to a comprehensive risk assessment which is still not available for this mycotoxin/matrix combination.

This study represents a contribution to risk assessment of OTA in dry-cured ham. The investigation was carried out by applying a fully validated analytical method based on UPLC/FLD. This method is based on a solid/liquid extraction of 10 g of homogenized sample with 40 mL of a methanol/water (80:20, v/v) mixture, followed by immunoaffinity SPE purification. The chromatographic separations were accomplished by using a Kinetex C18 analytical column eluted in isocratic mode with a mobile phase composed of acetonitrile/methanol/water/acetic acid (45:9:46:1, v/v/v/v). The wavelengths of fluorimetric excitation and emission were set to 333 nm and 463 nm, respectively.

Overall, 50 samples of dry-cured ham, characterized by different origin, maturation time (between 9 and 24 months), salt concentration and food additives (with and without nitrates), were collected in local stores following the randomness principle, and analysed. The samples were collected outside the official control plans in order to acquire information about this type of contamination. Each sample was composed of at least 50 grams and it was fully homogenized before the analysis.

Quantifiable amounts of OTA (> LOQ: 0.2 µg/kg) were detected in 6 samples (12% of total samples), with concentrations in the range 0.22 – 0.42 µg/kg. Traces of OTA (> LOD: 0.06 µg/kg) were detected in other 7 samples. Taking as reference the only available limit, established in the Circular n. 10 of 9 June 1999 of the Italian Ministry of health (1.0 µg/kg), all detected values were lower than the limit. The evaluation of Margin of Exposure (MoE) confirmed that these levels do not represent a significant food safety risk, since the values calculated were higher than 10,000 under both the low and high exposure scenario. The investigation confirmed as well the possible presence of OTA in dry-cured ham, making necessary the official control of such products, other than the establishment of specific legal limits at European level.

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Oral Communication(s)

THE ONE HEALTH STRATEGY OF THE REGION CAMPANIA TO FACE ENVIRONMENTAL CRISIS: A 10-YEARS LONG LASTING EXPERIENCE

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From 2008, the Government of the Region Campania had to face relevant cases of food and feed contamination as a consequence of heavy environmental pollution, above all caused by illegal traffic of wastes. The increasing episodes of burning wastes made the Region famous all over the world for the phenomenon known as the “Land of Fires”, considered the main cause of the high incidence of tumours and other severe pathologies among exposed population. Moreover, this phenomenon was considered a relevant source of dioxins, polychlorobiphenyls, polycyclic aromatic hydrocarbons, heavy metals and other xenobiotics contaminating mainly milk and vegetables. A crisis regarding buffalo milk contaminated by dioxins caused heavy economic losses in the Region. It was resolved by an extraordinary monitoring plan set by the Governor of Campania in cooperation with the Ministry of Health and the European Commission, introducing a modern strategy based on risk evaluation, buffer local georeferencing and feed selection. After this experience, some actions and plans were issued and funded by the Region Government, to correlate environmental pollution, food safety and health evaluation of exposed population, starting from 2014 to 2025:

a. 2014 –“QR Code Campania” was a program of in-depth analysis aiming to food safety, through a system of vouchers for farmers, to demonstrate their products were compliant to the EU laws.

b. 2015 – “Campania Trasparente” was a large integrated monitoring program to evaluate pollution of top soil, water and air all over the Region, and possible contamination of food, vegetables and wild life, leading to the classification of high, medium and low environmental impact areas.

c. 2016 – “SPES” was a multidisciplinary epidemiological Study on the Exposure of Susceptible Population, based on human biomonitoring (4.200 people) to evaluate correlations between pollution and health.

d. 2019 – the so-called project DRGC 180/2019, was an integrated study in cooperation with ARPA Campania, IRCCS Pascale and Naples University, to elaborate a model for public health prevention.

During these years, 150 air stations were monitored, and thousands of samples were tested: 4.000 top soils, 1.200 waters, 3.500 food, 2.600 vegetables. More than 5.000 persons were recruited for human biomonitoring.

The Project DRGC 180/2019 was organised in four main areas: Environment, Health, Research and Communication. Within each working area, the activities were organised in sub-projects, based on cooperation with other partners (universities, CNR), leading to an integrated mosaic and a large network of researchers. Thus, we started the following studies: SPEL regarding workers exposed to chemicals; SPEM to select new biomarkers in humans for environmental correlated pathologies; ERICA to correlate obesity with some endocrine disruptor chemicals; CRISALIDE for the screening of papilloma virus in the Region. Then, a follow up of the study SPES was also carried out.

Moreover, we introduced the CUI, the Unique Registry of Hydric Utilities, a census of the water resources of the Region, and the regional Water Safety Plans were issued. Other studies were carried out about heavy metals in PM10 particulate, environmental crisis in the valley of the rivers Sabato and Irno, the geotag of water resources used in agriculture.

These projects produced several outputs and scientific publications. The overall final results of the project are going to be presented.

The model SPES is now the basis for a project funded by the State known as IN-SINERGIA, involving 9 Italian Regions.

Oral Communication(s)

SUSPECT SCREENING ANALYSIS OF POLY-AND PERFLUOROALKYL SUBSTANCES IN WILD BOAR LIVER

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Per- and polyfluoroalkyl substances (PFAS) represent a large family of highly persistent and mobile chemicals, some of which are characterised for their toxicity in biota and humans. Their extensive industrial production and use in different manufacturing sectors have led to widespread environmental emissions and subsequent transfer to food webs [1]. Most bioaccumulative PFAS have the liver as primary target organ for toxicity associated with dyslipidemia and immunosuppression. Among terrestrial wildlife species, the wild boar has been reported to exhibit a notable capacity to bioaccumulate both legacy and emerging PFAS in edible tissues, representing a source of intake for frequent consumers of game meat [2]. Given the need to assess PFAS toxicity as mixtures, and that food safety regulation encompass only a limited subset of PFAS, this study aimed to perform a suspect screening analysis (SSA) to broaden the knowledge about the detectable and identifiable PFAS spectrum. SSA focuses on analysing so-called “known unknowns”, whose names and structures are clearly defined and suspected to be present in the sample. We analysed a pooled wild boar liver sample formed from 28 wild boar liver specimens collected from 5 different Italian areas (provinces of Savona, Spezia, Bologna, Alessandria and Potenza) characterised by different industrial, anthropogenic, and natural PFAS pressures. The liver pool derived from hunted animals intended for consumption (8 females and 20 males, between 6 and over 24 months of age, and between 30 and 98 kg of body weight). The pool was extracted by acetonitrile, followed by clean-up through solid-phase extraction (SPE) and dispersive SPE. After concentration, the extract was analysed by liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS). The workflow enabled the tentative identification of 29 additional PFAS beyond the 4 (PFOS, PFOA, PFNA, and PFHxS) covered by food safety regulation and the other 15 conventionally targeted legacy compounds. Notably, emerging PFAS classes, previously reported in environmental and biota samples, have been identified, according to the Schymansky grid [3]. Our results are in good agreement with those already reported for the same matrix and support the approach of monitoring PFAS-containing products throughout their life cycle until disposal, without limiting the study to industrial emission sources alone. The detection of these compounds in wild boar liver further supports the evidence of their wide environmental dissemination and trophic transfer. Although preliminary, these findings highlight the need to harmonise untargeted approaches for PFAS in food, thus supporting a comprehensive toxicological assessment of mixtures. Last but not least, this work reinforces the role of the wild boar as a valuable sentinel species for assessing terrestrial PFAS contamination in line with the European Union "safe soil" strategy.

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Oral Communication(s)

LABELLING ASSESSMENT AND MOLECULAR AUTHENTICATION OF SQUID-BASED PRODUCTS FROM TWO MAJOR EUROPEAN CITIES

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A total of 198 squid-based products were collected in Brussels (n=98) and Milan (n=100) from supermarkets, fishmongers and restaurants between February and May 2025. The study assessed labelling compliance and species authenticity of fresh, frozen and processed products by combining label evaluation with DNA barcoding for mono-species samples and DNA metabarcoding for multi-species products. Overall, mislabelling was detected in 13.3% of products in Brussels and 30.2% in Milan. However, when the analysis was restricted to products allowing species-level verification, comparable frequencies were observed in the two cities (27.3% and 26.5%, respectively), indicating that the apparent difference was largely driven by national seafood nomenclature systems. In Brussels, the widespread use of umbrella commercial designations hindered species-level traceability, whereas the more specific Italian naming system facilitated the detection of mismatches. Substitutions frequently involved the replacement of higher-value Mediterranean or Northeast Atlantic taxa with lower-priced imported species such as Dosidicus gigas, Doryteuthis gahi and Illex argentinus. Statistical analyses did not show significant associations between mismatch frequency and either product category or retailer type. Processed and multispecies products also revealed undeclared complexity, with metabarcoding detecting additional animal taxa in some samples. Species-level transparency was further limited because scientific names were only rarely provided voluntarily for products outside the scope of mandatory EU labelling requirements, such as processed products and restaurant items. These findings show that squid mislabelling is a relevant issue in both markets and support the need for harmonised and stricter EU labelling requirements, including mandatory species information for processed products and food service items.

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Oral Communication(s)

COMPARATIVE ANATOMICAL AND HISTOLOGICAL CHARACTERIZATION OF THE PUDENDAL NERVE IN MINIATURE AND FARM PIGS: REFINING PORCINE MODELS FOR TARGETED NEUROMODULATION

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Pig models are considered the gold standard for studying lower urinary tract (LUT) disorders and developing neurotechnological approaches, due to their close anatomical similarity to humans. This study aimed to comparatively characterize the anatomical accessibility, morphological variations, and histological organization of the pudendal nerve in mini-pigs and farm pigs, in order to optimize neuromodulation strategies.

Six mini-pigs and seventeen farm pigs were included in the study. No animals were sacrificed specifically for this study, as all specimens were obtained from animals previously enrolled in independent experimental protocols. A transgluteal approach was employed to access the pudendal nerve, which was dissected from the sacral plexus to its terminal branches. Tissue samples from four mini-pigs and four farm pigs were processed for histological examination using hematoxylin and eosin (H&E), Luxol Fast Blue, and Masson's Trichrome staining. Additionally, the neurochemical composition of the nerve fibers was evaluated by immunohistochemical analysis using NFP, ChAT, Substance P, and TH antibodies.

The transgluteal approach was found to provide safe and consistent access to the pudendal nerve with minimal tissue damage. Anatomically, the pudendal nerve was determined to originate from the L6 and S1–S3 spinal roots. A significant morphological difference in nerve formation was observed between the two models: in farm pigs, the pudendal nerve is formed by the convergence of two main branches, whereas in mini-pigs, it is formed by the simultaneous fusion of three distinct branches.

Histologically, the pudendal nerve in both species was found to consist of 30–50 fascicles. Immunohistochemical analyses revealed that NFP- and TH-positive fibers were distributed throughout all fascicles. In contrast, ChAT-positive (motor) and Substance P-positive (sensory) fibers were confined to specific fascicles, indicating a functional organization (somatotopy). Additionally, ChAT-positive fibers were predominantly localized in smaller fascicles, whereas Substance P-positive fibers were more frequently observed in larger fascicles.

These findings provide a detailed morphological and topographic map of the porcine pudendal nerve. Specifically, elucidating the differences in root junction patterns between mini-pigs and farm pigs provides a critical anatomical basis for bioengineering applications and targeted neuromodulation therapies.

Oral Communication(s)

FROM OVERNUTRITION TO BRAIN-GUT ALTERATIONS DURING AGING IN *NOTHOBRANCHIUS FURZERI*

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Overnutrition is associated with chronic inflammation and alterations involving both the central nervous system and the intestine. Hypercaloric diets can induce cognitive impairment through mechanisms including gut microbiota dysbiosis and activation of inflammatory pathways along the gut–brain axis [1]. Several of these changes overlap with hallmarks of aging, suggesting that prolonged caloric excess may promote aging-like features. In this context, *Nothobranchius furzeri*, a short-lived vertebrate model, represents an ideal system to investigate the long-term effects of overnutrition on the gut–brain axis during aging [2]. In a previous pilot study, we tested different overnutrition conditions and identified the one inducing the most marked central alterations in adult animals (20 weeks post hatching, wph). Based on these findings, in the second phase of the study we maintained this condition up to 27 wph, corresponding to the onset of aging in this species, to assess its long-term effects on brain and gut homeostasis. Fish from the MZM 04/10 strain were divided at 6 weeks of age into two groups and fed with *Chironomus spp.*: control (300 mg/day) and overfed (780 mg/day). The study was approved by the Italian Ministry of Health (protocol 77/2023-PR). For *ex vivo* analyses, animals were classified as adult control (AD-C), adult overfed (AD-OF), old control (OLD-C) and old overfed (OLD-OF), allowing the evaluation of the combined effects of diet and aging. Brain samples were analysed for the glial markers GFAP and LCP1 to assess glial and microglia-related responses and to evaluate whether overnutrition induces neuroinflammatory changes resembling or anticipating those typically associated with aging. GFAP was significantly increased in AD-OF compared with AD-C and OLD-C and showed a further increasing trend in OLD-OF fish. LCP1 followed a comparable pattern, with higher expression in overfed and aged groups, supporting the presence of a persistent glial response associated with chronic overnutrition. At intestinal level, IL-1β, IL-8 and IL-10 expression was analysed in AD-C, OLD-C and OLD-OF fish. Significant differences emerged mainly between AD-C and OLD-C animals, whereas OLD-C and OLD-OF groups did not differ significantly, suggesting that cytokine modulation is closely associated with aging and chronic overnutrition does not appear to further influence their expression during aging. Gut microbiota was analysed by 16S rRNA sequencing. In OLD-OF animals, *Proteobacteria* and *Actinobacteria* remained dominant, while *Firmicutes* were reduced and *Bacteroidetes* disappeared, indicating a dysbiotic shift that may contribute to gut barrier dysfunction and altered gut–brain axis signaling, potentially sustaining neuroinflammatory responses in the brain. Overall, these findings indicate that an integrated brain–gut profile may emerge in *Nothobranchius furzeri*, suggesting that prolonged caloric excess may interact with aging processes and contribute to neuroinflammatory alteration. Future studies will investigate hypothalamic circuits involved in the regulation of energy and metabolic homeostasis.

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Oral Communication(s)

ORAL SELF-DNA INDUCES SYSTEMIC ALTERATIONS IN ZEBRAFISH

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Fragmented extracellular self-DNA, derived from conspecific organisms, can exert species-specific inhibitory effects. This phenomenon has been widely documented in plants, where self-DNA accumulation in leaf litter induces negative plant–-soil feedback and metabolic disorders [1], and has also been reported in invertebrates, causing metabolic, developmental, and reproductive alterations in both Drosophila melanogaster [2] and Caenorhabditis elegans [3].

In this context, we investigated the effects of dietary self-DNA on vertebrates using zebrafish (Danio rerio) as model. Self-DNA was delivered through spirulina (Arthrospira platensis), a widely used feed supplement and biostimulant in aquaculture. Three experimental groups were established: C, a control group fed a standard diet; A-25, fed a diet containing 25% wild-type spirulina and 75% standard feed; and S-25, fed a diet containing 25% spirulina wild-type enriched with self-DNA and 75% standard feed.

Metabolomic profiling by Liquid Chromatography–Mass Spectrometry revealed that the S-25 group showed increased levels of deoxyguanosine, deoxyadenosine, guanine, xanthine and uric acid compared with the other groups, suggesting a dysregulation of purine metabolism with a shift toward increased catabolism. These findings are consistent with enhanced xanthine oxidase activity, which generates reactive oxygen species (ROS) as by-products and may contribute to oxidative stress. This hypothesis is further supported by the increased levels of cystathionine, a precursor involved in glutathione biosynthesis. Additionally, reduced levels of phospholipids, such as glycerophosphocholine, were detected.

Transcriptomic analysis revealed differential expression of genes associated with metabolic regulation and oxidative stress responses, supporting the occurrence of metabolic imbalance and activation of antioxidant pathways. In the S-25 group, a drastic reduction in reproductive performance was observed in the F1 generation, consistent with altered expression of gonadotropin-related genes along the brain–gonad axis. Proteomic analysis revealed reduced expression of proliferation markers together with increased levels of proteins associated with immune activation and glial reactivity, in the S-25 group compared with the other groups.
These findings suggest that dietary self-DNA induces metabolic stress, immune activation and reproductive impairment in zebrafish, highlighting an inhibitory role of extracellular self-DNA also in vertebrates.

Protocol authorization number: N° 260/2024-PR

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Oral Communication(s)

A NOVEL GTPBP2 IN-FRAME DELETION ASSOCIATED WITH NON-SYNDROMIC PROGRESSIVE RETINAL ATROPHY IN LABRADOR RETRIEVERS

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Progressive Retinal Atrophy (PRA) represents one of the most clinically relevant inherited retinal diseases in dogs, affecting numerous breeds and leading to complete blindness [1]. Identification of causative variants is essential for genetic testing and disease management. Recently, a naturally occurring form of non-syndromic PRA in a Labrador Retriever litter has been described. It was found that three of the six siblings developed retinal degeneration and visual impairment, while the parents and the remaining littermates were unaffected. Pedigree analysis supported an autosomal recessive mode of inheritance.

Using SNP-based homozygosity mapping combined with whole-genome sequencing, a homozygous 3-bp in-frame deletion in GTPBP2 (c.1606_1608del; p.Ala536del) as the only candidate variant segregating with the phenotype has been identified. Screening of over the 2,600 dogs from a Labrador Retriever sample pool and in a database of canine variants (Dog 10k multiple breeds) confirmed the extreme rarity of this variant, consistent with a novel breed-specific mutation. As a member of the GTPase superfamily, GTPBP2 has been associated with multiple cellular processes, including the regulation of key cellular processes such as proliferation, migration and ribosome-associated quality control [2].

To investigate the functional consequences of the A536del variant, exogenous expression of either WT or A536del mutant GTPBP2 was achieved by transfecting heterologous cell systems with the corresponding cDNA constructs. Western blot analysis performed on total cell lysates using anti-GTPBP2 antibodies revealed that protein levels of the A536del mutant were slightly decreased relative to WT, although this difference did not reach statistical significance. Subcellular localization of both WT and A536del GTPBP2 was then assessed by immunofluorescence staining followed by confocal microscopy. While the WT protein displayed a diffuse cytosolic distribution, the A536del variant induced the formation of cytoplasmic aggregates in approximately 70–80% of transfected cells.

These findings identify GTPBP2 as a novel candidate gene for PRA and support a pathogenic mechanism involving altered protein localisation, potentially impairing translational homeostasis and increasing photoreceptor susceptibility to cellular stress.

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Oral Communication(s)

GUSTATORY AND OLFACTORY SYSTEMS IN *NOTHOBRANCHIUS FURZERI*: PHENOTYPIC CHARACTERIZATION OVER AGING

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Taste and smell are essential sensory modalities for survival, guiding feeding behavior and social interactions in the animal kingdom. With aging, the ability to distinguish tastes and odors may decrease or undergo significant changes, potentially contributing to deterioration of the overall quality of life. Animal models allow researchers to test hypotheses and study potential treatments or interventions that could potentially benefit human health and longevity. Depending on the evolutionary position and adaptation, a discrete variation in the morphology of the gustatory and olfactory systems has been reported in numerous teleost species. Very recently, an in-depth anatomical characterization of these two sensory systems has been described in Nothobranchius furzeri [1]. Given the increasing use of this fish species as a naturally short-lived fish that recapitulates key phenotypic hallmarks of aging [2], we here investigate the age-related changes in the gustatory and olfactory organs. To this aim we used the short-lived strain GRZ. Three age groups were analyzed (6wph, 12wph and 18wph), corresponding to sexual maturity, adulthood, and advanced age. Behavioral assays were performed to assess feeding responses following exposure to two different food sources (bloodworms, as a common food used for housing [3], and a nutritionally comparable but dry proposal) for only 5 min after habituation in a novel tank (15 min). Trials were repeated four consecutive days for each time point. Feeding intention and ingestion were quantified through jaw and opercular movements. These analyses were complemented by molecular and immunohistochemical investigations on taste buds and olfactory epithelium. Behavioral results revealed an age-dependent decline in exploratory and feeding activity, while the preference for bloodworms was maintained across all age groups. We then investigated age-related changes in key components of the chemosensory signal transduction pathway, namely TRPM5 and PLCB2, known to be evolutionary well-preserved. Both mRNAs showed no significant changes in both olfactory epithelium (OE) and taste buds (TBs). In contrast, CD36 mRNA expression, a crucial component of chemosensory mechanisms involved in lipid detection, showed a significant drop in adults and old specimens only in TBs. We focused also on the age-associated regulation of Calbindin28DK, β-tubulin and Ki67/PCNA previously identified as cellular markers of both OE and TBs [1]. Interestingly, Calbindin28DK and β-tubulin protein levels decreased in the OE of adult and old animals, whereas only β-tubulin was significantly reduced in TBs. Comparable expression levels in the two target tissues were observed also for proliferating cells (PCNA). Overall, our preliminary findings on behavior and mRNAs expression suggest a preserve chemosensory regulation over aging, whereas an impairment in the structural proteins such as Calbindin28DK and β-tubulin is observed in both target tissues. Altogether, these observation open the avenue for further analysis on the chemosensory decay in vertebrates during aging.

Protocol authorization number: N° 291/2022-PR

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Oral Communication(s)

HEPATOPROTECTIVE EFFECTS OF SPIRULINA PRECONDITIONING AGAINST ISCHEMIA–REPERFUSION INJURY IN STEATOTIC LIVERS

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Hepatic ischemia–reperfusion (IR) injury is a significant clinical complication, particularly in steatotic livers, which are highly susceptible to oxidative and inflammatory cascades. This study evaluated whether spirulina (Arthrospira platensis) could attenuate IR-induced damage exacerbated by diet-induced steatosis in rats. The study was conducted in accordance with the Declaration of Helsinki and approved by the Research Ethics Committee for Life and Health Sciences of the Higher Institute of Biotechnology of Monastir-Tunisia (Approval reference: CER-SVS/ISBM 012/2024, approval date: 15 February 2025).

Male Wistar rats were divided into five groups: sham, IR, high-fat diet (HFD), HFD + IR, and a group treated with 1000 mg/kg/day of spirulina (SP1000), through oral gavage in random order and at the same time each day, for three weeks. Liver injury was assessed through serum transaminases (ALT, AST), histological analysis (Suzuki and steatosis scores), immunofluorescence assay, and markers of oxidative stress, inflammation, and inflammasome/pyroptosis signaling.

Steatosis significantly worsened IR outcomes, leading to higher transaminase release, enhanced oxidative stress (increased Malondialdehyde and decreased Superoxide Dismutase/Reduced Glutathione), and activation of inflammatory (TLR4, NF-κB, TNF-α, IL-1β) and inflammasome (NLRP3, IL-18, GSDMD) pathways. Spirulina supplementation markedly mitigated these effects, preserving hepatic architecture and restoring antioxidant defenses. Mechanistically, spirulina downregulated pro-inflammatory and pyroptotic gene expression while shifting lipid metabolism markers toward control levels.

These findings suggest that spirulina preconditioning is a viable nutritional approach to protect steatotic livers from IR injury by suppressing oxidative stress, downregulating inflammation, and inflammasome-mediated cell death.

Oral Communication(s)

GOJI BERRY SUPPLEMENTATION MODULATES GUT MORPHOLOGY, INFLAMMATORY RESPONSE AND METABOLISM IN FATTENING RABBITS

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In recent years, because of the ban on antibiotics in Europe, there has been a notable rise in research regarding the impact of feed supplements on the health and well-being of farm animals intended for human consumption. Among these, *Lycium barbarum* (goji berries, GB) is rich in bioactive compounds, including polysaccharides, carotenoids and polyphenols, which have been associated with antioxidant and immunomodulatory properties [1]. The aim of our work is the evaluation of the effects of dietary GB supplementation on metabolic parameters, intestinal morphology and inflammatory response in fattening rabbits. The study was approved by Ministry of Agriculture and Rural Development, NAVPP, Albania (prot. 824/2021) and was funded by the PRIN 2022 (prot. 2022YK8EM9). The 35-days old weaned rabbits were randomly divided into two groups according to the diet: the control group fed with standard feed and the GB group supplemented with 1.5% of GB. Metabolic hormones were analyzed, together with histological, histochemical and immunohistochemical evaluations of digestive tract and lymphoid tissues. Variations in circulating levels of NEFA (an indicator of fat mobilization) and insulin were observed in GB group, suggesting that GB could influence energy metabolism and insulin sensitivity during the fattening phase. Glycohistochemical analysis of digestive tract revealed no major alterations in complex carbohydrate production. However, localized modifications were observed in goblet cells of the duodenum and ileum, with an increased presence of heparin/heparan sulphate-like glycosaminoglycans and a reduction of chondroitin sulphate-like compounds. Through better hydration and barrier function, these modifications may lead to increased mucosal protection. In the digestive tract, GB supplementation induced significant morphological changes. Villus height was reduced in the proximal small intestine (duodenum and jejunum) but increased in the ileum and cecum, while epithelial thickness was significantly higher in the jejunum, ileum and cecum. These findings suggest a shift in absorptive activity toward distal intestinal segments. No significant differences were observed in the histomorphology of Peyer's patches and mesenteric lymph nodes, suggesting that GB did not alter the basal immune architecture. The expression of pro-inflammatory cytokines (TNF-α, IL-1β and IL-8) and the anti-inflammatory cytokine IL-10 was evaluated. TNF-α expression was significantly lower in the stomach, ileum and colon, while IL-1β and IL-8 were reduced mainly in the proximal gastrointestinal tract, including the stomach and duodenum. Conversely, the anti-inflammatory cytokine IL-10 was significantly increased in multiple regions, including the stomach, duodenum, jejunum and colon. No significant differences were observed in cytokine expression of Peyer's patches and mesenteric lymph nodes. Additionally, gastric leptin expression was higher in GB group, indicating a potential modulation of gastrointestinal endocrine activity. In conclusion, dietary supplementation with GB modulated metabolic responses and improved intestinal morphology while promoting an anti-inflammatory environment in the gastrointestinal tract, suggesting a beneficial role in supporting gut health and functional adaptation in fattening rabbits.

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Oral Communication(s)

IMPACT OF APPLE-DERIVED EXOSOMES ON HISTOLOGICAL FEATURES AND GHRELIN AND NPY ENTEROENDOCRINE CELLS IN BROILER PROVENTRICULUS

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Extracellular vesicles (EVs), which are released by all cell types, are now recognized as key mediators of cell-to-cell communication rather than simply being carriers of cellular waste. One specific subgroup, exosomes, are nano-vesicles (30–150 nm) that originate from endosomes and multivesicular bodies. Exosomes transport proteins, lipids, and nucleic acids (such as mRNA, lncRNA, and miRNA) and can deliver these molecules to recipient cells. Due to their small size, stability, biocompatibility and target specificity, exosomes have attracted the interest of the pharmaceutical and food industries. They show potential for use in the diagnosis and treatment of diseases, including cancer and neurodegenerative diseases, as well as for drug delivery [1]. However, research into the effects of exosomes on feed intake and productive performance in commercially important livestock species is limited. The chickens were divided into three experimental groups, namely a control group (CTR) and two groups that received diets supplemented with 2% (EXO2%) or 5% (EXO5%) apple-derived exosomes, respectively. After slaughter, proventriculi (n = 32; 12 per group) were collected, fixed in formalin, paraffin-embedded, and processed for Masson's trichrome staining and immunohistochemistry. Proventriculus morphometric evaluations showed that 5% exosome supplementation significantly increased total wall thickness compared with the CTR group (2.607 vs 1.924 µm; $P \leq 0.05$). The mucous epithelium and *lamina propria* were also higher in the EXO5% group than in CTR (2607.6 vs 1924.5 µm; $P \leq 0.05$), while the EXO2% group showed a trend ($P = 0.09$). Moreover, glandular diameter increased in both EXO2% (1376.3 vs 1072.6 µm; $P \leq 0.05$) and EXO5% groups (1538.6 µm; $P \leq 0.0001$). Similarly, compound gland circumference (EXO2% vs CTR: 4328.9 vs 3368 mm, $P \leq 0.02$; EXO5% vs CTR: 4831.3 vs 3368 mm, $P \leq 0.0001$) and area (EXO2% vs CTR: 1.6 vs 0.9 mm^2, $P \leq 0.02$; EXO5% vs CTR: 1.9 vs 0.9 mm^2, $P \leq 0.0002$) showed the same trend. Ghrelin (GHR) and neuropeptide Y (NPY) immunoreactive (-IR) enteroendocrine cells (EECs) in the proventriculus displayed both open-type (with cytoplasmic extensions reaching the lumen) and closed-type (lacking apical contact with the lumen) morphologies. NPY- and GHR-IR EECs were found intermingled with oxyntopeptic and epithelial cells, with most showing a rounded closed-type profile. The proventriculus contained more GHR-IR than NPY-IR cells. Both EXO2% and EXO5% diets significantly increased NPY-IR (83.9 ± 16.7; 87.8 ± 16.2) and GHR-IR cells (101.4 ± 18.8; 107 ± 6.9) compared with controls (34.3 ± 8.6 and 43.3 ± 5.7; $P \leq 0.0001$). The proventriculus morphology and EECs population in broiler chickens were positively influenced by dietary supplementation with exosome. Specifically, 5% exosome supplementation significantly increased total wall thickness, mucous epithelium, lamina propria and glandular size compared to the control group, whereas 2% exosome supplementation produced more modest effects. Furthermore, both the EXO2% and EXO5% diets markedly increased the number of NPY- and GHR-IR EECs, indicating enhanced endocrine activity in the glandular stomach. These results suggest that exosomes can modulate gastrointestinal structure and cellular function, highlighting their potential as dietary supplements to improve digestive physiology and productivity in poultry.

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Oral Communication(s)

HAZELNUT SKIN POLYPHENOLS DIETARY SUPPLEMENTATION IN BROILER CHICKENS: EVALUATION OF CECUM MORPHOMETRY, CONVENTIONAL HISTOCHEMISTRY, AND ADIPONECTIN RECEPTORS

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In the context of growing attention to more sustainable livestock practices, polyphenols represent a natural resource of great interest due to their antioxidant, anti-inflammatory, and immunomodulatory potential (1). Research conducted to date has highlighted how these compounds can positively influence the growth and quality of broiler chickens (2). Hazelnut skins (HS) are rich in bioactive compounds, including fibers, polyunsaturated fatty acids, tocopherols, and minerals, making them a promising resource within a circular economy framework. This study evaluated the effects of dietary supplementation with HS polyphenol extract on the intestine of broiler chickens. To this end, 72 male Cobb were divided into four experimental groups (18 animals per group) and fed diets containing different levels of HS polyphenol extract (0, 200, 400, or 800 mg/kg, respectively) during the finisher phase (22-42 days of age). Afterwards, cecal samples were collected, processed, and subjected to histomorphometric, histochemical, and immunohistochemical analyses. Immunohistochemical analysis aimed to evaluate the expression of the adiponectin receptors AdipoR1 and AdipoR2, considering that adiponectin is known for its role in energy metabolism and modulation of the inflammatory response (3). Histomorphometric analysis revealed no detrimental effects and a general trend of improved histological metrics in D1 and D2, suggesting a possible beneficial effect on intestinal morphology. In particular, a significant decrease in epithelium height in D3 (-4.78 µm; $p = 0.008$) was observed, while epithelium height was significantly higher in D1 (+8.42 µm; $p < 0.001$) and D2 (+5.59 µm; $p < 0.001$) compared to D[3] Conventional histochemistry showed a variable response of glycoconjugate secretory structures between the different groups. The D2 diet promoted higher tissue hydration while the D3 group had smaller positive effects than the D2 one. Immunohistochemical analysis highlighted the presence of the AdipoR1 receptor mainly in the cells of the connective tissue lamina propria and the AdipoR2 receptor mainly in the epithelium lining the intestinal crypts, showing a tendency towards greater immunopositivity for groups D1 and D[2] This pilot study highlights the sensitivity of the chicken caecum to polyphenols extracted from hazelnut skins. Despite the need for more in-depht analysis, the obtained results from the morphological investigation seem to indicate that administration of these substances has a potentially beneficial effect in relation to the doses administered.

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Oral Communication(s)

MORPHOLOGICAL AND MORPHOMETRIC STUDY OF THE ABDOMINAL AORTA AND ITS BRANCHES IN GUINEA PIGS

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The guinea pig (Cavia porcellus) is a species of increasing relevance in veterinary medicine, both as a companion animal and as an experimental model. In this context, detailed knowledge of the vascularization of the abdominal and pelvic cavities is essential for clinical, surgical, and diagnostic practice. Several pathological conditions, including gastrointestinal disorders, urolithiasis, and reproductive diseases, may require complex surgical procedures in which accurate identification of the arterial vessels is crucial to minimize intra and post operative complications. However, the available literature is often outdated, sometimes inconsistent, and frequently lacks a systematic morphometric analysis [1].

The aim of the present study was to provide a detailed morphological and morphometric characterization of the abdominal aorta and its main branches in the guinea pig, in order to supply updated data useful for veterinary practice. The study was conducted on 21 adult guinea pig cadavers (11 females and 10 males), with body weights ranging from 344 to 598 g. The animals, which died from causes unrelated to the cardiovascular, gastrointestinal, or urogenital systems, were subjected to anatomical dissection and vascular casting through the injection of colored polyurethane foam into the thoracic aorta. This technique, previously described in the literature [2], allowed a three-dimensional visualization of the arterial system and an accurate assessment of vessel course and branching patterns.

The point of origin of the main branches of the abdominal aorta was observed and documented, also the calibre of the vessels was measured, as was the distance between consecutive arteries. After measuring and observing the topography of the vessels, the gastrointestinal tract was isolated to enable detailed assessment of its vascularisation. The collected data were analyzed using descriptive statistics.

The results demonstrated the constant presence of a celiacomesenteric trunk in all examined subjects, confirming previous anatomical findings in this species. This vascular arrangement represents a distinctive feature of the guinea pig compared to most domestic mammals, in which the celiac and cranial mesenteric arteries arise separately. Additionally, anatomical variations were frequently observed, including duplication or triplication of the renal arteries, in agreement with previous reports [3]. Further variability was noted in the distribution of visceral arteries, particularly within the gastrointestinal and cecal regions, evidence of the important role it plays in the digestive physiology of this species.

In conclusion, this study provides an updated and comprehensive contribution to the knowledge of abdominal and pelvic arterial vascularization, integrating both morphological and morphometric data. Given the high prevalence of gastrointestinal and urogenital diseases in this species, these findings have direct clinical relevance for surgical planning, interpretation of diagnostic imaging, and comparative anatomical studies. Furthermore, the vascular casting technique proved to be a reliable and practical method, supporting its use in anatomical studies of small animal species.

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Oral Communication(s)

ANATOMY OF THE THORACOLUMBAR FASCIA: AN OVERLOOKED PLAYER IN CANINE BACK DISORDERS

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Thoracolumbar disorders are a common source of pain, reduced performance, and altered locomotion in dogs affected by musculoskeletal disease. Clinical assessment is traditionally focused on vertebrae, intervertebral discs, and paraspinal muscles; however, this approach may overlook the contribution of fascia, an integrated connective tissue system increasingly recognized as a key component of locomotor function.

Fascia is a continuous three-dimensional network of fibrous connective tissue that envelops and interconnects muscles, nerves, and vessels, contributing to force transmission, coordination, and proprioception [1]. It is organized into distinct but interconnected layers, including superficial and deep fascia, characterized by specific structural and biomechanical properties. Despite its relevance in human medicine, the role of fascia in canine thoracolumbar disorders remains poorly defined.

This project aims to characterize the thoracolumbar fascia in the dog through a combined anatomical and histological approach. Samples obtained from post-mortem donations were used to define a reference morphological model, avoiding invasive procedures in living animals.

Preliminary histological findings highlight a clear stratification of the fascial layers: superficial components show a looser organization with a higher content of elastic fibers, suggesting a role in tissue compliance, whereas deeper layers are composed of densely packed collagen bundles, consistent with mechanical stabilization and force transmission [2].

These findings support the concept of fascia as an active component of the locomotor system [3] and suggest its potential involvement in thoracolumbar dysfunction. The integration of anatomical data with ultrasound and magnetic resonance imaging in clinical cases will allow the identification of correlations between fascial structure, imaging features, and clinical presentation.

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Oral Communication(s)

MORPHO-FUNCTIONAL EVOLUTION AND ANTIOXIDANT CAPACITY OF MUSCLE FIBERS IN MARCHIGIANA CATTLE DURING POST-MORTEM AGING

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Oxidative stability and proteolytic processes significantly influence meat quality during post-mortem maturation [1-2]. Muscle fiber composition affects both oxidative susceptibility and enzymatic tenderization, yet the integrated dynamics of these processes in bovine muscles with differing metabolic profiles remain incompletely characterized [3]. This study investigated fiber composition, morphometry, antioxidant activity, and μ-calpain/desmin colocalization in the *Longissimus lumborum* (LL) and *Psoas major* (PM) muscles of six clinically healthy Marchigiana cattle raised in Benevento (Campania, Italy) and slaughtered at 15–18 months.

Muscle samples were collected at days 0, 1, 4, and 7 of controlled post-mortem aging. Antioxidant capacity was measured via DPPH, superoxide dismutase (SOD), and catalase (CAT) assays, while lipid oxidation was assessed as malondialdehyde (MDA). Fiber types were identified by succinate dehydrogenase (SDH) histochemistry, and morphometric parameters (fiber diameter, cross-sectional area) were quantified by digital image analysis. Immunofluorescence (IF) was employed to evaluate μ calpain/desmin colocalization and temporal protein degradation.

The immunofluorescence analysis revealed, in the LL, a predominance of glycolytic type IIb/IIx fibers with an intermediate fraction of oxidative type IIa/I fibers, whereas the PM was found to be composed mainly of oxidative fibers. DPPH values were high during the early post-mortem stages, likely supported by the availability of polyunsaturated fatty acids (PUFAs) and bioactive peptides, and then decreased sharply by day 7, concomitantly with the increase in MDA levels. This phenomenon was more pronounced in the PM, confirming a greater oxidative susceptibility of oxidative metabolism muscles compared to glycolytic ones (LL). Regarding proteolysis, at day 0 μ-calpain was detectable with structurally intact desmin. μ-calpain/desmin colocalization increased significantly between day 1 and day 4, preferentially localizing at the Z-line, marking the onset of cytoskeletal breakdown. The peak of colocalization and visible desmin fragmentation were observed between days 4 and 7. The results suggest a functional link between proteolysis and redox balance: desmin degradation, in addition to promoting tenderization, may facilitate the release of bioactive peptides with radical-scavenging properties, thereby modulating lipid oxidation during the early aging phase. In conclusion, the study demonstrates that in the Marchigiana cattle the first post-mortem week represents a critical time window: it is during this phase that the decisive “match” occurs between lipid compartment protection and proteolysis activation. While the LL shows greater metabolic resilience, μ-calpain/desmin colocalization emerges as a key marker. These findings provide a framework for understanding the evolution of meat quality in Marchigiana cattle during the first week post-mortem.

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Oral Communication(s)

POINT BREAK: MORPHOLOGICAL CARTILAGE CHANGES IN SOWS AS AN OSTEOARTHRITIS MODEL

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Osteoarthritis (OA) is a progressive cartilage degeneration characterized by phenotypic changes in chondrocytes and degradation of the cartilage extracellular matrix (ECM). Mechanical overweight and aging are major risk factors. OA involves complex cellular stress responses, alterations in protein homeostasis, and inflammatory signalling pathways, all contributing to cartilage degeneration.

In this context, heat shock protein 70 (HSP70) is a stress-inducible molecular chaperone involved in cytoprotection, protein folding, and cellular response to mechanical and metabolic stress. Protein gene product 9.5 (PGP9.5) is a neuronal marker also involved in protein turnover and proteostatic regulation that can increase during pathological conditions. Substance P (SP) has been implicated in inflammatory and catabolic processes in OA cartilage [1]. Together, these markers may reflect different molecular aspects of cartilage degeneration.

This study aims to evaluate whether the adult sow can be used as a model to study spontaneous OA in humans and pets through integrated macroscopic and microscopic analyses of the femoral condyles and tibial plateaus. Stifle joints of young pigs (n=5; 9 months old, 110 kg) and sows (n=5; 4 years, 250 kg) were obtained from a slaughterhouse in accordance with the principles of the 3Rs, samples underwent X-ray, computed tomography (CT), and magnetic resonance imaging (MRI). The articular cartilage surface of the medial femoral condyle and tibial plateaux was also evaluated according to [2], as the medial compartment is is more susceptible to OA. Samples of the central zone of articular cartilage were stained with Safranin-O staining to evaluate glycosaminoglycan (GAGs) content. HSP70, PGP9.5, and SP were assessed by immunohistochemistry.

X-ray showed no bone OA lesions, however CT detected structural alterations in the femoral condyle, while MRI highlighted early cartilage changes, suggesting a higher sensitivity of advanced imaging techniques. Young pigs showed mild macroscopic and microscopic evidence of cartilage degeneration, and a weak staining of HSP70, PGP9.5, and Substance P around chondrocytes. On the contrary, sows presented severe OA changes, including cartilage ruptures, fibrillation, loss of GAGs, and matrix disorganization. ECM exhibited diffuse immunostaining positivity for the three markers. Notably, no nerve fibers were detected by PGP 9.5.

Increased HSP70 expression in OA cartilage may reflect a cellular stress response induced by sustained mechanical overload. Its localisation in the ECM may indicate active secretion, passive release from damaged cells, or increased matrix permeability during degeneration. Enhanced PGP9.5 immunoreactivity could be related to an activation of proteostatic pathways linked to tissue degeneration. Similarly, strong SP staining is likely related to acquisition of a pro-inflammatory phenotype, potentially contributing to matrix degradation through autocrine and paracrine mechanisms.

Taken together, the adult pig emerges as a biologically relevant and translational model of naturally occurring OA, marked chondrocyte phenotypic changes, increased cellular stress, and altered proteostasis.

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Oral Communication(s)

UNDER HEAT, UNDER PRESSURE: TROUT MUSCLE ON THE EDGE AND BACK

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Climate change and water temperature fluctuations represent major challenges for aquaculture. Muscle satellite cells play a crucial role in growth and regeneration, making them a relevant cellular model for evaluating thermal stress responses in aquaculture species. We compared in parallel satellite cells and muscle tissue from rainbow trout (Oncorhynchus mykiss), using HSP70 as a common marker of stress. This approach allows assessment of whether in vitro models reflect the physiological behavior of intact muscle and satellite cells under thermal stress.

In this study, satellite cells and muscle tissue were exposed to 14°C (control), 25°C (thermal stress), or a recovery condition (3 days at 25°C followed by 4 days at 14°C) with measurements at T0, T3, and T7.

Satellite cell viability was evaluated by Live/Dead assay, while nuclear size and stress response were assessed through DAPI staining and HSP70 immunofluorescence. Muscle tissue temperature and morphology of in vitro cultured samples were monitored using infrared thermography, alongside HSP70 analysis. At 14°C, satellite cells maintained high viability (90%) and low baseline activity throughout the experiment. Exposure to 25°C induced an increase in cellular stress response (14.7 to 17.3°C) and triggered an early HSP70 response. However, prolonged exposure led to perinuclear redistribution of HSP70 and a reduction in viability (60%), indicating a transition from adaptive to chronic stress. Under recovery conditions, cells showed an activation of stress response during heat exposure followed by a rapid decline upon return to 14°C, with partial restoration of metabolic balance and improved viability (85%). These findings indicate that thermal elevation initially promotes adaptive responses that become detrimental if sustained, while recovery at optimal temperature supports restoration of cellular homeostasis.

Satellite cell morphology showed a transition from a rounded phenotype at T0 to an elongated morphology across all conditions and time points, consistent with early myogenic differentiation, while nuclear number remained relatively stable, with values at T7 comparable across conditions (e.g., 14–17 nuclei), indicating no substantial treatment-dependent effect. Muscle analysis showed that tissue morphology was preserved at 14°C and during the initial phases of stress; however, at T7 under 25°C conditions, structural alterations became evident, including increased fibrillation of the connective compartment and a tendency for cells to detach from the tissue architecture, suggesting progressive deterioration. At 14°C, tissue displayed stable, low surface temperatures as measured by infrared thermography. At 25°C, thermal stress induced a rapid increase in surface temperature, followed by partial stabilization over time. Under recovery conditions, temperature initially increased during heat exposure, then declined upon return to 14°C, gradually stabilizing alongside normalization of HSP70 distribution.

HSP70 dynamics were consistent with the observed thermal responses, which showed early induction under heat stress, redistribution under prolonged exposure, and partial recovery under optimal temperature, in both satellite cells and muscle tissue. Overall, elevated temperatures stimulate thermal response and HSP70 expression in both cells and tissue, whereas prolonged exposure leads to stress accumulation and reduced cellular viability. The comparison between satellite cells and muscle tissue demonstrates that in vitro cellular responses mirror tissue dynamics, providing a functional link between cellular adaptation and muscle physiology. Recovery at physiological temperature allows restoration of thermal response and stress protein distribution, highlighting the dynamic interplay between thermal balance, stress response, and cell survival. These findings provide a useful framework for understanding thermal stress responses in muscle biology and support the use of satellite.This approach has implications for aquaculture, through improved understanding of temperature resilience, and cellular aquaculture, by informing the optimization of culture conditions for muscle cell.

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Oral Communication(s)

TIMING AND HIERARCHY OF ERK PATHWAY DEFINE LINEAGE SPECIFICATION IN ICM OF RUMINANT BLASTOCYSTS

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The establishment of pluripotency and lineage segregation within the inner cell mass (ICM) is tightly coordinated during early mammalian development, yet the hierarchy and temporal integration of the signaling pathways involved remain poorly defined in ruminants. In particular, how ERK signaling regulates naïve epiblast pluripotency competence and hypoblast specification across developmental time remains unclear [1].

Here, we dissected the contribution of ERK signaling during the morula-to-blastocyst transition by applying stage-specific pharmacological inhibition in both sheep and bovine embryos. Embryos were treated at the compact morula stage (day 4 in sheep and day 5 in cattle), prior to epiblast and hypoblast segregation. ERK inhibition with the selective inhibitor Ulixertinib (BVD-523; VRT752271) resulted in blastocysts completely lacking SOX17-positive hypoblast cells. In contrast, inhibition at later stages (day 5 in sheep and day 6 in cattle) failed to induce complete hypoblast ablation, indicating a restricted temporal window during which ERK activity is required for hypoblast specification.

This phenotype was conserved across species, with minimal differences attributable to species-specific developmental timing rather than distinct molecular responses.

Furthermore, ERK inhibition led to the stabilization of SOX2 and KLF4 expression within the epiblast, maintaining the ICM in a developmentally immature, naïve-like state. Functionally, ERK-inhibited ICMs exhibited enhanced pluripotency and developmental plasticity in vitro, retaining the capacity to generate extra-embryonic lineages, including CDX2-positive trophoblast-like cells, a competence that is normally restricted in control embryos [2].

Lineage-specific marker expression (SOX2, KLF4, SOX17 and CDX2) was assessed by immunostaining.

Collectively, these findings support a hierarchical model in which ERK signaling acts upstream of hypoblast formation while simultaneously driving lineage restriction within the ICM.

By preventing hypoblast differentiation, ERK inhibition preserves an extended window of pluripotency and lineage competence [3]. These results have remarkable implications for the generation of stem cell-based embryo models, including blastoids, where controlled modulation of ERK signaling may enhance developmental potential and lineage flexibility in species with prolonged preimplantation development.

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Oral Communication(s)

ChREBP: A CENTRAL METABOLIC SENSOR DRIVING LIPID DROPLET RENEWAL IN PREIMPLANTATION MOUSE EMBRYOS

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Lipid droplets (LDs) are abundant during early embryogenesis; however, yet, the mechanisms that govern their synthesis, maintenance, and functional relevance remain poorly defined [1]. This study aimed to investigate how early mouse embryos sense lipid depletion and activate adaptive metabolic responses to sustain developmental progression. Mouse zygotes were mechanically delipidated and cultured under fatty acid free conditions, and LD recovery was quantified by BODIPY staining across development. RNA sequencing was performed at the 2-cell stage embryos to identify transcriptional responses to delipidation. Furthermore, immunofluorescence microscopy, quantitative assays, and functional studies were conducted by the use of inhibitors to investigate molecular actors of LDs de novo synthesis and their role in embryonic survival.

Delipidated embryos rapidly regenerated LDs and progressed to the blastocyst stage at rates comparable to controls. Transcriptomic profiling identified ChREBP (Mlxipl) as the principal gene upregulated in response to lipid depletion, with both mRNA and protein levels markedly increased in delipidated embryos. ChREBP displayed dynamic subcellular localization, including nuclear accumulation associated with LD biogenesis and the formation of cytoplasmic foci preferentially localized at the cell cortex and in proximity to endoplasmic reticulum–enriched regions. Partial co-localization of ChREBP with LDs was observed across stages of de novo LD synthesis following delipidation. Furthermore, an association with lipid LDs was observed during active LD regeneration following delipidation. Notably, the inhibition of ChREBP impaired lipid droplet regeneration and resulted in developmental arrest, identifying the morula-to-blastocyst transition as a critical window of sensitivity and the trophectoderm formation as a vulnerable bottleneck

These findings identify ChREBP as a key metabolic sensor that coordinates LD synthesis and supports normal developmental progression during early embryogenesis, highlighting LDs as critical regulators of early embryonic competence.

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Oral Communication(s)

DNA MEIOTIC RECOMBINASE 1 (*DMC1*) GENE EXPRESSION AND IMMUNOLOCALIZATION IN GONADS OF *XENOPUS TROPICALIS*

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Meiotic recombination is a crucial process for accurate chromosome segregation and genetic diversity, mediated by the DNA meiotic recombinase 1 (DMC1; 1-2). While its role has been studied in several vertebrate models and in some amphibian urodeles (e.g. 3), its expression dynamics in anurans is not characterised. Here, we present preliminary data on dmc1 gene expression and DMC1 protein localization in gonads of Xenopus tropicalis during early post-metamorphic development. Quantitative PCR revealed that dmc1 expression remained stable in females but increased progressively in males from 3-4 weeks post-metamorphosis, with a significant sex × stage interaction. Sequence analyses confirmed high conservation and amminoacidic positivity of X. tropicalis DMC1 with other vertebrate orthologs. Immunohistochemistry localised DMC1 protein specifically in germ cell nuclei: primary spermatocytes in males and early oocytes in females, whereas somatic cells were negative. Preliminary Western blot analyses indicate that this protein is conserved across multiple classes, orders, and/or species of vertebrates—including domestic, laboratory, and wild animals—while the primary antibody employed exhibits cross-reactivity among these taxa. These findings support a conserved role of DMC1 in meiotic entry and progression, and indicate sex-specific temporal dynamics in X. tropicalis. This study provides the first evidence of DMC1 expression in anurans and a foundation for future comparative analyses of meiotic regulation in amphibians and other vertebrates.

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Oral Communication(s)

DECELLULARIZED BOVINE MUSCLE ORGANOID AS A PLATFORM FOR RECELLULARIZATION

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Three-dimensional (3D) muscle organoids represent advanced in vitro systems capable of recapitulating key structural features of native skeletal muscle [1]. In this context, the preservation of extracellular matrix (ECM) composition and architecture is critical to ensure biological relevance and appropriate cellular responses.

In the present study, we aimed to standardize the production of a decellularized cylindrical scaffold (1 cm in diameter) derived from the bovine diaphragm and to perform a comprehensive assessment of ECM preservation and scaffold biocompatibility [2]. This tissue is characterized by a unique multilayered organization, with a regular alternation of muscle and connective laminae, making it particularly suitable for the development of structurally biomimetic models.

Decellularization protocols were optimized and compared, identifying a detergent-enzymatic treatment based on 4% sodium deoxycholate (SDC), DNase and trypsin-EDTA, followed by extended washing, as the most effective condition. This protocol ensured complete DNA removal and substantial depletion of muscle-specific proteins, including actin.

A detailed structural and compositional analysis demonstrated that the ECM architecture was preserved, maintaining the characteristic multilayered organization. Histological and immunofluorescence analyses revealed the presence of both ghost fibres and filled ghost fibres, indicating differential retention of matrix components within the scaffold. In particular, outer fibres appeared largely depleted (ghost fibres), whereas inner fibres retained ECM constituents (filled ghost fibres).

Glycosaminoglycan (GAG) content was reduced following decellularization; however, this reduction was consistent across all tested protocols. Given the role of GAGs in promoting cell differentiation, this partial depletion may influence the scaffold efficiency in recellularization step.

Residual detergent cytotoxicity, a critical and often overlooked aspect, was specifically evaluated. Cytocompatibility assays demonstrated that residual SDC, after the optimized washing phase, did not exert cytotoxic effects on C2C12 cells, confirming the effectiveness of the protocol in producing a biologically safe scaffold.

Preliminary recellularization approaches suggest the potential of this system for cell integration; however, further investigations are ongoing.

Overall, this study provides a standardized and reproducible strategy for generating decellularized muscle scaffolds with preserved ECM integrity and low cytotoxicity, strengthening their applicability as reliable platforms for in vitro muscle modelling and regenerative medicine.

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Oral Communication(s)

PHOTO-ELECTROCATALYSIS AND REARING DENSITY: EFFECTS ON WELFARE OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) REARED IN A RECIRCULATING AQUACULTURE SYSTEM

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Gills and liver are key organs in fish, directly involved in environmental interaction and metabolic regulation [1]. Due to their extensive surface area and thin epithelial barrier, gills are particularly responsive to water quality changes. The liver, as the main metabolic and detoxifying organ, plays a central role in processing environmental stressors and maintaining systemic homeostasis. The efficiency of a nanotube titanium dioxide–based photoelectrocatalytic (PEC) filter was evaluated in a recirculating aquaculture system (RAS). Its effects on the growth performance and health status of adult rainbow trout, *Oncorhynchus mykiss*, (mean weight: 160 g) were assessed at two stocking densities: 15 kg/m³ (low density, LD) and 30 kg/m³ (high density, HD) for 4 weeks. Control groups (CTR) were maintained using standard filtration, while the treatment groups were equipped with the PEC system. In the LD and HD groups, the PEC was installed alongside a conventional UV lamp. Water physical and chemical parameters were monitored throughout the trial.

At the end of the trial, gill and liver tissues (n=6) were collected for histological and biochemical analyses. In the gills, histometrical analyses were performed, including filament width and lamaellae length, to tackle structural changes. In the gills, the expression of three cytokines (IL-1β, IL-10, and TNF-α), heat shock protein 70 (hsp70), and claudine-30 (cldn30) was analysed to assess inflammation status, stress, and epithelium integrity, respectively. In the liver, the melano-macrophage centers (MMCs), indicators of environmental stress and activation of the mononuclear phagocyte system, were evaluated. In both tissues, the activity of endogenous antioxidant enzymes catalase, superoxide dismutase, and glutathione peroxidase, was measured. Authorization code: OPBA_20_2020.

Water parameters were not affected by the PEC system. All groups exhibited similar weight and length. No histological differences were found in the structure of the gills between treatments: primary lamellae appeared arranged in double rows, and secondary lamellae lined by squamous epithelial cells. Gill histometric parameters were not affected either, indicating preservation of the epithelium, confirmed by the claudin-30 gene expression. No signs of gill inflammation were noticed, as cytokines expression levels did not differ among groups. The PEC system did not alter the oxidative stress markers in the gills, which is consistent with HSP70 gene expression results, indicating a lack of cellular stress at the tissue level. Normal liver architecture was evident in all experimental groups. At both stocking densities, the percentage area of MMCs was significantly higher in the CTR than in the PEC groups (LD, $p<0.01$; HD, $p<0.05$), indicating an activation of the immune response in this group. From a morphological perspective, the reduction of MMCs in PEC groups suggests a lower degree of chronic environmental stress and immune stimulation. This difference may reflect improved environmental conditions in PEC tanks, possibly associated with lower nitrate levels. The findings suggest a protective role of this innovative water treatment system, even in fish reared at high densities. Although no evident improvements in water purification were observed, further research is needed to optimize the system and assess its potential under various farming conditions.

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Oral Communication(s)

EXPRESSION OF GROWTH AND STRESS RELATED GENES IN RAINBOW TROUT REARED IN AN ELECTRO-PHOTOCATALITIC BASED INNOVATIVE DEPURATION SYSTEM

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In the RAS systems it is essential that the recirculating water is appropriately filtered to ensure its reuse and to maintain optimal water quality for the fish environment. The present study is part of the PRIMA project Fish-PhotoCAT, which aims to evaluate the efficiency of a smart purification system in improving water quality, fish production, and environmental sustainability in aquaculture. The system utilizes an innovative TiO_2-based photo-electrocatalytic (PEC) filter for the remediation of nitrogen compounds in water. This new-generation, low-cost filter is derived from traditional aquaculture filtration systems, combining the purifying action of TiO_2 with the biocidal effect of UV radiation [1].

Juveniles and adult rainbow trout were reared at both low (LD, 5 kg/m3 for juveniles and 15 kg/m 3 for adults) and high (HD, 10 kg/m3 for juveniles and 30 kg/m 3 for adults) stocking densities. The trial for juveniles, conducted during the grow-up phase lasted three weeks. In the adult grow-out phase, fish were reared for four weeks at LD and two weeks at HD. For each age group, three tanks equipped with a conventional, mechanical and biological filtration and UV disinfection system were used as controls (CTRL), while three tanks were equipped with the PEC filter. At the end of the trial, fish were sampled and livers were taken and stored in RNAlater. To assess the effects of the PEC and CTRL systems on growth and response related to environmental stress, the relative expression of Insulin Growth Factor 1 (IGF-I) Myostatin (MSTN), Heat Shock Protein 70 (HSP70) and cytochrome CYP1A1 (Cytochrome P450, family 1, subfamily A) have been evaluated in the liver by RealTime PCR following the protocols reported in Bortoletti et al. (2026) [2].

No significant differences were observed between CTRL and PEC groups in the expression of the analysed genes although myostatin expression was not detected in juveniles at high stocking density. Comparable results were obtained at both stocking densities and in both juveniles and adult fish, indicating that the PEC system did not negatively affect growth- or stress-related molecular responses.

The trial was conducted in agreement with the Italian regulations on animal experimentation and ethics (DL 26/2014) and the European regulation (Dir. 2010/6). The trial was approved by the Animal Welfare Body of the University of Milan (number 62_2023).

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Oral Communication(s)

IN VITRO EVALUATION OF HYDROPONIC BARLEY FODDER AS A DIETARY STRATEGY TO MITIGATE ENTERIC METHANE EMISSIONS AND MAINTAIN RUMEN DIGESTIBILITY

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Livestock agriculture faces the challenge of reducing enteric methane (CH4) emissions without compromising animal performance and feed efficiency. Hydroponic barley fodder (HBF) is a biomass characterized by high enzymatic activity and readily fermentable carbohydrates. This study evaluated the *in vitro* CH4 mitigation potential and apparent organic matter digestibility (IVOMD) of HBF when replacing standard dietary components at two physiological maturities: Day 4 (HBF1) and Day 6 (HBF2). The Hohenheim Gas Test was utilized to simulate rumen fermentation during a 24-hour incubation. Treatments included a standard control diet (CTR; 60% hay, 40% concentrate), pure HBF substrates, and mixed diets where HBF replaced either the hay fraction at graded levels (LOW, MED, HIGH, REPL) or the concentrate fraction (10% and 20% inclusion). A quadrant analysis comparing treatments to the CTR baseline demonstrated that eight dietary formulations achieved the targeted criteria of simultaneously lowering CH4 emissions and improving overall digestibility. Specifically, pure HBF substrates exhibited significantly higher digestibility compared to the CTR (60.08%). HBF1 recorded an IVOMD of 73.56% due to its high content of readily available plant starch. As the plant matured to Day 6, HBF2 IVOMD naturally decreased to 67.99% due to the development of structural fiber. Correspondingly, both maturities successfully lowered the CH4/-CO2 ratio, achieving reductions of 5.8% and 9.0% respectively, when compared to CTR. In the hay-substitution diets, higher inclusion levels of both HBF1 (MED, HIGH, REPL) and HBF2 (HIGH, REPL) consistently shifted the fermentation into the targeted quadrant compared to the control. To statistically validate this relationship, a correlation analysis (n = 130) was performed, revealing a significant negative overall correlation between the CH4/CO2 ratio and IVOMD (Pearson r = -0.557, p < 0.001). This confirms that treatments improving digestibility quantitatively associate with reduced methane output. Furthermore, significant negative correlations were confirmed within the pure HBF1 (r = -0.791, p = 0.011) and HBF2 (r = -0.793, p = 0.019) substrates, as well as in specific HBF1 inclusion levels (LOW, REPL, and LC20). The decrease in CH_4 emissions seen in high-HBF diets is likely due to the rapid fermentation of plant starches. *In vitro* findings suggest that substituting fibrous forage with hydroponic forages at certain maturity stages may help reduce CH_4 emissions; however, further *in vivo* studies are needed to confirm these effects and determine optimal inclusion levels.

Oral Communication(s)

APPLICATION OF MACHINE LEARNING TO PREDICT ENTERIC METHANE EMISSIONS AND ENHANCE DAIRY CATTLE FARM MANAGEMENT

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Ruminant livestock are estimated to emit between 80 and 95 million tonnes of methane (CH4) annually, at global level. Approximately 80.7% of these emissions originate from enteric fermentation in dairy cattle. Effective monitoring and management of CH4 emissions are therefore crucial to enhance the environmental sustainability of livestock production systems. In this study, we developed two machine learning models: 1) a model to predict the daily enteric CH4 emissions of dairy cattles; 2) a probabilistic Bayesian model to support farmers in optimizing their daily farming practices, such as reducing CH4 emissions, while maintaining milk production yield. Both models were developed using a set of 25 variables measured at the DIMEVET experimental dairy cattle farm, housing approximately 100 dairy cows. Data were recorded over a period of 252 days and included enteric CH4 measurements, obtained from sniffers, environmental parameters, milk production yield and composition, animal variables, such as body weight and parity. In addition, detailed information on feed type and ingredients were also recorded. The first version of the two models showed good predictive performance, with further improvements expected as data collection continues. Upon completion and validation, both models will be available as simple and user-friendly web applications to support farmers, policy makers and other stakeholders in enhancing environmental sustainability of livestock production systems.

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Oral Communication(s)

MILK COAGULATION PROPERTIES IN RELATION TO INTRAMAMMARY INFECTION AND DRY-OFF ANTIBIOTIC TREATMENT IN ALPINE DAIRY GOATS

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Milk coagulation properties are key determinants of cheese-making efficiency. However, limited information is available on factors affecting these properties in goat milk, particularly in relation to intramammary infections (IMI). Half-udder milk samples were collected during the morning milking from 64 Alpine dairy goats one week before dry-off (T1). Samples were used for bacteriological culture (BC), and bacterial species were identified using MALDI-TOF. Based on BC results at T1, goats were classified as healthy (both halves negative; n = 35) or infected (at least one half positive; n = 29). At dry-off, goats were blocked by health status and randomly assigned to an intramammary rifaximin treatment (Fatroximin, Fatro, Bologna, Italy; TG: n = 32) or control (CG: n = 32). Milk samples (50 mL) were collected at T1 and one-week postpartum (T2) by pooling both udder halves and were used to assess rennet coagulation time (R) curd firming time (K20) and curd firmness at 30 minutes (A30) using a Formagraph. Each sample was analyzed in duplicate, and plate position was recorded to account for technical variability. Samples that did not coagulate within the observation time were classified as non-coagulating (FF), while K20 was considered not estimable when the curd did not reach an amplitude of 20 mm within 30 minutes. Data were analyzed using a two-step approach. First, the probability of coagulation (coagulating vs FF) and the probability of poor coagulation (K20 not estimable) were analyzed using mixed-effects logistic regression models (PROC GLIMMIX), including treatment, infection status, time, and their interactions as fixed effects, parity, number of kids, days from drying-off, and plate position as covariates, and goat as random effect. Second, R, K20, and A30 were analyzed only in coagulating samples using mixed-effects models for repeated measures, with the same fixed effects structure. Milk from healthy goats showed better coagulation properties compared to infected ones throughout the experimental period, with shorter R (11.25 vs 13.36 min; p = 0.07) and K20 (2.46 vs 2.92 min; p = 0.07), and higher A30 (56 vs 48 mm; p = 0.03). Moreover, the probability of milk coagulation at T2 was higher in healthy goats than in infected ones (94.0% vs 81.4%; OR = 3.6; p = 0.03). Among infected goats, antibiotic treatment improved some coagulation parameters, with shorter R (11.27 vs 15.45 min; p = 0.01) and lower A30 (40 vs 57 mm; p < 0.01) compared to controls. Interestingly, K20 at T2 was higher in treated goats compared to controls regardless of initial infection status (3.88 vs 2.95 min; p < 0.01). These findings may suggest that IMI detected before dry-off have a persistent negative effect on milk coagulation, and that antibiotic dry-off treatment represents an effective strategy to mitigate their negative effects in the new lactation.

Oral Communication(s)

HUMAN-SYSTEM INTERACTION IN ITALIAN DAIRY CATTLE FARMS: AQUALITATIVE STUDY OF HERD MANAGEMENT SOFTWARE USE

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The rapid automation of ruminant farming has led to the large-scale adoption of herd management software and precision livestock farming (PLF) technologies, generating significant volumes of data to support decision-making (Bovo et al., 2024; Cavallini et al., 2025). However, limited evidence is available on how farmers use these systems in real working conditions. This study aims to investigate the practical use, accessibility, and integration of herd management software on Italian farms through a qualitative, user-centred approach. Semi-structured online interviews with screen sharing were conducted to directly observe navigation patterns, workflows and decision-making during routine farming activities. Each interview was conducted by the same two researchers to ensure consistency, and responses were transcribed into a structured framework covering production, reproduction, health, behaviour, environmental conditions and software usability. At the time of submission, nine interviews had been completed and analysed.

Preliminary findings highlight a persistent gap between the technological potential of digital systems and their actual use. Farmers operate within highly fragmented digital ecosystems, often relying on multiple independent platforms with limited interoperability. Software are mainly used for operational control tasks, such as monitoring alerts, checking livestock lists and confirming events, whilst advanced analytical functions (e.g. multiparameter graphs, composite indices, long-term trends) are rarely used. These features are often perceived as complex, time-consuming or difficult to interpret. Browsing behaviour appears highly routine, with users relying on predefined paths instead of search or filtering tools, suggesting sub-optimal information retrieval strategies.

The interviews reveal recurrent issues, such as the high cognitive load associated with complex interfaces, duplication of data entry across systems, lack of transparency regarding sensor-derived indicators, and low confidence in results that are difficult to interpret. Farmers do not express a need for additional data, but instead for better integration, simplification of information and a clearer definition of indicator priorities. The desired features converge towards a single integrated platform, intuitive dashboards, minimal manual input and decision-oriented alerts.
These preliminary results highlight that current herd management software are mainly used as a monitoring tool rather than a true decision-support system. The findings underline the critical role of human-system interaction, usability, and interoperability in determining the effectiveness of digital technologies in livestock farming. This study provides early empirical evidence supporting the need for a paradigm shift from data-rich to user-centred PLF system design, with implications for future technology development and adoption strategies.

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Oral Communication(s)

EFFECT OF THI AND TIME OF DAY ON BASELINE METHANE AND CARBON DIOXIDE CONCENTRATIONS MEASURED BY A SNIFFER SYSTEM FOR DAIRY COWS

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In vivo measurement of methane emissions still represents a challenge to date, especially in commercial farms (Massaro et al., 2024). In this context, the sniffer technique is promising, because it enables large-scale, non-invasive data collection, although the large volume of raw high-frequency records requires standardized and efficient processing (Løvendahl et al., 2024). In these processing steps, baseline gas concentration is often calculated from intervals with no cow present within the measurement area of the sniffer system, in order to obtain a net estimate of bovine emissions (Ryczek et al., 2026).

The study aimed to evaluate the effect of Temperature-Humidity Index (THI) and time of day on baseline methane and carbon dioxide concentrations measured by the sniffer system.

A dedicated processing pipeline based on four Python scripts was specifically developed to handle the raw data generated by the two sniffers installed in the automatic milking systems (AMS). All intervals recorded while no cow was present in the AMS were grouped by hour, along with the environmental temperature and relative humidity values used to calculate THI. A mixed model including THI and recording hour as fixed effects was applied to mean gas concentrations and to the absolute residuals of log-transformed gas concentrations to evaluate their trend and variability, respectively.

Baseline gas concentrations remained stable throughout the day, except for a peak detected between 7:00 and 9:00 AM, during which methane and carbon dioxide increased significantly ($P < 0.05$) by 95% and 117%, respectively. After accounting for time of day, a THI value greater than 68, compatible with the activation of the ventilation system, led to a significant reduction ($P < 0.05$) in methane and carbon dioxide concentrations by 18% and 52%, respectively. The variability of baseline methane concentrations was significantly affected ($P < 0.05$) by recording hour and THI, with lower residuals dispersion observed when THI exceeded 68.

These findings indicate that both THI and time of day influence the baseline values measured using the sniffer technique and, consequently, the net methane emission values calculated for cows by subtracting baseline concentrations from gross measurements. Therefore, ventilation system activation and farm-specific management routines should be specifically taken into account to reduce the risk of biased measurements.

This study was carried out within the Agritech National Research Center and received funding from the European Union – NextGenerationEU (Task 5.3.2., Project CN00000022).

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Oral Communication(s)

CHESTNUT BIOCHAR AS A FUNCTIONAL FEED INGREDIENT IN MILK REPLACER TO IMPROVE GUT HEALTH AND REDUCE DIARRHOEA IN PRE-WEANING CALVES

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The dairy sector is increasingly challenged to enhance animal health and welfare while reducing antibiotic use and improving sustainability in line with the One Health approach. In this context, neonatal calf diarrhoea represents one of the main causes of morbidity and mortality, as well as a major driver of antimicrobial use. This scenario further underscores the urgent need for effective alternative strategies to antibiotics.

In recent years, increasing attention has been directed toward functional nutrition approaches, which aim not only to meet the nutritional requirements of the animal but also to modulate physiological processes, gut health, and immune function. Functional feed ingredients can exert beneficial effects within the gastrointestinal tract, improving microbial balance, enhancing barrier integrity, and reducing the impact of enteric pathogens, thereby supporting animal resilience during critical developmental stages, such as the pre-weaning period.

Biochar, a carbon-rich co-product obtained from plant biomass pyrolysis, has recently emerged as a potential functional feed ingredient due to its adsorptive, antimicrobial, and antitoxin properties. However, evidence regarding its application in calf nutrition, particularly during the pre-weaning phase, remains limited.

The aim of this study was to evaluate the effects of chestnut-derived biochar supplementation in milk replacer on health status and diarrhoea treatment in pre-weaning Holstein calves. The biochar was preliminarily characterized for its chemical and functional properties through compositional analyses and electron microscopy, revealing a highly porous structure and a large specific surface area potentially associated with its adsorptive capacity. In addition, in vitro assays demonstrated its antimicrobial and antioxidant activity against pathogenic strains of Escherichia coli, supporting its functional role in gastrointestinal modulation.

Subsequently, an in vivo trial was conducted on 10 calves with clinical signs of diarrhoea, randomly assigned to two groups: a control group (CTRL, n = 5) and a treated group (BIOCH, n = 5) receiving 4% biochar (on milk replacer basis) for 10 days from the onset of diarrhoea. Animals were monitored daily for milk intake, clinical treatments, and faecal consistency through the faecal score (0–3 scale; diarrhoea defined as score > 1) and faecal dry matter content.

In calves, biochar supplementation significantly reduced the duration of diarrhoea by approximately 62% in BIOCH compared to CTRL ($p < 0.05$), with a marked improvement in faecal consistency and increased faecal dry matter after 2 days of treatment. The BIOCH group showed a 51% reduction in total diarrhoea days, a 57% reduction in severe diarrhoea days, and a 47% reduction in moderate diarrhoea days compared with CTRL ($p < 0.05$).

Moreover, 80% of calves in the CTRL group required at least one therapeutic intervention (antibiotics, anti-inflammatory drugs, oral rehydration, or antidiarrheal treatments), whereas no animals in the BIOCH group required any additional intervention.

In conclusion, despite the limited number of animals in this pilot study, chestnut biochar supplementation in milk replacer displayed promising effects in improving gut health and reducing the severity and duration of diarrhoea in pre-weaning calves. Importantly, this approach may contribute to reducing antibiotic use and associated costs, thereby supporting more sustainable and responsible dairy production systems.

Oral Communication(s)

SERUM BIOCHEMICAL PARAMETERS AND ENDOMETRIAL CYTOLOGY IN SUBFERTILE DAIRY COWS

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Subfertility in dairy cows is a multifactorial condition increasingly associated with inadequate metabolic adaptation and immune–inflammatory dysregulation during the transition period. Several studies have focused on fertility-associated markers, particularly in bull semen and through genetic and genomic approaches, but the predictive potential of systemic biochemical indicators in dairy cows remains not fully defined. In this context, routine clincal chemistry profiling represents a practical and accessible tool for assessing animal health status at the farm level. The aim of this study was to identify biochemical patterns associated with reproductive outcomes, in order to support the early identification of cows at risk of subfertility.

The trial was conducted in two medium-sized commercial dairy farms and involved 137 multiparous Holstein cows monitored from dry-off until the second artificial insemination (AI). Cows were retrospectively classified according to reproductive outcome as Fertile 1 (F1; pregnant at first AI, n = 34), Fertile 2 (F2; pregnant at second AI, n = 28) or Subfertile (SF; non-pregnant after 2 AIs, n = 67). Eight cows were culled before the second AI and were therefore excluded from the data analysis. Blood samples were collected from the coccygeal vein into tubes without anticoagulant at 21 days before calving, 10 and 35 days in milk (DIM), at first AI and at second AI when applicable. Serum was obtained after centrifugation at 3000 rpm for 15 minutes and stored at -80°C until analysis. In addition, endometrial cytology samples were collected at 10 and 35 DIM and at first and second AI using a cytobrush technique. Polymorphonuclear cell (PMN) percentages were determined to assess uterine inflammatory status. Data distribution was assessed using the Shapiro-Wilk test. As variables were non-normally distributed, group comparisons (F1 vs SF and F2 vs SF) across timepoints were performed using the Wilcoxon rank-sum test. Analyses were conducted with JMP v19 (SAS Institute Inc.), with significance set at $p < 0.05$.

No significant differences between pregnant and non-pregnant cows emerged from data collected between -21 days before calving and +35 DIM. Conversely, significant differences were evident at first and second AIs, between F1 vs SF and F2 vs SF. Endometrial cytology revealed a significant higher PMN percentage in SF cows both at first (SF 3.99% vs F1 0.65%, $p < 0.001$) and second AI (SF 5.10% vs F2 0.68%, $p = 0.001$), indicating a higher uterine inflammatory status compared to Fertile animals. Regarding biochemical variables, significant differences were observed at first AI for albumin (SF 3.43 g/dL vs F1 3.51 g/dL, $p = 0.046$) and calcium (SF 9.2 mg/dL vs F1 9.5 mg/dL, $p = 0.020$), and at second AI for albumin only (SF 3.55 g/dL vs F23.71 g/dL, $p = 0.009$). Lower albumin and calcium concentrations in SF cows, support the association between systemic inflammation and impaired reproductive outcomes.

These findings suggest that alterations in biochemical and inflammatory variables at the moment of insemination may be associated with lower probability of pregnancy, highlighting their potential role in the understanding of factors underlying impaired reproductive performance.

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Oral Communication(s)

VISUAL IDENTIFICATION OF DAIRY COWS USING THE LINK BETWEEN DEEP LEARNING AND RFID TAGS

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Monitoring changes in the feeding behaviour of dairy cows is crucial for evaluating their feeding preferences, milk production, and overall health status. Cows affected by illness often display altered feeding patterns, such as reduced feeding time and frequency, making early detection essential for effective farm management.

Traditional methods for monitoring feeding behaviour are labour-intensive, time-consuming, and susceptible to human error. To overcome these limitations, precision livestock farming technologies have gained increasing attention. Although wearable sensors, such as accelerometers and RFID tags, can provide accurate data, they present certain drawbacks, including high costs and the potential to cause stress to the animals. In contrast, computer vision-based approaches offer a non-invasive and efficient alternative for monitoring feeding behaviour. Deep learning techniques, particularly the YOLO (You Only Look Once) object detection model, have been widely applied in animal husbandry. However, despite significant progress in object detection, recognizing individual cows in real farm environments remains challenging due to the lack of standardized and robust methodologies.

The primary objective of this study is to evaluate the reliability and validate a deep learning-based computer vision model for the automatic identification of individual cows at the feeding lane under real-world conditions. By identifying each cow, it becomes possible to measure feeding time, duration, and daily frequency. This paper outlines the entire workflow, from data collection to analysis and validation of an enhanced YOLOv8n model. After fine-tuning on the collected video dataset, the model achieved a precision of 85% and a recall of 62% (F1 score of 0.72) at an IoU threshold of 0.5, while processing 640×640 pixel frames in just 12 ms on an NVIDIA RTX 2080.

The promising results contribute to the advancement and validation of computer vision applications in herd monitoring, supporting the commercial adoption of these technologies for analysing cow behaviour, ultimately improving animal welfare and the sustainability of livestock production.

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Oral Communication(s)

IN VITRO EVALUATION OF NEUTRAL DETERGENT FIBER DIGESTIBILITY AT 24H AND 30H AND THEIR CORRELATION ACROSS DIFFERENT FORAGE TYPES

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Neutral detergent fiber digestibility (dNDF) is a key parameter for evaluating forage nutritive value and plays a central role in ration formulation models describing fiber degradation kinetics. Mechanistic nutrition models characterize fiber digestion using fermentation time points at 30, 120, and 240 h to estimate the fast, slow, and indigestible NDF fractions [1]. However, dNDFom at 24 h analysis is commonly evaluated in laboratories, raising questions about its comparability with the 30 h reference point. This study evaluated the relationship between in vitro dNDF at 24 h and 30 h across 33 forage samples, including alfalfa hay (n=9), maize forage (n=15), and wheat hay (n=9), selected to represent a wide range of fiber digestibility. Samples were incubated *in vitro* according to the Tilley and Terry method [2] at 24 h and 30 h using rumen fluid from lactating cows across three independent fermentation runs, with duplicate measurements per run. Neutral detergent fiber digestibility at each time was calculated [3]. Linear regression and Pearson correlation analyses were performed using JMP software (SAS Institute Inc., Cary, NC, USA) to evaluate the relationship between dNDF24h and dNDF30h. Results indicated a strong linear association between the two points (R^2 = 0.842, $p < 0.05$), described by the equation: dNDF30h = 9.461 + 0.9077 * dNDF24h. Relative differences among samples were largely preserved between time points. Mean dNDF at 24 h was 36.47 ± 10.9% for alfalfa, 46.67 ± 10.0% for maize forage, and 44.79 ± 6.9% for wheat hay, increasing at 30 h to 42.14 ± 10.6%, 54.95 ± 10.9%, and 50.83 ± 5.9%, respectively. Differences among forage types reflect structural variability in fiber composition, although the predictive relationship remained consistent across matrices. From a practical perspective, the ability to estimate dNDF30 h from 24 h measurements could reduce laboratory time, improve analytical throughput, and support more efficient database development and NIR calibration. This approach may provide nutrition models with robust fiber digestibility inputs and enable the effective use of existing 24 h datasets in nutritional model systems.

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Oral Communication(s)

MAIN COMPOSITIONAL DIFFERENCES AMONG THE PRIMARY FORAGES ANALYZED IN THE PO VALLEY OVER THE PAST CROP YEAR

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In the Po Valley, forages represent the basis of dairy cattle diets. Ration formulation begins with the nutrients supplied by forages, while additional ingredients are added to achieve proper balance. Typically, diets for milk production consist of 40–60% forage. The main challenge in formulating these diets lies in accurately assessing forage quality and understanding its impact on feed intake, digestion, and milk yield. This system is currently experiencing significant variability in forage composition and quality, due to extreme weather conditions and management choices. Therefore, our study aims to evaluate the main compositional and nutritional differences in hay and silages analyzed in the Po Valley over the past year. The data analyzed in this work derive from the analyses carried out by the Regional Association of Livestock Breeders of Lombardy (ARAL) for farmers during the last crop season (11/2024-11/2025), that included 4250 feed samples, of which more than 40% were silages and hays. Specifically, 1202 samples of corn silage, 163 samples of sorghum silages, 292 samples of meadow hay and 140 samples of alfalfa hay were taken into consideration. Forages compositional and nutritional features were determinate by using the Near-Infrared Reflectance Spectroscopy (NIRs) while dry matter was determined by oven drying. Result have presents as mean standard deviation, from which the coefficient of variation (CV) was deducted and expressed as a percentage. The results showed very different compositional and nutritional profile between the two conservation methods and within each category. Corn silage had a high-energy profile, mostly due to the starch content that was 30.1% ± 6.3 (mean±DS), with a neutral detergent fiber (NDF) content of 41.4% ± 5.3, both on a dry matter (DM) basis. Sorghum silage revealed a starch content that was 7.2% ± 6.1 (mean±DS), with a NDF content of 54.8% ± 5.6, expressed on DM basis. The distribution of the data was relatively concentrated around the mean, indicating moderate variability for most of the variables considered in the silage group, with the exception for starch in the case of sorghum (high CV above 40%). In contrast, dried forages showed different profiles: alfalfa hay was high in protein (18.1% ± 2.8) with moderate NDF (51.6% ± 7.7), while mixed meadow hay had lower protein (7.9% ± 3.5) but the highest NDF (62.6% ± 7.0). We also noticed some variability even within the hay categories, especially for protein in meadow hays (CV 44%). In conclusion, this study confirms substantial differences in nutrient composition between fermented and dried forages, along with notable variability within each category for specific parameters, such as starch in silages and protein in hays. These characteristics highlight the need for regular analytical monitoring that are essential for precise diet formulation and consistent dairy cow performance across production systems.

Oral Communication(s)

EFFECTS OF LIVE *TENEBRIO MOLITOR* LARVAE SUPPLEMENTATION ON GROWTH PERFORMANCE AND HEALTH IN WEANED PIGLETS FED DIETS DIFFERING IN CRUDE PROTEIN LEVELS

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Weaning represents a critical phase in swine farming, often associated with reduced feed intake, immature gut and immune functionality, and increased susceptibility to disease. In parallel, the need to reduce antibiotics use and dietary crude protein (CP) levels and reliance on conventional protein sources highlights the importance of sustainable and functional feeding strategies. In addition, improving feed palatability and stimulating feeding behaviour are key factors in promoting early feed intake and supporting post-weaning adaptation. Live insects may act not only as a nutrient source but also as a form of edible environmental enrichment, potentially enhancing animal welfare and reducing stress-related behaviours. Furthermore, the functional properties of insects (e.g., antimicrobial peptides and chitin) may contribute to gut health modulation and improved resilience during this critical phase. However, despite growing interest in insect meals, evidence on the use of live larvae in pig nutrition remains scarce, particularly in relation to their interaction with dietary crude protein levels.

In this context, the present study evaluated the effects of live Tenebrio molitor larvae supplementation in post-weaning piglets fed diets with moderate (15% CP) or standard (17% CP) protein levels.

A total of 48 weaned piglets (28 ± 2 days of age) were allocated to four dietary treatments (ethical authorization OPBA n° 41_2025): CTRL15 and CTRL17 (basal diets without larvae) and INS15 and INS17 (basal diets supplemented daily with 50 g/pig of live larvae) over a 42-day period. The 17% CP diet was formulated with a higher inclusion of soybean meal compared to the 15% CP diet. In the CTRL groups, an equivalent amount of protein was provided daily through soybean meal supplementation to balance the protein contribution provided by the live larvae. Growth performance, feed intake, feed efficiency, health status, larvae consumption time, nutrient digestibility, serum metabolic profile and antioxidant status content were assessed.

Larvae supplementation significantly improved growth performance, particularly during the early post-weaning phase. Piglets in the INS17 group achieved higher final body weight compared with both control groups ($p < 0.05$), while INS15 piglets reached comparable performance to CTRL17 despite the lower dietary protein level. Feed conversion ratio was significantly improved during the first two weeks in both INS groups ($p < 0.05$), indicating enhanced feed efficiency.

Live larvae were rapidly accepted and consistently consumed, with consumption time decreasing to less than 2 minutes in the final phase, whereas soybean meal supplementation showed low palatability and frequent refusals.

Health status was positively affected by larvae supplementation. Piglets receiving live larvae showed a significantly lower incidence of diarrhoea, respiratory disorders, and reduced vitality compared with control groups ($p < 0.05$). Notably, no mortality or antibiotic treatments were recorded in the INS17 group.

No significant differences were observed in apparent total nutrient digestibility, serum biochemical parameters, antioxidant capacity at the end of the trial.

In conclusion, live T. molitor larvae could represent a promising functional feeding strategy to stimulate early feed intake, improve growth performance, and enhance health status in weaned piglets. Importantly, their use may allow for moderate reduction of dietary crude protein without compromising performance, contributing to more sustainable pig production systems.

Oral Communication(s)

EXPLORING UNCONVENTIONAL FIBER SOURCES AS NOVEL PREBIOTIC INGREDIENTS IN A CANINE GUT MODEL

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Dietary fiber modulates gut health through its effects on intestinal microbiota composition and metabolic activity. While conventional fiber sources are well studied, unconventional substrates remain largely unexplored in canine nutrition. Therefore, alternative fiber sources may offer new opportunities to support gut health in dogs.

The aim of this study was to evaluate in vitro the effects of beet pulp (BP), pomegranate peel (PP), prickly pear cladodes (PC), yellow pea hulls (YP), potato fiber (PF), apple fiber (AF), and lemon fiber (LF) on canine fecal microbiota metabolism. Substrates were subjected to in vitro digestion simulating the upper gastrointestinal tract[1]. To recover the soluble dietary fiber, the soluble fraction was precipitated with ethanol and recombined with the undigested residue. Eight treatments were tested: a control diet (CTRL) and CTRL supplemented with BP, PP, PC, YP, PF, AF, or LF (based on digestibility coefficients to simulate a 2% inclusion level in the diet). Fermentation vessels (5 per treatment) contained the undigested residue (10 g/L) of the in vitro-digested control dry diet and were inoculated with canine fecal suspension[2]. Incubation was performed anaerobically at 39 °C for 24 h. Samples of fermentation fluid were collected at 6 and 24 h. Data were analyzed using Kruskal–Wallis with Dunn's post hoc test or one-way ANOVA with Dunnett's test, as appropriate.

After 6 h, acetate increased (+30%; P<0.0001) in PP and LF, while PP and PC decreased propionate (-11% and -16%, respectively; P=0.0004). PC also decreased butyrate (-18%; P=0.0014), branched-chain fatty acids (BCFA, isobutyrate -30%; isovalerate -25%), and total volatile fatty acids (VFA, -15%) (P<0.01). BCFA were reduced by PP (isobutyrate -35%; isovalerate -25%;) and PF (isovalerate -19%) (P<0.01). At 24 h, acetate was increased (P<0.0001) by all treatments: +23% for BP, +10% for PC, +16% for PP, +9.4% for YP, + 32% for PF, + 23% for AF, + 48% for LF. Propionate was increased (P<0.0001) by BP (+11%), PF (+17%), AF (+8.7%), and LF (+9.7%), while butyrate was unaffected. Isobutyrate was lowered by PP (-28%) and LF (-22%), while isovalerate was decreased by BP (-19%), PP (-22%), and LF (-19%) (P=0.0002). Total VFA increased in BP (+11%), PF (+17%), AF (+9.8%), and LF (+22) (P<0.0001). A reduction in pH units was observed at 6h (P<0.0001) in BP (-0.08) and both at 6 and 24h (P=0.0002) in PF (-0.13 and -0.22), AF (-0.10 and -0.16), LF (-0.09 and -0.16). Ammonia concentrations were unaffected at 6 h, while a decreasing trend was observed at 24 h for PP (−63%) and YP (−58%) (P = 0.06).

Unconventional fiber sources exhibited distinct fermentation profiles in a canine gut model. Potato fiber, apple fiber, and especially lemon fiber showed the greatest prebiotic potential, increasing VFA production and reducing pH. In contrast, prickly pear cladodes appeared less fermentable. Pomegranate peels showed selective effects, including reductions in BCFA and ammonia, suggesting reduction of proteolytic fermentation. Overall, these substrates may represent functional ingredients for canine nutrition, with effects depending on fiber type. The next step of this study will be to characterize key bacterial taxa associated with these fermentation patterns using qPCR.

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Oral Communication(s)

IMMUNOPROTECTIVE POTENTIAL OF *FUCUS VESICULOSUS* AGAINST ENNIATIN B1 TOXICITY IN A PORCINE MACROPHAGE MODEL

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Enniatin B1 (ENNB1) is an emerging mycotoxin produced by *Fusarium* species, frequently detected in cereals and animal feed, where it exerts toxic effects in livestock, including oxidative damage, mitochondrial impairment, and pro-inflammatory signalling. Despite their widespread occurrence, enniatins remain unregulated in food and feed, highlighting a critical gap in current mycotoxin risk management. Given this context, there is growing interest in natural bioactive compounds that can mitigate their adverse effects. Marine macroalgae such as *Fucus vesiculosus*, a phlorotannin- and fucoidan-rich brown macroalga, have demonstrated antioxidant, anti-inflammatory, and immune-regulatory activities, offering promising applications in nutritional strategies aimed at enhancing cellular resilience. This study evaluated the protective effects of *F. vesiculosus* against ENNB1-induced damage in porcine alveolar macrophages (PAMs) through both pre-treatment and co-treatment experimental designs. Cell viability was assessed by a crystal violet-based adhesion assay, while immune and antioxidant responses were evaluated by RT-qPCR analyses of key gene transcripts. When administered alone, the extract exhibited concentration-dependent cytotoxicity, reducing cell adhesion below 25% at 100 µg/mL after 24 h. However, pre-treatment significantly improved cellular resilience to ENNB1, resulting in a more than 4-fold increase in adhesion at 100 µg/mL compared with ENNB1-treated controls ($p<0.05$). Gene expression analysis showed a marked downregulation of IL-6, IL-8, and COX-2 at early time points, alongside upregulation of GPX-2, consistent with stimulation of cellular antioxidant pathways. Co-treatment provided moderate cytoprotection, restoring cell adhesion to approximately 82% of baseline and partially attenuating ENNB1-driven transcriptional activation. Overall, the obtained results support the immunoprotective potential of *F. vesiculosus*, particularly when administered preventively, and highlight its relevance as a sustainable functional feed additive to mitigate mycotoxin-associated immune stress in swine production systems.

Oral Communication(s)

STUDY OF THE IMPACT OF DIFFERENT DIET SUPPLEMENTATION ON THE MICROBIOTA AND GENE EXPRESSION OF CYTOKINES, GUT VASCULAR BARRIER AND REDOX-RELATED MOLECULES IN THE GUT WALL OF RABBIT

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Rabbits play an important role as livestock, pet and laboratory animal. Their sensitivity to bacterial infections presents challenges. Although antibiotics are commonly used to treat these infections, alternative solutions are being sought to prevent the development of antibiotic resistance. In this study we evaluated the impact of different diet supplementations on the microbiota and gene expression of immune-, gut vascular barrier- and red-ox- related molecules in rabbits. Bovine colostrum (BC) has been investigated for its potential in treating and preventing various diseases [1]; Goji barriers (GB) for their antioxidant and immunomodulating effect [2]; fish oil (FO) and flaxseed (FS) as different sources of PUFA [3]; Padina pavonica algae extract (PP) and extruded lineseed (ELS) for its antioxidant and antibacterial activities [4].

The aim of the study was to assess effects of the different diet supplementation on the microbiota of rabbit intestinal tract evaluating also the gene expression of immune (*IL1B*, *CXCL8*, *TGFB1*), gut vascular barrier (*PLVAP*, *CTNNB1*) and red-ox related (*SOD1*, *GSR*) genes via RT-qPCR.

Rabbits were divided into groups (7-14 animals/group) according to the diet: control group (commercial feed), 2,5% BC (commercial feed + 2,5% of BC), 5% BC (commercial feed + 5% of BC), FS (commercial feed + 10% of FS), FO (commercial feed + 3.5% of FO), GB (commercial feed + 1% of GB) or LPP (commercial feed + 3.5% of ELS and 0.2% of PP). The rabbits were weaned at 35 days and were fed with the different diets from 35 to 90 days of age. Digestive tracts were removed at slaughter, tissue samples from jejunum, cecum, colon, collected for RNA extraction. The content of the different tracts was collected for the microbiota analysis by 16S rRNA Gene Sequencing.

The results indicate that the different supplementations modulate both the microbiota and the gene expression in the gut of rabbits. Dietary effects were largely segment-specific, with small intestine showing the most pronounced responses in gene expression modulation. The different supplementations were also able to modify the Firmicutes:Bacteroidetes ratio, the relative abundance of specific bacterial families, the alpha and beta diversity. The modifications of microbiota were specific for different gut tracts under study.

These results suggest that diet supplementation has significant effects on the rabbit gastrointestinal tract's microbiota and inflammatory, antioxidant, and gut-barrier responses, supporting their potential as a dietary strategy to enhance gut health through nutritional immunomodulation and microbial metabolic reprogramming. Further research is required to fully understand their histological and physiological impact and possible combinations to reach the best results, also meeting quality and reproductive performances.

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Oral Communication(s)

A STABLE SPRAY-DRIED FORMULATION OF MILK EXTRACELLULAR VESICLES ATTENUATES EXPERIMENTAL COLITIS

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Milk extracellular vesicles (mEVs) have therapeutic potential in inflammatory bowel disease (IBD), reducing cytokine production, limiting oxidative stress, and supporting gut homeostasis [1]. In livestock, their immunomodulatory properties could offer a promising adjunct or alternative to antibiotic therapy. However, a major limitation to their clinical use is preservation (requires storage at -80°C). Our research group recently developed a stable, room-temperature dry powder formulation of mEVs by spray drying with two excipients [2]. This study aimed to assess the effect of this spray-dried mEVs (SDmEVs) formulation in models of IBD. The biological activity of SDmEVs was compared with a spray-dried excipient-only formulation (SDExc) and evaluated: *in vitro* in inflamed human colonic epithelial cells (HCEC-1CT) and human colonic organoids; *ex vivo* in inflamed colonic biopsies from patients with ulcerative colitis; and *in vivo* in a DSS-induced mouse model of colitis following oral administration. Mucosal and inflammatory genes and proteins were assessed by immunofluorescence and qPCR. Disease severity, histological damage, and transcriptomic profiling by Illumina sequencing of mRNAs and small RNAs were performed on mouse colon tissues. In inflamed epithelial monolayers, SDmEVs preserved epithelial architecture and maintained ZO-1 and occludin localization compared with excipient-treated controls. Consistently, treatment of human colonic organoids and *ex vivo* cultures from ulcerative colitis biopsies restored epithelial organization and increased ZO-1 and occludin expression. *In vivo* oral administration of SDmEVs significantly ameliorated DSS-induced colitis, reducing disease severity and histological damage and attenuating mucosal inflammation. SDmEV treatment restored the tight junction protein expression and was associated with increased phosphorylation of AMP-activated protein kinase (AMPK), supporting barrier-repair pathways. Transcriptomic analysis showed that, compared with DSS mice, SDmEV-treated mice displayed a coordinated reduction in inflammation-associated miRNAs, including miR-122, miR-132-3p, miR-155, miR-212, and miR-223-5p, together with downregulation of inflammatory effector genes such as *S100a8*, *S100a9*, *Cxcl3*, *Mmp8*, and *Trem1*. These changes were accompanied by increased expression of epithelial repair-associated markers, including *Ang4*, *Lgr5*, and *Muc2*, consistent with partial restoration of epithelial homeostasis. Notably, this molecular profile was not observed in mice treated with SDExc, which largely retained a DSS-like signature. These findings demonstrate that spray drying effectively preserves mEVs in a stable dry powder formulation, enabling room-temperature storage while maintaining bioactivity. Overall, this formulation represents a stable, non-invasive, translational approach to restore epithelial integrity and modulate mucosal inflammation in intestinal inflammatory disorders.

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Oral Communication(s)

EFFECT OF AN INTRAMAMMARY DRY-OFF TREATMENT IN ALPINE DAIRY GOATS WITH HEALTHY OR INFECTED HALF UDDERS

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Intramammary infections (IMI) represent a major concern in dairy goats, and the effectiveness of antibiotic dry-off therapy in this species remains controversial, particularly regarding bacteriological cure, prevention of new IMIs, and somatic cell count (SCC) response. Half-udder milk samples were collected during the morning milking from 69 Alpine dairy goats one week before dry-off (T1), at dry-off (T2) and one week postpartum (T3), and the California Mastitis Test (CMT) was performed contextually. Samples were used to assess SCC (expressed as linear score, LS) and to perform bacterial culture (BC), identifying isolated bacterial species using MALDI-TOF. Based on BC results at T1, half-udders were classified as healthy (no bacterial growth) or infected (≥1 bacterial species isolated) and goats were classified accordingly as healthy (both healthy halves) or infected (at least one infected half). At dry-off, goats were blocked according to health status and randomly treated with an intramammary antibiotic therapy (TG: n = 36 goats, 72 half-udders) or used as controls (CG: n = 33 goats, 66 half-udders). Data were analyzed at the half-udder level to explore cure and new infection rates, LS and CMT responses to antibiotic treatment at the resumption of lactation (T3). Half-udders classified as infected at T1 were further categorized as confirmed or non-confirmed IMI based on BC results at T[2] Only confirmed cases (n = 21) were included in the analysis of bacteriological cure at T[3] Samples classified as polymicrobial were not excluded but reclassified using a deterministic longitudinal rule based on adjacent time points. Bacteriological cure and incidence of new IMIs were analyzed using mixed-effects logistic regression models (PROC GLIMMIX), including treatment as fixed effect, parity, number of kids, and days from dry-off (DFD) as covariates, and goat as a random effect. LS and CMT were analyzed using linear mixed models for repeated measures (PROC MIXED), including time, treatment, infection class, and their interactions, with goat as a random effect. No significant effect of antibiotic treatment on IMI cure was observed (OR = 1.00; 95% CI: 0.06–17.44; p = 0.998). Among initially healthy half-udders (n = 89), the incidence of new IMIs was numerically lower in the treated group compared to controls (7.0% vs 13.1%; OR = 2.00; 95% CI: 0.44–9.22; p = 0.36). LS and CMT were significantly associated with IMI status ($p < 0.01$ for LS and $p < 0.10$ for CMT). Half-udders with confirmed IMIs showed higher values compared to healthy ones (LS: 3.95 vs 2.53; CMT: 0.83 vs 0.58), while non-confirmed IMIs showed intermediate values (LS: 3.07; CMT: 0.61). No consistent effect of treatment over time was detected on LS or CMT. Antibiotic dry-off therapy did not significantly improve bacteriological cure of IMI in this study, likely due to the limited sample size. However, a numerical reduction in new IMIs suggests a potential preventive effect against minor pathogens. SCC-related indicators were primarily associated with IMI status rather than treatment.

Oral Communication(s)

MOLECULAR PROFILING OF EXTRACELLULAR VESICLES IN BUFFALO COLOSTRUM AND MILK ACROSS LACTATION BY AN INTEGRATED MULTI-OMICS APPROACH

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Milk-derived extracellular vesicles are bioactive nanostructures involved in intercellular communication and immune regulation through their cargo of proteins, lipids, metabolites and RNA, which contribute to their immunomodulatory potential. Although bovine milk has been extensively investigated, buffalo extracellular vesicles are still poorly characterized, particularly across lactation. 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 (15 and 60 days post-parturition) by integrating transcriptomics (small RNA next-generation sequencing) and metabolomics (high-performance liquid chromatography-mass spectrometry analyses). Vesicle preparations were characterized by nanoparticle tracking analysis and scanning electron microscopy, confirming the expected size distribution and morphology. Mean particle size was about 175.6 ± 3.2 nm in colostrum-derived extracellular vesicles and 174.0 ± 1.5 nm in milk-derived extracellular vesicles, with concentrations in the order of 10^{12} particles/mL. Flow cytometry, performed using both single-vesicle and bead-based approaches, showed expression of the canonical tetraspanins CD9, CD81 and CD6[3] Colostrum samples contained a higher abundance of extracellular vesicles than mature milk, whereas milk extracellular vesicles collected at both time points showed greater molecular diversity and shared the largest number of detected features. Small RNA sequencing identified 51 microRNAs enriched in colostrum-derived vesicles, 81 in milk vesicles at 15 days and 79 at 60 days. Metabolomic analysis revealed 379 molecules specifically enriched in colostrum-derived vesicles, while 619 and 764 were enriched in milk vesicles at 15 and 60 days, respectively. Preliminary integrative multi-omics analysis highlighted a clear separation among the three groups in principal component analysis and integrated heatmaps, together with strong microRNA-metabolite associations (r = 0.85), supporting a stage-dependent remodeling of vesicular cargo throughout lactation. Functional analysis of class-specific microRNA targets indicated enrichment of pathways related to immune and stress responses, nucleic acid metabolism, protein signaling and intracellular transport, whereas metabolite pathway analysis suggested dynamic changes in amino acid, lipid, carbohydrate and signaling metabolism. These findings provide a systems-level view of buffalo milk extracellular vesicles and support their potential role in neonatal development, immune modulation and future clinical applications.

Oral Communication(s)

COMPARATIVE MODULATION OF THE HIPPO SIGNALLING PATHWAY IN BOVINE PAPILLOMAS AND EQUINE SARCOIDS ASSOCIATED WITH BOVINE PAPILLOMAVIRUS INFECTION

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Papillomaviruses (PVs) are DNA viruses that infect epithelial cells of a wide range of vertebrate species. In humans, viral infections are estimated to account for approximately 15–20% of cancers worldwide, with papillomaviruses contributing to about 5% of all malignancies [1]. Among domestic animals, cattle and horses represent particularly relevant models because bovine papillomaviruses (BPVs) are among the few papillomaviruses capable of crossing the species barrier. In cattle, BPVs induce cutaneous papillomas and fibropapillomas, whereas in horses they are associated with the development of equine sarcoids, characterized by locally aggressive behaviour and frequent recurrence [2].

Recent studies have highlighted the potential role of the Hippo signalling pathway in tumour development. This evolutionarily conserved pathway regulates cell proliferation, apoptosis and tissue homeostasis. In mammals, the Hippo cascade controls the activity of the transcriptional co-activators YAP and TAZ capable to promote the expression of genes involved in proliferation, survival and tissue remodelling [3,4]. Evidence from human papillomavirus (HPV) models suggests that viral oncoproteins can interfere with regulators converging on the Hippo network, promoting pro-proliferative transcriptional programs. Although direct evidence in BPV-associated lesions is still limited, the interaction between viral oncogenic signals, extracellular matrix remodelling and mechano-transduction may represent a relevant mechanism in papillomavirus-driven tumorigenesis.

In this study, the expression of genes belonging to the Hippo and Wnt signalling pathways were investigated by Real-Time PCR in 25 equine sarcoids and 18 bovine papillomas. In equine sarcoids, a significant transcriptional deregulation of Hippo pathway was observed, with down-regulation of APC2, CTNNA3 and FZD3, and up-regulation of WNT5A, YAP1, TAZ, SAV1 and LATS2, suggesting a possible involvement of the Hippo–YAP/TAZ axis in the fibroproliferative behaviour of these lesions. Conversely, bovine papillomas did not show a global deregulation of the Hippo pathway, although a different trend of WNT5A expression, was observed.
Overall, these results suggest that the Hippo–YAP/TAZ axis may represent a relevant molecular node in the pathogenesis of equine sarcoids, while its role in bovine papillomas appears more limited and possibly influenced by interactions with other signalling pathways such as Wnt and TGF-β. The comparative analysis between the two species provides new insights into the molecular mechanisms underlying BPV-associated lesions and highlights biological differences between epithelial papillomatous lesions and fibroproliferative tumours.

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Oral Communication(s)

IMMUNO-HEMATOLOGICAL CHANGES FOLLOWING THERAPEUTIC VACCINATION IN BPV-POSITIVE EQUINE SARCOIDS

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Equine sarcoids are the most common cutaneous tumors in horses and are strongly associated with infection by bovine papillomavirus (BPV) types 1, 2 and 13 [1]. Despite multiple therapeutic approaches, recurrence and treatment resistance remain major clinical challenges [2]. Therapeutic vaccination has been proposed as an immunomodulatory strategy; however, its systemic immunological effects remain poorly characterized.

This study aimed to evaluate the hematological, biochemical and immunogenetic modulation induced by vaccination in 12 BPV-positive horses with sarcoids. Peripheral blood samples were collected before vaccination (T0) and at multiple post-vaccination time points (7, 14, 21, 28, 35 and 42 days). Hematological and biochemical parameters were analyzed using both non-parametric tests and mixed-effects models to account for repeated measures. In parallel, gene expression of selected immune mediators was evaluated in peripheral blood monucleate cells (PBMCs).

Neutrophil counts showed a significant time-dependent reduction in mixed-effects models (global $p = 0.0036$), with marked decreases at days 7, 14, 28, 35, and 42 compared to baseline. This pattern was consistent across subjects and suggests a progressive attenuation of the innate inflammatory component. Lymphocytes did not display a significant overall time effect; however, a transient increase was observed at day 42 ($p = 0.008$), indicating a delayed and time-specific adaptive response. Eosinophils exhibited a biphasic pattern, with significant elevations at day 7 ($p = 0.002$) and day 48 ($p < 0.001$), consistent with immunomodulatory activation. Monocytes showed high inter-individual variability without a uniform temporal trend.

Plateletcrit demonstrated a consistent reduction at several time points, suggesting a mild systemic modulation of platelet mass, whereas alanine aminotransferase changes observed in non-parametric analysis were not confirmed in mixed models, indicating absence of clinically relevant hepatic effects.

Gene expression analysis in PBMCs revealed significant temporal modulation of *IL18* ($p < 0.001$) and *TNFA* ($p < 0.05$), with an early increase after vaccination followed by return toward baseline levels. No significant changes were observed for *CXCL8*, *IL1B*, *TGFB*, *IL17*, *IL12A*, *IL12B*, *IL23* or *EBI3*, although transient trends were noted.

Overall, vaccination was associated with a coordinated immunological shift characterized by reduction of neutrophil-driven inflammation, transient activation of pro-inflammatory cytokines linking innate and adaptive immunity, and delayed lymphocyte modulation. These findings provide evidence that therapeutic vaccination induces systemic immune remodelling in BPV-associated equine sarcoids.

This project has been founded by Italian Ministry of Health (RC23C03).

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Oral Communication(s)

WEARABLE DEEP PHENOTYPING AND CIRCULATING MICRORNAS AS FIELD MARKERS OF EXERCISE AND HEAT-STRESS RESILIENCE IN EQUINE ATHLETES

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Climate change increases the frequency of heat load during training and competition, challenging equine welfare and performance. Within the PRIN 2022 ECOS project, we implemented a field-ready framework combining wearable-derived deep phenotyping (DEPH) and liquid biopsy to quantify physiological strain and regulatory signatures associated with exercise and heat stress (HS).

Cardiovascular and locomotor variables (heart rate and heart rate variability, locomotor speed/stride metrics and environmental data) were recorded with the Equimetre® wearable system and paired with hematology/biochemistry and serum small-RNA sequencing for circulating microRNAs (miRNAs). Three complementary datasets were collected: (i) six Arabian endurance horses performing field Standardized Exercise Tests (fSETs) under thermoneutral (TN) and HS conditions (Perugia); (ii) fifty-eight Thoroughbred racehorses monitored during routine training across cold, intermediate (TN) and hot (HS) windows (Capannelle racetrack, Rome); and (iii) eighteen elite endurance horses evaluated during a controlled canter on deep sand (Dubai, United Arab Emirates, UAE). ANOVA and Student's T test evaluated differences in blood parameters and wearable device measures. miRNA profiles were analyzed by sequencing total RNA isolated from serum through the Illumina® NGS approach. A set of differentially expressed small RNAs in response to temperature and/or exercise (|log2 fold change| >1 and adjusted $p<0.05$), were obtained with DESeq2 software; a protein-protein interaction (PPI) network for targets of miRNAs was built and submitted for a gene ontology (GO) enrichment analysis.

HS amplified exercise strain in fSETs (higher lactate, increased packed cell volume (PCV) and creatinine post-test, and higher muscle-related biomarkers) and reshaped circulating miRNAs, including miR-206, miR-3613-3p, miR-142 and miR-144, associated with energy metabolism, cytoskeletal remodeling and immune/stress pathways. In Thoroughbreds, hot conditions reduced mean speed and increased post-exercise white blood cells (WBC), hematocrit (HCT) and hemoglobin (HB); miR-122 was consistently upregulated after exercise across weather windows. In Dubai, stride length modulation supported higher speed on deep sand, and HRV recovery indices indicated autonomic recovery status; post-exercise miRNA shifts (19 differentially expressed miRNAs) highlighted targets involved in extracellular matrix remodeling and vascular/immune regulation.

Integrating wearable DEPH with circulating miRNAs provides sensitive, non-invasive markers of resilience relevant to welfare-oriented monitoring of sport horses across climates and disciplines, and supplies actionable inputs for predictive models under increasing environmental temperatures.

Oral Communication(s)

SEROPREVALENCE OF Q FEVER IN CATTLES AND WATER BUFFALOES IN THE CAMPANIA REGION

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Q fever is a widespread zoonosis caused by the obligate intracellular bacterium *Coxiella burnetii*. The pathogen is characterized by a broad host range, with buffalo and cattle serving as the primary reservoir for human infection. Considering that in southern Italy, particularly in Campania, there are an estimated 303.000 buffalo, with milk production of around 410.000 tons/year, and 137.000 cattle, mainly distributed in the provinces of Salerno and Caserta, is essential to monitor the prevalence of *C. burnetii* for both animal health and public safety.

Within the framework of the Italian national eradication programs for Brucellosis, a total of 3,692 blood samples were collected in the provinces of Salerno and Caserta: 2,039 from 67 farms in the province of Salerno (677 cattle and 1,362 buffaloes) and 1,653 from 9 buffalo farms in the province of Caserta.

The analyzed subjects were divided into three age groups using cut-off points derived from the age distribution: <3 years, 4–8 years, and >8 years.

The serum samples were analysed using the ID Screen® Q fever Indirect Multi-species ELISA kit (ID, France) developed for ruminants for the detection of antibodies against *C. burnetii*.

Descriptive statistical analyses were performed to estimate prevalence rates, stratified by geographic location, age, and species.

It can be observed a prevalence of 7% of positive cases distributed across both provinces.

Specifically, in the province of Salerno, the distribution appears to be wider and characterized by greater variability, with a more consistent presence of herds located in the intermediate prevalence ranges, with a prevalence rate of 6,57%.

In contrast, in the province of Caserta, the distribution is more concentrated in the lower prevalence classes, with a prevalence rate of 7,74%.

As regards the comparison between species, however, a prevalence of 8.2% can be observed in cattle compared to 6.8% in buffaloes.

Finally, if we consider prevalence by age group, we see a prevalence of 1.2% among young, 7.9% among adults, and 13.1% among older.

Preliminary results confirm the circulation of *C. burnetii* in livestock farms in Campania, suggesting the presence of potential risk factors associated with species and age, similar to those reported in previous studies. [1]

The descriptive analysis highlights some differences in the distribution of values between the two provinces, with greater apparent variability in the province of Salerno than in Caserta, but with a higher prevalence in the province of Caserta than in Salerno and in cattle than in buffalo.

It is also possible to observe an increase in prevalence directly proportional to the advancing age of the animals, as already highlighted in previous studies, linked to the rate of contact with the pathogen, which tends to increase with age because of the increasing probability of contact over the course of a lifetime.

These data suggest the need for targeted surveillance programs and more rigorous management practices, especially in areas of high prevalence.

However, due to the small number of samples, these data should be considered preliminary. Larger studies are needed, including genotype characterization and identification of active infection in positive herds using direct diagnostic techniques, in order to assess the risk factors and the real relevance of *C. burnetii* infection.

Funded by the Italian Ministry of Health (RC IZSME 10/2024).

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Oral Communication(s)

THE INTERFERON GAMMA RELEASE ASSAY: CHARACTERIZATION AND TEMPORAL DYNAMICS OF THE IMMUNE RESPONSE IN THE BUFFALO SPECIES

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Bovine tuberculosis (TB) is a chronic, infectious, and contagious disease caused primarily by Mycobacterium bovis, a member of the Mycobacterium tuberculosis complex, and has significant health, economic, and trade implications. The Interferon Gamma Release Assay (IGRA) and the intradermal test (IDT) are the official tests for the in vivo diagnosis of TB for disease eradication and are primarily based on measuring the cell-mediated immune response. The performance of these tests may depend, in the case of the IDT, on: animal species (i.e., skin thickness), animal age, the tuberculins (PPD_S) used, co-infection, or prior exposure to environmental mycobacteria; in the case of the IGRA, on: the PPD_S used, their concentration, and the animal's physiological status [1]. The aim of this study is to evaluate the temporal dynamics of the IGRA following the skin test and the effect of the used PPD_S concentration. With authorization from the Italian Ministry of Health (No. 103/2025-PR), we analyzed 151 buffaloes by collecting blood samples for the IGRA at T0 (before bovine Tuberculin (PPDbov) inoculation) and then at T3, T21, T42, and T63 days after PPDbov inoculation, and divided them into the three age groups: 1–4, 4–7, and >7 years. For the blood sensitization phase, the following were used: phosphate-buffered saline (PBS), bovine and avian PPD_S at concentrations of 20 and 10 µg/mL each, non-specific mitogen (PWM) at 5 µg/mL, and a mix of recombinant ESAT6_CFP10 antigens, each at 4 µg/mL. The concentrations refer to the respective sensitization wells, each containing 1 mL of blood. After incubating the samples at 37°C for 18–24 hours, the ELISA phase of the IGRA was performed on the recovered plasma. All samples tested were negative for IDT at T3 and for IGRA at T0 using PPDbov at 20 µg/mL (100% agreement); with PPDbov at 10 µg/mL and with ESAT6_CFP10, we obtained 1 (99.3%) and 2 positive results (98.7%), respectively, in different animals. The apparent specificity (Sp_a) of IGRA performed with PPDbov-10, PPDbov-20, and ESAT6_CFP10 was generally very good at all five time points considered, particularly at T0 and T2[1] The poorest performance was observed with PPDbov-20 at T3, with 12 positive results (Sp_a 92%, lower limit 86.5%). In the other cases, Sp_a is always greater than 95%, and the lower limit is always greater than 90%. Preliminary data show no significant differences for PPDbov-10, PPDbov-20, and ESAT6_CFP10 among the age groups 1–4 years, 4–7 years, and >7 years. Apart from the control on day 0, the Sp_a of PPDbov-10 appears higher, although not significantly, than that of PPDbov-20. In conclusion, in our experimental setup, no significant differences were observed between the two concentrations used in the IGRA (10 vs. 20 µg/mL). Our data also indicates that the age of the animals tested does not influence the Sp_a of the IGRA under the different conditions considered.

Funded by the Italian Ministry of Health (RC IZSLER 02/2023).

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Oral Communication(s)

APPLICATION OF QUADRATUS LUMBORUM BLOCK AND TRANSVERSUS ABDOMINIS PLANE BLOCK DURING LAPAROSCOPY IN THE HORSE: A PRELIMINARY STUDY

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Standing laparoscopic surgery in horses is a minimally invasive technique performed under sedation and local anesthesia. The common indications for performing standing laparoscopy include cryptorchidectomy and inguinal hernia repair in males and ovariectomy in mares. During these procedures, local anesthesia is typically achieved by infiltration of lidocaine at the planned portal sites, including the skin, subcutaneous tissue, muscle layers, and peritoneum. Additional techniques, such as line blocks or inverted L blocks. In human medicine and small animal practice, the quadratus lumborum (QL) block is an ultrasound (US)-guided regional anesthetic technique involving injection of local anesthetic into the fascial planes surrounding the QL muscle, providing somatic and visceral analgesia for abdominal and pelvic procedures [1]. Conversely, the transversus abdominis plane (TAP) block consists in the US-guided injection of local anesthetic between the internal oblique and transversus abdominis muscles, blocking the ventral branches of thoracolumbar nerves and providing analgesia to the abdominal wall [2]. To the authors' knowledge this is the first report describing the US landmarks for the application of the QL and TAP blocks in horses undergoing standing laparoscopic surgery.

Five horses were included in the study: three horses underwent laparoscopy for removal of a left cryptorchid testicle and they received both QL and TAP blocks (group QLTAP). Two additional horses underwent laparoscopy for ovarian tumour removal and cryptorchidectomy, respectively, and received only the QL block (group QL).

The horses were restrained in stocks and sedated with detomidine (0.01 mg/kg IV) and morphine (0.1 mg/kg IV) and then maintained with a detomidine infusion (0.005 mg/kg/h). The US anatomical landmarks were identified: the transverse processes of L1, the longissimus dorsi muscle, the QL muscle, and the interfascial plane between the QL and psoas minor muscles. The QL block was performed using an US-guided transverse approach, injecting 50 mL of ropivacaine 0.125% into the interfascial plane at the level of L1 with 20G spinal needle. The TAP block was performed under US guidance by injecting 50 mL of ropivacaine 0.125% between the internal oblique and transversus abdominis muscles, caudal to the last rib and distal to the line of the iliac crest, using a 20G spinal needle.
The identification of the anatomical landmarks for performing the QL and the TAP blocks was feasible in all the horses. None of the horses showed any signs of ataxia. The two horses that received only the QL block, require additional infiltration of 20 mL lidocaine 2% at the abdominal due to significant reaction during surgical incision. After the opening of the surgical portal, in the group QL and TAP no reaction was observed when the viscera were pulled with forceps, while in the group QL an infiltration of 10 ml 2% lidocaine was required in the testicle ligament to reduce the horse's reaction.

In conclusion, the preliminary findings of this study suggest that the combination of the QL block and the TAP block is a promising technique for improving analgesia during standing laparoscopy in horses; QL and TAP block provided more effective control of both somatic and visceral pain, reducing the need for additional intraoperative local anesthetic infiltration.

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Oral Communication(s)

INTRANASAL LIDOCAINE ADMINISTRATION PREVENTS LARYNGOSPASM AND IMPROVES OROTRACHEAL INTUBATION IN RABBITS UNDERGOING GENERAL ANAESTHESIA FOR ELECTIVE SURGERY

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Orotracheal intubation is essential in rabbits undergoing general anaesthesia, yet it remains challenging due to species-specific anatomical features and their predisposition to laryngospasm (1). In both human and veterinary medicine, topical lidocaine reduces laryngospasm by attenuating airway reflexes, allowing orotracheal intubation with minimal or no systemic hypnotic drugs (2,3). Rabbits' obligate nasal breathing and the epiglottis positioned above the soft palate may allow laryngeal desensitisation via intranasal administration of lidocaine, which flows toward the laryngeal inlet. However, this approach has not been investigated in rabbits. This study aimed to determine whether intranasal lidocaine prevents laryngospasm and facilitates orotracheal intubation in client-owned rabbits undergoing general anaesthesia for elective surgeries.

The study was approved by the Institutional Ethical Committee for Animal Care at the University of Milan (OPBA_24_2025). Fifty rabbits were enrolled (25 per group) with written owner consent. All animals received intramuscular dexmedetomidine (0.08 mg/kg) and ketamine (5 mg/kg). Sedation was assessed, and alfaxalone was administered intravenously to effect if rabbits remained insufficiently sedated for orotracheal intubation after 15 minutes. Animals were randomly assigned to two groups according to the intranasal solution administered before intubation: lidocaine 2% (Group L) or 0.9% NaCl (Group C). Total intranasal volume was adjusted to body weight in both groups: 0.2 mL for rabbits weighing 0.5-1.5 kg, 0.3 mL for rabbits weighing > 1.5-3 kg, and 0.4 mL for those > 3 kg. Intubations were performed by three experienced operators blinded to treatment allocation, using a video-endoscope. Clinical efficacy of intranasal lidocaine was evaluated by total procedure time (from intranasal administration to successful orotracheal intubation), number of intubation attempts, and occurrence of laryngospasm. Intubation quality was assessed using a simple descriptive scale from 0 to 3, where 0 indicated impossible intubation, and 3 a smooth, uncomplicated procedure.

Total procedure time (minutes) was significantly longer in Group C (median 11; range 6-18) than in Group L (median 8; range 5-18) ($p < 0.01$). The median number of intubation attempts was significantly lower in Group L (1; range 1-2) compared with Group C (2; range 1-3) ($p < 0.01$). Laryngospasm occurred significantly more often in Group C than in Group L ($p < 0.0001$). Additionally, rabbits in Group C required significantly higher doses of alfaxalone to allow orotracheal intubation compared with rabbits in Group L ($p < 0.01$). Overall intubation quality was significantly higher in Group L (3, range 1-3) than in Group C (1, range 0-3) ($p < 0.001$).

Intranasal lidocaine significantly facilitates orotracheal intubation in rabbits by reducing procedure time, minimizing the attempts, decreasing the incidence of laryngospasm, and lowering the requirement for additional anaesthetic induction agents. This minimally invasive technique may represent a valuable adjunct to airway management protocols in rabbits and overall anaesthetic safety in this species.

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Oral Communication(s)

NOVEL TECHNIQUE FOR THE PUDENDAL NERVE BLOCK: PRELIMINARY RESULTS OF A CADAVERIC AND CLINICAL STUDY

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Perineal surgical procedures in dogs are commonly associated with significant intra and postoperative pain, making effective locoregional analgesia a key component of multimodal anesthetic protocols. The pudendal nerve block represents a promising technique; however, limited data are available regarding ultrasound-guided approaches in clinical settings [1]. The aim of this study was to evaluate the analgesic efficacy of an ultrasound-guided pudendal nerve block in dogs undergoing perineal surgery. The study was conducted at the Veterinary Teaching Hospital and consisted of a cadaveric and a clinical phase. In the cadaveric phase, two canine cadavers, donated for research purposes and deceased due to causes unrelated to the study, were used to investigate the sono-anatomy of the pudendal nerve. A linear probe was positioned transversely along the midline between the anus and the ischial tuberosity. After bilateral identification of the pudendal nerve, a spinal needle was advanced in-plane from a lateral approach, and 0.05 mL/kg of 1% methylene blue was injected. The procedure was then repeated contralaterally to confirm technique reproducibility. The clinical phase (OPBA approval number 11/2025 of 12/02/2025) included eight client-owned dogs of various breeds and ages undergoing perineal surgical procedures, including perineal herniorrhaphy, anal sacculectomy, and excision of perineal masses. Premedication consisted of intramuscular injection of dexmedetomidine (5 µg/kg), ketamine (1 mg/kg), and methadone (0.2 mg/kg). General anesthesia was induced with intravenous propofol or alfaxalone and maintained with inhalational agents. The pudendal nerve block was performed using the ultrasound-guided technique established in the cadaveric phase, administering 0.5% ropivacaine 1 mg/kg per point. Intraoperative monitoring was performed at 5-minute intervals. Rescue analgesia with fentanyl (1 mcg/kg IV) was administered if an abrupt increase of >15% in heart rate and blood pressure was recorded. Postoperative pain was assessed hourly using the 4Vet Pain Scale, and methadone was administered if scores reached ≥5. Robenacoxib 2 mg/kg was administered with methadone rescue analgesia or 12 hours after the block. Eight dogs were included in the study, of these 2/8 dogs (25%) required intraoperative rescue analgesia. One dog was excluded from postoperative analysis due to incomplete data. Among the remaining seven dogs, four required postoperative methadone, whereas three did not require any additional analgesia during the observation period. Methadone administration occurred at variable time points: 6 h 18 min, 17 h 45 min, 12 h 15 min, and 10 h 20 min after block execution. By integrating these data with those from dogs not requiring rescue analgesia, the estimated mean minimum duration of block efficacy was approximately 12 h 40 min. In conclusion, the ultrasound-guided pudendal nerve block appears to provide effective and prolonged analgesia in dogs undergoing perineal surgery. Although limited by the small sample size and descriptive design, these findings are consistent with current veterinary literature and support the clinical applicability of this technique within multimodal perioperative analgesic protocols. Further studies with larger populations and standardized methodologies are warranted to confirm and expand these results.

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Oral Communication(s)

COMPARISON OF TRANSVERSUS ABDOMINIS PLANE BLOCK, QUADRATUS LUMBORUM BLOCK, AND SYSTEMIC ANALGESIC MANAGEMENT FOR POSTOPERATIVE PAIN CONTROL IN DOGS UNDERGOING MAJOR ABDOMINAL SURGERY

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In dogs, major abdominal surgeries are associated with moderate to severe pain, involving both somatic and visceral components [1]. Interfascial plane blocks, particularly the transversus abdominis plane (TAP) block and the quadratus lumborum (QL) block, provide effective control of somatic pain; however, their efficacy in managing visceral pain remains debated [2]. While in humans these techniques reduced the requirement for systemic analgesics and their associated side effects [3], their use in the postoperative period in dogs is rarely reported. This study aimed to evaluate the postoperative analgesic efficacy of TAP and QL blocks, compared with systemic analgesia alone, in dogs undergoing major abdominal surgery (OPBA 26_2026). All patients were premedicated with intramuscular (IM) methadone and, if additional sedation was required, dexmedetomidine. Anesthesia was induced with intravenous (IV) propofol and ketamine (1:1 admixture) and maintained with isoflurane in oxygen. At the end of surgery, patients were randomly assigned to one of three groups: the TAP group received an ultrasound (US)-guided TAP block; the QL group received an US-guided QL block (both performed with 0.25% ropivacaine); the control group (C) received no block. Postoperative pain was blindly assessed at scheduled time points over 24 hours, using the Short-form Glasgow Composite Pain Scale (SF-GCMPS) and the Visual Analogue Scale (VAS). In addition, sedation level, heart rate, respiratory rate, non-invasive blood pressure, rectal temperature, and the time elapsed from extubation to spontaneous feeding and urination were recorded. Methadone (0.2 mg/kg; IM) was administered as rescue analgesia when required. A total of 21 dogs were included in the study (7 per group). SF-GCMPS scores were significantly higher in the C group compared to both TAP and QL groups at 4, 5, 9, and 12 hours post-extubation, and compared to the QL group only at 6 and 24 hours ($p < 0.05$). Similarly, VAS scores were significantly higher in the C group compared to both TAP and QL groups at 1.5 hours and from 3 to 24 hours after extubation ($p < 0.05$). No significant differences were found among groups regarding sedation scores, physiological parameters, or time to spontaneous feeding and urination. Rescue analgesia was required in 5 dogs in the C group, with a significant difference among groups ($p = 0.003$). Both TAP and QL blocks provided superior postoperative analgesia compared to the C group, supporting the inclusion of interfascial plane blocks in multimodal analgesic protocols for major abdominal surgery in dogs. Analgesic quality was comparable between the two techniques, with no apparent differences in the control of somatic versus visceral pain. In conclusion, TAP and QL blocks are effective for postoperative analgesia, allowing reduced opioid consumption and improved pain control in dogs undergoing major abdominal surgery.

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Oral Communication(s)

SMARTPHONE-BASED ELECTROCARDIOGRAPHY IN CATS RECOVERING FROM ANAESTHESIA: ACCURACY OF TWO DEVICES FOR HEART RATE AND RHYTHMASSESSMENT

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Assessment of heart rate and cardiac rhythm in cats can be challenging [1], particularly in anesthesia and emergency settings where rapid and reliable monitoring is required. Smartphone-based electrocardiogram (ECG) devices represent a portable alternative to standard ECG [2], but their accuracy in feline patients remains insufficiently investigated and warrants further evaluation.

This prospective pilot study evaluated the accuracy and feasibility of two smartphone-based ECG devices for heart rate (HR) and rhythm monitoring in cats recovering from anesthesia, using a standard ECG as gold standard (GS) reference.

Eighteen client-owned cats undergoing general anesthesia for clinical or surgical procedures were included. ECG recordings were collected for 30 seconds during recovery, with three traces acquired simultaneously in right lateral recumbency. Recordings included Kardia, Ekuore, and a standard ECG. Hair clipping was not performed, and alcohol was applied to improve skin–electrode contact. Trace readability was assessed independently by two emergency clinicians with different levels of experience (one junior and one senior). Inter-observer agreement was evaluated using Cohen's kappa. HR measurements from smartphone devices were compared with the GS ECG.

The median age was 5.7 years (range 0.5–13.8); one cat had a cardiac murmur. Readable ECG traces were obtained in 18/18 recordings (100%) with the GS, 12/18 (67%) with Kardia, and 13/18 (72%) with Ekuore. All cats showed sinus rhythm; one had a right bundle branch block. Two cats exhibited tremors during recording; smartphone-based tracings remained readable. HR measurements showed strong correlation with the GS ECG for both devices (Kardia $r = 0.95$; Ekuore $r = 0.94$). Inter-observer agreement for trace readability was excellent (Cohen's $\kappa = 0.91$).

While overall readability was moderate, particularly for Kardia, the compact size and portability of both devices make them attractive for perianesthetic monitoring where standard equipment is impractical. Smartphone-based ECGs can serve as a useful adjunct for rhythm assessment in such contexts. Further studies are warranted to assess performance in cats with arrhythmias and to better define their role in diverse clinical and emergency settings.

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Oral Communication(s)

INTERFASCIAL PLANE BLOCKS FOR PERIOPERATIVE ANALGESIA IN DOGS UNDERGOING LAPAROSCOPIC OVARIECTOMY: A RETROSPECTIVE STUDY (2020-2025)

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Ultrasound-guided interfascial plane blocks are increasingly used to improve perioperative analgesia in dogs undergoing laparoscopic procedures, although comparative clinical data remain limited. This retrospective study evaluated 97 client-owned dogs undergoing laparoscopic ovariectomy to compare transversus abdominis plane (TAP), quadratus lumborum (QL), and caudal thoracic paravertebral (C-TPV) blocks in terms of execution time, analgesic requirements, and perioperative safety. Medical records from 109 dogs were reviewed; 12 cases were excluded due to conversion to open surgery or incomplete records. Intraoperative fentanyl requirements differed significantly among groups ($p < 0.01$), with the highest requirements observed in the TAP group, intermediate values in the QL group, and the lowest in the C-TPV group. Block execution time also differed significantly ($p < 0.01$), with QL showing the shortest duration, followed by C-TPV and TAP. Postoperative methadone requirements were minimal across all groups, with a statistically significant difference detected only between C-TPV and TAP ($p = 0.03$), although absolute differences were clinically negligible. The incidence of complications and intraoperative hypotension was low and comparable among groups. Overall, C-TPV was associated with reduced intraoperative opioid requirements, whereas QL provided the fastest execution, while TAP showed less favorable intraoperative analgesic performance. These findings highlight clinically relevant differences among techniques, supporting the preferential use of C-TPV when opioid-sparing strategies are prioritized and QL when procedural efficiency is critical. Prospective controlled studies are warranted to confirm these results.

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Oral Communication(s)

EFFICACY OF THE COCCYGEAL EPIDURAL FOR THE PERI-OPERATIVE MANAGEMENT OF BITCHES UNDERGOING CYSTOSCOPY: PRELIMINARY RESULTS

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The epidural coccygeal (EpC) is a local anaesthetic technique effective for post-operative pain management in dogs after cystoscopy [1]. The aim of the study was to assess the effects of the EpC on the anaesthetic management of bitches undergoing cystoscopy. We hypothesised that EpC might improve the anaesthetic stability and pain control. Female dogs undergoing cystoscopy were included in the study. Dogs were premedicated with acepromazine (0.02 mg/kg) and methadone (0.1 mg/kg). Anestesia was induced with propofol and maintained with isoflurane. Dogs were randomly assigned to an EPI group (a EpC was performed at the S3-C1 intervertebral space with 0.1 mL/kg 0.3% ropivacaine) and a CON group (EpC was omitted). At the end of the cystoscopy 0.25% bupivacaine was instilled in the urethra in all dogs. Before premedication (baseline), before the EpC (Tepi), 5 min before starting the cystoscopy (T0) and 1 hour after extubation (T1) the following parameters were recorded by a blinded observer: heart rate (HR), respiratory rate (fR), blood pressure (BP) and temperature (T). Intraoperatively, the HR, the BP, the fraction of expired isoflurane (FE'Iso) and CO2 (FE'CO2) were evaluated by an anaesthesist blinded to the treatment. A 20% increase of the HR or the BP with respect to T0 was considered indicative of insufficient analgesia and fentanyl was administered accordingly. Post-operatively, at T1, pain was assessed using a Visual analogue scale (VAS) and the Glasgow composite pain scale sort form (GCPS-SF), motor block [2], the sedation and the recovery score were evaluated. Finally, the procedural difficulty score was graded. Data were evaluated for normality by using a Shapiro-Wilk test and compared between the two groups with a Student-t test or a Mann-Whitney test. Differences over time in each group were evaluated with a one way ANOVA.

Eleven dogs were included: 5 in group EPI and 6 in group CON. No statistically significant differences were observed between the two groups at any time point. Intraoperatively, the mean HR was 87 ± 18 bpm in the EPI group and 86 ± 19 bpm in the CON group; the mean MAP was 64 ± 7 mmHg in the EPI group and 66 ± 13 mmHg in the CON group. The mean FE'Iso % was 1.1 ± 0.1 in the EPI group and 1.1 ± 0.2 in the CON. The recovery and the pain score did not differ significantly between groups: at T1 the median recovery score was 3 (3-4) in both groups, the median VAS was 3 (1-3) in the EPI group and 2 (1-4) in group CON, the median GCPS score was 3 (1-6) in group EPI and 3 (1-5) in the CON group. None of the animals developed motor block and the overall procedural difficulty did not differ significantly between the two groups.

In conclusion, in bitches undergoing cystoscopy, balanced anaesthesia was effective in obtaining a stable anaesthetic plane and a sparing effect on isoflurane requirement with respect to the minimum alveolar concentration described in dogs [3]. The EpC was not associated with motor block but did not improve the post-operative pain management compared with the local-anaesthetic instillation alone.

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Oral Communication(s)

EVALUATION OF CLINICAL EFFECTS AND PLASMA METABOLOMIC PROFILES AFTER THE ADMINISTRATION OF GRAPIPRANT IN DOGS AFFECTED BY OSTEOARTHRITIS: A PROSPECTIVE, OFF-ON–OFF, CLINICAL STUDY

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Osteoarthritis (OA) is a progressive and degenerative joint disorder that affects the entire joint organ and represents one of the most prevalent chronic musculoskeletal diseases in dogs, with up to 20% of animals over one year of age being affected [1]. Clinically, it manifests with stiffness, reduced mobility, and a decline in quality of life. Large breed dogs show a higher predisposition, with contributing factors such as genetic background, previous trauma, obesity, and aging. Growing evidence indicates that OA should be regarded not only as a degenerative disorder but also as a condition characterized by persistent low grade inflammation and metabolic dysregulation. The primary treatment for canine OA relies on NSAIDs, which provide analgesia but may cause gastrointestinal, renal, or hepatic adverse effects with prolonged use. Grapiprant, a first in class EP4 receptor antagonist, provides targeted inhibition of PGE_2 mediated pain and inflammation with a favorable safety profile. Clinical trials show it effectively reduces pain and improves mobility in dogs with OA compared to conventional NSAIDs [2]. OA is associated with alterations in amino acids, lipids, lactate, and energy related metabolites, reflecting mitochondrial dysfunction, hypoxia, and inflammation. Metabolomics represents a robust approach for investigating the biochemical alterations that arise during OA. Through ^1H NMR spectroscopy, the metabolomic profiling of serum and synovial fluid enables the assessment of systemic and local metabolic changes. The aim of this prospective, randomized study is to evaluate the clinical efficacy of grapiprant in dogs affected by moderate to severe OA and to assess its effects on the metabolomic profile [3].

Thirty-six dogs with mild to moderate OA were enrolled in a prospective off-on–off study (30 days pre-treatment, 60 days grapiprant treatment at 2 mg/kg, 30 days post-treatment). Outcomes included owner questionnaires (LOAD, CBPI), pressure-sensitive walkway gait analysis (GLS), orthopedic evaluation, and serum metabolomics.

Grapiprant significantly reduced LOAD scores at T30 and T60 compared with baseline ($^*p < 0.05$), with partial regression at TPOST. GLS improved significantly, exceeding the 15% threshold considered clinically relevant at T60 ($^{**}p < 0.01$). Metabolomic analysis revealed distinct separation between pre- and post-treatment serum samples, with significant reductions in lactate, N-acetyl glycoproteins, and formate, and an increase in citrate ($p < 0.05$).

Grapiprant improved mobility and pain control in dogs with OA and induced metabolomic shifts consistent with reduced inflammation and improved energy metabolism. This is the first study to integrate clinical outcomes with metabolomics in canine OA, supporting the translational value of metabolic biomarkers for monitoring therapeutic response.

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Oral Communication(s)

EFFECTS OF MAROPITANT-MEDETOMIDINE-BUTORPHANOL VS. KETAMINE-MEDETOMIDINE-BUTORPHANOL ON CARDIORESPIRATORY VARIABLES, SEDATION AND RECOVERY QUALITY IN HEALTHY SHELTER CATS SEDATED FOR ECHOCARDIOGRAPHIC SCREENING FOR HEARTWORM DISEASE. PRELIMINARY RESULTS

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Intramuscular (IM) sedation is often preferred in cats due to their uncooperative nature [1]. Aim of the study was to evaluate the cardiorespiratory effects and the sedative and recovery quality of maropitant vs ketamine, both in combination with medetomidine and butorphanol in healthy shelter cats. The study was approved by the Ethical Committee of the University of Sassari (prot. N.0101145/2023). Twenty healthy shelter cats were IM administered with medetomidine 20 µg/kg and butorphanol 0.4 mg/kg and randomly divided into two groups of 10 cats each, to receive either maropitant 1 mg/kg (MBM group) or ketamine 3 mg/kg (MBK group). Each cat was monitored in a light- and noise-protected cage to assess sedation and detect any adverse effect. Oscillometric noninvasive mean arterial pressure (MAP), heart rate (HR), and respiratory rate (RR) were recorded at the sedation achieved (T0) and after 5, 10, 20, 30, 40 and 50 minutes (T1-T6). Sedation and recovery quality were evaluated according to the scale described by Volpato [2]. At sedation achieved, blood collection from jugular vein for hematological screening and cephalic cannulation for fluid infusion and for propofol rescue sedation were performed. Transthoracic standard echocardiographic evaluation was performed between 10 and 40 min. Atipamezole IM 10 µg/kg was administered in cats still sedated after 70 min. All data of each cat were detected by two operators unaware of the drugs combination administered, and comparison between the two groups at each time point by Mann Whitney test was performed, with significance for $P<0.05$. Median MAP values from T0 to T6 were significantly higher in MBK group than in MBM group (157, 156, 154, 149, 144, 135, 129 and 129, 128, 125, 120, 116, 114, 112 mmHg respectively) while median HR values were significantly higher only from T0 to T4 in MBK group than in MBM group (123, 122, 119, 118, 120 and 102, 100, 99, 97, 95 beats/min respectively). On the contrary, median RR values were significantly higher at each time point in MBM group than in MBK group (37, 38, 37, 36, 37, 36, 36 and 31, 31, 32, 32, 30, 31, 30 breaths/min respectively). Propofol administrations and total doses were higher in MBK group than in MBM group without significance. Sedation was achieved significantly earlier in MBK than in MBM group (6 and 9 min respectively). Sedation depth was found intense in MBK group (score 3) and moderate in MBM group (score 2), without significance. Sedation length was significantly shorter in cats of MBM group than in MBK group (58 and 68 min respectively), and atipamezole administration was required in 2 cats of MBM group and in 5 cats of MBK group. Recovery quality was excellent (score 0) in all cats of MBM group, both antagonized and non-antagonized; in MBK group it was excellent (score 0) in the 5 non-antagonized cats and satisfactory (score 2) in the antagonized ones. Both ketamine and maropitant, in combination with butorphanol and medetomidine at the doses used here, contribute to obtaining good sedation in the cat, of sufficient duration for the echocardiographic examination. If medetomidine is antagonized, recovery is better with maropitant than with ketamine.

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Oral Communication(s)

SAFETY AND EFFICACY OF NON-INVASIVE PRESSURE SUPPORT VENTILATION VIA CPAP HELMET IN DOGS RECOVERING FROM GENERAL ANESTHESIA: A PRELIMINARY STUDY

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Non-invasive respiratory support using continuous positive airway pressure (CPAP) delivered via helmet is increasingly used in veterinary medicine in both pathological and perioperative settings.[1] Recovery from general anesthesia is critical even in healthy dogs, as anesthetic and analgesic effects may transiently impair respiration, causing ineffective breathing and postoperative hypoxemia, requiring ventilatory support. While the effectiveness of CPAP delivered via helmet is well documented in the literature, the application of pressure support ventilation (PSV) as a non-invasive ventilation approach (NIV) in veterinary patients remains poorly investigated. The aim of this clinical study was to evaluate the safety and efficacy of non-invasive PSV delivered through a CPAP helmet in dogs during recovery from general anesthesia. Normoxemic dogs meeting inclusion criteria (body weight >5 kg, ASA I–III, no major cardiopulmonary disease) were enrolled. Following extubation, the AirTest (measurement of peripheral haemoglobin saturation -SpO_2- at room air) was performed, and each normoxemic dog (SpO_2>95%) underwent three consecutive 10-minute NIV phases with an inspired oxygen fraction of 0.21: CPAP (5 cmH_2O), PSV (ΔP 7 cmH_2O + CPAP), and CPAP again, followed by a post-treatment AirTest. Parameters recorded included SpO_2, heart rate (HR), respiratory rate (RR), mean arterial pressure (MAP), temperature, sedation score, recovery quality, and interface tolerance. Respiratory mechanics were continuously monitored and recorded, as well as the leak percentage of the system. Diaphragmatic excursion and slope were also measured using M-mode ultrasound before to start (pre-NIV), during each study phase, and 5 min post-NIV. Statistical analysis was performed using appropriate parametric and non-parametric tests, with significance set at $p < 0.05$. Seven dogs completed the study, with no significant differences in HR, RR, MAP, or SpO_2 across study phases, confirming the safety of the technique. Peak airway pressure (Ppeak) was significantly higher during PSV compared to both CPAP phases (12.6±1.3 vs 6.6±2.6 and 5.9±0.4, $p < 0.001$). Importantly, tidal volume (VTe spn) significantly increased during PSV compared to both CPAP phases (439.6±199.2 vs 204.7± 3, $p = 0.006$ and 210.5±107.5, $p = 0.017$), indicating improved ventilatory effectiveness. Leak percentage did not differ significantly between phases, suggesting adequate system performance. Ultrasound evaluation revealed a significant increase in diaphragmatic movement during PSV compared to pre- and post-NIV measurements (1.59±0.63 vs 0.64±0.07, $p = 0.008$ and 0.56±0.01, $p = 0.004$), supporting a physiological enhancement of respiratory mechanics. No significant differences were observed in diaphragmatic slope, sedation score, or tolerance score over time. Four dogs were excluded due to helmet intolerance or failure of the helmet to maintain CPAP, highlighting potential limitations related to interface fit and patient variability. In conclusion, PSV via CPAP helmet was found to be a feasible, safe, and effective NIV technique in dogs recovering from general anesthesia, improving tidal volume and diaphragmatic function without affecting vital parameters. These preliminary findings support further investigation of non-invasive PSV in veterinary patients, including those with respiratory disease or postoperative hypoxemia.

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Oral Communication(s)

VOLUME SUPPORT PLUS (VS+), A NOVEL ASSISTED VENTILATORY MODE, IMPROVES CO_2 CONTROL DURING PNEUMOPERITONEUM IN CATS

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Volume Support Plus (VS+) is a novel assisted ventilation mode in veterinary medicine. It is a pressure support, volume-guaranteed modality with the additional capability to regulate end-tidal carbon dioxide ($ETCO_2$) within a predefined range by automatically adjusting tidal volume and respiratory rate based on measured CO_2 values. It supports spontaneous inspiratory efforts through an automatic trigger and provides backup ventilation in case of apnoea. This clinical study evaluated the performance of VS+ in anaesthetized cats undergoing laparoscopic ovariectomy, assessing its ability to maintain normocapnia while preserving spontaneous breathing compared with spontaneous ventilation (SV). We hypothesized that VS+ would improve ventilatory function during pneumoperitoneum. Fifteen female cats were enrolled (SV: n = 8; VS+: n = 7), with a mean body weight of 3.17 ± 0.47 kg and a mean age of 18.5 ± 15.1 months. In the VS+ group, ventilator settings included an $ETCO_2$ target of 35–45 mmHg, peak inspiratory pressure adjusted to maintain a driving pressure ≤ 10 cmH_2O, a baseline tidal volume of 10 mL/kg, and a backup respiratory rate of 6–8 breaths/min. Respiratory and physiological variables were recorded every 5 minutes. Data were analyzed at predefined time points relative to pneumoperitoneum: before insufflation (PPRE), and at 5 (PP5), 10 (PP10), 15 (PP15), and 20 minutes (PP20) after pneumoperitoneum, as well as after desufflation (PPOST). Non-parametric statistical methods were used. Variables differing at baseline were analyzed as changes from baseline. Between-group comparisons were performed using the Mann–Whitney U test, and within-group analyses for compliance and driving pressure were performed using the Wilcoxon signed-rank test. Baseline $ETCO_2$, respiratory rate (RR), and tidal volume (VT) differed between groups. Baseline $ETCO_2$ was higher in the VS+ group ($p = 0.006$). When changes from baseline were analyzed, $ETCO_2$ values were significantly higher in the SV group at PP5 ($p = 0.015$), PP15 ($p = 0.003$), PP20 ($p = 0.009$), and PPOST ($p = 0.006$). RR differed at baseline ($p = 0.015$) and was higher in the VS+ group at PP20 ($p = 0.031$) and PPOST ($p = 0.012$). Baseline VT was higher in the VS+ group ($p = 0.002$). No significant differences were observed for the remaining variables, including haemodynamic parameters. VS+ ventilation provided improved control of CO_2 elimination compared to spontaneous ventilation, without inducing clinically relevant haemodynamic alterations. These findings suggest that VS+ represents an effective and well-tolerated ventilatory strategy during pneumoperitoneum.

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Oral Communication(s)

COMPARISON OF SPONTANEOUS BREATHING AND VOLUME SUPPORT PLUS MODE VENTILATION ON CARDIOPULMONARY FUNCTION IN ANESTHETIZED NEW ZEALAND RABBITS

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General anesthesia in rabbits is associated with a high risk of hypoventilation and hypercapnia, especially when spontaneous breathing is maintained. Providing adequate ventilatory support in this species remains challenging [1]. The aim of this study was to evaluate the effects of two ventilation modalities in rabbits: spontaneous breathing (SB) and volume support plus mode (VS+) ventilation, focusing on hemodynamic stability and respiratory function.

This study received the approval of the Health Ministry (n° 236/2023-PR) in accordance with current Italy legislation on Animal experimentation (D.lgs. 26/2014).

Twelve healthy adult male New Zealand rabbits, homogeneous for age (12 months) and body weight (3.11 ± 0.12 kg), were enrolled in a randomized crossover study with a two-week washout period. Sedative drugs were administered subcutaneously (SC) in the interscapular region. Each animal received two sedation protocols: ketamine (7 mg/kg), dexmedetomidine (30 µg/kg), and buprenorphine (15 µg/kg) (KDB), and alfaxalone (2.5 mg/kg), dexmedetomidine (30 µg/kg), and buprenorphine (15 µg/kg) (ADB).

After sedation, a venous catheter was placed in the auricular vein for intravenous administration of drugs and fluids, and endotracheal intubation was performed using a blind technique with an appropriately sized endotracheal tube. Correct placement was confirmed by measuring end-tidal carbon dioxide ($EtCO_2$, mmHg).

Invasive arterial blood pressure was monitored via catheterization of the auricular artery using the PRAM monitor sistem. Systolic (SAP, mmHg), mean (MAP, mmHg), and diastolic (DAP, mmHg) arterial pressures, heart rate (HR, bpm), $EtCO_2$, respiratory rate (RR, breaths/min), oxygen saturation (SpO_2, %), and expiratory tidal volume (VTe) were recorded from baseline every 5 minutes to 30 minutes. Body temperature was maintained within the normothermic range using a forced-air warming system (Bair Hugger). Arterial blood gases ($PaCO_2$, PaO_2) were measured at the end of ventilation using CG8+ cartridges with the i-STAT analyzer.

Two ventilation strategies were evaluated: SB and mechanical ventilation using the VS+ mode of the Veta 5 Plus ventilator (Mindray), which adjusts ventilatory parameters based on real-time $EtCO_2$ monitoring. In the VS+ group, $EtCO_2$ was setted within 45–60 mmHg [2].

Statistical analysis was performed using GraphPad Prism. Two-way repeated-measures ANOVA followed by Bonferroni post-hoc test was used. A p-value < 0.05 was considered significant. SAP, MAP, and DAP remained stable throughout the study period, with no significant differences between sedation protocols, ventilation strategies, or their interaction. SAP ranged from 71.5 ± 2.7 to 81.3 ± 13.6 mmHg, MAP from 57.7 ± 3.97 to 66.3 ± 7.8 mmHg, and DAP from 47.7 ± 6.05 to 58.5 ± 6.5 mmHg. No difference in HR was observed between ventilation strategies (132.8 ± 21.4 bpm in SB vs 136.1 ± 21.7 bpm in VS+, $p = 0.17$).

$EtCO_2$ increased significantly over time during spontaneous breathing (68.4 ± 2.9 mmHg) and remaining lower and more stable under VS+ ventilation (60.3 ± 3.04 mmHg, $p = 0.007$).

$PaCO_2$ was significantly lower in VS+ (63.6 ± 12.4 mmHg) compared to SB (72.2 ± 8.3 mmHg, $p = 0.0003$), while PaO_2 was higher in VS+ (409.5 ± 55.9 mmHg) compared to SB (336.5 ± 88.09 mmHg) but not significantly ($p = 0.34$).

VTe was significantly higher in VS+ (35 ± 4.7 mL) compared to SB (15.5 ± 1.2 mL, $p < 0.05$), RR was lower in VS+ (9.7 ± 1.7 vs 17.8 ± 2.5 breaths/min, $p = 3.1$).

Ventilation using the VS+ mode significantly improved ventilation and gas exchange compared to spontaneous breathing in New Zealand rabbits, reducing hypercapnia and improving oxygenation without affecting hemodynamic stability. These findings support adaptive ventilation strategies to optimize respiratory management during anesthesia in this species.

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Oral Communication(s)

EX VIVO PORCINE CYSTIC DUCTS SEALED WITH BIPOLAR VESSEL SEALING SHOWED LESS THERMAL INJURY AND BETTER HISTOLOGIC FUSION THAN ULTRASONIC ENERGY

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Laparoscopic cholecystectomy is widely recognized as the standard approach for gallbladder removal in humans [1] and is increasingly being adopted in selected cases in dogs and cats. A crucial step in the procedure is the secure cystic duct ligation to prevent complications such as bile leakage, hemorrhage, or clip migration [2]. Although titanium clips are traditionally used, risks associated with clip displacement and long-term foreign body reactions have driven the exploration of alternative closure techniques [3].

This study aimed to compare ex vivo porcine cystic duct closure achieved with a bipolar vessel sealing device versus an ultrasonic dissector–coagulator, using histopathologic evaluation and measurements of thermal injury extent. Twelve cystic ducts were harvested from 12 female Large White pigs (Sus scrofa domesticus) aged 3–4 months. Between June and July 2025, the ducts were collected immediately after euthanasia and randomly assigned to closure with either a bipolar vessel sealing device or an ultrasonic dissector–coagulator; both instruments were new and unused. The sealed segments were then fixed, paraffin-embedded, sectioned, and stained with hematoxylin and eosin. A blinded pathologist applied a semiquantitative scoring system to assess local tissue reaction, wall necrosis, edema, epithelial integrity (each scored from 0 to 3), and fusion quality (scored from 0 to 3 on a reverse scale). The extent of thermal injury (µm) was quantified using histomorphometric analysis.

All specimens demonstrated complete gross closure without visible bile leakage. Compared with bipolar sealing, ultrasonic energy produced significantly greater overall histologic damage (median total score 10 [IQR 9–11] vs 2 [IQR 2–2]; P = .004) and a larger extent of thermal injury (mean ± SD 1,308 ± 129 µm vs 974 ± 35 µm; P = .001). Fusion quality scores were significantly higher in the bipolar group (P = .006).

In this ex vivo porcine model, bipolar vessel sealing resulted in less thermal injury and more uniform histologic fusion of the cystic duct than ultrasonic energy. However, as burst-pressure testing and in vivo validation were not performed, these findings should be considered preliminary morphologic evidence requiring further functional and clinical confirmation.

Keywords: cystic duct; bipolar vessel sealing device; ultrasonic energy; histopathology; laparoscopy.

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Oral Communication(s)

PREVALENCE OF PROXIMAL TIBIAL RADIOLUCENCIES IN DOGS AFFECTED BY CRANIAL CRUCIATE LIGAMENT DISEASE: A RETROSPECTIVE RADIOGRAPHIC EVALUATION

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Proximal tibial radiolucencies identified on mediolateral radiographs of certain canine stifles have been described in a single prior study and were proposed to represent retained cartilage cores. In the affected dogs, an association with medial patellar luxation and a decreased odds of cranial cruciate ligament rupture (CCLR) was found. Our retrospective study aimed to determine the prevalence of proximal tibial radiolucencies in a population of dogs, predominantly affected by CCLR. Mediolateral radiographs of single or both stifles from 110 dogs presented for CCRL at the veterinary hospital of the University of Padova between 2019 and 2026 were reviewed for the presence or absence of proximal tibial radiolucency. When identified, lesions were graded using a three-point scoring system based on diameter and severity of radiolucency. In our study, proximal tibial radiolucency was identified in a higher proportion of dogs than in the previously reported study. The relatively high prevalence observed in dogs with CCLR suggests a potentially different association than previously described. Further investigations are warranted to clarify the underlying etiology and clinical significance of these radiographic findings, particularly in dogs with high-grade proximal tibial lesions that may undergo tibial plateau leveling osteotomy (TPLO) for the management of CCL disease.

Oral Communication(s)

IMPACT OF MECHANICAL UNLOADING ON THE IRISIN PRECURSOR, FNDC5, IN SKELETAL MUSCLE OF SHEEP AS A LARGE ANIMAL EXPERIMENTAL MODEL

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Skeletal muscle is a highly dynamic tissue that responds to various stimuli, especially changes in mechanical load. Consequently, the disuse of skeletal muscle directly affects the phenotype of muscle fibers, influencing morphological characteristics and protein expression. One of the muscle proteins that is a key load-sensitive molecule is the precursor of the myokine irisin, the transmembrane protein Fibronectin type III domain-containing protein 5 (FNDC5). Irisin is produced by skeletal muscle during contraction, as a secreted portion of FNDC5. Studies in mouse models have shown that treatment with recombinant irisin prevents the onset of muscle atrophy caused by disuse or denervation. Considering the numerous preclinical validations in mouse models and in view of the results obtained from observational studies in humans, the development of an irisin-based pharmaceutical formulation could be a future perspective. This type of drug therapy could be used in all patients in whom immobilization, or metabolic and neurodegenerative diseases result in severe decline of the musculoskeletal system. In the present study, we evaluated over the period of 1-2 weeks following immobilization the impact of the absence of mechanical loading on the expression of FNDC5 and functional proteins in quadriceps muscle of a sheep model. The selected sheep (N=20), 3-year-old sheep weighing 45±5.1 kg. At the end of the authorized experimental period and after euthanasia of the sheep, muscle biopsy samples were collected from the quadriceps femoris at the time of sacrifice. The first group of sheep (N=8) was sacrificed 1 week after surgery, and the second group of sheep (N=12) was sacrificed 2 weeks after surgery. The right limbs were always operated for an implant of a medial meniscal prothesis, while the left limb when was used as a control (contralateral). Muscle biopsy samples consisted of 10 unloaded hindlimb (HU) and 6 contralateral hindlimb at the 1-week time point, and 19 HU and 5 contralateral at the 2-week time point. The samples were histologically and immunohistochemistry examined and PCR assessment were conducted . The results showed that mechanical unloading caused a reduction of FNDC5 in sheep quadriceps, which was already evident after 1 week, but became significant after 2 weeks. The decrease in FNDC5 was accompanied by a significant reduction in the expression of myosin isoforms MyHC1 and MyHC2x and an increase in muscle fibrosis. A positive correlation between FNDC5 and myosins was also observed, suggesting that the irisin/FNDC5 system is a driving force for muscle function also in this large animal model.The results of this study, despite some limitations, can improve the translational pathway. The sheep model has the advantage of improving musculoskeletal translation, particularly due to the body size, long lifespan, and similarities in anatomical structures of sheep with other animals and to humans.

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Oral Communication(s)

RAPID OPEN APPROACH FOR BILATERAL KIDNEY ISCHEMIA AND REPERFUSION IN A SWINE MODEL OF ISCHEMIA REPERFUSION INJURY

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In renal Ischemia reperfusion injury (IRI) the animal experimental models are crucial, as there are only a limited number of in vitro models. As experimental model of IRI in literature are documented mouse, rats, miniature pigs or farm pigs. Indeed, preclinical studies using pig can play an important role in the evaluation of new medical devices and pharmacological therapy efficacy before their use in clinical studies. To produce IRI it is necessary to close all kidney vessels or to close the arteries alone. In the open access these procedures significantly lengthen surgical times and increase the risk of visceral damage for the presence of bowels and the necessity of large intestinal retractors. In this study our primary aim was the reduction of surgical time for legation of vein and artery for both kidneys. Our secondary aim was to evaluate the variation of mean arterial blood pressure (MAP) during the procedure. After approval by the Italian Ministry of Health (n° 986/2023-PR 25) 30 pigs of 40□2 Kg., were divided randomly in two group (Group A, Group B). With animals in general anaesthesia, an approximately 15 cm incision was made on the linea alba cranial and posterior to the umbilical scar. In Group A we used the traditional technique; in group B the small and large intestine were removed from the abdominal cavity and place them in a bag and move them away from the operating field. With the new technique used the intestinal package was extracted from the first Teflon pocket and gently passed into the second Teflon pocket on the opposite side. The kidneys were highlighted to reach the renal vessels, the same was detached with a vascular dissector to free the vessels which were closed together with a vessel loop passed twice around the vein and artery. To complete the closure, the two ends of the vessel loops were passed inside a piece of Foley catheter which was then pushed and tensioned at the level of the vessels and blocked with a hemostatic clamp. Then small and large intestine were repositioned into the abdominal cavity which was temporarily closed with a laparotomy gauze until the end of the ischemic period (1 hour). During the procedures the aneshesiological parameters, the ischemic time, the time of surgery were monitored. The hemodynamic changes due to secondary venous stasis of the mesenteric vessels were also assessed at the time of intestinal displacement within the pouches. After 60 minutes from the ischemia, the vessel loupes on both sides were removed and the laparotomic incision was closed routinely. The data were analyzed with GraphPad. We obtained a statistically significant reduction of the time of surgery (T Student test for means confrontation) while the MAP values remain similar (74,40 vs 77,40) during the displacement of the intestine. This paper provides a simpler open surgical approach for the bilateral closure of kidneys vessels for bilateral ischemia and reperfusion that makes the surgical porcine model easier and fast. During the surgery we did not have any complication so we can assume this new approach can be a valid alternative to the traditional open ischemia and reperfusion surgical techniques in a swine model.

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Oral Communication(s)

CUSTOM-DESIGNED SCAFFOLD FOR BONE TISSUE ENGINEERING: AN *IN VIVO* STUDY

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Critical-sized bone defects often cannot be fully healed with conventional grafting techniques. Bone tissue engineering offers alternative strategies using natural or synthetic materials to enhance bone regeneration. The objective of this study was to evaluate the biological performance of custom-designed 3D-printed scaffolds made from a novel composite material for potential orthopedic applications. All the laboratory animal procedures in this study were approved by the Italian Ministry of Health (authorization number 224/2022-PR). Twenty Sprague-Dawley rats were used, each receiving two critical-sized calvarial defects: one treated with the scaffold and one left untreated as a control. Animals were euthanized at four and eight weeks for micro-CT and histological analyses. Results of both analyses showed significantly greater bone regeneration in the scaffold-treated defects compared to controls at both time points ($p < 0.05$). Histological evaluation also revealed significantly lower inflammation and hemorrhage in the scaffold group ($p < 0.05$). These findings indicate that the composite scaffold is biocompatible and promotes osteogenesis, suggesting its potential as an alternative bone substitute. Further studies are needed before clinical application.

Oral Communication(s)

DAILY ORAL ADMINISTRATION OF ANCHOVY COLLAGEN HYDROLYSATES MODULATES INFLAMMATORY RESPONSE IN MURINE COLLAGEN-INDUCED ARTHRITIS

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Arthritic disorders, including osteoarthritis and rheumatoid arthritis, are among the leading causes of chronic pain, functional limitation and reduced quality of life in both humans and animals. Despite the availability of several therapeutic options, long-term use of conventional drugs may be associated with relevant adverse effects. Therefore, the identification of innovative and safe complementary strategies able to modulate joint inflammation represents a key objective of translational veterinary research. In this context, the potential nutraceutical applications of marine-derived bioactive compounds are being increasingly investigated. These peptides can capture and neutralize free radicals, preventing oxidative damage to cells and tissues. The aim of this study was to investigate the protective effect of marine collagen hydrolysates obtainedfrom anchovy waste (ACHs) on the modulation of the inflammatory response in mice with collagen-induced arthritis (CIA). Hydrolysates were produced through controlled enzymatic hydrolysis and were preliminarily characterized in vitro on murine cell lines for their antioxidant and anti-inflammatory properties. The mice were randomly divided into 4 groups (n=12 for each group): CIA Group: mice with CIA not treated with ACHs; Sham Group: mice kept under the same experimental conditions not subjected to CIA; CIA-ACHs low dosage: mice treated with a low concentration of ACHs (3,8 mg/Kg/day for 4 weeks); CIA-ACHs high dosage: mice treated with a high concentration of ACHs (38 mg/Kg/day for 4 weeks). CIA was induced by two intradermal injections of 100 µl of an emulsion of chicken collagen (CII) and complete Freund's adjuvant on day 0 and 2[1] At 49-day post CIA induction, the animals were sacrificed. CII injection caused erythema and edema in the paws, histological alterations with lymphocytes, macrophages and neutrophils cells infiltration of joints, hyperplasia of synoviocytes, bone and cartilage erosion with increased osteoclasts and pannus formation, as well as behavioral changes. Oral administration of ACHs starting at the onset of arthritis ameliorated the clinical signs, improved behavioral deficits as well as decreased histological and radiographic alterations. Inflammation of synovia as well as bone and cartilage damages were reduced. The transcript levels of the target genes associated with oxidative stress markers, Superoxide dismutase (SOD), Glutathione peroxidase (GSH-Px), Catalase (Cat), and inflammatory markers (iNOS, COX-2, NF-κB, ICAM-1, VCAM-1, IκBα, IκBγ, TNF-α and IL-1β) were quantified by quantitative Real Time PCR. The observed molecular modulations indicate that ACHs supplementation exerts a protective effect on oxidative stress pathways and anti-inflammatory activities by inducing the downregulation of master pro-inflammatory regulators that are increased in CIA animals. Therefore, ACHs could represent a potential supplement to support joint health in the treatment of rheumathoid arthritis.

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Oral Communication(s)

MODIFIED TRANSANAL PROCTECTOMY IN THE MANAGEMENT OF CHRONIC “SO CALLED” PERIANAL FISTULAE IN DOGS: A PRELIMINARY STUDY WITH LONG TERM FOLLOW-UP

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Surgical treatment of ulcerative lesions constantly present in the circumanal region in the so called perianal fistulas disease in dogs has historically been considered therapeutic since 1984 [2]. The improvement in knowledge of the disease and the consequent resolution of the terminological confusion about the lesion classification, has profoundly changed diagnostic and therapeutic approaches over the years with the adoption of topical protocols using immunosuppressive drugs like Tacrolimus [1]. However, there are no studies in the literature with a high level of scientific evidence that definitively establish guidelines for the management of ulcerative lesions, and treatment with immunosuppressive drugs may not guarantee effective responses in all patients, with the presence of "partial remissions" which determine a slow and constant progression of the disease [1]. It is clear that in such patients, a multimodal approach protocol based also on surgery is necessary (2,3).

The determining factor in the elective surgical technique to be employed is essentially linked to the extent of the ulcerative lesions in the anal region itself, as well as to secondary lesions related to the evolution of the pathological process [2].

The surgical technique recommended for extensive, chronic lesions is complete excision with transanal proctectomy. The technique described involves excision of the anal sphincter and rectum, reaching the columnar region, removal of anal sacs, followed by a rectocutaneous anastomosis. Significant complications of this technique include fecal incontinence or inappropriate defecation, and rectal stenosis [2,3].

The aim of this study is to propose a proctectomy procedure for patients with chronic ulcers with secondary proliferative lesions who are unresponsive or partially responsive to immunosuppressant treatment. This technique involves anatomical dissection of the rectum, identifying the insertion and extension of the internal sphincter muscle and performing the proctectomy while preserving the greatest extent of the muscle.

The results of a follow-up of at least 6 months are discussed in five male canine patients with evidence of extensive bilateral ulcerative processes, partially or totally unresponsive to cyclosporine-based protocols, who underwent modified transanal proctectomy at the University Veterinary Hospital of Lodi between January 2024 and July 2025.

All treated patients had histopathologic confirmation of chronic ulceration with concurrent septic inflammation of anal sacs and showed complete resolution of the process without the use of topical immunosuppressive therapies. No patient included in the study presented complications such as incontinence, inappropriate defecation or rectal stenosis.

The choice to anatomically preserve the internal sphincter muscle appears to guarantee better functional surgical results than the proctectomy technique extended to the columnar region.

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Oral Communication(s)

ACUTE HYDRATED NUCLEUS PULPOSUS EXTRUSION IN DOGS WITH SEVERE NEUROLOGICAL STATUS: COMPARISON BETWEEN CONSERVATIVE AND SURGICAL TREATMENT

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Hydrated nucleus pulposus extrusion (HNPE) is a form of intervertebral disc herniation in dogs characterised by extrusion of hydrated or partially degenerated nucleus pulposus, leading to spinal cord compression. Magnetic resonance imaging (MRI) is the gold standard for diagnosis, enabling evaluation of compression and spinal cord changes. Optimal treatment remains debated. Recent studies suggest conservative management may achieve outcomes comparable to surgery, but these studies included dogs with a wide range of neurological severity.

This retrospective multicenter study compared outcomes in non-ambulatory paretic or plegic dogs with HNPE treated either conservatively or surgically, and aimed to identify prognostic factors.

Dogs with MRI-confirmed HNPE were included. Data collected included signalment (age, sex, breed type, body weight), neurological grade (from grade 3: non-ambulatory paresis to grade 5: paralysis without deep pain), neurolocalisation (C1–C5, C6–T2, T3–L3, L4–S3 spinal cord segment), MRI findings (location and appearance of extruded disc material, intramedullary hyperintensity, degree of spinal compression), and treatment type. Conservative management consisted of steroidal or non-steroidal anti-inflammatory drugs, analgesia, strict rest, and physiotherapy. Surgical techniques included ventral slot and hemilaminectomy/mini-hemilaminectomy, based on HNPE location.

Outcome was assessed at 1-4 weeks and 6 months. A satisfactory outcome was defined as recovery of voluntary urination and independent ambulation. Recovery time was the number of days to regain unassisted walking.

Eighty-six dogs were included: 60 (70%) received conservative treatment and 26 (30%) underwent surgery. HNPE was cervical in 83% and thoracolumbar in 17%. At presentation, 72% were non-ambulatory paretic, 26% plegic with intact nociception, and 2% plegic without nociception. Satisfactory outcome was achieved in 85% of conservatively treated dogs and 100% of surgically treated dogs. Median recovery time was 7 days for conservative treatment and 9 days for surgery.

In the conservative group, dogs with more severe neurological status at admission were more likely to have worse neurological grades at 1 week ($p=0.002$) and 4 weeks ($p=0.023$); non-chondrodystrophic breeds ($p=0.034$) and those with thoracolumbar herniations ($p<0.001$) were more likely to present severe neurological status 6 months after diagnosis. Additionally, dogs treated medically with heterogeneous extruded material had worse outcomes at 1 month ($p=0.036$) and 6 months ($p=0.009$) compared to those with homogeneous material. In both treatment groups, intramedullary hyperintensity was associated with poorer outcomes at 1 week ($p=0.001$ conservative; $p=0.027$ surgical) and 4 weeks ($p=0.017$ conservative; $p=0.048$ surgical), and remained significant at 6 months in the conservative group ($p=0.04$).

In conclusion, both conservative and surgical treatments can result in favourable outcomes in dogs with compressive HNPE and severe neurological deficits. However, surgical decompression may be preferable, particularly in dogs with severe deficits, non-chondrodystrophic breeds, thoracolumbar lesions, or heterogeneous disc material.

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Oral Communication(s)

NODAL LYMPHATIC MAPPING IN 26 DOGS AND 3 CATS BEARING SOLID MALIGNANT TUMORS AND ENLARGED REGIONAL LYMPH NODES: A DESCRIPTIVE STUDY (2022–2026)

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In human oncology, lymph node enlargement due to neoplastic infiltration or inflammatory conditions may alter lymphatic flow and compromise sentinel lymph node (SLN) mapping by disrupting normal drainage and blocking tracer migration [1-3]. While SLN mapping in non-palpable or normal-sized lymph nodes has been widely investigated in veterinary oncology, no data are currently available regarding its reliability in the presence of enlarged regional lymph nodes (eRLN) in dogs and cats.

The present study aimed to evaluate the influence of eRLN on SLN mapping in canine and feline patients with solid malignancies and to describe potential alterations in lymphatic drainage patterns and tracer distribution.

Client-owned dogs and cats diagnosed with solid tumors and presenting at least one clinically or imaging-detected eRLN were prospectively enrolled. All patients underwent complete staging to exclude distant metastasis and subsequently SLN mapping using lymphoscintigraphy and/or near-infrared fluorescence imaging (NIRF). The number of SLNs, qualitative tracer uptake (total, incomplete, or absent), and histopathological nodal status were recorded and analyzed descriptively.

A total of 29 animals (26 dogs and 3 cats) were included. Two main lymphatic mapping patterns were identified. In 10/29 cases (34%), mapping detected exclusively the eRLN (Pattern 1), with no detection of additional SLNs. In the remaining 19/29 animals (66%) (Pattern 2), at least one additional non-palpable or normal-sized SLN was identified alongside the eRLN. A total of 33 adjunctive non-palpable or normal-sized SLN were detected and removed. Among the latter, 6/33 (18%) were located within the same lymphocentrum of the eRLN and 27/33 (82%) in a different lymphocentrum. Notably, 15/33 (45%) adjunctive non-palpable or normal-sized SLN were metastatic at histopathology, despite lacking clinical or imaging abnormalities. Alterations in tracer uptake were also observed: in four Pattern 2 cases, the eRLN showed incomplete or absent tracer uptake, with concurrent visualization of one or more alternative SLNs, consistent with a lymphatic rerouting phenomenon toward so-called neo-sentinel lymph nodes (neo-SLNs).
These findings support the hypothesis that metastatic or inflammatory involvement of lymph nodes may disrupt normal lymphatic architecture, potentially increasing intranodal pressure and redirecting lymphatic flow to alternative pathways. Overall, these results indicate that lymphatic drainage in the presence of eRLN is often unpredictable and cannot be reliably inferred based solely on lymph node size or palpation. Consequently, limiting lymphadenectomy to enlarged nodes alone may result in underestimation of metastatic spread.

In conclusion, based on this sample population, SLN mapping using lymphoscintigraphy and/or NIRF is strongly recommended in dogs and cats with eRLN, as it enables the identification of additional occult metastatic nodes, including neo-SLNs, thereby reducing the risk of residual microscopic nodal disease.

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Oral Communication(s)

EFFICACY OF AN OZONE AND UV-C STERILIZATION SYSTEM ON VETERINARY SURGICAL INSTRUMENTS AND COMPLEX MEDICAL DEVICES CONTAMINATED WITH MDR *KLEBSIELLA PNEUMONIAE* AND *STAPHYLOCOCCUS PSEUDINTERMEDIUS*

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The rising prevalence of multidrug-resistant (MDR) healthcare-associated infections (HAIs) necessitates robust veterinary antimicrobial stewardship, where rigorous instrument reprocessing is fundamental to mitigating nosocomial transmission and reducing antimicrobial reliance [1,2]. This study evaluates the antimicrobial efficacy of a novel sterilization system combining gaseous O_3 and UV-C radiation against MDR *K. pneumoniae* and *S. pseudintermedius*. Two distinct equipment sets were inoculated with fresh clinical isolates (1.5×10^8 CFU/mL) and allowed to form dry surface biofilms to simulate real-world clinical challenges where organic matter protects pathogens. Set 1 comprised 10 stainless-steel surgical instruments featuring complex mechanical joints (box-locks and ratchets). Set 2 comprised 10 heterogeneous devices characterized by complex geometries, internal lumens, and porous materials (laryngoscopes, clipper blades, endotracheal tubes, high-flow nasal cannulas, and non-invasive blood pressure cuffs). Three pre-treatments were assessed before O_3/UV-C exposure: Protocol A (direct packaging, simulating non-compliant omission of pre-treatment), Protocol B (standard decontamination and mechanical cleaning), and Protocol C (complete protocol including mechanical cleaning and high-level chemical disinfection). To isolate the specific bactericidal efficacy of the O_3/UV-C system, a parallel control phase was integrated: devices were microbiologically swabbed immediately after the decontamination phase of Protocols B and C, without subsequent O_3/UV-C exposure. Sterility was assessed via qualitative and quantitative (CFU) microbiological analysis. To detect a significant difference between treatment protocols, an a priori power analysis was conducted. Assuming an expected ozone efficacy of 90% (α error = 0.05), a minimum of 4 devices per group was required; the sample size was increased to 10 to ensure statistical robustness. Under the non-compliant Protocol A, Set 1 (surgical instruments) showed a 40% failure rate, with bacterial persistence localized exclusively within the box-locks and ratchets of articulating tools. Set 2 (complex medical devices) exhibited a 100% failure rate under the same protocol, with significant bacterial proliferation detected across all porous and luminal geometries (median > 100 CFU). Conversely, Protocols B and C achieved total sterilization (0 CFU) across both sets. Post-decontamination controls demonstrated that mechanical cleaning alone was insufficient for sterility, establishing the O_3/UV-C advanced oxidation process as the essential factor for complete bacterial eradication. Statistical analysis via the Friedman test confirmed that the reduction in bacterial load between Protocol A and Protocols B/C was highly significant ($p<0.001$) for both datasets. No statistically significant difference in antimicrobial efficacy was observed between Protocol B and Protocol C ($p>0.05$).

Synergistic O_3/UV-C effectively eradicates MDR *K. pneumoniae* and *S. pseudintermedius*, supporting veterinary antimicrobial stewardship. As organic bioburden shields against O_3 diffusion and UV-C radiation, rigorous pre-cleaning remains a prerequisite for sterilizing instruments with articulating joints, internal lumens, and porous matrices.

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Oral Communication(s)

PILOT VALIDATION OF A NOVEL INLINE DEVICE FOR REAL-TIME MONITORING OF ABDOMINAL MECHANICS DURING PNEUMOPERITONEUM

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The abdominal pressure–volume (P–V) relationship during laparoscopic insufflation is curvilinear and subject to substantial inter-individual variability [1].

Although the importance of characterizing individual P–V curves has been widely acknowledged [2], translating this concept into clinical practice has been constrained by the absence of validated real-time monitoring tools. Consequently, current clinical practice continues to rely on standardized pressure targets derived from population-level guidelines [3].

The Smart Inline Compliance Module (SICM) is a novel inline retrofit device that acquires intra-abdominal pressure and insufflation gas flow through physically separated sensing circuits, reconstructs insufflated volume by numerical integration of the flow signal, and derives the abdominal P–V curve and its biomechanical parameters in real time.

This study reports the first two-arm pilot benchmark validation of the SICM system.

Arm A comprised a controlled biomechanical phantom with three defined stiffness levels (Soft, Medium, Rigid) tested under Continuous and Stepwise insufflation protocols (30 curves).

Arm B comprised three female feline cadavers assessed under the same dual-protocol design (18 curves).

Signal quality was consistently high across both arms (Curve Quality Index: 1.0000 in the phantom arm; 0.9974 ± 0.0009 in the cadaveric arm).

Volume integration accuracy was confirmed against an independent offline reference (mean absolute percentage difference: 0.07%).

The system extracted reproducible biomechanical parameters under the Continuous protocol: in the cadaveric arm, maximum compliance (Cmax) ranged from 116.8 to 191.4 mL/mmHg across subjects, with intra-session coefficients of variation below 16%; Knee Pressure (Pknee), defined as a working operational index of the compliance transition, was 3.33–4.17 mmHg with coefficient of variation (CV) below 8%.

The Rigid phantom condition produced parameter values consistent with the cadaveric dataset, supporting cross-arm pipeline coherence.

The Stepwise protocol revealed the boundary conditions of the current parameter extraction pipeline and identified methodological targets for the next development iteration.

These results are interpreted exclusively within the scope of technical feasibility and preliminary biomechanical characterization; clinical applicability and optimal pressure guidance require adequately powered in-vivo studies.

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Oral Communication(s)

EVALUATION OF AN ANTISEPTIC SOLUTION FOR THE TREATMENT OF SURGICAL SITE INFECTION IN DOGS AND CATS UNDERGOING ORTHOPEDIC SURGERY: A PRELIMINARY PROSPECTIVE STUDY

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Infectious osteomyelitis represents a challenging complication in orthopedic practice, particularly when associated with implantable devices. A significant proportion of osteo-articular infections is sustained by biofilm-forming microorganisms, whose structural organization enhances bacterial survival and reduces the effectiveness of both antimicrobial therapy and host immune responses. In addition, the increasing prevalence of multidrug-resistant organisms further complicates management, highlighting the necessary for integrated approaches combining surgical strategies and targeted local treatments. In this setting, intraoperative control of contamination is crucial.

Preventia® Surgical Irrigation is a solution containing poloxamer and 0.1% polyhexamethylene biguanide (PHMB). Poloxamer promotes disruption of the biofilm matrix and mechanical removal of microorganisms, while PHMB provides broad-spectrum antiseptic activity through membrane interaction. This combination ensures both detergent and antimicrobial effects, with good local tolerability.

The primary objective of this study was to evaluate the efficacy of intraoperative irrigation with Preventia® compared to normal saline in reducing bacterial load in in infected orthopedic surgical sites.

Between January 2025 and March 2026, 15 patients with microbiologically confirmed implant-associated infections were enrolled. To evaluate the effect of irrigation on bacterial load, two swabs were collected for each patient: the first prior to debridement and intraoperative irrigation, and the second immediately before wound closure, after intraoperative irrigation. Intraoperative irrigation was performed using Preventia® and saline respectively in 9 (group P) and 6 (group C) patients. For microbiological analysis, a total of 30 swabs were cultured in nutritive and selective agar media, and bacterial colonies were identified by MALDI-ToF MS. Moreover, total bacterial counts (CFU/swab) were determined in triplicate on Columbia Blood Agar (5% sheep blood) and incubated aerobically at 36±1 °C for 48 hours. Antibiotic susceptibility profiles were also assessed. Statistical analysis was conducted both within each study group and between groups, and p-value < 0.05 was considered significant.
Preliminary results indicate a statistically significant reduction in bacterial load in group P, with decreases ranging from 2 to 9 log units, in some cases associated with culture negativity ($p < 0,05$). Statistical analysis further demonstrated a significantly lower load in group P compared with group C ($p < 0,05$). The results further suggest that the antiseptic effect may be observed even in the presence of multidrug-resistant organisms.

This study supports a potential role for Preventia® as an innovative strategy against implant-associated infections and preventing intraoperative contamination in veterinary medicine.

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Oral Communication(s)

EVALUATION OF TUMOR ELECTRICAL RESISTANCE IN ELECTROCHEMOTHERAPY-TREATED FELINE CUTANEOUS SQUAMOUS CELL CARCINOMA

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Tumors represent complex microenvironments in which uncontrolled proliferation alters not only biochemical pathways but also the physical and electrical properties of tissues [1]. Electrochemotherapy (ECT) exploits electroporation to transiently increase cell membrane permeability, enhancing intracellular delivery of cytotoxic agents such as bleomycin [2]. This prospective observational pilot study aimed to evaluate in vivo changes in tumor electrical resistance during ECT in feline cutaneous squamous cell carcinoma (fSCC) and to investigate potential correlations between resistance variations and treatment response. This study was approved by the Ethics Committee of the CESA Department of Veterinary Medicine-University of Bari with number protocol 31/2021

Inclusion criteria were confirmed diagnosis of cutaneous fSCC in the region of the head, absence of metastases, and completed treatment. ECT was performed under general anestesia and intravenous bleomycin followed by biphasic electric pulses (1200 V/cm 1 Herz). Tumor electrical resistance (Ω) was recorded in real time during each pulse. Adverse events included pruritus, alopecia, erythema, ulcerations, and necrosis. Overall survival (OS) was analyzed in relation to tumor stage, localization, number of ECT sessions, and treatment response. A generalized linear mixed model (GLMM) assessed the effect of sessions, impulses, response, recurrence, and stage on resistance values.

Thirty-nine cats met the inclusion criteria and were classified as T1 (28.2%), T2 (41%), T3 (18%), and T4 (12.8%). Lesions were located on the nasal planum (76.9%) and on the eyelids (23.1%). One ECT session was performed in 53.8% of cats, two in 33.3%, and three in 12.8%. At the end of treatment, 87.2% achieved complete response (CR) and 12.8% partial response (PR) with an overall response rate (ORR) of 100%. Adverse effects were mild and self-limiting. Resistance ranged 600–4900 Ω and decreased across sessions ($p < 0.001$: 2391.2 Ω [1st], 1431.8 Ω [2nd], 900.9 Ω [3rd]) and impulses ($p < 0.001$: 1978.7 Ω [1st], 1629.4 Ω [2nd], 1281.2 Ω [3rd], 1086.7 Ω [4th]). It was higher in palpebral (1811.5 Ω) vs nasal planum (1169.7 Ω; $p < 0.001$) and lower in CR (1323.5 Ω) vs PR (1601.0 Ω; $p = 0.004$). Resistance increased with fSCC stage ($p < 0.001$): T1 1086.7 Ω, T2 1410.6 Ω, T3 1690.8 Ω, T4 1732.1 Ω, with a grading × localization interaction ($p < 0.001$): lower in nasal planum than palpebral. OS was associated with ECT sessions ($p = 0.002$), location ($p = 0.020$), response ($p < 0.001$), and stage ($p < 0.001$).

The observed decrease in resistance is consistent with electroporation-induced formation of aqueous membrane pores, leading to increased ionic conductivity. The association between lower resistance and improved response suggests that resistance may reflect the extent and homogeneity of effective electroporation. Real-time monitoring of tumor electrical resistance may therefore represent a non-invasive surrogate marker of treatment adequacy and a potential predictive indicator of therapeutic outcome. These findings provide the first clinical evidence of in vivo resistance dynamics during ECT in veterinary oncology and support further investigation into the integration of electrical parameter monitoring to optimize treatment protocols.

Oral Communication(s)

STAPHYLECTOMY WITH CARBON DIOXIDE LASER VS. BIPOLAR SEALING DEVICE IN DOGS: A PROSPECTIVE, RANDOMIZED, CLINICAL AND HISTOLOGICAL-IMMUNOHISTOCHEMICAL TRIAL

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Elongated soft palate is a common airway abnormality in dogs. In most cases, it represents one of the primary alterations observed in dogs affected by brachycephalic obstructive airway syndrome. It can also be observed in non-brachycephalic dogs, although more often as a single alteration and with milder symptoms and a less severe clinical course. This condition is often surgically treated by staphylectomy. Staphylectomy is traditionally performed using a scalpel or scissors with subsequent mucosal suturing. Recent evidence suggests that electrosurgical techniques may reduce complications and minimize iatrogenic tissue damage. This study aimed to compare clinical efficacy and tissue invasiveness of two staphylectomy techniques: the CO_2 laser and bipolar sealing device (LigaSure®).

Thirty-six dogs with elongated soft palate and no other primary or secondary abnormalities to the upper airways were included in a prospective, randomized clinical trial and allocated into two groups: 18 in Group CO_2 (CO_2 laser) and 18 in Group BSD (Bipolar Sealing Device). In this study, non-brachycephalic dogs were also included, and their outcomes were compared with brachycephalic dogs in a stratified subgroup analysis. Clinical parameters (clinical score, surgical duration, owner satisfaction), histological features (oedema, thermal damage, hyperaemia, margin regularity), and immunohistochemical markers (Caspase-3, Heat Shock Proteins, T.U.N.E.L expression) were used to evaluate the effect of surgery and the degree of surgery-induced iatrogenic injury. Each dog underwent clinical evaluation before surgery (T0) and during follow-up at 24 hours (T1), 30 days (T2) and 6 months (T3) after surgery.

Clinical outcomes were satisfactory and similar for both devices ($P>0.05$), with a significant improvement in the clinical trend observed over time within each group ($P<0.0001$). The duration of surgery was significantly shorter in the Group BSD ($P<0.0001$). Histological ($P<0.0001$, both in the total score and in each parameter) and immunohistochemical analyses (HSP: $P<0.0001$; T.U.N.E.L: $P=0.0177$; Caspase-3: $P=0.1089$) consistently indicated that BSD was less invasive than CO_2 laser, causing reduced collateral tissue damage. The subgroup analysis showed no significant differences between brachycephalic and non-brachycephalic dogs, except for a longer duration of surgery with CO_2 laser in brachycephalic dogs, and a greater clinical improvement at T2 and T3 in non-brachycephalic dogs regardless of the surgical technique.

Although both instruments proved effective in performing staphylectomy in dogs with elongated soft palate, the results of our study suggest that Bipolar Sealing Device allows for a faster procedure and is a less aggressive approach on pharyngeal tissues compared to the CO_2 laser.

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Oral Communication(s)

WHEN TO CONSIDER PATIENT-SPECIFIC 3D-PRINTED GUIDES FOR CANINE ANTEBRACHIAL DEFORMITY CORRECTION

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Antebrachial angular limb deformities (ALDs) in dogs can be hard to correct because malalignment is often multiplanar and may include axial torsion; therefore, small technical errors can lead to clinically relevant residual deformity. CT-based 3D planning helps describe the deformity and can be paired with patient-specific 3D-printed instrumentation (PSI), usually a cutting guide and a reduction guide, to reproduce the virtual planning during surgery. PSI has been increasingly reported for canine antebrachial deformity correction in experimental accuracy studies and clinical series [1-3]. Because PSI requires CT imaging, design/manufacturing time and additional costs, clinicians need practical criteria to decide when PSI is worth using rather than a free-hand approach. We suggest considering PSI when one or more of the following are present: (1) biapical/multiapical deformity or marked multiplanar angulation; (2) clinically relevant torsion on CT; (3) osteotomies close to the joint, where the margin for error is small; (4) planned correction that prioritizes limb-length preservation (opening or length--neutral wedge planning) and therefore requires tight reduction control; and/or (5) cases where the plan requires simultaneous correction of angulation and torsion. Freehand correction may remain reasonable for straightforward uniapical deformities with minimal torsion and good exposure, especially in experienced hands and when a rapid procedure is needed. In practice, this guidelines can be applied after CT-based classification and choice of correction strategy; it is meant to support, not replace clinical assessment. Preliminary observations from our ongoing doctoral in vitro work are consistent with this approach: guide-assisted workflows mainly improved reliability in the most complex biapical model, while differences in mean angular error were small. This may help allocate PSI to cases with the highest expected benefit and guide future clinical studies on outcomes and cost-effectiveness.

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Oral Communication(s)

FLUORESCENCE-GUIDED SURGERY OF THE BILIARY TRACT IN CATS: PRELIMINARY STUDY FOR THE OPTIMIZATION OF INDOCYANINE GREEN ADMINISTRATION

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Indocyanine green fluorescence cholangiography (ICG-FC) enables real-time visualization of the extrahepatic biliary tree and has been validated in humans and dogs [1]. No species-specific pharmacokinetic or imaging data are available for cats, a species characterized by distinctive hepatic metabolism, that may substantially influence ICG tracer kinetics and signal-to-background ratio (SBR). This knowledge gap is clinically relevant given the increasing application of minimally invasive biliary surgery in feline patients.

The objective is to characterize the dose-dependent fluorescence kinetics of the extrahepatic biliary structures following ICG administration in cats, as a secondary observational endpoint of a prospective study on gonadal vascular anatomy.

A prospective pilot study was conducted on nine healthy female domestic cats undergoing elective laparoscopic ovariectomy. ICG was administered intravenously at the end of the positioning of the second laparoscopic trocar, as part of an approved protocol primarily designed to assess vascularization of the feline reproductive tract. Biliary fluorescence data were collected as a pre-specified secondary observational endpoint. Subjects were allocated into three dosing groups: ultra-low-dose (UL 0.0119 mg/kg), low-dose (L 0.05 mg/kg), and high-dose (H 0.25 mg/kg). Laparoscopic fluorescence imaging was performed using the RUBINA Karl Storz 4K NIR/ICG system. Fluorescence intensity of liver, gallbladder, cystic duct, and duodenum was quantified at five timepoints (5, 20, 30, 45, and 60 minutes post-injection) using ImageJ.

The data were analyzed using descriptive statistics and inferential tests. For each subject and variable, means and standard deviations were calculated at the various time points. Independent samples t-tests (Anova) were performed to compare the three groups at each time point. A linear mixed-effects models with repeated measures (Anova) was used to assess the overall trend over time and the effect of treatment. To estimate statistical power (power analysis) a Monte Carlo simulation was conducted based on the variance parameters estimated in the study.

All cats completed the protocol without adverse events attributable to ICG administration. The gallbladder demonstrated the earliest and highest fluorescence peak across all doses. Cystic duct and duodenum reached optimal visibility at later timepoints (45–60 min). A clear dose-dependent gradient in hepatic background fluorescence was observed: the ICG-UL group showed the lowest hepatic signal and earliest biliary visualization, while the ICG-H group displayed the highest background fluorescence, with a delayed improvement in biliary discrimination. The ICG-L group showed intermediate behavior.

Observational analysis demonstrates that the technique is safe and informative across a wide dose range (0.0119–0.25 mg/kg IV). The ultra-low dose provided the superior signal-to-background profile, enabling earlier and cleaner identification of extrahepatic biliary structures. These findings constitute the first species-specific reference dataset for ICG-FC in cats and provide the rationale for dedicated prospective trials in patients with hepatobiliary disease.

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Oral Communication(s)

ASSOCIATION BETWEEN CLINICAL ONSET AND THORACOLUMBAR-LUMBOSACRAL PAIN EXPRESSION ACROSS NEUROLOGICAL GRADES IN CATS

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Spinal pain (SP) is a common clinical and neurological finding in cats and may result from a variety of spinal diseases (SD), including infectious myelitis, neoplasia, spinal fracture and luxation, intervertebral disc disease (IVDD), and ischaemic myelopathy. These conditions are associated with different treatment options and prognoses and are characterised by variable clinical presentation, lesion localisation, and pain expression. Few studies have evaluated the prevalence of SP and its relationship with the onset of clinical signs in cats with SD. This single-centre retrospective study aimed to describe signalment, clinical and neuroanatomical features, and diagnostic categories and to assess the association between clinical onset and SP in cats with SD. Medical records of cats diagnosed with SD at the Ospedale Veterinario San Francesco were retrospectively reviewed. Data collected included breed, sex, age, lesion localization, onset of clinical signs, neurological grade, presence of spinal pain, and final diagnosis. Acute onset was defined as clinical signs developing within 7 days, whereas chronic onset was defined as signs persisting for more than 7 days (2). The presence of SP was assessed during the neurological examination and was defined as pain caused by palpation or manipulation of the spine. In addition, each cat underwent a pain assessment using the Feline Grimace Scale (FGS). Diagnoses were grouped into five categories: intervertebral disc disease (IVDD), neoplasia, inflammatory, infectious, and other disorders. In cats with neurological grades 2–5, the association between onset (acute vs chronic) and spinal pain was evaluated using odds ratios (OR). Sixty-two cats were included. Domestic shorthair cats were the most represented breed (50/62, 80.6%). There were 36/62 males (58.1%) and 26/62 females (41.9%), with a mean age of 6.64 years (median 8.0; range 0.25–15). Neuroanatomical localization was most frequently T3–L3 (39/62, 62.9%), followed by L4–S3 (23/62, 37.1%). Onset was acute in 33/62 cats (53.2%) and chronic in 29/62 (46.8%). Neurological grade at presentation was grade 1 in 5/62 cats (8.1%), grade 2 in 20/62 (32.3%), grade 3 in 20/62 (32.3%), grade 4 in 5/62 (8.1%), and grade 5 in 12/62 (19.4%). The SP was observed in 26/62 cats (41.9%). The most common diagnostic categories were IVDD (21/62, 33.9%) and neoplasia (20/62, 32.3%). Among cats with neurological grades 2–5, SP was present in 18/29 (62.1%) cats with acute onset and in 3/28 (10.7%) cats with chronic onset. Cats with acute onset had significantly higher odds of spinal pain compared to those with chronic onset (OR 13.64, 95% CI 3.32–56.03; p = 0.00008). SP was most frequently associated with compressive IVDD, whereas it was uncommon in neoplastic disorders and absent in cats diagnosed with ANNPE. In this cohort, thoracolumbar (T3–L3) disease predominated, and IVDD and neoplasia were the most frequent diagnoses. Acute onset was strongly associated with SP in cats with neurological grades 2–5, particularly in compressive disc disease. These findings may aid clinicians in refining differential diagnoses and anticipating pain presentation in cats with SD.

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Oral Communication(s)

FEMORAL TROCHLEAR PROSTHESIS ASSOCIATED WITH TIBIAL TUBEORSITY TRANSPOSITION TOOL TECHNIQUE IN NINE DOGS AFFECTED BY PATELLAR LUXATION

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Patellar luxation (PL) is a common canine orthopedic disorder affecting the pelvic limb, which compromises weight bearing and eventually leading to pain, lameness and osteoarthritis (OA). The PL diagnosis is generally made during an orthopedic examination; however, radiographic and/or tomographic assessment of the hind limb deformities can be challenging. Main traditional surgical techniques for PL management included trochlear wedge osteotomy, tibial tuberosity transposition (TTT) and capsulorraphy. In order to mitigate complication associated, in the last years new implants have been proposed. Thus, the aim of this case-series study was to evaluate clinical outcomes and complication rates of dogs undergoing trochlear prosthesis and tibial tuberosity transposition tool (4T) application for PL.

Inclusion criteria were dogs affected by medial (MPL) or lateral (LPL) patellar luxation, in which trochlear ridge prosthesis associated with 4T technique were used. Each dogs underwent the same work up including orthopedic examination, blood work followed by femoral and tibial radiographic evaluation. Lameness was subjectively assessed by a 0-4 scale. The implant size was determined using V-pop system template. Surgical management was performed using patellar groove replacement (PGR) and Mamamia (MM). A medial or lateral parapatellar approach was performed allowing the trochlear ridge osteotomy. The prosthetic implant was applied according to the manifacturer guidelines. Then, an incomplete tibial tuberosity osteotomy was performed to transpose the tibial tuberosity (TT) following the PL side. The transposition allowed the use of large pin size monocortically applied parallel to tibial plateau (TP) in a cranio-caudal direction as a spacer in order to allow the TT fixation. Capsulorraphy with imbrication of the opposite side to the luxation was performed. Subcutis and skin were routinely sutured. Post operative management included strict cage rest for six weeks associated with wound care and anti-inflammatory medication drug administration for two weeks. Follow up recheck included orthopedic examination and orthogonal stifle radiographs performed within one year after surgery.

Complications were classified as major or minor in case revision surgery was required or not.

Nine dogs met the inclusion criteria. They were six male and three female dogs. The mean body weight was 7.4 kg (range 3-14 kg) and the median age was 3 year-old (range 1-6 year-old).

Of the 9 cases, MPL was present in 8/9, of which five were grade III luxation and three were a grade II; LPL was present in 1/9 as grade III luxation. No incompetence of cranial cruciate disease was observed. Implants included PGR in 7/9 and MM in 2/9. The sizes of prosthesis used in these cases were as follows: 4 (n = 5), 5 (n = 4). Pin size for 4T was 2.5 mm (6/9) and 3mm (3/9). No major intra-operative or post-operative complications were reported. Post-operative gait improvement was observed in all dogs. In one case medial PGR application was radiographically observed without evidence of clinical signs or patellar luxation recurrence.

Radiographic bone healing was achieved in all dogs and no implant removal was performed up to date.

The main limitation of the present study is the small population size and the absence of a control group. Further studies are encouraged in order to better understand outcomes and complications.

According to the these preliminary results, combination of femoral trochlear prosthesis (PGR or MM) associated with 4T technique may be consider a possible option for surgical management of PL in dogs.

Oral Communication(s)

3D VIRTUAL SURGICAL PLANNING FOR CANINE FEMORAL AND TIBIAL DEFORMITIES: WORKFLOW AND CLINICAL APPLICATION IN 11 CASES

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Femur and tibia deformities are complex musculoskeletal disorders that can lead to patellar luxation and stifle instability during growth, with varying degrees of lameness (1). Surgical treatments require deformity characterization in the sagittal, frontal and transverse planes, accurate preoperative planning and precise intraoperative execution (2). Computed tomography (CT) images can be exported to computer-aided design (CAD) software for three-dimensional (3D) virtual surgical planning (VSP) of corrective osteotomies, improving deformity assessment and surgical decision-making (3). The aim of this study was to describe a standardized workflow for 3D VSP using dedicated software and to report its clinical application in dogs with femoral and/or tibial deformities. Eleven dogs with femoral and/or tibial deformities associated with medial or lateral patellar luxation and/or cranial cruciate ligament rupture were retrospectively evaluated. All patients underwent clinical and orthopedic examination followed by CT study of the pelvic limbs with standardised positioning to ensure reproducible limb alignment. All CT images were reconstructed at a slice thickness of 0.6 mm using a bone tissue algorithm and exported to the VSP software BonaPlanner (©Bonabyte LLC, Moscow, Russia) for segmentation and 3D reconstruction. Multiplanar assessment of angular and torsional deformities was performed, and corrective osteotomies were virtually simulated by adjusting the type, level, and orientation of bone cuts until target alignment was achieved. The VSP workflow enabled detailed characterization of complex deformities and clear visualization of the relationship between angular and torsional components. Iterative simulations facilitated comparison of different corrective strategies, supporting the selection of the optimal osteotomy configuration, fixation construct and surgical approach. In dogs with bilateral tibial torsion, iterative virtual corrections enabled restoration of a straight tibial axis and symmetrical alignment between limbs, while in dogs with distal femoral deformities the planned postoperative angles closely matched physiological values. In patients undergoing postoperative CT, measured femoral and tibial angles were within 5° of the planned values in all planes, confirming the accuracy of the VSP. Clinically, all dogs showed improved limb function and reduction or resolution of patellar luxation at postoperative follow-up. According to our results, 3D VSP with BonaPlanner proved to be a valuable tool for the assessment and correction of complex femoral and tibial deformities in dogs. The software used in this study improved deformity understanding and supported accurate and reproducible surgical planning. This approach may provide a reliable basis for the implementation of patient-specific 3D-printed surgical guides and implants in veterinary orthopedic surgery.

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Oral Communication(s)

IMPLEMENTATION AND EVALUATION OF A STANDARDIZED HANDOVER PROTOCOL TO IMPROVE CLINICAL COMMUNICATION IN A VETERINARY TEACHING HOSPITAL: A PILOT PROSPECTIVE OBSERVATIONAL STUDY

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Effective communication during the transfer of clinical cases between different units is a critical step for patient safety in veterinary hospitals. Incomplete or unstructured handovers may result in information loss, diagnostic delays, or incorrect therapeutic decisions being made. Although structured communication models have been largely adopted in human medicine, their application in veterinary clinical practice continue to be limited. This prospective observational study aims to investigate the effectiveness of implementation of a standardized handover protocol on the improvement of information transfer quality and staff compliance in a Veterinary Teaching Hospital (VTH). The study was conducted at the VTH of the Department of Veterinary Medicine and Animal Production at the University of Napoli "Federico II". This study does not involve clinical trials or invasive procedures on animals, does not alter standard diagnostic or therapeutic pathways and the study was approved by the CERSUB (PG/2026/0025382). In the first phase, handovers between clinicians, categorized as senior (full and associate professors, and clinical unit heads) and junior (clinical fellows and PhD students), were prospectively observed without prior notification. Data completeness during handover were assessed using structured checklists. Based on these findings, a standardized handover form was developed and adapted to fit clinical workflows. In the second phase, the handover form was implemented in daily practice following targeted staff training. Its impact was assessed through repeat observation and an anonymous survey to determine staff feedback. Descriptive statistics and Fisher's exact test were used to analysed categorical variables. Preliminary results from the first part of the study indicate that, considering each data point, the completeness is an average of 52%. Reduced completeness is associated with interruptions during handover (OR = 0.25; p = 0.11). Handover sessions conducted by senior staff show a trend towards greater completeness compared to those conducted by junior staff (OR=3.14; p ≈0.23). The introduction of a structured handoff protocol is expected to improve the quality and accuracy of information transfer and increase staff members communication and satisfaction. In conclusion, introducing a structured handover protocol could be a feasible and effective strategy to improve communication and continuity of care in veterinary hospital settings.

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Oral Communication(s)

COMPARISON OF LAPAROSCOPIC VERSUS FLANK OVARIECTOMY IN GUINEA PIGS (*CAVIA PORCELLUS*)

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In guinea pigs, the assessment of pain is inherently challenging due to their prey species behaviour and the lack of validated scoring systems [1]. For this reason, surgical techniques should aim to minimise tissue trauma and nociceptive stimulation. In this context, laparoscopic surgery may represent a promising approach, as several studies in dogs and cats have shown that laparoscopic procedures are associated with reduced surgical stress and postoperative pain compared to open surgery [2]. Although laparoscopic ovariectomy has been described in guinea pigs, its clinical advantages over conventional open approaches have not been fully investigated [3].

The aim of this study was to compare intraoperative variables and postoperative recovery between laparoscopic ovariectomy (LapOVE) and open flank ovariectomy (OVE) in guinea pigs.

A total of 12 guinea pigs were randomly assigned to two groups (OVE, n = 6; LapOVE, n = 6). In the laparoscopic group, ovariectomy was performed using a two-port technique, whereas, in the open group, a bilateral flank approach was used. Recorded variables included surgical time, anaesthetic time, total incision length, intraoperative bleeding and complications, time to first spontaneous food intake, and time to first faecal output. Postoperative discomfort was indirectly assessed through functional and behavioural parameters, including food intake, faecal output, and general attitude.

Data were analysed using non-parametric statistical methods (Mann–Whitney U test). No significant differences were observed between groups in surgical time, anaesthetic time, or total incision length ($P > 0.05$). Median surgical time was lower in the laparoscopic group compared to the open group (LapOVE: 17 min; OVE: 21.5 min). Postoperative recovery parameters, including time to first spontaneous food intake and time to first faecal output, did not differ significantly between groups ($P > 0.05$). Minor intraoperative complications were observed in both groups and were related to the technical characteristics of each approach, with no major complications recorded. No relevant postoperative complications were observed.

The absence of significant differences between techniques may be related to the small sample size and to the use of a flank approach in the open group, which is considered minimally invasive and associated with reduced surgical trauma compared to ventral midline laparotomy. In the present sample, the shorter median surgical time observed in the laparoscopic group may suggest a clinical advantage despite the lack of statistical significance.

Both techniques proved to be feasible, safe, and well tolerated in the early postoperative period, with no evidence of major complications. However, guinea pigs are herbivorous animals characterised by large and fragile abdominal organs, such as the caecum and colon, which may increase the risk of intra-abdominal injury, particularly during laparoscopic procedures [3]. Further studies with larger sample sizes are warranted to better evaluate postoperative discomfort and long-term outcomes.

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Oral Communication(s)

DELAYED REPAIR OF LARGE INCISIONAL HERNIAS IN HORSES: A THREE-CASE SERIES COMPARING OUTCOMES WITH AND WITHOUT MESH

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Incisional hernia (IH) is a recognised complication of ventral midline celiotomy in horses, with a reported incidence of 8-16% [1,2]. Development of IH is strongly associated with surgical site infection, re-laparotomy, and impaired incisional healing. While many IHs are primarily of cosmetic concern, large or thin-walled defects may require repair due to the risk of progressive enlargement and potential evisceration [1-3].

This case series describes the surgical management and outcome of three horses with large IHs following celiotomy. Horses underwent herniorrhaphy (HR) under a standardized anaesthetic protocol and received systemic antimicrobials and NSAIDs for 5 days postoperatively. The hernial defect was closed using polyglactin 910 (USP 6). Interrupted sutures were applied in areas under greater tension, while tension-free regions were closed with a simple continuous pattern. In one case, a polypropylene mesh reinforced the abdominal wall. Subcutaneous tissue was closed with Glycomer™ 631 (USP 2-0) and skin with polyester (USP 0) in a cross-mattress pattern. A stent bandage of laparotomy gauze secured with staples was applied over the incision, followed by a belly bandage for 10 days. Horses recovered uneventfully with no postoperative complications. Stall rest was maintained for 8 weeks, followed by turnout in a restricted enclosure for 4 weeks, then gradual return to activity. Abdominal wall healing was satisfactory and horses returned to their previous level of use. In two horses, despite the large hernia size, HR was successful without prosthetic mesh. Avoiding mesh may reduce complications such as infection, dehiscence, foreign body reaction, and intra-abdominal adhesion formation. In these cases, the interval between laparotomy and HR (9 months and 2 years) likely allowed stabilization of the hernia and maturation of the fibrous ring, facilitating primary closure. Delayed repair allows resolution of inflammation, better definition of fibrous margins, and tissue strengthening, enhancing suture holding capacity and reducing recurrence risk. In contrast, mesh reinforcement was used in the third horse, in which HR was performed five months after laparotomy. This horse was an actively breeding Thoroughbred stallion, characterised by a more excitable temperament, a potential risk factor for increased postoperative strain. Mesh was elected to provide additional mechanical support. This case highlights how repair timing and individual patient factors may influence surgical decision-making. While prosthetic mesh may be indicated in selected cases with large defects or high tension, this series supports successful primary closure in horses when proper case selection, surgical technique, and postoperative management are applied. Delayed HR appears to promote tissue maturation, increasing the likelihood of repair success without mesh. Limitations include the small number of cases and descriptive nature of the observations. Nevertheless, these findings provide clinical insight into managing equine IHs and support primary closure as a safe and effective option in selected horses.

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Oral Communication(s)

ONCOLOGICAL HYPERTHERMIA USING TECAR FOR THE TREATMENT OF MELANOMA IN HORSES: NEW PERSPECTIVES

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Cutaneous melanoma is a common neoplasm in gray horses [1] and poses a clinical challenge, particularly in extensive forms or when located in anatomically complex sites. Oncological hyperthermia, widely used in human medicine, exploits controlled heating to selectively target tumor tissue and enhance adjunct therapies. Its application in veterinary medicine remains limited, despite growing biological evidence supporting further investigation [2,3]. TECAR technology, based on capacitive and resistive energy transfer at 447 kHz, offers a precise method to induce localized hyperthermia within neoplastic tissues.

This preliminary study evaluated the feasibility, safety, and efficacy of TECAR-mediated hyperthermia for equine melanomas, focusing on tumor volume reduction. Horses with histologically confirmed cutaneous or mucosal melanomas were enrolled. Each tumor underwent ten treatment sessions at 72-hour intervals, maintaining tissue temperature at 41–45°C as verified by thermal imaging. Tumors were assessed before the first session (T0) and after the last (T1) using calipers, B-mode ultrasonography, and Power Doppler, and standardized photographs digitally analyzed with ImageJ software to quantify area, width, height, and perimeter. TECAR device parameters, including delivered power and tissue impedance, were systematically recorded. Pre- and post-treatment measurements were statistically analyzed ($p < 0.05$).

Four Connemara ponies, aged 9–18 years (15 ± 3.5 years), with seven cutaneous or mucosal melanomas were included and tolerated the TECAR protocol without adverse effects. Caliper measurements showed significant reductions in both longitudinal and transverse tumor dimensions ($p = 0.003$). Ultrasonography confirmed a reduction in internal tumor size despite structural heterogeneity, and Power Doppler showed minimal vascularization. Digital analysis demonstrated a significant decrease in tumor area and width ($p = 0.003$ and $p = 0.01$), while height and perimeter changes were not statistically significant, though a decreasing trend was observed. Intra-operator reproducibility of ImageJ measurements was excellent (ICC > 0.90). Device data indicated consistent energy delivery, with mean capacitive power 46.97 ± 19.58 VA and mean resistive power 11.03 ± 7.57 W.

These results suggest TECAR-mediated hyperthermia is a non-invasive, safe, and potentially effective strategy for reducing equine melanomas. The therapy may be applied outside hospital settings and integrated into multimodal protocols, including preoperative tumor reduction or as an alternative when surgery is challenging or contraindicated. Consistent reductions across caliper, ultrasonographic, and digital measurements support the reliability and objective quantification of the effect. Limitations include the small sample size, homogeneous breed and age, lack of long-term follow-up, and absence of a control group, restricting generalizability. Future studies should include larger, more diverse populations, different tumor stages, and follow-up to determine durability. Such studies could refine TECAR parameters, optimize protocols, and explore combination strategies with chemotherapy or radiotherapy.

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Oral Communication(s)

ANESTHETIC MANAGEMENT IN MARE UNDERGOING THIRD-DEGREE PERINEAL LACERATION REPAIR: A RETROSPECTIVE CLINICAL STUDY

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Rectovestibular lacerations in mares occur as a consequence of strong expulsive forces during parturition, resulting in a persistent communication between the rectum and vestibule. This condition leads to chronic fecal contamination of the reproductive tract, and is commonly associated with reduced reproductive performance. Surgical repair is typically performed in standing, sedated animals and requires an effective combination of sedation and locoregional anesthesia to ensure adequate analgesia, immobility, and patient safety. This retrospective clinical study describes the anesthetic management in 15 mares undergoing rectovestibular lacerations repair between 2020 and 2025. All procedures were performed with the mare standing and restrained in stocks, with the tail elevated and secured to facilitate optimal surgical access. Sedation protocols consisted of intravenous administration of an α2-agonist combined with an opioid, followed by a continuous rate infusion (CRI) to maintain a stable level of sedation throughout the procedure. The most commonly used protocol was detomidine (0.01–0.02 mg/kg IV) combined with butorphanol (0.02 mg/kg IV), followed by CRI (0.01 and 0.02 mg/kg/h, respectively) (n. 10 cases). Recently, the authors replaced this protocol by romifidine (0.08 mg/kg IV) combined with morphine (0.1 mg/kg IV), followed by CRI (0.03 and 0.05 mg/kg/h, respectively) (n. 5 cases), with the aim of improving sedation quality and analgesic efficacy. Epidural anesthesia was routinely performed using a caudal approach (Co1–Co2 space). This technique was preferred due to its ease of access, safety, and suitability also for field conditions, as well as its lower risk of motor blockade and ataxia compared to the lumbosacral approach. The injection site was identified by palpation of the first mobile intervertebral space at the tail head. Subcutaneous lidocaine bleb at the site of injection helped the insertion of the needle. Different analgesic agents can be chosen for epidural use. The authors preferred to use epidurally lidocaine (0.22 mg/kg) combined with either xylazine (0.17 mg/kg) diluted to 10 ml with 0.9% saline solution, providing effective and reliable perineal analgesia. Correct needle placement was confirmed using the hanging drop technique. Spinal needles ranging depended on the size and body condition of the horse. Hyoscine butylbromide and metamizole were commonly used as an antispasmodic and analgesic agents to relieve gastrointestinal smooth muscle spasm and to enhance pain control. All mares tolerated the procedure well, maintaining adequate posture without ataxia and cooperation throughout surgery. The romifidine–morphine protocol was associated with improved cardiovascular stability, reduced ataxia, and a more consistent depth of sedation and analgesia compared to detomidine–butorphanol. Epidural anesthesia provided satisfactory intraoperative analgesia in all cases, with no significant complications or inadvertent recumbency. In conclusion, the combination of standing sedation with CRI of α2-agonists combined with opioids and caudal epidural anesthesia represents a reliable, safe, and effective anesthetic approach for rectovestibular laceration repair in mares, allowing optimal surgical conditions while minimizing intraoperative and perioperative risks.

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Oral Communication(s)

PRE-EMPTIVE ORAL PARACETAMOL COMBINED WITH LOW-DOSE PHENYLBUTAZONE FOR PERIOPERATIVE ANALGESIA FOR EQUINE ARTHROSCOPY: A PILOT STUDY

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Effective perioperative pain management is essential to improve welfare, reduce complications, and prevent maladaptive sensitization. Multimodal analgesia is increasingly advocated to enhance efficacy while minimizing adverse effects. Paracetamol, a centrally acting analgesic, showed synergistic effects with non-steroidal anti-inflammatory drugs (NSAIDs) in other species [1], but its role in equine perioperative pain remains unclear. This prospective, single-blinded pilot study evaluated the clinical effects of pre-emptive oral (PO) paracetamol combined with intravenous (IV) phenylbutazone compared with IV phenylbutazone alone in horses undergoing arthroscopy.

Eight healthy horses were randomly assigned to treatment (P: n=4) or control (C: n=4) (OPBA_27_2026). Paracetamol (30 mg/kg; PO) was administered 30 minutes before sedation. All horses received a standardized protocol including acepromazine, xylazine and phenylbutazone (2.2 mg/kg IV), followed by induction with ketamine and diazepam (T0-i) and maintenance with isoflurane. Intraoperative rescue analgesia (ketamine 0.25 mg/kg IV) was administered in case of intraoperative nociception. Postoperative pain was evaluated using a composite pain scale (CPS) [2] up to 24 hours following standing after general anaesthesia (T0-p), while residual sedation was assessed using the EquiSed scale. Rescue analgesia (phenylbutazone 2.2 mg/kg IV) was administered when CPS ≥13/39.

Intraoperative heart rate was significantly higher in P group at T10-i (P: 47±3; C: 37±4 bpm; p=0.01) and T20-i (P: 47±3; C: 40±3 bpm; p=0.02), while arterial pressures were significantly higher in C group at T10-i (systolic: C: 115±7; P: 80±15 mmHg; p=0.01; mean: C: 89±7; P: 66±7 mmHg; p=0.003; diastolic: C: 77±6; P: 57±7 mmHg; p=0.006) and T30-i (systolic: C: 109±8; P: 91±10 mmHg; p=0.04). Ketamine requirement (P: 0.5, 0–1; C: 2, 0–5 boluses) and mean dobutamine dose (P: 0.5±0.4; C: 0.5±0.3 µg/kg/min) did not differ. EquiSed scores significantly decreased over time (p=0.001, both). CPS scores were significantly lower in P group at T8-p (P: 1.5, 1–6; C: 7.5, 6–9; p=0.02). Rescue analgesia was required in one horse in C group at T6-p (CPS: 13/39).

A transient paracetamol-induced hypotensive effect has been described in humans, but it is clinically insignificant in healthy patients. Similarly, cardiovascular differences observed were mild and did not affect dobutamine requirement. The multimodal anaesthetic protocol provided adequate intraoperative analgesia, likely masking any additive analgesic effect of paracetamol. Postoperatively, the prolonged analgesic action of paracetamol became evident at intermediate time points, consistent with sustained plasma concentrations up to 8 hours in horses [3]. Pre-emptive oral paracetamol combined with low-dose IV phenylbutazone in horses undergoing arthroscopy did not enhance intraoperative analgesia but reduced postoperative pain eight hours after surgery, with lower rescue NSAID requirement. Findings are preliminary but promising, further trials are warranted.

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Oral Communication(s)

SURGICAL SAFETY PROCESSES AND EARLY POSTOPERATIVE OUTCOMES IN EQUINE SURGERY: PRELIMINARY RESULTS

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Postoperative complications remain a key issue in equine surgery due to the lack of standardized perioperative protocols and validated safety tools [1,2]. Literature calls for harmonized terminology and structured reporting, while evidence from human and small animal surgery shows that standardization improves patient safety and outcome assessment [1,2]. This retrospective study aimed to describe postoperative complications in equine surgical patients over an 8 year period and assess perioperative adherence to a 36 item checklist. Records from 2018–2025 at the Equine Teaching Hospital of the University of Córdoba were reviewed. Eligible cases were horses undergoing elective or emergency surgery with at least one postop complication within 15 days. Collected variables included demographic data, surgical diagnosis, urgency, complication type (gastrointestinal, incisional, infectious inflammatory, respiratory, vascular, orthopedic, systemic), severity (major: reoperation, organ dysfunction or death; minor: self limiting), and checklist items. Checklist adherence was quantified by scoring documented items as completed and non reported fields as uncompleted.

Fifty equids were included (49 horses, 1 donkey). Males (38/50; 76%) and PRE horses were most represented (26/50; 52%). Ages ranged from neonatal to geriatric (median 6 years). Gastrointestinal surgeries were most frequent (17/50; 34%), followed by hernia repairs (12/50; 24%), orthopedic procedures (10/50; 20%) and upper airway surgery (6/50; 12%). Most surgeries were emergencies (33/50; 66%). Postop complications were dominated by gastrointestinal events (25/50; 50%), infectious–inflammatory (18/50; 36%), incisional (8/50; 16%), systemic (10/50; 20%), respiratory (7/50; 14%) and orthopedic issues (6/50; 12%); vascular complications were rare (3/50; 6%). Major complications occurred in 22/50 cases (44%) and minor in 28/50 (56%).

Overall adherence was high, with 22/36 items completed in all records. However, several items showed substantial gaps: tetanus vaccination was no/unreported in 36/50 cases (72%), shoe removal in 50/50 (100%), biological sampling in 27/50 (54%), and postop briefing in 11/50 (22%). The least compliant item was the preop gauze check (4/50; 8%). Adherence was similar between major and minor cases, with consistent documentation of core items. Some fields still showed gaps: tetanus vaccination was no/unreported in 75% of major and 64% of minor cases, biological sampling remained inconsistent, shoe removal was never documented, and the gauze check showed the lowest compliance (7% major; 9% minor). Postop briefing showed moderate adherence (79% major; 77% minor).

Postop complications were common, mainly gastrointestinal or infectious–inflammatory. Core perioperative practices were well documented, but several ancillary items showed poor adherence, reflecting recording gaps rather than links to complication severity. Findings highlight the need for an equine specific validated checklist to strengthen documentation, standardization, and surgical safety.

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Oral Communication(s)

SHORT- AND LONG-TERM OUTCOME IN HORSES UNDERGOING LAPAROSCOPIC NEPHROSPLENIC SPACE ABLATION

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Left dorsal displacement of the large colon (LDDLC) is a common cause of colic in horses and is frequently associated with recurrent episodes, which may negatively affect animal welfare, athletic performance, and management costs [1]. Preventive surgical strategies have therefore been proposed to reduce the risk of recurrence. Among these, laparoscopic nephrosplenic space (NSS) ablation has gained increasing interest as a minimally invasive technique aimed at preventing entrapment of the large colon between the spleen and the left kidney [2].

The aim of this retrospective study was to evaluate short- and long-term outcomes of horses undergoing laparoscopic NSS ablation in two Italian Veterinary Teaching Hospitals between 2016 and 2024. Medical records were reviewed to collect signalment, clinical history, number of previous colic episodes or hospitalisations, previous colic surgery, and details of the surgical procedure. Short-term outcomes included perioperative complications, postoperative colic, and discharge status. Long-term follow-up data were obtained through structured telephone interviews with horse owners and included survival, recurrence of colic episodes, need for additional surgical interventions, return to athletic or breeding activity, and owner satisfaction with the procedure.

A total of 48 horses met the inclusion criteria. Most horses (91.7%) were discharged without postoperative complications, confirming the safety of the standing laparoscopic approach. The duration of long-term follow-up ranged from 20 days to 8.8 years. At the last follow-up, 81.3% of horses were alive and 70.8% had returned to athletic or reproductive activity. Post-discharge colic episodes were reported in 31.2% of horses, and suspected recurrence of LDDLC was reported in a subset of cases. Kaplan–Meier analysis estimated a one-year post-discharge survival probability of 83.3%. Horses that required laparotomy during the follow-up period showed significantly reduced survival compared with horses that did not require further surgery. Bayesian modelling suggested that a history of recurrent colic was associated with an increased probability of postoperative complications, whereas previous colic surgery showed a tendency towards improved survival.

Overall, owner satisfaction with the procedure was high, with 83.3% of respondents reporting a positive outcome, mainly related to clinical improvement and return to activity.

In conclusion, laparoscopic NSS ablation appears to represent a safe and clinically useful preventive procedure for horses considered at risk of recurrent LDDLC. The technique allows favourable long-term survival and a high rate of functional recovery, although postoperative colic episodes may still occur and require ongoing clinical monitoring.

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Oral Communication(s)

TREATMENT OF PROXIMAL DIGITAL ANNULAR LIGAMENT DESMITIS USING AN OPEN SURGICAL TECHNIQUE WITH PARTIAL LIGAMENT EXCISION

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The desmopathy of the palmar annular ligament (PDAL) has been described in several different conditions in the horse. The classic presentation of the disease is characterized by lameness associated with the fetlock bowed at the level of PAL and the proximal edge of the DFTS. Numerous techniques for ligament resection have been described, including tenovaginoscopic, minimally invasive, and ultrasound-guided approaches [1]. Despite this, the success rate of the procedure, in terms of lameness reduction, ranges from 50% to 70% [2]. The aim of this study is to describe an open surgical technique involving partial ligament excision and to evaluate the outcome one year after surgery. This study included 24 horses that underwent surgery for PDAL between 2023 and 2025. The surgical technique used was the open approach, involving the removal of a strip of ligament approximately 0.3–0.5 cm wide. The subcutaneous tissue was sutured in two layers using size 0 polyglycolic acid suture. The use of two layers was due to the abundant fibrotic subcutaneous tissue often present and to prevent the development of a synovial fistula.

The skin was sutured using size 0 nylon. The limb was bandaged for three weeks after surgery, with the bandage changed every three days. Skin sutures were removed 12–15 days postoperatively.

The horses were hand-walked for 15 minutes per day starting four days after surgery to prevent adhesion formation. After three weeks, the horses were returned to work unless an underlying tendinopathy was present. In those cases (3/24), the horses resumed work only once the tendon was ultrasonographically healed. One year after surgery, the attending veterinarians were interviewed to determine whether lameness had resolved and, if it had recurred, whether the cause was attributable to a condition related to PDAL.

Lameness recurred in 1 out of 24 horses (4.17%) at three months post-surgery, associated with the presence of a DDFT lesion and a positive tendon sheath anesthesia. The remaining 23 out of 24 horses (95.83%) showed no signs of lameness one year after surgery. No horses experienced postoperative complications. Therefore, it can be stated that, although the technique does not offer the advantages of minimally invasive approaches, it is safe and truly effective in treating PDAL, with a success rate higher than those reported in the literature. According to the authors, the high success rate is likely due to the fact that the PAL does not risk reconnecting after surgery, and adhesions are probably prevented.

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Oral Communication(s)

COMPUTED TOMOGRAPHY FEATURES OF ADRENOCORTICAL CARCINOMA IN CATS

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Adrenal tumors in cats are rare, with a reported prevalence of approximately 0.2% of all feline neoplasms[1]. They are classified as cortical (adenomas and carcinomas) or medullary tumors (pheochromocytomas). In dogs, several computed tomography (CT) studies have identified imaging criteria useful for differentiating tumor type based on histopathological correlation[2,3]. In contrast, feline studies have mainly focused on clinical and pathological aspects, and detailed CT descriptions remain limited in this species. The aims of this retrospective multicentric study were to describe the CT features of adrenal tumors in cats and to assess their correlation with histopathological findings. Cases were collected from the Veterinary Teaching Hospitals of the University of Bologna, the University of Pennsylvania and the Anicura – Istituto Veterinario di Novara. Inclusion criteria were the presence of an adrenal mass on CT and cytological or histopathological confirmation of adrenal neoplasia. For each case, signalment (breed, age, sex, body weight), endocrine testing and ultrasonographic and surgical findings (when available) were recorded. For each adrenal lesion, CT parameters evaluated included: size, shape, margins, pre- and post-contrast attenuation (including presence of foci of mineralization), contrast-enhancement pattern, presence of locoregional vascular invasion, appearance of the perilesional retroperitoneal space and sign of suspected distant metastases. The contralateral adrenal gland was also assessed. Ten cats were included: seven neutered males (70%) and three spayed females (30%), with a median age of 13 years (range 10-15 years) and a median body weight of 6.7 kg (3,5 – 8,2 kg). A diagnosis of adrenocortical carcinoma was established in all cases, based on histopathology in 80% of cats and cytology in 20% of cases. Most tumors were suspected to be functional (80%): four aldosterone-secreting, two cortisol-secreting and two with combined secretion. Tumors were more frequently located in the right adrenal gland (60%), and were predominantly oval (50%) or round (40%) in shape, with well-defined margins (80%). Median attenuation values were 40 (24-50 HU) Hounsfield Units (HU) on pre-contrast images, 51.5 HU (29-123 HU) in the early post-contrast phase, and 83 HU (42-129 HU) in the delayed phase. Pre-contrast attenuation was heterogeneous in most cases (70%), and intralesional mineralized foci were observed in one case. Contrast enhancement was mainly heterogeneous (90%), with occasional rim enhancement (30%). No definitive CT evidence of vascular invasion was identified; however, invasion of the phrenicoabdominal vein was observed intraoperatively in one case. The contralateral adrenal gland was normal in size in eight cats (70%, median short axis 4 mm; range 3,1-5,4 mm), reduced in volume in one cat (1,7 mm in short axis) and enlarged in one case (6,3 mm in short axis). This study provides a preliminary characterization of CT features of feline adrenocortical carcinomas and highlights imaging patterns that may support their diagnosis in clinical practice.

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Oral Communication(s)

SEEING BEYOND THE LUMEN: FUNCTIONAL CARDIAC CT FOR DYNAMIC (CINE) ASSESSMENT OF CORONARY ARTERIES AND MYOCARDIAL STATIC PERFUSION IN DOGS

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Coronary artery anomalies are extensively described in dogs, especially in English Bulldog, but also in other breeds [1]. Although coronary artery anomalies represent a relevant clinical application for advanced cardiac imaging, the present study was focused on the technical and methodological aspects of retrospectively ECG-gated cardiac Computed Tomography (CT), highlighting the feasibility of multiphasic reconstruction for CT dynamic (cine) and functional cardiac assessment in veterinary patients. The aim of this study was to describe the technical feasibility and potential diagnostic value of retrospectively ECG-gated cardiac computed tomography with multiphasic reconstruction for dynamic (cine) assessment of coronary arteries and myocardial static perfusion in dogs. Cardiac CT examinations were performed using a Siemens SOMATOM go.Top multidetector CT system with 128 slices. The scanner was set up for high-resolution with 70 kVp, 0.6 mm slice thickness, a temporal resolution of 165 ms, optimized for small animal cardiac imaging. All studies were acquired using a ECG-gated protocol, enabling full coverage of the R–R interval. Raw data were reconstructed into multiple cardiac phases, allowing phase-resolved analysis across both systolic and diastolic segments of the cardiac cycle. This approach enabled consistent temporal sampling of coronary enhancement and myocardial static perfusion dynamics. Image reconstruction included standard multiplanar reformations (MPR), maximum intensity projections (MIP), and dedicated cardiac reconstructions. A dynamic evaluation framework was applied, based on comparison of attenuation values (HU) across different cardiac phases, enabling semi-quantitative assessment of coronary perfusion behavior, especially for the identification of phase-dependent variations in contrast distribution, which may reflect functional flow characteristics rather than fixed anatomical abnormalities. This protocol was specifically designed to move beyond static morphological assessment, introducing a reproducible method for phase-resolved functional analysis of coronary circulation in canine and feline patients. Retrospective ECG-gated acquisition provides multiphasic datasets covering the entire cardiac cycle, allowing dynamic visualization of cardiac structures through cine reconstructions. This CT dynamic (cine) approach enables phase-resolved assessment of coronary artery opacification, myocardial static perfusion enhancement, and ventricular wall motion during both systolic and diastolic phases, without the need for additional contrast administration or separate functional imaging studies. Additionally, it facilitates assessment of myocardial static perfusion, highlighting possible focal or diffuse alterations of the cardiac muscle. The technology can detect the best diastolic phase and the best systolic phase so that the comparison of myocardial contraction is shown as soon as the scan is over. Ten dogs were included in the study; in all of them, the coronary arteries were clearly visualized and consistently tracked along their course. Anatomical variations and anomalies were recorded. Myocardial static perfusion was adequately assessed in all examinations. Setting the machine at 70 kVp enhanced contrast medium attenuation, allowing radiation dose reduction while maintaining diagnostic image quality. Moreover, this setting improved temporal resolution and trigger performance, avoiding motion artefacts even in dogs with irregular heart rates. The pulsing system enabled scans from peak R to peak R, allowing evaluation of myocardial contraction from end-systole to end-diastole. Real-time VRT, MIP, and stretched vessel reconstruction (including elongated and isolated vessels) were automatically generated within a few seconds, significantly facilitating image interpretation and diagnostic workflow. This integrated anatomical and functional framework supports the role of CT dynamic (cine) as a technical and methodological imaging tool that extends cardiac CT beyond static morphological assessment, providing comprehensive cardiac evaluation within a single examination.

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Oral Communication(s)

DIAGNOSTIC IMAGING FINDINGS IN 29 HORSES WITH ESOPHAGEAL DISEASE

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Equine esophageal disorders include obstruction, stenosis, ulceration, diverticula, and megaesophagus. While clinical presentation and outcomes are widely described, imaging findings are less consistently reported. This retrospective study describes the imaging findings in horses with esophageal disorders, aiming to improve characterization of their imaging features in clinical practice.

Medical records were retrospectively reviewed. Horses that had undergone oesophageal radiography and ultrasonography, with or without contrast esophagography were included. Radiography and contrast studies evaluated size and content abnormalities and perforations, ultrasonography assessed esophageal wall structure, integrity and thickness, luminal content, motility, vascularization, and paraoesophageal abnormalities.

Twenty-nine horses were included. The population consisted mainly of ponies (7/29), warmblood-type horses (6/29), and Friesians (4/29), with other breeds also represented. The sex distribution included 15 mares, 8 geldings, and 6 stallions. Age ranged from less than 1 year to 34 years, with a median of 15 years.

Radiography confirmed a dilated esophagus in 24/29 horses (83%). Air–fluid interfaces and mixed gas/soft tissue intraluminal content were observed on plain radiographs in 19/29 horses (66%). Paraoesophageal emphysema was present in 15/29 horses (52%), and adjacent soft tissue swelling suggestive of cellulitis or abscessation in 12/29 horses (41%). Secondary aspiration pneumonia was present in 9/29 horses (31%).

Ultrasonography revealed esophageal wall thickening in 22/29 horses (76%), and luminal dilation in 18/29 horses (62%), with air–fluid interfaces in 14/29 horses (48%). Paraoesophageal fluid collections containing gas were detected in 9/29 horses (31%), frequently extending into cervical soft tissues, and cervical emphysema was present in 7/29 horses (24%). Reduced or absent peristalsis was observed in 15/29 horses (52%).

Contrast esophagography was performed in 5/29 horses and demonstrated focal or segmental dilation, mainly at the caudal cervical esophagus and thoracic inlet. In 2 cases, contrast leakage confirmed partial esophageal tears, whereas in 3 cases delayed bolus transit suggested esophageal dysmotility or stricture.

Multimodal imaging, used alongside routine endoscopy, provides the most comprehensive evaluation of equine esophageal disease and significantly boosts diagnostic confidence. By complementing endoscopy, ultrasound offers superior assessment of the wall, motility, and paraoesophageal tissues in the cervical esophagus, radiography clarifies the intrathoracic portion and easily detects gas, and together, they screen for aspiration pneumonia. This combined approach solidifies the diagnosis and refines the clinical prognosis.

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Oral Communication(s)

COMPARATIVE STUDY BETWEEN COMPUTED TOMOGRAPHY AND ULTRASONOGRAPHY IN THE EVALUATION OF LUMBOSACRAL DISC ABNORMALITIES IN HORSES: DIAGNOSTIC POTENTIAL ON SPECIMENS

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Low back pain and poor performance in horses are frequently associated with disorders of the lumbosacral region [1]. Abnormalities of the lumbosacral intervertebral disc may impair locomotion and represent a relevant cause of poor athletic performance [2]. Ultrasonography is widely used in equine practice, but its diagnostic reliability compared with computed tomography (CT), considered the gold standard, remains incompletely defined [3]. A retrospective study was performed on 23 equine cadaver specimens. Ultrasonographic findings of the lumbosacral intervertebral disc were compared with CT images. Parameters assessed included disc morphology, echogenicity, mineralization, protrusion, spondylolisthesis, vertebral canal visualization, and morphometric measurements. Agreement between imaging modalities was evaluated using descriptive statistics and Pearson correlation coefficients. Ultrasonography showed good agreement with CT for disc morphology in 61% of cases. The vertebral canal was visualized in 78% of specimens, indicating that clinically relevant structures can be assessed in most cases. Strong correlations were found for morphometric measurements ($r = 0.640–0.874$; $P < 0.05$), supporting the reliability of ultrasonography for quantitative evaluation. Mineralization showed high concordance (87%), although ultrasonography underestimated its prevalence compared with CT, likely due to partial volume effects and reduced sensitivity to small mineral deposits. Disc protrusion was detected in 61% of cases on CT but showed lower agreement with ultrasonography (53%), indicating limited ability to identify dorsal or centrally located lesions. Spondylolisthesis, identified in 13% of specimens on CT, was not detected ultrasonographically, highlighting a critical limitation in the assessment of vertebral instability. These findings indicate that ultrasonography is a useful first-line imaging modality for evaluating lumbosacral disc morphology and morphometry in horses, particularly in field conditions due to its accessibility and real-time capability. However, its limited sensitivity for deeper structures and inability to detect instability may result in underestimation of clinically relevant lesions. Discrepancies between imaging modalities likely reflect acoustic limitations and reduced ultrasound penetration in deeper regions. Therefore, ultrasonography should be combined with advanced imaging when protrusion or instability is suspected. CT remains essential for comprehensive evaluation and accurate characterization of lumbosacral pathology, especially in performance horses. Clinically, imaging findings should always be interpreted alongside physical and functional assessment, as ultrasonographic changes may not reflect the full extent of pathology.

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Oral Communication(s)

COMPUTED TOMOGRAPHY MEASUREMENT OF PRESUMPTIVELY HEALTHY THORACIC ESOPHAGEAL WALL IN BRACHYCEPHALIC AND NON-BRACHYCEPHALIC DOGS: A PRELIMINARY ASSESSMENT OF POSSIBLE BREED-RELATED THICKNESS VARIABILITY

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Brachycephalic dog breeds have experienced a marked rise in popularity in recent years and are characterized by specific anatomical features that often lead to particular disorders (e.g., BAOS) [1]. When assessing CT images from these breeds, it is of paramount importance to clearly distinguish strictly pathological findings from breed-related features, as may occur in cases such as lower HU attenuation of the thyroid parenchyma [2] or enlarged lateral ventricles [3].

This study aimed to assess possible differences in esophageal wall thickness in presumptively healthy dogs between brachycephalic and non-brachycephalic breeds. A total of 261 CT examinations were retrospectively reviewed, and dogs were divided into group 1 (brachycephalic) and group 2 (non-brachycephalic).

Exclusion criteria included known esophageal disease, evident hiatal hernia, collapsed esophageal lumen, and incomplete CT studies. For each examination, the following data were recorded: signalment, reason for CT examination, cranial thoracic esophageal wall thickness (at the thoracic inlet) and caudal thoracic esophageal wall thickness (before the esophageal hiatus) (mm), degree of distension (mild, moderate, severe), and HU values pre- and post-contrast.

Paired and unpaired t-tests were used for statistical analysis, with significance set at $P < 0.05$.

Twenty-two and sixty-eight dogs met the inclusion criteria for groups 1 and 2, respectively.

In group 1, the mean wall thickness was 2.4 mm, standard deviation (SD) 0.79 for the cranial portion and 4.6 mm, SD 1.3 for the caudal portion, with a significant difference between them ($P < 0.0001$). In group 2, the mean wall thickness was 2.0 mm, SD 0.72 for the cranial portion and 2.8 mm, SD 1.0 for the caudal portion, also with a significant difference ($P < 0.0001$).

Brachycephalic dogs showed a higher wall thickness in the caudal portion compared to non-brachycephalic dogs ($P < 0.0001$).
No significant differences were observed based on the degree of distension in either group.

Based on these results, both brachycephalic and non-brachycephalic dogs may exhibit increased thickness in the caudal thoracic portion without disease-related anatomical abnormalities or gastrointestinal disorders reported in their history.

Under the same conditions, brachycephalic dogs exhibit a significantly higher caudal thoracic wall thickness compared to other dogs.

This finding could be considered a potential normal variant during CT evaluation and should not be misinterpreted as an exclusive sign of esophageal disease.

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Oral Communication(s)

PREDICTIVE VALUE OF COMPUTED TOMOGRAPHY AND SCINTIGRAPHY IN THE CHARACTERISATION OF THYROID LESIONS IN HYPERTHYROID CATS

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Feline hyperthyroidism is one of the most common endocrinopathies in geriatric cats, typically associated with proliferation of functional thyroid tissue, most frequently due to adenomatous hyperplasia or adenoma, and less commonly to carcinoma[1]. Diagnostic imaging plays a key role in lesion characterisation, with computed tomography (CT) providing anatomical detail and scintigraphy with technetium-99m pertechnetate representing the gold standard for functional assessment[2].

The aim of this study was to assess the predictive value of CT and scintigraphy in the characterisation of feline thyroid lesions and to determine whether their combined use improves diagnostic accuracy.

Twenty-nine hyperthyroid cats with 40 thyroid lesions were retrospectively enrolled. All patients underwent scintigraphy followed by pre- and post-contrast CT, and imaging characteristics were compared with cytological (n=10) or histopathological (n=20) diagnoses.

Evaluated CT variables included lesion localisation, morphology, margins, volume, attenuation and contrast enhancement (CE) characteristics. Scintigraphic parameters comprised radiotracer uptake pattern, ectopic thyroid tissue, lymphatic and vascular spread, and quantitative indices, including thyroid-to-salivary (T/S), thyroid-to-trachea (T/T), thyroid-to-background (T/A) ratios, and technetium uptake percentage.

Statistical analysis was performed using Shapiro–Wilk testing, chi-square test, and univariate logistic regression, with ROC curve analysis for quantitative variables.

A strong concordance was observed between CT and scintigraphic localisation of the lesion ($P < 0.0001$); however, CT failed to reliably differentiate unilateral from bilateral disease, confirming its limited functional capability, as previously reported in the literature[3].

Most morphological parameters, including lesion type, margins, volume, heterogeneity, and lymphatic and vascular spread, were not predictive (all P-values > 0.05), highlighting the limited diagnostic value of conventional morphological assessment. Conversely, quantitative parameters demonstrated significant diagnostic performance. Mean attenuation values were highly predictive ($P < 0.0001$), with parathyroid cysts exhibiting significantly lower values than all other lesions, while post-contrast attenuation allowed differentiation between adenoma and hyperplasia ($P = 0.01$).

Scintigraphic quantitative parameters showed the highest diagnostic performance. The T/A ratio and technetium uptake percentage were significantly higher in hyperplasia ($P = 0.002$ and $P = 0.005$) with good diagnostic accuracy (AUC 0.83–0.85), high sensitivity (100%) and moderate specificity (68%), indicating preserved functional activity compared to neoplastic lesions. Ectopic thyroid tissue was also significantly associated with hyperplasia ($P = 0.036$), supporting a systemic proliferative process.

In conclusion, CT and scintigraphy provide complementary but non-interchangeable information. Quantitative imaging parameters, particularly scintigraphic indices, show good diagnostic performance and predictive value, while a multimodal approach improves lesion characterisation and supports more appropriate clinical decision-making.

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Oral Communication(s)

COMPUTED TOMOGRAPHY FEATURES OF DACRYOPS IN DOGS: A CASE SERIES

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Dacryops is a rare cystic ectasia of the lacrimal gland or its ductal system, infrequently reported in dogs, with only a limited number of cases described in veterinary literature. It is typically characterized by a fluid-filled, expansile lesion arising in close association with the nasolacrimal system. Imaging plays a key role in lesion characterization; however, the computed tomography (CT) features of dacryops in dogs remain poorly described. [1, 2]

The aim of this retrospective study was to describe the CT characteristics of orbital lesions consistent with suspected dacryops in dogs and to correlate imaging findings with clinical presentation.

Sixteen dogs with a CT diagnosis of dacryops were included, with a mean age of 8.1 years (range 3–13) and a slight male predominance (9/16, 56%) with females accounting for 7/16 (44%). The most represented breeds included mixed breed (7/16, 43.75%), Labrador Retriever (3/16, 18.75%) and French Bouledogue (2/16, 12.5%), while the remaining breeds were each represented by a single dog.

Clinical presentation was equally divided between incidental findings (8/16, 50%) and symptomatic cases (8/16, 50%). In symptomatic patients, the most commonly reported clinical signs included periocular swelling (6/16, 37.5%), exophthalmos (5/16, 31%), and epiphora (2/16, 12.5%). Lesions detected incidentally were smaller compared to those associated with clinical signs as assessed by transverse measurements (median 13.5 mm, range 6–23 mm vs median 25.5 mm, range 16–78 mm).

Lesions were consistently located along the course of the nasolacrimal duct, typically positioned rostroventrally and medially to the ipsilateral ocular globe. All lesions were unilateral (16/16, 100%), with an even distribution between the right (8/16, 50%) and left sides (8/16, 50%).

On CT, lesions were predominantly round to ovoid and well-defined, characterized by a thin hyperattenuating wall and hypoattenuating fluid content. A mild contrast enhancement of the wall was observed in most cases (14/16, 87.5%). Bone resorption was present in all cases, most commonly involving the lacrimal and maxillary bones, and nasal turbinates. In only one case, mild, smooth, and continuous periosteal proliferation was also observed.
Dacryocystography was performed in two cases: in one case, contrast medium progression was interrupted at the level of the lesion, while in the other case the contrast medium progressed through the nasolacrimal duct, which appeared in close association with the lesion. In neither case was contrast filling of the lesion observed.

Computed tomography provides valuable information for the characterization of dacryops in dogs. Lesions are typically unilateral, well-defined, fluid-filled cysts located along the nasolacrimal duct, associated with bone resorption. Larger lesions are more likely to produce clinical signs, whereas smaller lesions may be incidental findings. Dacryocystography may serve as a complementary imaging tool to assess lesion communication with the nasolacrimal system for supporting diagnosis and management.

Oral Communication(s)

DIAGNOSTIC AND PROGNOSTIC VALUE OF ABDOMINAL CONE-BEAM CT: A MULTICENTRE STUDY FOR THE EVALUATION OF INTRA-ABDOMINAL TUMOURS IN SMALL ANIMALS

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Diagnostic imaging plays a key role in small-animal clinical practice, supporting non-invasive diagnosis and decision--making. To overcome the limitations of conventional imaging, cross-sectional modalities such as multidetector computed tomography (MDCT) and cone-beam CT (CBCT) have been developed [1]. CBCT is increasingly used in dogs and cats, particularly in dentistry and maxillofacial imaging, due to its high spatial resolution for high-contrast structures [2]. Although historically limited by poor soft tissue contrast, its application has recently expanded to the assessment and treatment planning of neoplastic lesions. Recent studies have also highlighted its potential when combined with other diagnostic techniques, improving the detection of peripheral lung lesions [3]. This retrospective study aimed to evaluate the clinical utility of contrast-enhanced abdominal CBCT for assessing abdominal masses in dogs and cats. Fifty-six cases (49 dogs and 7 cats) from four veterinary practices between January 2022 and February 2026 were retrospectively reviewed. Animals with suspected abdominal neoplasms or lesions affecting the abdominal organs for which first-line imaging, including radiography and ultrasonography, was inconclusive for clinical management or treatment planning, were included in the study. All patients underwent abdominal CBCT with intravenous contrast medium administration, including native scans, arterial phase at 20 seconds, and venous phase at 25 seconds. Images were evaluated by a single observer for lesion origin, size, extent, attenuation characteristics, enhancement patterns, and presence of secondary lesions or comorbidities. Lesions were identified in the liver (24 cases), spleen (20 cases), adrenal glands (6 cases), and other sites including the intestinal wall, lymphatic system, peritoneum, abdominal wall, kidneys, prostate, and bladder. Image assessment was based on the tissue's response to the contrast medium, with attenuation parameters considered as relative values rather than direct measures of standard Hounsfield units (HU). These results suggest that contrast-enhanced abdominal CBCT can be a useful diagnostic tool for the evaluation of abdominal masses, providing clinically relevant information on lesion characteristics and extent. This technique may support diagnostic decision-making and contribute to prognostic and preoperative assessment in veterinary patients.

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Oral Communication(s)

GENITOURINARY GRASS AWN MIGRATION IN DOGS AND CATS: A RETROSPECTIVE ULTRASONOGRAPHIC STUDY OF 35 CASES

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Although the migration of grass awns has been widely described in the literature—as highlighted in the 2023 review by Cavani et al.—data regarding involvement of the genitourinary tract remain limited and are mainly based on small case series or single case reports. In light of this, the present study aims to assess the consistency of the available evidence by analyzing a larger population. This is a retrospective study conducted at the Ultrasound Service of the Veterinary Teaching Hospital of the University of Pisa and by an independent veterinary sonographer, covering the period from January 2018 to December 2025. Through the review of ultrasound reports, dogs and cats with a definitive diagnosis of foreign bodies located in the genital or urinary tract or in adjacent tissues, were selected. The study population comprised 35 animals, 31 dogs and 4 cats, 29 females and 6 males, for which data on signalment, clinical signs, ultrasonographic findings, and removal techniques were collected. In the analyzed population, the most frequently affected site was the vestibulovaginal, (14 cases) followed by the vulvar and perivulvar region (6 cases), and less frequently the uterine horn (4 cases), uterine stump (4 cases), and cervix (1 case). In one subject, two grass awns were identified within the vagina. All affected females presented with vulvar discharge; cystitis was additionally reported in two cases. On ultrasonographic examination, 7 out of 8 grass awns located in the uterine horn or uterine stump were associated with purulent collections. In contrast, grass awns located in the more distal portions of the genital tract resulted in focal fluid collections in only two cases, accompanied by wall thickening and perilesional reactivity. In two subjects, mild enlargement of the medial iliac lymph nodes with decreased echogenicity was also observed. Three out of 6 male dogs presented with dysuria and hematuria associated with grass awns located in the penile urethra, prostate and urinary bladder, respectively. One dog showed perineal fistulas caused by grass awns originating from the scrotum; in another case, a grass awn located in the balanopreputial fold led to the formation of a peripenile fistula, characterized by corpuscular content, hyperechoic foci, and marked reactivity of the surrounding tissues on ultrasound. Clinically, all affected dogs exhibited swelling and purulent-hemorrhagic discharge. In the last dog, a parapenile abscess was identified, without associated fistulas. No significant predisposition related to reproductive status was identified. In all cases, grass awns appeared as linear, lanceolate, hyperechoic structures, consistent with intact foreign bodies; a posterior acoustic shadow was observed in 9 cases. Therapeutic data were available for 28 patients: 11 underwent surgical removal, 2 transvaginal endoscopic removal, and the remaining cases ultrasound-guided removal (transvaginal, transurethral, or through the fistulous tract). Ultrasonography therefore proved to be not only an essential tool for identifying the primary lesion, but also an effective means of assessing the extent of the pathological process and, in many cases, guiding foreign body removal.

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Oral Communication(s)

CLINICAL OUTCOMES AND LONG-TERM EFFICACY OF ELECTROCHEMOTHERAPY IN CATS WITH ADVANCED (T3–T4) NASAL PLANUM SQUAMOUS CELL CARCINOMA

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Feline squamous cell carcinoma (fSCC) is a common malignant cutaneous tumour in cats, typically affecting unpigmented or lightly pigmented skin, frequently involving the nasal planum, eyelids, and pinnae, and is characterized by marked local invasiveness and a low metastatic rate[1]. Electrochemotherapy (ECT), is a locoregional treatment with a favourable safety profile and promising efficacy, combining cytotoxic drugs with electroporation to enhance intracellular uptake[2]. Despite increasing clinical use, data on its long-term efficacy as a sole treatment modality in advanced-stage nasal planum fSCC remain limited. This retrospective study evaluated the efficacy of ECT as a sole treatment in cats with advanced-stage nasal planum fSCC, with long-term follow-up to better define its clinical outcomes and potential palliative benefit.

Inclusion criteria required cytological or histopathological diagnosis, absence of metastasis, no additional treatments, and a minimum one-year follow-up. ECT was performed under general anaesthesia with intravenous bleomycin. Outcomes included overall response rate (ORR), progression-free survival (PFS), overall survival (OS), and time to progression (TTP). Pruritus, alopecia, erythema, skin ulcerations, tissue necrosis, and pain (evaluated through palpation of the tumor area), and anorexia and lethargy were assessed.

Eighteen cats met the inclusion criteria. Clinical signs including respiratory noise, pain, anorexia, and sneezing. Tumour stage was $T_3N_0M_0$ in 55.6% of cases and $T_4N_0M_0$ in 44.4%. ECT was administered in 1 (38.9%), 2 (44.4%), or 3 (16.7%) sessions. At the end of treatment, 72.2% achieved complete response (CR), 16.7% partial response (PR), and 11.1% progressive disease (PD), yielding an ORR of 88.8%. Treatment response significantly influenced PFS ($p = 0.006$), OS ($p < 0.001$), and TTP ($p = 0.008$), with longer outcomes in CR (median 820 days) compared with PR (56 and 365 days, respectively) and PD (65 and 311 days, respectively).

Recurrence negatively affected PFS ($p = 0.013$) and OS ($p = 0.002$); Cats without recurrence had median PFS and OS of 820 days vs 151 and 373 days in those with recurrence, respectively. At one year, 61.1% of cats were still in CR. The estimated recurrence risk in the second year was 57.1%. After the first session, pain (72.2%, grade 2), erythema/crusting (33.3%, grade 2), pruritus (27.8%, grade 1), alopecia (5,5% grade 1) and anorexia (5,5% grade 2). After the second session, pain (12.5% grade 1), erythema/crusting (62.5% grade 1), pruritus (37,5% grade 1), after the third session, 33.3% of cats achieved alopecia (grade 1), pruritus (grade 1) and anorexia and lethargy (grade 2).

ECT was generally well tolerated, with adverse events mostly mild and transient; only a few cases required additional treatment. Temporary worsening of pre-existing clinical signs was likely due to an acute local inflammatory response induced by ECT, which may transiently intensify tumour-associated inflammation, rather than to treatment-related toxicity. The subsequent resolution of symptoms, together with high response rates and durable local disease control, indicates that ECT is an effective treatment for advanced nasal planum fSCC, with a clear benefit for quality of life.

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Oral Communication(s)

DISTRIBUTION OF MALIGNANT TUMOURS AND THEIR ASSOCIATION WITH DIAGNOSTIC IMAGING MODALITIES IN DOGS AND CATS: A RETROSPECTIVE SINGLE-INSTITUTION STUDY

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Neoplastic diseases are commonly investigated in small-animal practice using diagnostic imaging, which plays a crucial role in lesion detection, characterisation, staging, and follow-up. However, the relationship between tumour histotype and the choice of the imaging modality used in clinical veterinary populations has not been well characterised. This retrospective, single-institutional study aimed to describe the distribution of malignant neoplastic lesions by histogenetic classification, species, and imaging modality in dogs and cats examined at a referral diagnostic imaging centre between 2021 and 2025. All included cases had a confirmed malignant diagnosis based on cytology and/or histopathology. Tumours were classified as epithelial, mesenchymal, round cell, neuroendocrine, melanocytic, or germ cell. Species, breed, age, body weight, and imaging modality were recorded. Descriptive statistics were performed, and the association between tumour histotype and imaging modality was

assessed using a chi-square test. A total of 5,977 imaging examinations were performed, of which 343 cases (5.74%) had a confirmed to be malignant neoplasm. The population included 291 dogs (84.84%) and 52 cats (15.16%). In dogs, 137 were males (47.08%) and 154 females (52.92%); in cats, 24 were males (46.15%) and 28 females (53.85%). Median age was 10 years (range 1–17) in dogs and 11 years (range 2–17) in cats. Overall, epithelial tumours were most frequent (34.40%), followed by round cell (27.11%) and mesenchymal (25.95%) tumours. Neuroendocrine, germ cell, and melanocytic tumours accounted for 3.21%, 3.50%, and 5.83% of cases, respectively. Species-specific differences were observed: in dogs, epithelial tumours predominated (33.68%), followed by mesenchymal (27.15%) and round cell tumours (25.09%); in cats, epithelial and round cell tumours were equally represented (38.46%), with mesenchymal tumours less frequent (19.23%). In dogs, neuroendocrine, melanocytic, and germ cell tumours accounted for 3.44%, 5.84%, and 3.78% of cases, respectively. In cats, neuroendocrine, melanocytic, and germ cell tumours accounted for 3.85%, 5.77%, and 1.92%, respectively. Computed Tomography (CT) was the most used single modality (32.94%), followed by combined radiography and ultrasonography (22.16%) and ultrasonography alone (18.95%); radiography alone accounted for 11.95%. A significant association was found between tumour histotype and imaging modality (χ^2 = 36.61, $p<0.001$), with CT more frequently used in epithelial and mesenchymal tumours, ultrasonography in round cell tumours, and multimodal approaches in complex cases. In conclusion, epithelial and round cell tumours were the most prevalent malignant neoplasms. Differences between species were observed, with round cell tumours relatively more common in cats. CT was the most frequently used modality, while radiography and ultrasonography were widely applied for staging and follow-up. These findings may help to optimise diagnostic strategies in veterinary oncology practice.

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Oral Communication(s)

APITHERAPY IN VETERINARY MEDICINE: USE AND PERCEIVED EFFICACY IN SOUTHERN ITALY

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In the context of rising antibiotic resistance and the increasing demand for natural alternatives in veterinary medicine, bee products are attracting significant clinical interest due to their documented pharmacological and nutraceutical properties, including antibacterial, anti-inflammatory, and wound-healing effects. Furthermore, apitherapy offers a valuable adjuvant support for certain therapies at a significantly lower cost than conventional medications. However, this therapeutic practice suffers from a lack of clear regulation and data on its clinical application; data from Eastern Europe remain sparse, particularly in Italy. Therefore, to address these limitations, this cross-sectional study, conducted between March 2023 and January 2024 in the Catanzaro province (southern Italy), assessed the use, perceived efficacy, and acceptance of apitherapy through structured questionnaires administered to 70 veterinarians and 100 pet owners, following the STROBE-Vet guidelines. The veterinarians were recruited voluntarily from the provincial register, with the requirement of having worked in the area for at least one year. Of the 178 registered veterinarians, 70 were found to be eligible. Of these, 85.7% reported using bee products: honey was the most used (71.4%), followed by propolis (10%) and royal jelly (4.3%); none used bee venom. Use was almost exclusively for companion animals (71.7%), with only 6.7% using bee products on livestock, while the remainder treated non-traditional companion animals and exotic species. Regarding clinical indications, 68.3% used apitherapy to treat wounds, ulcers, and skin lesions, 11.7% for dermatological conditions, 8.3% for oral lesions or gingivitis, 5% to stimulate appetite, and 13.3% for other conditions. Inferential analysis confirmed a significant association between the use of honey and management of skin lesions (OR = 17.14; p-value < 0.01). Regarding perceived efficacy, 45% of veterinarians rated the results as good or excellent, 41.7% reported a slight improvement, and 13.3% reported no improvement. No adverse effects were reported. Among owners, 73% were willing to use honey on their animals, and trusted that the veterinarian emerged as the strongest predictor of treatment acceptance (OR = 73.44; p-value < 0.001). A significant gap in perception emerged: although 87% of pet owners considered natural products to be superior or equivalent to conventional medications, only 45% of veterinarians reported good or excellent clinical improvement (OR=0.12; p-value <0.001). Regarding practical issues, 14.3% of veterinarians reported difficulties with application, with insect attraction being the main problem (60%). Notably, 84% of veterinarians had no university training in apitherapy, relying instead on self-study and empirical experience. In conclusion, apitherapy is an established practice in veterinary clinics for companion animals in Southern Italy and demonstrates a favorable safety profile, particularly for honey. However, the heterogeneity of clinical protocols, the absence of standardized guidelines, significant training gaps, and marked divergence between owner expectations and outcomes show the urgent need for evidence-based protocols, targeted professional training, and product standardization.

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Oral Communication(s)

ENDOSCOPIC APPROACH TO THE DIFFERENTIAL DIAGNOSIS AND TREATMENT OF COMPRESSIVE PARAPARESIS DUE TO PELVIC OBSTRUCTION IN CHELONIANS

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Neurological dysfunctions in reptiles pose a diagnostic challenge for veterinarians due to the anatomical, physiological, and behavioral variability of these species and the frequent nonspecificity of clinical signs. In chelonians, hindlimb paraparesis may be associated not only with primary nervous system disorders but also with compressive conditions secondary to gastrointestinal, urinary, or reproductive diseases [1].

The aim of this study was to describe the diagnostic and therapeutic approach in a retrospective case series of chelonians presenting with neurological symptoms characterized by hindlimb paraparesis. Six specimens belonging to the species Testudo hermanni, Emys orbicularis, and Trachemys scripta scripta were evaluated at the Veterinary Teaching Hospital of the Department of Veterinary Sciences of the University of Messina between September 2022 and July 2023.

The subjects underwent a complete clinical examination, a neurological assessment focused on the hindlimbs (including evaluation of response to nociceptive stimuli), radiographic investigations, and subsequent cloacoscopic examination. Cloacoscopy was performed using a rigid endoscope (4 mm diameter, 0°, 8.5 cm length; Olympus Medical, Japan). Animals were manually restrained in dorsal recumbency and received alfaxalone (Alfaxan 10 mg/ml, Jurox Limited), administered intramuscularly at a dose of 15 mg/kg. The endoscope was inserted into the cloaca in a caudo-cranial direction, and a warm sterile saline solution was infused to improve visualization. This study did not require ethics committee approval, as it is a retrospective case series based on clinical cases managed under routine veterinary practice without experimental interventions, and written informed consent was obtained from all animal owners prior to the procedures.

The main pathologies diagnosed were severe constipation with fecal impaction (n = 3), egg retention (n = 1), and bladder urolithiasis (n = 2). In all cases, pelvic or cloacal compression was considered the likely cause of neurological dysfunction. Treatment was performed using a minimally invasive endoscopic technique with hydrodissection and lavage to fragment fecal impaction and uroliths, and endoscopic fragmentation of the retained egg [2,3].

All subjects showed immediate improvement in response to nociceptive stimuli and hindlimb mobility, with complete recovery of ambulation within 15 days following treatment.

These findings suggest that cloacoscopy may represent a useful minimally invasive diagnostic and therapeutic option in selected cases of chelonians presenting with paraparesis associated with pelvic obstructive disease. Furthermore, they highlight the importance of considering extracranial compressive causes in the differential diagnosis of neurological dysfunctions in reptiles.

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Oral Communication(s)

TOPICAL SUCRALFATE REDUCES BACTERIAL BURDEN AND MODULATES HEALING IN FULL-THICKNESS CUTANEOUS WOUNDS: EVIDENCE FROM A PORCINE MODEL

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This blinded clinical study investigated the effects of a topical sucralfate formulation on wound healing and bacterial burden in full-thickness cutaneous wounds using an experimental porcine model (Ministry of Health authorization n. 162/2025-PR, 06/03/2025).

Five 3-month-old Duroc pigs were initially enrolled; one animal was excluded due to post-procedural dermatitis. Under general anesthesia and standardized conditions, eight full-thickness square wounds (4×4 cm) were created on the dorsal thoracic region of each animal, symmetrically distributed on both sides.

Wounds on the left side were assigned to the treated group and received daily topical application of a 20% sucralfate spray following cleansing with sterile saline, while contralateral wounds served as controls and were managed with saline irrigation and moist dressings only. All wounds were covered with standardized bandaging, replaced daily, and animals were housed under controlled conditions. Wound healing was assessed weekly (T0–T28) using a digital planimetric imaging application, until complete re-epithelialization. Microbiological evaluation was performed at each time point by collecting wound swabs before and after treatment procedures. Samples were processed within two hours and subjected to quantitative culture on selective and non-selective media, with bacterial load expressed as CFU/swab. Bacterial isolates were identified at species level using MALDI-TOF MS. Statistical analysis included parametric and non-parametric tests, as well as Kaplan–Meier survival analysis to evaluate time to healing ($p < 0.05$). Both groups showed a significant reduction in wound area over time, with near-complete closure by day 28, and no significant differences at individual time points. However, Kaplan–Meier analysis revealed a significantly higher probability of healing over time in the treated group. Complete re-epithelialization occurred in a higher proportion (87.5%) of treated wounds compared to controls (75%; $p=0.6539$). No bacterial growth was detected at baseline. At subsequent time points, the control group exhibited an increase in bacterial load following routine wound management, whereas treated group consistently showed a reduction after sucralfate application. Overall, considering all time points, these differences were significant, with a trend toward increased bacterial burden in controls and decreased load in treated wounds. Moreover, post-treatment bacterial counts were significantly lower in the treated group, with an approximate 5-log reduction. In both groups, bacterial load decreased over time, likely reflecting the physiological progression of wound healing.

These findings suggest that topical sucralfate, while not significantly affecting wound area reduction compared to standard care, may enhance healing dynamics and exert a short-term local antimicrobial effect. This property may support its use in wound management in veterinary medicine, potentially contributing to reduced reliance on topical antibiotics.

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Oral Communication(s)

ISOLATION OF *ESCHERICHIA COLI* FROM JOINT SWABS IN COMMERCIAL BROILERS WITH LAMENESS: A FIELD STUDY

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Diseases affecting the musculoskeletal system, along with infections caused by *Escherichia coli*, constitute a major health challenge for the global poultry industry. In particular, Avian Pathogenic Escherichia coli (APEC) is implicated in the development of extraintestinal infections that may progress into respiratory or systemic disease, commonly referred to as colibacillosis [1]. Affected birds frequently develop lesions such as perihepatitis, airsacculitis, pericarditis, peritonitis or salpingitis, and arthritis. These conditions have a substantial impact, leading to significant economic losses for the global poultry industry [2].

Arthritis in broilers occurs between 14 and 48 days of age. The most common aerobic bacterial cause is *Staphylococcus aureus*; however, Gram-negative bacteria such as *Escherichia coli, Salmonella spp., Pasteurella multocida, Pseudomonas aeruginosa,* as well as Gram-positive bacteria including *Streptococcus spp., Enterococcus spp.*, and *Listeria monocytogenes* are also frequently isolated from affected joints [3].

This field study was carried out over a 15-month period, from January 2025 to March 2026,

in six commercial broiler farms, comprising a total of 48 houses. During this period, approximately 1.328.800 broilers were raised in the houses under investigation. Animals showing clinical signs of musculoskeletal impairment were subjected to clinical examination following which joint swabs were collected for bacteriological analysis. This study did not require ethics committee approval, as it was an observational field study and all procedures were performed within routine veterinary practice without additional experimental interventions.

A total of 60 joint swabs were collected from animals showing lameness and musculoskeletal abnormalities. Of these, 41 joint swabs tested positive for *E. coli*. Positive animals were observed at various ages throughout the production cycle. Gross lesions, which could be unilateral or bilateral, included tarsal arthritis characterized by serohaemorrhagic or purulent exudate and necrosis of the femoral head, ranging in severity from mild to severe.All 41 *Escherichia coli* isolates were subjected to bacterial typing and antimicrobial susceptibility testing, allowing the identification of multidrug-resistant strains. Antimicrobial susceptibility was evaluated against 12 antimicrobial agents; overall, the isolated *E. coli* strains exhibited resistance in 54.53% of cases, susceptibility in 28.57%, and intermediate susceptibility in 16.90% across all isolate–antimicrobial combinations. These findings highlight the involvement of *E. coli* in the musculoskeletal disorders observed in intensive broiler farms and emphasize the importance of systematic bacteriological monitoring of lameness, together with a more targeted and prudent use of antimicrobials in intensive poultry production. Furthermore, lameness can also be considered a relevant indicator of animal welfare, since the proportion of affected animals within a flock reflects both the effectiveness of farm management and the environmental conditions of the housing system. Taken together, these results support the need for integrated health monitoring programs aimed at improving both animal welfare and antimicrobial stewardship in intensive poultry farming.

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Oral Communication(s)

OCCUPATIONAL INJURIES AND RISK PERCEPTION IN VETERINARY MEDICINE: ACROSS-SECTIONAL PRELIMINARY INVESTIGATION

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The veterinary profession is an inherently complex activity characterized by high levels of occupational risk. This multifaceted landscape of hazards - encompassing biological risks, physical trauma, and psychological strain - arises from the heterogeneous nature of clinical practice and persistent interaction with different animal species and potentially pathogenic agents. Indeed, during diagnostic, therapeutic, and prophylactic procedures, veterinarians are exposed to diverse occupational hazards. Risk management responsibilities vary significantly by employment status; while employers bear comprehensive protective obligations for staff, self-employed practitioners assume a greater burden for risk self-management under Article 21 of Legislative Decree no. 81 of 9 April 2008. This study primarily aimed to evaluate the correlation between the incidence of occupational injuries and the risk perception expressed by diverse categories of veterinary professionals.

To achieve this, a cross-sectional preliminary investigation was conducted using an anonymous 25-item survey designed to collect demographic data, injury history, and risk self-assessment. The study cohort comprised 170 veterinarians with a varied academic backgrounds (PhD, Postgraduate School of Specialization, etc.), stratified into clinical facility practitioners (41.3%), self-employed professionals (31.6%), and public health employees (27.1%).

The investigation revealed significant gap in safety compliance: 34.8% of respondents lacked valid safety training certification, and 52.9% admitted to inconsistent use of Personal Protective Equipment. Data regarding injury prevalence are particularly striking, with 80.7% of the total sample having experienced at least one occupational injury. When stratified by professional category, injury rates reached 90.7% for self-employed practitioners and 100% for those operating within clinical facilities. Specifically, 80.6% reported physical trauma from animals, 52.3% suffered emotional distress linked to euthanasia or culling procedures, and 47.1% faced zoonotic exposure. Approximately half of the participants (50.3%) attributed these incidents to the animal's behavioural state or lack of habituation to handling. Regarding risk perception, 61.3% of respondents considered themselves moderately or highly exposed to physical injury, whereas 24.5% reported a high perceived risk regarding biological exposure. These preliminary findings demonstrate a significant correlation between reported injury frequency and risk perception. The hierarchy of events largely aligns with the order of perceived threats, suggesting a high level of professional awareness regarding daily operational hazards. Ultimately, the predominance of injury risk confirms that animal handling and restraint remain the most immediate and concrete safety challenges for the veterinary profession.

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Oral Communication(s)

ADVANCED VALIDATION OF A MULTIDIMENSIONAL WELFARE ASSESSMENT PROTOCOL FOR OSTRICHES (*STRUTHIO CAMELUS*) IN SEMI-INTENSIVE SYSTEMS: A DATA-DRIVEN RISK STRATIFICATION AND CLUSTERING APPROACH

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Modern agricultural trends are increasingly characterized by a heightened sensitivity toward animal welfare, extending the focus from conventional livestock to emerging sectors such as ostrich (Struthio camelus) farming. As the world's largest ratite, the ostrich represents a recently domesticated species whose production has transitioned from historical feather exploitation to a contemporary focus on high-quality leather and nutrient-dense, low-fat meat. Despite this industrial growth, a significant regulatory and methodological gap persists: unlike traditional poultry or cattle, there is currently no specific European legislation or standardized protocol to objectively assess the welfare of farmed ostriches. Consequently, management often relies on subjective interpretations of generic guidelines, which may fail to respect the unique physiological and behavioral demands of the species.

This study describes the validation of a multidimensional welfare protocol specifically tailored for ostriches in semi-intensive systems, bridging the gap between production efficiency and ethical requirements. The assessment tool integrates 42 indicators categorized into indirect (resource and management-based) and direct (animal-based) measures, including physiological, physical, and behavioral parameters. Field validation was conducted across nine semi-intensive ostrich farms in Sicily (n = 201 birds), representing 41% of the region's facilities. To transition from a descriptive scoring system to a robust diagnostic tool, a data-driven risk classification framework was developed using one-dimensional k-means clustering (k = 2). This statistical approach allowed for the identification of empirical cut-off values to stratify facilities into "Low-Risk" (optimal management) and "High-Risk" (requiring intervention) categories. The analysis identified specific thresholds: 21.58 for direct animal-based measures, 32.15 for indirect resource/management measures, and a composite score of 51.42 as the definitive benchmark for overall facility categorization.

Results demonstrated that resource-based measures showed the highest variability between farms, particularly regarding the provision of environmental enrichment (e.g., mud baths, shrubs) and shelter adequacy. Direct animal-based measures revealed generally stable health conditions, although localized issues such as feather pecking and suboptimal BCS were recorded in 22% of the population. By applying these thresholds, 66% of the surveyed farms were classified as Low-Risk, while 33% were identified as High-Risk, signaling a mandatory requirement for structural or management refinements. Sensitivity analysis performed on the full record-level dataset confirmed the stability and repeatability of the clustering-derived thresholds, supporting the reliability of the protocol across different analytical units. This validated protocol provides a standardized, objective baseline for veterinarians and regulatory bodies to monitor welfare, ensuring high ethical standards while mitigating management-related risks in non-conventional poultry sectors.

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Oral Communication(s)

DOG AGGRESSION PREVENTION AND THE ROLE OF VETERINARIANS IN ITALY: A NATIONAL SURVEY

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Dog aggression toward humans represents a relevant public health issue with important clinical, social, and regulatory implications. In Italy, severe and fatal attacks occur predominantly in domestic environments and mainly involve owned dogs, highlighting the importance of early prevention and risk identification. Within this context, private veterinary practitioners may play a strategic role; however, limited information is available on how this preventive function is perceived and implemented in routine clinical practice. This study aimed to investigate the role of private veterinarians working in the companion animal sector in Italy in the prevention of dog aggression, focusing on regulatory knowledge, risk perception, and professional practices. A cross-sectional observational study was conducted through an anonymous online questionnaire administered between November 2025 and February 2026. Of the 1,229 questionnaires collected, 1,088 met the eligibility criteria and were included in the final analytical sample. The survey explored professional characteristics, clinical experience with aggressive dogs, knowledge of the current regulatory framework, attitudes toward reporting to Local Health Authority Veterinary Services, and opinions on preventive tools. Data were analyzed using descriptive statistics and chi-square tests, with Cramer's V used to estimate effect size. Aggression emerged as a frequent component of clinical activity: 99.0% of respondents reported having observed aggressive behavior during clinical procedures, 96.1% had examined dogs with a history of aggression, and over 60% had managed at least five such cases in the previous year. Most veterinarians reported informing owners about behavioral support or training programs (82%), investigating the dog's behavioral history in the domestic context (89%), and assessing child–dog interactions in households with minors (82%). Overall, 80% recognized an active preventive role for veterinarians. However, only 55% reported being familiar with the Ministerial Ordinance of 6 August 2013, and only 19% had ever submitted a report to Veterinary Services. Specific training in behavioral medicine was reported by 12.96% of respondents and was significantly associated with greater knowledge of the regulatory framework, greater awareness of reporting procedures, and a higher likelihood of having submitted at least one report. Most respondents supported structured preventive strategies, including mandatory owner training programs (89.99%) and mandatory educational pathways for dogs and owners in the presence of risk factors (99%). These findings indicate that private veterinary practitioners are in a privileged position to contribute to the prevention of dog aggression in Italy, but also reveal gaps between the preventive role assigned by regulation and its implementation in practice, suggesting the need to strengthen training in behavioral medicine and improve integration between private practitioners and public veterinary services to enhance the effectiveness of prevention.

Oral Communication(s)

ESSENTIAL OILS AND HONEY BEE HEALTH: EVIDENCE FROM OUR LABORATORY AND FIELD STUDIES

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In recent decades, there has been a growing interest in the use of natural substances, such as essential oils (EOs), to fight infections in apiaries. EOs are mixtures of terpenes, terpenoids and phenolic compounds and are known to have bactericidal, virucidal, fungicidal and antiparasitic activities [1]. EOs and their monoterpenes are widely studied as alternatives to synthetic acaricides for controlling pests and diseases in honey bees. However, few EO-based products have entered the market because of the discrepancy between independent field and laboratory studies, even for the same plant species [1]. Further investigations on EOs are therefore needed to establish effective field protocols, to prevent or reduce diseases in honey bees. The following two studies were conducted with the aim of investigating the beneficial properties of EOs in relation to bee pathogens, both under field conditions and within the laboratory setting.

The EOs of cinnamon and oregano were administered to honey bees as dietary supplements, and their beneficial properties on bee colony health were explored in a field study [2]. Concurrently, the EOs of cinnamon and oregano, along with 16 additional EOs, were examined for their antimicrobial activity against the causative agents of foulbrood diseases, specifically *Melissococcus plutonius* and *Paenibacillus larvae*, and for their toxicity towards bees, under laboratory conditions [3]. Under field conditions, the oral administration of oregano and cinnamon EOs did not affect mite infestation levels, viral (DWV, CBPV and BQCV), fungal (*Nosema ceranae*) and bacterial (*M. plutonius*) infection levels, population density, and honey yield. Conversely, the EOs impacted the expression of *vitellogenin*, which was found to be overexpressed [2]. In a laboratory setting, oregano, juniper, sage, thyme, cinnamon, cumin, clove and black pepper were identified as the most effective EOs in combating the foulbrood bacteria. Among the various EOs examined, the bees demonstrated a heightened level of tolerance to the vapour of cinnamon and oregano following a one-hour exposure period. As the duration of exposure increased to a period of three hours, an escalation in toxicity was observed [3].

The field study demonstrated that the oral administration of cinnamon and oregano EOs was safe for honeybees, as it did not affect either the adult bee density or honey production. Furthermore, cinnamon and oregano have been demonstrated to enhance the gene expression of *vitellogenin*, though the consequences of this enhancement remain to be elucidated. The *in vitro* study demonstrated that EOs possess antimicrobial properties against the foulbrood pathogens, yet concurrently exerted toxicity towards bees. However, further studies are required to validate their clinical effectiveness and safety. Both studies were funded by Associazione Apicoltori Siena Grosseto Arezzo, Italy, grant CA87-2022 to CM, and by the Istituto Superiore di Sanità, Italy.

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Oral Communication(s)

EFFICACY OF DOMPERIDONE IN MAINTAINING STABLE OR IMPROVING THE CLINICAL PICTURE OF DOGS WITH CHRONIC KIDNEY DISEASE AND POTENTIAL IMPACT ON OWNER WELL-BEING: PRELIMINARY RESULTS

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Chronic Kidney Disease (CKD) is a common renal disease in dogs and is characterized by progressive loss of renal function, often associated with clinical signs that negatively affect both patient quality of life and owner well-being. Domperidone, a peripheral dopamine (DA2) receptor antagonist, has been reported to slow CKD progression in dogs affected by leishmaniosis [1, 2]. Since DA2 receptors are also expressed in renal vasculature, their blockade may improve renal hemodynamic enhancing glomerular filtration rate, renal plasma flow and filtration fraction [3]. The present study aims to evaluate the efficacy of domperidone plus standard therapy in slowing the progression of nephropathy in dogs affected by CKD, and the impact of managing CKD patients on owners' well-being. This therapeutic, prospective, randomized, and controlled clinical study (Approval Number UNIBA 27/23) included client-owned dogs of any age or sex, affected by CKD (IRIS stages 1-3a) and negative for vector-borne pathogens. Dogs were randomly allocated to Treatment Group (T) and Control Group (C). Both groups received renal diet and standard medical therapy according to IRIS guidelines, while Group T additionally received domperidone (2 cycles of 0.5 mg/kg SID for 28 days with intervals of 3 months-long periods without treatment). After enrolment, dogs were followed up after 4 (T4) and 8 (T8) months. Physical examination, blood pressure measurement, complete blood count, biochemical profile including symmetric dimethylarginine, and urinalysis including urinary protein/creatinine ratio evaluation have been performed. Owners' well-being was monitored monthly using a validated 10-item Likert scale questionnaire. Preliminary data are available for 7 dogs (Group T, n=4; Group C, n=3; 8.7±4.3 years; 5 females and 2 males). Out of 7 animals, 5 completed the T8 evaluation (2/4 in Group T and 3/3 in Group C). At enrolment, 6 dogs were classified as IRIS stage 2 (n=3 in each group), while one patient in Group T was classified as stage [1] At T4, in Group T, 3/4 dogs maintained their baseline IRIS stage, while one improved from stage 2 to stage 1, with this change persisting to T8. In Group C, IRIS stage remained unchanged at both T4 and T8. In Group T, serum creatinine remained stable between T0 and T4 (median 1.56 vs. 1.32 mg/dL), with no consistent individual trend. In contrast, Group C showed a progressive increase over time (median 1.57 mg/dL at T0, 1.91 at T4, and 1.97 at T8), with all dogs exhibiting rising values. At T4 and T8, creatinine was lower in Group T than in Group C (median 1.32 vs. 1.91 mg/dL and 1.47 vs 1.97 mg/dL, respectively). Owner-reported burden scores were lower in group T compared to group C at both timepoints. These preliminary findings, although limited by the small sample size, suggest a potential effect of domperidone in a short period of time on renal function in dogs with CKD, and an impact on the owner's well-being. The observed trends support further investigation in larger, adequately powered studies to confirm its clinical utility.

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Oral Communication(s)

BACTERIAL DNA SIGNALS IN CULTURE-NEGATIVE URINE OF CATS WITH FELINE IDIOPATHIC CYSTITIS: UROBIOME CHARACTERIZATION AND CLINICAL IMPLICATIONS

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Feline idiopathic cystitis (FIC) is one of the most prevalent conditions within feline lower urinary tract disease (FLUTD) and has traditionally been regarded as a "sterile" inflammatory syndrome.

The use of culture-independent methods has challenged the long-standing assumption of urinary sterility in both human and veterinary medicine. In cats, the role of bacterial DNA detected in culture-negative urine, including in FIC, remains unclear. Few studies report no viable bacteria or bacterial DNA in urine from healthy cats and cats with FIC, supporting the concept of urinary sterility. In contrast, other studies have detected bacterial DNA in cats with urinary tract disorders, including FIC, suggesting that culture-negative urine may still contain detectable bacterial signatures [1, 2].

The aim of this cross-sectional observational study was to investigate urinary bacterial biomass and microbiota composition in cats with FIC, compared with cats with urinary tract infection (UTI) and healthy controls (CON). All cats underwent clinical and laboratory evaluation. Urine samples, collected by cystocentesis, were analysed by quantitative culture, 16S rRNA gene qPCR and V3–V4 16S rRNA amplicon sequencing. Forty-one cats were enrolled, including 21 CON, 11 FIC and 9 UTI. Cats with UTIs had positive cultures ($\geq 10^3$ CFU/mL), whereas FIC and CON cats were culture-negative. Extracted DNA concentration and 16S qPCR-estimated bacterial load showed an increasing trend from healthy to FIC cats, reaching the highest levels in UTIs cases. UTI samples showed a high biomass, low complexity in the urinary microbiota and the dominance of one or a few taxa, with concordance between culture and sequencing in 7/9 cases. In contrast, in FIC samples intermediate biomass and high interindividual heterogeneity were identified. Notably, within FIC samples, 8/11 samples showed elevated relative abundance (≥10%) of uropathogenic taxa despite negative culture, including *Staphylococcus pseudintermedius*, *Proteus mirabilis* and *Enterococcus* spp., despite negative urine cultures. Healthy control urine represented an extremely low-biomass sample type with the lowest extracted DNA yield and 16S rRNA qPCR-estimated bacterial load, and 16/21 control samples did not reach the minimum sequencing depth threshold.

From a clinical standpoint, the detection of bacterial DNA in culture-negative urine from FIC cases does not demonstrate bacterial viability or active infection and does not support the use of empirical antimicrobial therapy [3]. Current recommendations remain unchanged, with antimicrobial treatment reserved for culture-confirmed UTIs in cats with lower urinary tract signs, while FIC management should focus on multimodal strategies addressing its multifactorial nature [3]. Longitudinal studies are needed to determine whether these microbial signals represent transient colonization or clinically relevant host–microbe interactions, as well as to evaluate recurrence risk and explore non-antibiotic strategies such as probiotics or prebiotics.

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Oral Communication(s)

ULTRASONOGRAPHIC CHARACTERIZATION OF GASTROINTESTINAL ALTERATIONS IN DOGS WITH ACUTE KIDNEY INJURY

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Canine Acute Kidney Injury (AKI) is a critical clinical condition that, beyond compromising renal excretory function, triggers a complex multi-organ dysfunction syndrome due to the accumulation of uremic toxins, electrolyte imbalances, and the release of inflammatory mediators [1]. Among extra-renal complications, the gastrointestinal (GI) tract emerges as a primary target, although veterinary literature still lacks a systematic ultrasonographic characterization of the associated morpho-functional alterations. The aim of this prospective observational study was to systematically characterize the GI morpho-functional alterations in dogs with AKI through B-mode ultrasonography. Specifically, the study wanted to determine the prevalence of structural abnormalities across different GI parts as stomach, small intestine, colon and to quantitatively assess gastrointestinal motility to identify specific patterns of dysmotility associated with uremia. This study enrolled 39 client-owned dogs diagnosed with AKI based on IRIS (International Renal Interest Society) guidelines [1]. Each patient underwent a standardized clinical workup and a comprehensive B-mode abdominal ultrasound, performed by the same operator on conscious animals, using high-frequency linear or micro-convex probes. The methodology focused on measuring wall thickness and evaluating the echogenicity and integrity of the parietal layers. Furthermore, 2D ultrasound was performed to detect sonographically visible GI contractions to quantitatively assess gastric and intestinal peristalsis. The cohort predominantly presented at advanced disease stages, with IRIS Grade IV being the most prevalent (33.3%). The GI tract was the most frequently affected system (97.4%). The small intestine was the most commonly involved segment (92.3%), followed by the stomach (61.5%) and colon (56.4%). Principal ultrasonographic abnormalities included parietal thickening and increased mucosal echogenicity across segments. Dynamic evaluation demonstrated profound motor impairment; peristalsis was completely absent in the stomach of 66.6% of evaluated patients and the small intestine of 50%. When present, contractions consistently exhibited a non-propulsive, bidirectional oscillatory pattern. These findings, including the early presence of hyperechoic mucosal bands in the stomach [2], suggest that uremic gastropathy is a common feature of acute injury. Furthermore, the high prevalence of accessory signs like mesenteric fat reactivity (41%) and biliary sludge (59%) underscores the activation of the renal-intestinal axis [3]. In conclusion, abdominal ultrasonography should not be limited to renal parenchyma but must include a detailed analysis of the GI tract, as the early identification of atony and mucosal changes is fundamental to guiding therapeutic choices and nutritional support in dogs with AKI.

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Oral Communication(s)

ANALYTIC PERFORMANCE OF A RAPID QUANTITATIVE ASSAY FOR MEASURING ANTI-*LEISHMANIA* ANTIBODIES IN DOG SERUM SAMPLES

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Canine leishmaniosis (CanL) is a complex disease caused by *Leishmania infantum*. In endemic areas the proportion of infected dogs is much higher than that of animals with CanL. Quantitative antibody (ab) measure is the first step in the diagnostic approach of suspected cases and in post-therapy follow-up [1]. Antibody detection is also required in healthy dogs in case of annual health check, blood donors, anti-*Leishmania* vaccination, relocation from/after travelling to endemic areas). The immunofluorescence ab test (IFAT) is a reference quantitative method for measuring anti-*Leishmania* abs. Qualitative serological point-of-care (POC) tests are available and their accuracy is variable, particularly in terms of sensitivity. A quantitative assessment of dog anti-*Leishmania* abs is obtained by a POC fluorescence immunochromatographic assay (cLSH-Ab, GUANGZHOU EXCBIO TECHNOLOGY CO., Ltd, Guangzhou, PRC) and automated reading of results [test units per milliliter (TU/mL)] by an immunofluorescence analyzer (FIAVETTM). A categorical semi-quantitative interpretation of results (negative, suspicious, weak positive, medium positive, strong positive) is provided.

No published studies have validated this test under field conditions, and the aim of this pilot study was to compare the analytic performance of the cLSH-Ab immunoassay with conventional IFAT, evaluating agreement and diagnostic performance at the two IFAT cut-off dilution mostly used for diagnosing CanL (1:80 and 1:160).

The sample size was calculated based on a statistical power of 95%, a type I error of 5%, and an expected disagreement rate between 20% and 28%. Agreement between the two tests was evaluated using Cohen's kappa coefficient (κ), and diagnostic performance parameters [accuracy (Acc), sensitivity (Sen), specificity (Spe), positive predictive value (PPV), and negative predictive value (NPV)] were calculated.

A total of 69 convenience serum samples of dogs from Sardinia were double-blind tested for anti-*Leishmania* abs with the cLSH-Ab immunoassay (according to the manufacturer's instructions) and IFAT [2]. Leftover serum aliquots from clinically healthy dogs tested prior to *L. infantum* vaccination, suspected cases, and from post-therapy follow-up were used.
Based on the cLSH-Ab immunoassay, 52 samples were negative and 17 tested positive (two medium positive and 15 strong positive). At the 1:80 IFAT cut-off, one false positive (medium positive) and eight false negatives (IFAT titers 1:80-1:160) were found (Acc 87.0%, Sen 66.7%, Spe 97.8%, PPV 94.1%, NPV 84.6%), and substantial agreement was seen (κ= 0.681). At the 1:160 cut-off, one false positive (medium positive) and one false negative (IFAT titer 1:160) were found (Acc 97.1%, Sen 94.1%, Spe 98.1%, PPV 94.1%, NPV 98.1%) and a strong agreement (κ= 0.922) was seen.

Most discrepancies (12%) occurred at cut-off IFAT level (1:80), whereas the cLSH-Ab immunoassay reliably identified positive samples with higher titers (≥160). Further studies, including also "suspicious" and "weak positive" samples, are needed to confirm that cLSH-Ab immunoassay provides a reliable POC quantitative assessment of anti-*Leishmania* abs in clinical settings.

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Oral Communication(s)

SHORT-TERM AND LONG-TERM OUTCOME OF CATS WITH BIG KIDNEY-LITTLE KIDNEY SYNDROME UNDER MEDICAL MANAGEMENT

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The “Big Kidney-Little Kidney” (BKLK) syndrome has been firstly introduced in feline medicine in 2011, to describe a significant size asymmetry between the kidneys. At present, BKLK is considered one of the leading causes of acute on chronic kidney disease (ACKD). Although ureteral surgery is usually considered the gold-standard, medical management is often attempted as first-line treatment [1,2,3]. The aim of the present study was to retrospectively investigate possible association between haemato-biochemical findings, ultrasonographic parameters, and survival in cats with ACKD due to BKLK. We included 54 cats referred to the nephrology and urology service of our hospital (2015-2025) for ACKD due to BKLK [2]. Signalment, haemato-biochemical, urinary, ultrasonographic parameters, and survival time were collected. According to survival time, cats were classified as short-term survivors (ST) if they survived <30 days of hospital admission, and long-term survivors (LT) if they lived > 30 days. Statistical analysis was conducted by GraphPad Prism 10 (statistical significance for $p<0.05$). Overall survival was 120 days (7-2190). Ultrasonographic signs of ureteral obstruction were found in 93% of cats, with unilateral obstruction in 28% of cats, and bilateral obstruction in 72% of cats. According to survival, 37 cats (69%) were in LT group, and 17 cats (31%) in ST group. ST group showed significantly higher values of median urea ($p=0.001$), creatinine ($p=0.002$), and phosphate ($p=0.01$), and higher number of cats with severe pelvic dilation, compared to LT group. No significant difference between the two groups was found in terms of cause of obstruction, number of cats with resolution vs failure, unilateral vs bilateral obstruction, localization of ureteral dilation. Cats with BKLK under medical management show elevated survival time also in case of partial resolution, regardless the cause, and localization of obstruction. However, the finding of severe pelvic dilation might be a decisive factor to consider surgery.

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Oral Communication(s)

SPURIOUS THROMBOCYTOSIS IN A DOG WITH PARAPROTEINEMIA DUE TO LEISHMANIA INFANTUM INFECTION

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A 3-year-old mixed-breed spayed female dog recently diagnosed with Leishmania infantum infection was referred to the University Veterinary Hospital of the University of Bologna for weakness and hyperthermia. Poor body condition, generalized lymphadenomegaly, lameness and scaly skin lesions were present at physical examination. A complete blood count was performed using the Advia 2120i analyzer on a EDTA blood sample and revealed severe thrombocytosis with a platelet count (PLT) of 1’642’000/µL (Reference interval, RI, 150’000-500’000/µL). On peripheral blood film evaluation, the estimated PLT was within RI, with an average of 11 platelets per 1000X field. Spurious thrombocytosis was suspected. There were abundant accumulations of proteinaceous, lavender material in the background, and blood cells did not show any morphologic abnormalities. Given that the serum total protein concentration was 10.10 g/dL with a severe polyclonal gammopathy (gamma-globulins 5.20 g/dL; RI 0.42-0.78), the presence of cryoglobulins/paraproteins was suspected as a potential cause of platelet misclassification by ADVIA 2120i. Other documented causes of spurious thrombocytosis (e.g., lipemia) were excluded. The same sample was analyzed using the BC-75R Vet analyzer yielding a PLT of 217’000/µL. To assess the presence of cryoglobulins, blood aliquots were analyzed with both analyzers after a 1-hour incubation at 20°C, 37°C, and 4°C, respectively. The PLT was 1’642’000 and 217’000/µL at 20°C, 1’351’000 and 225’000/µL at 37°C, and 790’000 and 220’000/µL at 4°C, counted by Advia 2120i and BC-75R Vet, respectively. Multiple myeloma was ruled out based on clinicopathological findings and follow-up. Cytologic examination of a lymph node aspirate confirmed Leishmania infection. The spurious thrombocytosis was not present after the first 3 weeks of anti-Leishmania treatment.

Paraproteinemia has been described in human medicine as a rare cause of pseudothrombocytosis, but it is poorly documented in veterinary medicine [2]. Canine leishmaniosis has been associated with hyperproteinemia and paraproteinemia, with cryoglobulinemia resulting in spurious thrombocytosis [2]. Advia 2120i uses an optical laser to identify platelets based on their volume and complex refractive index. Structures with similar size and composition sometimes can be misread as platelets, such as RBC fragments and, as in this case, protein precipitate [1]. The BC-75R Vet uses both impedance and a 3-laser fluorescence (SF-cube cell 3D) method, which detects platelet size, complexity, and nucleic acid content and enables platelet identification through a 3D scatter plot. A high efficiency of the V-7 FR fluorescence dye is reported to capture the nucleic acid content in platelets, resulting in their identification, and potentially minimizing the interference from other structures, such as paraproteins, as seen in this case [3]. In conclusion, causes of spuriously increased PLT have a different impact in the laboratory results based on the type of analyzer used. Platelet estimate on the blood smear remains mandatory.

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Oral Communication(s)

QUALITATIVE AND QUANTITATIVE CHANGES IN EXTRACELLULAR PARTICLES BEFORE AND AFTER TREATMENT IN DOGS WITH CANINE EHRLICHIOSIS

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Canine ehrlichiosis is a tick-borne disease in dogs caused by *Ehrlichia canis (E. canis)*. Understanding pathogenic mechanisms and performing accurate diagnosis is challenging due to non-specific clinical signs and multistage disease pattern [1]. The research into extracellular particles (EPs), which are of particular interest as mediators in intercellular communication and because of their close association with platelets [2], may contribute to understanding of the disease. The aim of this study was to investigate the qualitative and quantitative variations in EPs using interferometric light microscopy (ILM) (Videodrop, Myriade, Paris, France), that measures concentration (ILMc) and diameter (Dh) of EPs, and ultraviolet spectrophotometry (UV-vis) (NanoDrop One, Thermo Fisher Scientific, USA), that analyse nucleic and protein material, and to identify differences and correlations between healthy and thrombocytopenic *E. canis*-positive dogs. 34 healthy dogs (CTRL) and 23 positive dogs (EP), evaluated before (EP-BT) and after 28 days of treatment (EP-AT) with doxycycline at 10 mg/kg/day, were enrolled. Eligibility criteria were healthy condition, according to clinical assessment, and negative for infectious disease screening tests for CTRL, and first diagnosis of *E. canis* by PCR, negative for other infectious disease, and no previous treatment for ehrlichiosis for EP. From each dog, plasma, serum, and EDTA blood was used to perform hematobiochemical, ILM and UV-vis evaluations. The study was approved by the Ethical Animal Care and Use Committee of the University of Naples Federico II (Protocol n. PG/2025/0133615). Both hematobiochemical and ILM and UV-vis results were compared between groups (CTRL vs EP-BT, CTRL vs EP-AT, EP-BT vs EP-AT) and correlated. Mann-Whitney and Wilcoxon tests were used to highlight differences between groups, while correlation between variables was assessed using Spearman's rank correlation coefficient (ρ). Statistically significant results were considered for p value < 0.05. Significant differences were found regarding platelets ($p < 0.001$), and all protein fraction ($p < 0.001$) between groups. In the CTRL, ILMc was higher than in EP ($p < 0.001$), while Dh ($p < 0.04$) and UV-vis measurements were lower ($p < 0.001$). Comparing EP-BT with EP-AT, Dh ($p < 0.001$) and globulins ($p = 0.001$) were higher in EP-BT, while PLT ($p < 0.001$), serum proteins ($p = 0.005$), and UV-vis measurements ($p < 0.001$) were lower. A positive correlation was found between platelets and ILMc ($\rho = 0.521$, $p < 0.001$), while ILMc was positively correlated with triglycerides ($\rho = 0.241$, $p < 0.05$) and cholesterol ($\rho = 0.303$, $p < 0.01$) and negatively with globulins ($\rho = -0.412$, $p < 0.001$). Regarding Dh, a negative correlation was found with platelets ($\rho = -0.356$, $p < 0.01$). The differences observed between healthy and positive dogs in terms of the concentration and size of EPs may suggest that these nanostructures behave differently during *E. canis* infection. The correlation observed between platelets and the characteristics of EPs confirms that the latter are closely linked to and influenced by platelet count and activation, suggesting that EPs may represent a promising biomarker of disease activity and treatment response in canine ehrlichiosis.

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Oral Communication(s)

IDENTIFICATION AND CHARACTERIZATION OF SMALL RNA AS INFECTION MARKERS IN CANINE LEISHMANIASIS

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Leishmania infantum, the causative agent of canine leishmaniasis (CanL), establishes persistent infection by altering host immune and metabolic pathways. Evidence indicates that *Leishmania* modulates host gene expression via epigenetic mechanisms, especially through microRNA (miRNA) dysregulation, to support parasite survival and disease progression. Infection-driven miRNA modulation reshapes pathways linked to inflammation, macrophage activation, lipid metabolism, and cell survival. Circulating miRNAs are promising CanL biomarkers, showing altered levels in plasma, PBMCs, and tissues of infected animals, supporting their diagnostic and prognostic potential. This study aimed to identify dysregulated plasma miRNAs associated with different clinical outcomes, as candidate biomarkers to support early diagnosis and prognosis. Recruited dogs (n.80) were classified into four groups based on a modified Leishvet group staging [1]: Group 0 (n.27) healthy controls; Group I (n.22) mild infection, seronegative or with low antibody titers and minimal clinical signs; Group II (n.16) low to high antibody titers and broader clinical manifestations (skin lesions, lymphadenopathy, weight loss, anemia, hyperproteinemia , mild liver enzyme alterations) without renal involvement; Group III (n.15) severe clinical signs, medium to high antibody titers and renal impairment (at least one: creatinine >1.5 mg/dL, SDMA >18 µg/dL, or UP/C >0.5). Preliminary miRNA sequencing was performed on 12 plasma samples of dogs belonging to Groups 0, I, and III. RNA was extracted from 200 µL of plasma using the miRNeasy Serum/-Plasma Kit (QIAGEN), and libraries were prepared with the QIAseq miRNA Library Kit (QIAGEN). Hemolysis was assessed by qPCR (miR-23a/miR-451a ΔCt); samples with ΔCt < 7 were included, minimizing erythrocyte-derived miRNA bias. Differential analysis identified miRNAs with log2 FC >0.5 and FDR <0.05. In summary, 8, 31, and 6 miRNAs were found dysregulated in mild disease vs healthy, severe disease vs healthy, and severe disease vs mild disease, respectively. Sequencing validation was done on 24 miRNAs by qPCR using custom TaqMan Array MicroRNA Cards (ThermoFisher) on a larger cohort of 80 dogs. Among validated targets, the overexpression of miR-146a and miR-21 in Groups II and III was particularly relevant. The miR-146a attenuates inflammatory signaling by targeting adaptor proteins (i.e. IRAK1 and TRAF6), reducing downstream cytokine production. miR-21 is linked to anti-inflammatory and immunoregulatory responses, modulating macrophage activation and promoting immune suppression and tissue remodeling. The upregulation of miR-21 has been previously reported in dogs with canine visceral leishmaniasis [2]. However, our results suggest that these miRNAs are not merely associated with infection status, but rather correlate with the clinical manifestation and progression of the disease. Thus, miR-21 and miR-146a may represent promising biomarkers for distinguishing between subclinical infection and active disease, as well as for monitoring disease progression toward more severe forms. Overall, these findings suggest that miRNA dysregulation is more pronounced in severe conditions, supporting the presence of condition-specific miRNA signatures.

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Oral Communication(s)

METHOD COMPARISON BETWEEN JAFFÈ REACTION AND ENZYMATIC ASSAY FOR SERUM CREATININE CONCENTRATION IN DOGS AND CATS: DIAGNOSTIC ACCURACY AND CLINICAL APPLICATION

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Renal function in dogs and cats is commonly assessed by measuring serum creatinine concentration (sCr), which is considered a precise and accurate method to estimate the glomerular filtration rate for both clinical and research purposes. sCr can be measured using different methods such as alkaline picrate colorimetric assay (Jaffè method, JCr) or enzymatic methods (EnCr). The comparison of these two methods for application in dogs and cats is scarcely described[1].

Aim of this study was to compare analytical performance of JCr and EnCr applied on an automated chemistry analyzer (Beckman Coulter AU480 with specific reagents). A retrospective study was conducted on 32'455 and 11'996 serum samples collected from dogs and cats, respectively, in which JCr and EnCr were measured simultaneously. Samples were analyzed at the Clinical Pathology Service of the Department of Veterinary Medical Sciences, University of Bologna (2020-2025). Difference between JCr and EnCr was calculated and expressed as "delta". Data were reported as median and range (min-max). Passing-Bablok regression, Bland-Altman, and K- Cohen analyses were performed to compare the results. JCr was used as the reference method. Samples with delta >0.3 mg/dL were tested with Fisher's exact test to identify possible causes of this discrepancy. P<0.05 was considered significant.

Delta was 0.06 mg/dL (-3.19-1.67) and 0.04 mg/dL (-5.42-1.2) in dogs and cats, respectively. Excellent correlation between the two assays in dogs (r=0.98; slope=1.07; intercept=-0.14) and cats (r=0.99; slope=1.09; intercept=-0.19) was detected. The Bland-Altman plot showed a trend in the discrepancy between the two methods. In dogs, the mean difference between methods was 0.05 mg/dL (95% CI, -0.19-0.30), and the trend of delta progressively decreased as the mean concentration of sCr increased. Similarly, in cats, the mean difference was 0.004 mg/dL (95% CI, -0.51-0.50). Samples with delta >0.3 mg/dL were significantly associated with icterus (p<0.01) and lipemia (p<0.01) in cats and with icterus, lipemia, and hemolysis (p<0.01) in dogs.

Concordance at predefined JCr threshold values corresponding to preserved renal function (1.4 mg/dL for dogs; 1.6 mg/dL for cats)[2] has been tested. In both cases, optimal concordance (99%) was observed when the value fell below the set threshold; however, in 6.65% of dogs and in 7.16 % of cats, when the value fell over the set threshold, methods produced differing results. This implies that in these cases, according to the method used, the diagnostic interpretation would be different. The results of this study suggest that JCr and EnCr have similar characteristics; however, the discrepancy between the two assays may reach clinically relevant impacts, mainly when interferents are present, as reported in human medicine[3]. In addition, it is important to note that in research settings, particularly in studies involving serum creatinine values, the results could be significantly different according to the method used.

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Oral Communication(s)

ANTIARRHYTHMIC EFFICACY AND SAFETY OF INTRAVENOUS LIDOCAINE IN DOGS AND CATS WITH ARRHYTHMIAS: A RETROSPECTIVE ANALYSIS OF 157 CASES

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Lidocaine is a class Ib intravenous antiarrhythmic agent. In dogs and cats, it is predominantly used to treat hemodynamically relevant ventricular arrhythmias (VAs); additionally, it can be used to interrupt abnormal supraventricular rhythms (SVRs), such as orthodromic atrioventricular reciprocating tachycardia (OAVRT) and vagally mediated atrial fibrillation (vAF) [1]. Despite lidocaine having been widely used in small animal practice for decades, original studies evaluating the effects of this drug are scarce in the veterinary literature [2,3]. Therefore, the aim of this study was to evaluate the efficacy rate and safety of lidocaine in dogs and cats with arrhythmias.

For the purpose of this retrospective study, databases from a university center were searched for dogs and cats receiving intravenous lidocaine for the treatment of naturally acquired arrhythmias between January 2014 and March 2026. Signalment, clinical, diagnostic, and therapeutic data were retrieved. Antiarrhythmic efficacy was assessed electrocardiographically for VAs by comparing the modified Lown-Wolf (LW) grade before and after lidocaine administration, and for SVRs by the occurrence of cardioversion following lidocaine administration. Treatment-related side effects were investigated in all subjects. Data analysis was descriptive.

One hundred fifty-seven subjects were included (147 dogs and 10 cats). In dogs, the median age and body weight were 10 years and 28.5 kg, respectively. Lidocaine was administered mainly to treat VAs (144/147 [98%] dogs; LW grade 5 = 132; grade 4 = 12), while its use in SVRs was limited to 3/147 (2%) dogs (OAVRT = 1; vAF = 1; atrial dissociation = 1). Lidocaine was administered as a single bolus followed by a constant rate infusion (CRI) in 118/147 (80.3%) dogs, as boluses not followed by a CRI in 13/147 (8.8%), and as a CRI not preceded by a bolus in 16/147 (10.9%). The median number and dose of boluses were 1 and 2 mg/kg, respectively. The median duration and starting dose of CRI were 24 hours and 50 µg/kg/min, respectively. Efficacy was documented electrocardiographically in 113/147 (76.9%) dogs, including 112/144 (77.8%) with VAs and 1/3 (33.3%) with SVRs. Side effects were documented in 19/147 (13.2%) dogs, namely mental obtundation (18/19 [94.7%]) and sinus bradycardia (1/19 [5.3%]).

In cats, the mean age and body weight were 8.6 years and 4 kg, respectively. Rhythm disturbances were exclusively represented by LW grade 5 VAs. Lidocaine was administered as a single bolus not followed by a CRI in 7/10 (70%) cats, and as a single bolus followed by a CRI in 3/10 (30%). The median dose of boluses was 1 mg/kg. The duration of CRI was 12 hours in two cats and 8 hours in one cat. The median starting dose was 20 µg/kg/min in two cats and 30 µg/kg/min in one cat. Efficacy was documented electrocardiographically in 5/10 (50%) cats. Side effects were documented in 1/10 (10%) cat, consisting of mental obtundation associated with sinus bradycardia.

These findings provide novel data on the efficacy and safety of intravenous lidocaine in dogs and cats with naturally acquired arrhythmias.

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Oral Communication(s)

PROGNOSTIC SIGNIFICANCE OF CARDIORENAL AND ENDOTHELIAL BIOMARKERS IN DOGS WITH MYXOMATOUS MITRAL VALVE DISEASE

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Myxomatous mitral valve disease (MMVD) is the most common cardiac disease in dogs, and several echocardiographic variables are recognised predictors of outcome [1]. However, the prognostic value of circulating biomarkers reflecting systemic pathophysiological mechanisms has been less extensively investigated. The aim of this study was to evaluate the prognostic significance of biomarkers related to nitric oxide metabolism and renal function, including symmetric dimethylarginine (SDMA), asymmetric dimethylarginine (ADMA), L-arginine, and the L-arginine/ADMA ratio, in dogs with MMVD.

The protocol of this prospective multicenter study was approved by the Animal Welfare Ethics Committee of the University of Padua. Small breed dogs with clinical and echocardiographic findings consistent with MMVD were enrolled. The severity of MMVD was assessed according to the ACVIM guidelines [1]. Asymptomatic dogs without radiographic or echocardiographic evidence of cardiac remodeling were considered as stage B1; dogs with evidence of cardiomegaly with left atrial and ventricular enlargement were considered as stage B[2] Finally, symptomatic dogs in which at least one episode of heart failure had occurred and dogs with end-stage disease refractory to standard cardiac treatment were considered as stage C and D, respectively. In addition to cardiological evaluation, each dog underwent a complete blood count and determination of plasmatic ADMA, SDMA and L-arginine levels using high-performance liquid chromatography [2]. Mortality data were obtained from clinical records or, if unavailable, through owner interviews. Survival analyses were performed using Kaplan–Meier curves and Cox proportional hazards models to identify variables associated with cardiac death and all-cause mortality. In particular, the prognostic value of SDMA and ADMA were evaluated in different models as continuous and dichotomized variables according to receiver operating characteristic (ROC) curve analysis.

Seventy-seven dogs with MMVD with different disease severity, 30 females and 47 males with a mean age of 11.6 years, were included in the study. During the follow-up period (median = 440 days, minimum-maximum = 0-2373 days), 53 dogs died, including 30 from cardiac causes. The Kaplan-Meier survival curve showed that increased SDMA and ADMA and decreased L-arginine were significantly associated with a reduced survival for cardiac death and all-cause mortality. In multivariate analysis, SDMA was independently associated with cardiac death when analysed as a continuous variable. For all-cause mortality, SDMA and ADMA showed significant associations with the outcome, particularly when analysed using ROC-derived thresholds. Furthermore, the combined evaluation of SDMA and ADMA identified groups of dogs with markedly different mortality risks.

These findings suggest that biomarkers reflecting renal dysfunction and endothelial impairment may provide additional prognostic information in dogs with MMVD. In particular, the combined assessment of SDMA and ADMA may help identify dogs with a poorer prognosis and highlights the potential role of systemic pathophysiological mechanisms in the progression of MMVD.

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Oral Communication(s)

LEFT ATRIOVENTRICULAR COUPLING INDEX: A NOVEL ECHOCARDIOGRAPHIC MARKER OF ATRIOVENTRICULAR DYSFUNCTION IN CATS WITH HYPERTROPHIC CARDIOMYOPATHY

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The close physiological interaction between the left atrium (LA) and left ventricle (LV) suggests that indices integrating both chambers could provide useful information about disease severity. The atrioventricular coupling index (LACi) has recently been proposed in dogs with myxomatous mitral valve disease, showing a progressive impairment of left atrioventricular function as disease stage advances. However, no data are available for feline patients. Therefore, this study aimed to evaluate LACi in healthy cats and cats affected by hypertrophic cardiomyopathy (HCM).

Sixty-three cats (30 healthy cats and 33 cats with HCM - 15 ACVIM stage B1/B2 and 18 ACVIM stage C) were retrospectively included in the study. The LACi was calculated as LA volume/LV volume*100 at LV end-diastole (LACi-ED) and end-systole (LACi-ES), using a monoplane Simpson's method of discs from the left apical four-chamber view. Three measurements were averaged for each variable, and mean values were used for the statistical analysis.

In healthy cats, LACi-ED and LACi-ES showed no relationship with body weight and heart rate ($R^2 < 0.01$, for all variables). Age was poorly correlated with LACi-ED ($R^2 < 0.2$). In cats with HCM, both LACi-ED and LACi-ES were significantly higher in ACVIM stage C compared to B1/B2 ($P < 0.0001$, for both). LACi-ES did not differ between healthy cats and cats in B1/B2 ($P = 0.40$). The LACi-ED had excellent accuracy in identifying cats with congestive heart failure (cut-off >55%, AUC 0.95, sensitivity 89%, specificity 93%), higher than LACi-ES (cut-off >120%, AUC of 0.87, sensitivity of 83%, specificity of 80%). Left-atrial-to-aortic ratio (LA:Ao) and left atrial diameter normalized (LADn) showed AUCs of 0.94 (sensitivity 78%, specificity 87%) and 0.92 (sensitivity 83%, specificity 80%), respectively.

Our data suggest that LACi-ED represents a promising echocardiographic index for evaluating left atrioventricular function in cats with HCM, with excellent diagnostic accuracy for identifying congestive heart failure, outperforming conventional indices such as LA:Ao and LADn. Prospective studies are needed to assess its prognostic value in cats affected by HCM.

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Oral Communication(s)

PRIMARY CILIARY DYSKINESIA WITH SECONDARY PSEUDOMONAS INFECTION IN A DOG: ENDOSCOPIC AND DIAGNOSTIC FINDINGS

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A 7-month-old, 3.6 kg, intact male Dachshund was referred for a history of chronic cough, sneezing, and nasal discharge present since birth, with partial or complete response to multiple courses of antibiotic therapy and recurrence of clinical signs following treatment discontinuation. Physical examination revealed macrocrania, bilateral mucous nasal discharge, diffuse fine crackles on thoracic auscultation, and expiratory dyspnea. Hematological and diagnostic exam (radiographs of thorax) was consistent with pneumonia. Endoscopic examination of the upper airways revealed the presence of abundant dense mucus within both nasal cavities and in the left caudal bronchus (LB2). A bronchoalveolar lavage (BAL) was performed for cytological, bacteriological analysis and Polymerase Chain Reaction (PCR) testing of *Pneumocystis carinii*. Tracheal and nasal biopsies were collected for histopathological and microscopic examination. A nasal swab was obtained for bacteriological culture. Cytological examination of BAL fluid revealed septic neutrophilic inflammation, whereas histological examination of the trachea and nasal mucosa was unremarkable. PCR testing for *P. carinii* was negative. Bacteriological analysis of both samples yielded *Pseudomonas spp.* Transmission electron microscopy (TEM) revealed morphological alterations of the axonemal architecture in approximately 50% of the respiratory cilia. Overall, these findings were consistent with primary ciliary dyskinesia (PCD) complicated by secondary infection with *Pseudomonas spp.* Based on the antibiogram, marbofloxacin was administered for 30 days, in combination with anti-inflammatory therapy with prednisolone. Mucus fluidification was supported with aerosol therapy using NaCl and oral N-acetylcysteine. After discontinuation of the antibiotic and anti-inflammatory therapy, the dog exhibited regular breathing; thoracic auscultation revealed only reinforced vesicular sounds, with sporadic cough and persistent nasal discharge. Fluidification strategies were maintained to prevent recurrence of infections. PCD is a rare congenital disorder characterized by ultrastructural abnormalities of the cilia, leading to chronic rhinosinusitis, bronchitis and bronchopneumonia. Respiratory cilia play a central role in protecting the respiratory tract from infectious diseases by clearing mucus that traps pathogens and foreign particles. For this reason, animals affected by PCD are prone to recurrent secondary infections, and *P. aeruginosa* is frequently reported in cases of PCD in humans. Hydrocephalus, which may be associated with PCD, could explain the macrocrania observed in this dog. Following diagnosis, genetic testing for the CCDC39 gene mutation, previously associated with PCD, was performed in both the dog and its sibling, which showed similar clinical signs, and yielded negative results. These findings highlight the central role of endoscopy and TEM in the diagnosis of PCD. The management of PCD is challenging; antibiotic therapy for recurrent infections and ongoing mucus fluidification are fundamental for improving prognosis and prolonging life.

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Oral Communication(s)

PRELIMINARY RESULTS OF A GENOMIC ANALYSIS IN A DOG POPULATION WITH A HIGH INCIDENCE OF IDIOPATHIC CHYLOTHORAX

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Chylothorax is a relatively uncommon condition characterized by the accumulation of chyle within the thoracic cavity. It has been documented in association with a variety of thoracic pathologies and, when these conditions are treated, chylothorax may resolve, supporting a presumptive cause–effect relationship between the underlying disease and chylous pleural effusion. However, in the majority of cases, chylothorax is considered idiopathic, as no underlying cause can be identified [1]. Over the last decade, on San Pietro Island (Sardinia, Italy), a local population of stray mongrel dogs has been partially rescued and puppies have been given up for adoption. Local veterinarians reported a relatively high incidence of idiopathic chylothorax (7 cases out of 82 dogs). Given the characteristics of this population (closed and geographically isolated population, with frequent mating among related individuals), a genetic predisposition has been hypothesized. A total of 33 dogs (4 cases and 29 controls) were sampled and genotyped using the Illumina CanineHD BeadChip. Population structure was investigated through Principal Component Analysis (PCA) and ADMIXTURE to assess the presence of stratification. Runs of Homozygosity (ROH) were identified and used to estimate the genomic inbreeding coefficient (F_{ROH}). Additionally, ROH islands were analysed to detect potential selection signatures associated with idiopathic chylothorax. For case dogs, only ROH islands shared by all four individuals were retained, whereas in controls, ROH islands were considered if present in at least 50%+1 of the individuals [2]. Both PCA and ADMIXTURE analyses revealed the presence of population stratification, identifying three subgroups: two corresponding to ancestral populations and one comprising admixed individuals. No association was observed between affected dogs and these subgroups. No significant differences in F_{ROH} were detected between cases and controls. A total of 233 ROH were identified in case dogs and 1,080 in control ones. Nine and 27 ROH islands were detected in cases and controls, respectively. Six ROH islands were exclusive to case dogs. A total of 448 protein-coding genes located within ROH islands were retrieved using Ensembl BioMart. Functional enrichment analysis revealed several biological processes at nominal significance ($p < 0.05$), mainly related to protein ubiquitination, endoplasmic reticulum processing, immune response, and vesicle-mediated transport. In particular, multiple components of the ubiquitin–proteasome system were consistently represented, suggesting a potential involvement of protein turnover and quality control mechanisms. Additionally, enrichment of interferon-related genes and cytokine signalling pathways points to a possible contribution of immune and inflammatory processes. The concurrent enrichment of endoplasmic reticulum-associated and vesicle trafficking components further supports a role for altered protein processing and secretion pathways. Overall, these findings suggest that ROH regions specific to affected dogs may harbour genetic variants influencing protein homeostasis and immune-related pathways, providing preliminary insights into the molecular mechanisms underlying idiopathic chylothorax in this population, although further studies are needed to validate these associations.

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Oral Communication(s)

RADIOGRAPHIC FEATURES OF PERICARDIAL EFFUSION IN CATS: A RETROSPECTIVE MULTICENTRIC STUDY

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Pericardial effusion (PE) in cats is an uncommon condition, typically mild and often incidental, resulting in limited radiographic characterization compared to dogs [1]. Thoracic radiography is widely available in clinical practice; however, its diagnostic value for detecting PE in cats remains poorly defined. The aim of this retrospective multicentric study was to describe qualitative and quantitative radiographic features of cardiac silhouette changes in cats with echocardiographically confirmed PE.

Clinical records from the Veterinary Teaching Hospitals of the University of Padua and the University of Bologna (2015–2025) were reviewed. Inclusion criteria were echocardiographic diagnosis of PE and availability of complete thoracic radiographs (two orthogonal views) performed within 48 hours. Seventy-five cats met the inclusion criteria. Signalment, aetiology, and severity of effusion were recorded. Qualitative radiographic features assessed included increased cardiac size, rounded cardiac silhouette, dorso-caudal convexity, and margin definition. Quantitative analysis included measurement of vertebral heart size (VHS) and sphericity index (SI) [2,3].

Cardiomyopathy was the most common underlying condition (65.3%). Other causes were rare, each accounting for a single case (1.3%), including pulmonary disease, systemic inflammation, intrathoracic neoplasia, congenital heart disease, and valvular degeneration. In 28.0% of cats, the aetiology remained undetermined. The vast majority of cases presented with mild or mild-to-moderate effusion (98.7%). An increase in cardiac silhouette size was the most frequent radiographic finding (85.3%). Dorso-caudal convexity was observed in 73% of cases, while a rounded cardiac silhouette was present in 73.4% of cats when considering both projections. Clearly defined cardiac margins were less consistent, being present in at least one projection in 73% of cases. Mean VHS values were increased compared to reference ranges (8.9 ± 0.9 vertebrae in lateral projection; 10.0 ± 1.2 vertebrae in sagittal projection). In contrast, SI values (1.3 ± 0.1 lateral; 1.2 ± 0.2 sagittal; 1.3 ± 0.1 global) indicated no marked tendency toward a globoid cardiac shape.

In conclusion, increased cardiac silhouette size represents the most consistent radiographic finding in cats with PE, whereas other qualitative features are variable and less reliable. Quantitative assessment using VHS supports subjective evaluation, while SI appears to have limited diagnostic utility in predominantly mild effusions. These findings provide a basis for future studies investigating the diagnostic accuracy of thoracic radiography in feline PE.

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Oral Communication(s)

CLINICAL-THERAPEUTIC MANAGEMENT OF CHRONIC ENTEROPATHIES IN DOGS AND CATS IN THE ITALIAN LANDSCAPE

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Chronic enteropathies (CE) in small animals are a leading cause of veterinary consultation. This study aimed to evaluate clinical and therapeutic management of CE in Italy through a questionnaire survey of veterinarians (DVMs). The questionnaire, administered online in 2025, included 74 multiple-choice and Likert-scale questions covering diagnostic and therapeutic approaches, and adherence to international guidelines.

The questionnaire was completed by 104 DVMs practicing in small animal clinics (33%), practices (46.6%), and hospital facilities (12.6%). Approximately 36% of veterinarians held advanced qualifications, including PhDs (6.7%) and I/II Master's degrees (27.7%), while over half (56%) were general practitioners. A substantial proportion reported specialized practice in internal medicine (26%), gastroenterology (16%) and endoscopy (17%). Only 29.6% of DVMs agreed with the current clinical classification of CE, and many emphasized the need for structured, stepwise diagnostic pathways based on sequential testing and therapeutic trials. The most useful ancillary investigations include blood chemistry (including trypsin-like immunoreactivity TLI, vitamin B12, and folate), abdominal ultrasonography, and endoscopy. Although histopathological examination is considered valuable, it is used selectively by only 16% of DVMs, with 90.7% preferring the endoscopic approach as a sampling method. Dietary trials were recognized as effective by 95% of DVMs, with hydrolyzed diets preferred (55.4%). Microbiota modulation is widely used (79.2%), often combined with diet (52.2%). Although fecal microbiota transplantation (FMT) is regarded as a promising outpatient therapy by 93.9% of DVMs, its clinical use remains limited (33.7%) and lacks standardized protocols. Immunomodulatory therapy is considered useful by 90.9% of DVMs, with prednisolone, budesonide, cyclosporine, and chlorambucil most commonly used as mono- or dual therapy. Antibiotics, mainly macrolides and nitroimidazoles, are reserved as a last-line treatment strategy. The antibiotic trial is used by only 7.2% of DVMs, and its selection is guided by antibiogram results in 25.3% of cases, mainly obtained from intestinal biopsies (63.9%). Clinical response is expected within 1–2 weeks in food-responsive enteropathies (FRE), whereas longer response times (2–4 weeks) are observed in microbiota-modulation-responsive enteropathies (MrMRE) and immunosuppressive-responsive enteropathies (IRE) cases. A multimodal therapeutic approach is considered useful in 72.4% of cases. The use of stem cells and exosomes is perceived as experimental and not useful by the majority of DVMs. Their adoption is limited to only 2% of cases and is directed at patients affected by non-responsive enteropathies (NRE).

A total of 95.5% of DVMs endorsed a dynamic view of CE, recognizing the potential progression across its various forms. The results indicate that clinical management of CE is predominantly multimodal, reflecting a compromise between scientific evidence, international guidelines, and individual clinical experience.

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Oral Communication(s)

COLONIC IRRIGATION WITH CLEAN COLON DEVICE (iCLEAN 14®) AS A BOWEL PREPARATION TOOL PRIOR TO CANINE COLONOSCOPY

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Colonoscopy is a widely used diagnostic tool in human and veterinary clinical practice for the assessment of intestinal diseases. Bowel preparation (BP) is essential to achieve colonoscopy success, often involving the use of purgative solutions and/or prolonged fasting, leading to patient discomfort. In human medicine, colonic irrigation is a well-tolerated and effective alternative BP method. The Boston Bowel Preparation Scale (BBPS) is used to evaluate the adequacy of human BP by assigning a value (0-3) to each colonic segment (descending/transverse/ascending colon) based on the cleansing quality and mucosal visualization. BP is adequate if the sum of segment scores is ≥ 6. Considering the absence of a standardized BP protocol in dogs, this prospective study aimed to evaluate the tolerability of colonic irrigation using a clean colon device (iClean 14®) and the adequacy of bowel preparation, assessed by BBPS, in dogs undergoing colonoscopy, compared with dogs not receiving colonic irrigation. The present study received favourable approval by the Ethical committee (52/2024, University of Pisa). Sixty-four dogs were prospectively included and randomized into two groups: 36 received colonic irrigation (HYDR) and 28 only received a low-residue diet and a fasting of 12-14 hours (CTR). Dogs undergoing colonic irrigation weighing less than 10 kg and presenting with colorectal mass were excluded. Signalment (age, sex, and breed), weight (≤20 kg/>20 kg), fecal score index (FSI 1-3/4-5/6-7), heart rate (HR) and respiratory rate (RR) before $_{(T0)}$ and after colonic irrigation $_{(T1)}$, BBPS (each colonic segment/sum), and success in the visualization of ileocecocolic valve (ICC) were registered. Regarding CTR group, if needed, intraoperative colonic washing using instillation of water through the endoscope working channel and its duration were recorded. Regarding tolerability, within HYDR group, no difference was found between HR (HR_{T0} 64.3 bpm ± 19.3 bpm vs HR_{T1} 61.4 bpm ± 16 bpm; p=0.07) and RR (RR_{T0} 21.9 bpm ± 9.1 bpm vs RR_{T1} 20.2 bpm ± 6.8 bpm; p=0.07]. Within CTR group 21/28 (75%) dogs needed intraoperative colonic washing with a median duration of 15 minutes (5-30 minutes). For HYDR group no association was found between FSI (p=0.16) or weight group and BBPS≥6 (p=0.22). BBPS≥6 was statistically different between groups [HYDR 33/36 (91.7%) vs CTR 10/28 (35.7%); p<0.001, OR 19.7]. ICC visualization differed between study groups [HYDR 35/36 (97.2%) vs CTR 9/28 (32.1%); p<0.001, OR 73.9]. Colonic irrigation is well tolerated and significantly improves bowel preparation quality in dogs undergoing colonoscopy, resulting in a markedly higher rate of adequate preparation compared to standard protocols. Furthermore, in the study population, its effectiveness appeared consistent regardless of fecal consistency or patient size. Additionally, colonic irrigation significantly increased the success rate of ileocecocolic valve visualization, thereby potentially improving biopsy sampling.

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Oral Communication(s)

PRELIMINARY EVALUATION OF URINARY NGAL TO DIFFERENTIATE URINARY TRACT INFECTION FROM BACTERIURIA IN DOGS WITH NEUROGENIC BLADDER

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Bacterial urinary tract disease is a common cause of morbidity in dogs and cats and a major driver of antimicrobial use. According to current ISCAID guidelines, antimicrobial therapy is recommended only when bacteriuria is associated with clinical signs of urinary tract inflammation (UTI). In dogs with neurogenic bladder (NB), clinical signs of UTI may be absent or undetectable, complicating the decision to treat and increasing the risk of unnecessary antimicrobial use. In human medicine, urinary neutrophil gelatinase-associated lipocalin (NGAL) has been investigated as a potential biomarker to differentiate true UTIs from subclinical bacteriuria (SB). This prospective observational study assessed urinary NGAL concentrations in dogs with NB and compared them with healthy dogs, dogs with UTI, and dogs with SB. Thirty-three dogs were initially enrolled. Based on physical and neurological exam, they were divided in healthy dogs (HCG) and neurological dogs with NB. All dogs underwent abdominal ultrasonography, complete blood count, serum biochemistry, urinalysis and cystocentesis urine culture. Urinary NGAL was measured using a semi-quantitative rapid ELISA (negative, 0–3, 4–19, 20–89, >90 ng/dL) and a quantitative ELISA; uNGAL/uCrea was also calculated. Three healthy controls, one dog with UTI and one dog with NB were excluded due to physical, laboratory or imaging abnormalities. Thereafter, the remaining 28 dogs were divided into healthy controls (HCG, 9/28), dogs with UTI (UTIG, 5/28), dogs with NB (NBG, 13/28), and 1 with SB (SBG). The NB group was subdivided according to urine culture into NBGa (NB + UTI, 7/28) and NBGb (NB without UTI, 6/28). All procedures, including cystocentesis in healthy control dogs, were approved by the institutional ethics committee (Prot. n. 660-III/13), and informed owner consent was obtained. Semi-quantitative urinary NGAL test was negative for all dogs with negative urine culture. Mean NGAL levels were 10.92 ng/dL in HCG, 55.03 ng/dL in UTIG, 55.56 ng/dL in NBGa, and 9.93 ng/dL in NBGb. The single dog with SB displayed a value of 8.15 ng/dL, overlapping with non-UTI groups. Due to non-normal distribution, group comparisons were performed using the Kruskal–Wallis test, which showed a significant difference among groups ($H = 16.9$, $p = 0.0007$). Post-hoc Dunn analysis showed significantly higher NGAL values in both UTI groups (UTIG and NBGa) compared with healthy controls and NBGb ($p < 0.05$), while no significant difference was observed between HCG and NBGb. Median uNGAL/uCrea values were 0.04 (HCG), 0.36 (UTIG), 0.56 (NBGa), and 0.06 (NBGb).

These preliminary findings suggest that urinary NGAL may be a potential useful biomarker for distinguishing UTI from bacteriuria in dogs, including those with NB. The semi quantitative NGAL test could assist the clinician, whilst awaiting urine culture results, in deciding whether or not to initiate antibiotic treatment. A negative result strongly suggests that the urine culture will also be negative, and would therefore limit the unnecessary use of antibiotics. Creatinine-normalized NGAL showed higher intra-group variability and did not improve discriminatory performance. Given the limited numbers, particularly in the SBG, these results should be interpreted with caution.

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Oral Communication(s)

EVALUATION OF A DAILY CLINICAL DIARY FOR MONITORING CHRONIC ENTEROPATHY IN DOGS

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In clinical practice, follow-up is commonly based on Canine Inflammatory Bowel Disease Activity Index (CIBDAI) scores assessed by veterinarians during recheck visits; however, this approach may not fully capture day-to-day clinical variability. The aim of this study was to evaluate the usefulness, acceptability and feasibility of a daily clinical diary administered to owners of dogs with chronic enteropathy (CE).

This prospective, observational, longitudinal, multicenter study was conducted at the University of Parma, the University of Bologna and a private veterinary clinic between August 2025 and March 2026. The diary consisted of a simplified CIBDAI score plus additional clinical parameters: mucus and blood in feces, flatulence and licking behavior (O--CIBDAI). The diary was completed daily by owners for 30 consecutive days and CIBDAI scores were assessed by a veterinarian (V-CIBDAI) at enrollment (T0) and after 30 days (T30). Longitudinal clinical trend was defined by counting the number of days of improvement and worsening relative to the baseline (T0) CIBDAI score over the 30-day period; dogs were then classified as improved or worsened based on the predominance of improvement versus worsening days. At the end of the study, owners completed a questionnaire assessing the acceptability and usability of the diary, including ease of use, sustainability in daily routine, perceived usefulness and preference for electronic formats, as well as open-ended comments for qualitative feedback. The Wilcoxon signed-rank test was used to compare CIBDAI scores between T0 and T30, and McNemar's test was used to assess agreement between T30 classification and longitudinal clinical trends. Results were reported as median (range). Significance was set at p-value < 0.05.

Forty-three dogs with confirmed CE were enrolled, of which 35 completed the 30-day clinical diary. Median V-CIBDAI did not differ between T0 and T30 (3 [range 0–11] vs 2 [range 0–10]; p = 0.21). Similarly, median O-CIBDAI did not differ between T0 and T30 (1 [range 0–12] vs 1 [range 0–9]; p = 0.60). At T30, 11 (31%) were classified as improved, 15 (43%) as worsened, and 9 (26%) as unchanged compared to T0 based on O-CIBDAI scores. Agreement between T30 classification and longitudinal clinical trends was moderate (62%), with no evidence of systematic bias (p = 0.23), although 38% of dogs showed discordance between single time-point and longitudinal assessment. Owner feedback indicated high acceptability of the diary, 92% of respondents rated it as easy to use, 78% as sustainable within daily routines and 84% as helpful for monitoring clinical condition. Overall, 51% of respondents reported a positive perception, while 33% were indifferent and 15% reported a negative opinion. Lower or negative ratings were frequently associated with requests for additional space to record clinical details.

In conclusion, the daily clinical diary appears to be a feasible and well-accepted tool for continuous monitoring of dogs with CE. It highlights the limitations of single time--point assessment and supporting the use of longitudinal owner-reported data to better capture disease dynamics.

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Oral Communication(s)

PROTEIN-LOSING ENTEROPATHY PROGRESSING TO HIGH-GRADE INTESTINAL T-CELL LYMPHOMA IN A WHITE SWISS SHEPHERD DOG

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Protein-losing enteropathy (PLE) is a severe syndrome in dogs, commonly associated with chronic enteropathy, lymphangiectasia, or intestinal lymphoma, and often carries a guarded prognosis [1]. High-grade intestinal lymphoma also has a poor outcome, with short survival times reported in affected dogs [2]. We describe a dog diagnosed PLE that showed prolonged clinical stabilization under medical and dietary management, followed by progression to high-grade intestinal T-cell lymphoma.

A 9-year-old intact male White Swiss Shepherd Dog was referred to Veterinary Teaching Hospital of Teramo for PLE, presumed secondary to lymphangiectasia based on results of endoscopic biopsies. At the presentation, the dog showed chronic diarrhea, weight loss (bodyweight 29 Kg, BCS 3/9) despite preserved appetite, and marked hypoalbuminemia (1.6 g/dL; RI 2.5–3.7). An ultra-low-fat (ULF) homemade diet based on a novel protein source was initially introduced, resulting in partial improvement. Prednisolone (1 mg/Kg/die) was subsequently added, followed by cyclosporine (6 mg/Kg/die), leading to progressive clinical improvement, weight gain (until 40 Kg, BCS 5/9), and resolution of gastrointestinal signs, with a gradual increase in albumin, although not fully normalized (from 1.6 to approximately 1.9 g/dL; RI 2.5–3.7). Over time, cyclosporine was discontinued and corticosteroids were tapered to a low immunomodulatory dose, while maintaining ULF dietary management and the dog remained clinically stable for approximately 20 months.

After this period, clinical signs recurred progressively over several weeks, including vomiting, diarrhea, hyporexia, and weight loss. Examination revealed marked loss of body condition (bodyweight 32 Kg, BCS 3/9) and focal small intestinal thickenings. Serum albumin was 1.9 g/dL. Abdominal ultrasonography revealed marked focal, circumferential, asymmetric transmural thickening of the jejunum, non-obstructive in nature, with suspected mural ulceration, accompanied by mesenteric lymph adenomegaly and abdominal effusion.

Computed tomography confirmed the presence of three intestinal masses, one of which was located at the ileocecocolic valve, associated with moderate mesenteric lymph adenomegaly and abdominal effusion. Laparotomy was performed, and biopsy samples were collected from all intestinal lesions and one lymph node. Histopathology and immunohistochemistry are consistent with high-grade large T-cell lymphoma involving all sampled lesions.

Despite initiation of chemotherapy and an increased corticosteroid dosage, the clinical condition continued to deteriorate and, due to progressive disease and lack of therapeutic response, euthanasia was elected 72 hours after starting treatment.

This case documents progression from clinically controlled PLE to high-grade intestinal lymphoma over nearly two years. Given the prolonged clinical response, the presence of aggressive lymphoma at initial diagnosis appears unlikely. Possible explanations include true disease progression or initial underdiagnosis related to mucosal biopsy limitations. This case highlights the importance of long-term monitoring in dogs with PLE and supports reconsideration of intestinal lymphoma when gastrointestinal signs recur, even after prolonged periods of clinical control.

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Oral Communication(s)

CLINICAL EVALUATION OF A CANINE-SPECIFIC FECAL OCCULT BLOOD IMMUNOASSAY IN DOGS WITH ACUTE DIARRHEA: PRELIMINARY DATA

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Fecal occult blood (FOB) testing has traditionally been limited by poor specificity and dietary interference. Recently, immunochromatographic assays based on monoclonal antibodies against canine hemoglobin have been developed, but their clinical performance in dogs remains unexplored.

The aim of this study was to preliminarily evaluate the clinical applicability of a canine-specific FOB immunoassay in dogs with acute diarrhea and to explore its association with markers of intestinal and systemic inflammation, as well as hematological variables related to erythropoietic response.

Client-owned dogs hospitalized for acute diarrhea between April 2024 and February 2025, without macroscopic blood in the feces, were prospectively enrolled at the University of Parma. A group of healthy controls was included for fecal analysis only. At admission (single timepoint), fecal samples were collected and stored and subsequently analyzed in batch at University of Milan, using a monoclonal antibody-based immunochromatographic assay specific for canine hemoglobin (FASTest® FOBT canine). Fecal calprotectin (fCal), hematological parameters, and biochemical variables were also assessed. Statistical analysis was performed using Mann–Whitney U test for continuous variables and chi-squared test for categorical variables, with significance set at $p < 0.05$.

Included dogs (n=42) were categorized based on FOB result and compared accordingly. FOB positivity (26/42) was observed exclusively in dogs with acute diarrhea, while all healthy controls tested (n=10) were negative. FOB results were not associated with clinical severity. No differences were observed in erythrocyte parameters. However, FOB--positive dogs showed higher reticulocyte counts (median 49,000/mm³ (range 16,200–177,100) vs 26,400/mm³ (range 19,600–56,600); $p = 0.0358$). Markers of inflammation were significantly increased in FOB-positive dogs, including fCal (median 361.5 µg/g (range 24.4–3979.9) vs 46.2 µg/g (range 2.7–827.8); $p = 0.0014$) and C-reactive protein (median 13.35 mg/dL (range 0.01–39.86) vs 5.25 mg/dL (0.88–10.31); $p = 0.0174$). A progressive increase in fCal concentrations was observed according to FOB signal intensity ($p = 0.0036$). No differences were found in serum biochemical parameters.

This preliminary study supports the clinical applicability of a canine-specific fecal occult blood immunoassay in dogs with acute diarrhea. The absence of positive results in healthy controls and the association with inflammatory biomarkers suggest analytical specificity. The increased reticulocyte counts in FOB-positive dogs, in the absence of changes in erythrocyte indices, further support the presence of a mild but relevant erythropoietic response. This assay may provide a rapid, point-of-care tool complementary to established biomarkers. Further studies are warranted to define its diagnostic accuracy and clinical utility.

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Oral Communication(s)

TWO-DIMENSIONAL SHEAR WAVE ELASTOGRAPHY OF THE CANINE GASTROINTESTINAL TRACT: FACTORS INFLUENCING WALL STIFFNESS IN HEALTHY DOGS

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Two-dimensional shear wave elastography (2D-SWE) enables quantitative, non-invasive assessment of tissue elasticity. In human medicine, elastographic measurements in solid organs are influenced by intrinsic and extrinsic factors, whereas studies of the gastrointestinal (GI) tract in healthy subjects show no significant effect of body mass index, sex, age, depth or wall thickness on ileal and colonic stiffness (1). In veterinary medicine, GI elastography remains poorly investigated; only one study assessed jejunal mucosal stiffness in dogs without evaluating influencing factors (2). Conversely, studies in other organs suggest that variables such as body weight, sex and depth may affect elastographic measurements (3). Therefore, factors influencing GI wall stiffness in dogs remain unclear.

The aim of this study was to establish reference intervals for GI wall stiffness assessed by 2D-SWE in healthy dogs and to investigate variables influencing stiffness, including body weight, age, sex, depth, scanning plane, and peristalsis.

B-mode ultrasonography and 2D-SWE (Philips EpiQ Elite, ElastQ, L5–18 probe) were performed by a single operator. Intestinal stiffness was quantified as Young's modulus (kPa) in the duodenum (D), jejunum (J), and colon (C). For each segment, measurements were obtained in transverse and longitudinal planes, with three acquisitions per plane; mean values were calculated. Only acquisitions with homogeneous, stable colour-coded elastographic maps and adequate confidence maps were included. Body weight, age, and sex were recorded.

Measurement depth and peristalsis were recorded at acquisition. Reference intervals were calculated parametrically (Horn's method). Variables were analyzed with Kruskal–Wallis tests and Bland–Altman analysis with Spearman correlation. The study was approved by the Ethics Committee (protocol no. 0836114); owner consent was obtained.

58 healthy dogs were included (1–13 y; 1.8–37.9 kg; 16 males, 42 females), mostly mixed breed, with few Labradors and French Bulldogs. Preliminary reference ranges (kPa; transverse/longitudinal) were: D 5.35–9.65/5.83–10.12; J 5.57–10.12/5.74–10.02; C 4.20–10.73/4.99–10.95. No differences were found by sex, body weight, depth or peristalsis. Age significantly influenced colonic stiffness, particularly in transverse scans ($P = 0.003$), with no effect in D or J. No significant differences were found between scanning planes.

These preliminary results indicate 2D-SWE is a promising ultrasonographic technique for assessing GI stiffness in healthy dogs. SWE values were not significantly influenced by the evaluated variables, except for age. Increased colonic wall stiffness in older dogs may reflect age-related collagen deposition and fibrotic changes, even without B-mode abnormalities. These findings support potential application of 2D-SWE in both healthy and diseased patients, as reported in human medicine. Further studies with larger sample sizes and additional variables are required to standardize this technique.

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Oral Communication(s)

HYPOTHYROIDISM RISK IN NEUTERED AND EARLY AGE NEUTERED GOLDEN RETRIEVERS

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Hypothyroidism is one of the most diagnosed endocrine disorders in adult dogs, typically resulting from primary idiopathic or immune-mediated causes. The disease is characterised by a progressive course and non-specific, often multisystemic clinical signs, with predominantly dermatological and metabolic manifestations [1]. Recent studies have focused on the potential influence of reproductive hormones on thyroid endocrine function. Neutering may alter thyroid homeostasis by disrupting the hypothalamic–pituitary–gonadal axis. Sex steroid hormones appear to modulate thyroid hormone secretion and action. The identification of luteinising hormone (LH) receptors in canine thyroid tissue suggests that chronic increased LH concentrations following gonadectomy may contribute to thyroid dysfunction [2]. Although evidence in dogs remains limited, these observations support the hypothesis of complex endocrine interactions influencing thyroid pathophysiology, particularly in predisposed breeds such as the Golden Retriever [3]. The aim of this retrospective study was to evaluate the association between reproductive status and hypothyroidism in Golden Retrievers, including the effects of sex, neutering and age at neutering (<12 vs >12 months). Medical records of dogs with complete reproductive history were reviewed. Hypothyroidism was diagnosed based on compatible clinical signs, serum concentrations of thyroxine (T4), including total (T-T4) and free (fT4), thyroid-stimulating hormone (TSH) concentrations, thyroglobulin autoantibodies (TgAA), and a subsequent clinical response to levothyroxine therapy. Dogs were classified as affected or controls and stratified according to sex and reproductive status. A total of 2,807 Golden Retrievers were included, of which 465 (16.6%) were diagnosed with hypothyroidism. In males, the prevalence was higher in neutered dogs (22.3%, 121/543) than in intact individuals (11.2%, 82/734), corresponding to a significantly increased risk (OR = 2.28; 95% CI 1.68–3.10; $p < 0.001$). Age-stratified analysis demonstrated a strong association in male dogs neutered before 12 months (40.7%, 85/209; OR = 5.45; 95% CI 3.81–7.80; $p < 0.0001$), whereas males neutered later showed a risk comparable to intact dogs (10.8%, 36/334; OR = 0.96; 95% CI 0.63–1.45; $p = 0.93$). In females, the prevalence was higher in neutered dogs (21.4%, 140/655) than in intact individuals (13.9%, 122/875), indicating a significant overall increase in risk (OR = 1.68; 95% CI 1.28–2.19; $p < 0.001$). Similarly to males, age at neutering influenced the disease occurrence with a significantly higher risk in females neutered before 12 months (31.2%, 91/292; OR = 2.8; 95% CI 2.04–3.82; $p < 0.0001$), compared to those neutered later (13.5%, 49/363; OR = 0.96; 95% CI 0.6–1.3; $p = 0.83$). Our results suggest that early deprivation of gonadal hormones may represent a risk factor for hypothyroidism in Golden Retrievers. These findings highlight the importance of neutering timing within a breed-specific preventive medicine framework and support the need for prospective studies to clarify the underlying mechanisms and identify appropriate replacement therapeutic strategies.

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Oral Communication(s)

SUSPECTED OSTEOGENESIS IMPERFECTA IN A DOG: RESPONSE TO ALENDRONATE AND MEDIUM-TERM OUTCOME

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Osteogenesis imperfecta (OI) is a rare inherited collagen disorder causing skeletal fragility, bone deformities, and pathological fractures. In dogs, OI has been reported in different purebred (i.e., Dachshund, Chow Chow, Golden Retriever, Beagle, Collie, Norwegian Elkhound, Bedlington Terrier, Lagotto Romagnolo) and mixed-breed dogs [1,2]. Prognosis is often unfavorable, with severe orthopedic impairment and fractures occurring after minimal or no recognized trauma [2]. In this report, the clinical course, diagnostic work-up, and response to alendronate in a young dog with suspected OI was described.

A 4-month-old male mixed-breed puppy was presented for plantigrade stance, lameness, reduced growth, and dental abnormalities including pink teeth. Initial laboratory testing revealed hyperphosphatemia and ionized hypercalcemia (2.80 mEq/L, RI 2.44-2.70). Radiographic studies showed diffuse skeletal abnormalities consistent with generalized bone fragility, including long-bone deformities, previous rib fractures, bilateral ulnar fractures, a right femoral fracture, bilateral radial procurvatum, and symmetrical ventral deviation of the ischia. Endocrine work-up was performed to investigate differential diagnoses associated with stunted growth and skeletal abnormalities: total thyroxine (TT4) and TSH were within the reference interval (TT4 1.46 µg/dL, RI 1.00-4.00; TSH 0.20 ng/mL, RI <0.40), and IGF-1 did not support pituitary dwarfism (298.90 ng/mL, RI >120). The PTH was low (1.60 pg/mL, RI 1.8-14.7), excluding PTH-dependent ionized hypercalcemia, while vitamin D metabolites were within normal limits, making major endocrine-metabolic causes less likely. Canine TLI concentrations were initially low to equivocal (2.48-5.94 ng/mL) but later normalized (8.83 ng/mL) without pancreatic enzyme supplementation. Thus, although a partial loss of acinar cells cannot be completely excluded, the overall clinical course was not consistent with exocrine pancreatic insufficiency or nutritional bone disease as a primary cause.

In the days following initial evaluation, the puppy sustained a further right radius-ulna fracture after minimal trauma, strengthening the suspicion of OI or a closely related inherited skeletal fragility disorder. Oral alendronate (0.5 mg/kg/die) was started, and early callus formation was observed shortly thereafter, with no further fractures occurring for the following 14 months. Treatment was later discontinued due to foreign body surgery, and shortly after the dog developed an iliac fracture following minor trauma. After alendronate was reinstated, rapid callus formation was again observed. At the time of writing, approximately 18 months after diagnosis and with ongoing treatment, no new fractures have been recorded. Although a definitive genetic diagnosis was not obtained, the clinical presentation and evolution supported a presumptive diagnosis of OI. A previous report described a French Bulldog and an Akita with suspected OI treated with bisphosphonate, showing rapid clinical improvement and no further fractures during medium-term follow-up [3]. Together with the present case, these findings suggest that alendronate may be a useful therapeutic option in dogs with pathological fractures and suspected OI.

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Oral Communication(s)

CAREGIVER BURDEN AND USE OF CONTINUOUS GLUCOSE MONITORING IN CANINE AND FELINE DIABETES MELLITUS: A SURVEY-BASED STUDY

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Diabetes mellitus (DM) in dogs and cats requires lifelong management and monitoring, and its impact extends beyond the patient to the caregiver. The aim of this study was to evaluate caregiver burden and the use of continuous glucose monitoring (CGM) systems (FreeStyle Libre) in owners of DM dogs and cats using data derived from two questionnaire-based studies inspired by previously published quality-of-life assessment tools in diabetic pets [1,2].

Overall, 55 caregivers of DM dogs and 56 of DM cats were included; in both groups, respondents were predominantly female (>90%). Daily management was frequently performed by a single individual, with 41.8% of dog owners and 53.6% of cat owners managing the disease alone, while shared management with a family member was reported in 50.9% and 39.3% of cases, respectively.

A substantial caregiver burden emerged in both populations. Concern about the disease was common, but more pronounced in cat owners, with 83.9% reporting frequent concern, compared with 45.5% of dog owners reporting being very worried about their pet's condition. Daily routine was markedly affected, particularly in cats, with 80.4% of caregivers reporting a strong impact, whereas in dogs approximately one-third perceived their life as significantly limited by disease management. Economic burden was also relevant, with costs considered high or moderately high by over 80% of dog owners, while in cats 73.2% of respondents reported a strong concern about expenses.

Limitations in delegating care were evident in both populations, with a substantial proportion of owners reporting a change in their habits after diagnosis. In dogs, 18.2% of owners stated that they no longer entrusted their pet to third parties, while in cats this proportion reached 26.8%, highlighting the impact of the disease on daily life and social flexibility.

Use of CGM differed between species. In dogs, all respondents answering the FreeStyle Libre section reported having used the device at least once, indicating widespread adoption of CGM. Use was mainly on a case-by-case basis: 38.2% of owners used it when requested by the veterinarian and 36.4% at diagnosis, whereas continuous use was limited (7.3%). Sensor duration exceeded 10 days in 53.3% of cases, and glucose data were accessed through LibreView in 56.7% of owners using the device. In cats, use was less frequent, with 66.1% of owners reporting no use. Among users, application was mainly occasional; sensor duration exceeded 10 days in 17.9% of cases, although greater variability was observed. LibreView was used by 14.3% of owners using the device.

Overall, DM in companion animals places a considerable burden on caregivers, with important effects on emotional wellbeing, daily routines, financial resources, and the practical organization of care. CGM seems to be more widely integrated into canine DM management, while its use in cats remains more limited [3]. Together, these findings support the adoption of a caregiver-centered approach in veterinary DM care, emphasizing clear communication, practical guidance, and measures to enhance the long-term sustainability of treatment for owners.

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Oral Communication(s)

ERYTHROGRAM ALTERATIONS IN DOGS WITH CHRONIC LIVER DISEASE: A RETROSPECTIVE STUDY

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Erythrogram alterations are widely studied in human chronic hepatic disease. In veterinary medicine, studies about erythrogram alterations mainly focus on canine vascular hepatopathies, highlighting the occurrence of microcytosis and iron dysmetabolism, whereas data on canine chronic liver disease (CLD) remain scarce. This retrospective study aims to evaluate frequency and characteristics of erythrogram alterations in dogs with CLD. Our hospital database was searched for dogs with persistent (≥ 2 months) elevations of at least two of the following enzymes: ALP, GGT, ALT, or AST with concurrent ultrasonographic hepatic abnormalities. Dogs affected by acute liver disease, hepatic or extrahepatic neoplasia, endocrinopathies, and renal diseases were excluded. A total of 91 dogs were included. Signalment and RBC, HCT, HGB, MCV, MCHC, RDW, RETIC, RETIC-HGB were registered for each dog. Semi-quantitative evaluation of blood smear abnormalities (anisocytosis, poikilocytosis, polychromasia) was retrieved from medical records. Concurrent serum albumin/globulin (A/G) ratio and iron were recorded when available. Anemia was defined as HCT<37% and classified based on grading and erythrocytes indices. Median age was 9.2 years (range 0.7–15.5 years); 52 dogs (57.1%) were female and 39 (42.9%) male. Breed distribution was wide, with mixed-breed dogs (28.6%) most represented. Anemia was found in 19 dogs (20.8%) and it was mostly mild [12/19 (63.2%)] normocytic [11/19 (57.9%)], normochromic [17/19 (89.5%)], and non-regenerative [13/19 (68.4%]. Poikilocytosis was found in 18 dogs (19.8%), specifically among these cases, schistocytes were observed in 8 (44.4%), acanthocytes in 6 (33.3%), echinocytes in 4 (22.2%), and target cells in 3 (16.7%). Anisocytosis was present in 35 dogs (38.5%), while polychromasia was found in 10 dogs (11%). Among non-anemic dogs, decreased RETIC-HGB was identified in 27/72 (37.5%), whereas decreased serum iron was found in 5/59 (8.5%). In the overall population, MCV was positively correlated with HGB, RETIC-HGB, A/G ratio, and serum iron (r=0.21 p=0.04; r=0.67 p<0.001; r=0.38 p<0.001; r=0.32, p=0.006, respectively). RETIC-HGB positively correlated with serum iron (r = 0.32, p=0.006) and it was significantly lower in anemic vs non-anemic dogs [20.2pg (17.4–24.9pg) vs 22.8pg (16.9–26.7pg); p=0.005], whereas serum iron did not differ between groups. Based on our results, in dogs with CLD, erythrogram abnormalities seem to be relatively common and are mainly characterized by mild, normocytic, non-regenerative anemia and erythrocyte morphological changes despite the presence of the anemia. A substantial proportion of our non-anemic CLD dogs showed decreased RETIC-HGB despite normal serum iron concentrations, which may suggest a potential role of RETIC-HGB as an early marker of iron availability in the context of a chronic disease.

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Oral Communication(s)

PARR AS A UNIQUE DIAGNOSTIC TEST IN A CASE OF ERYTHROPHAGOCYTIC LOW-GRADE SPLENIC T-CELL LYMPHOMA IN A CAT

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Lymphoma is a common neoplasm in cats. Extranodal lymphomas are common in cats, with the intestinal, nasal, mediastinal and renal forms more frequently identified whereases the hepato-splenic form rarely diagnosed. This case report describes a case of feline erythrophagocytic low-grade splenic T-cell lymphoma with peripheral blood involvement never described before.

A six-year-old neutered male cat was referred to the Veterinary Teaching Hospital of the University of Perugia for severe chronic anemia. When referred a complete blood count, biochemical analysis, abdominal ultrasound examination, splenic and bone marrow fine-needle aspiration for cytology and immunophenotyping examination, biomolecular test for Mycoplasma spp., Feline immunodeficiency virus (FIV), Feline Leukemia Virus (FeLV) and Feline Coronavirus, liver and spleen biopsy for histologic examinations and PCR for antigen receptor rearrangements (PARR) of splenic pulp were performed.

Blood analysis confirmed a nonregenerative macrocytic normochromic anemia and blood smear evaluation revealed the presence of erythrophagocytic mononucleated cells. Biochemistry showed mild hyperbilirubinemia and ultrasound examination revealed solely moderate splenomegaly. Splenic cytology evidenced extramedullary erythropoiesis and round-cell proliferation exhibiting marked erythrophagocytosis. Splenic flow cytometry identified a reactive population with no immunophenotypic abnormalities. Bone marrow cytology and PCR testing for infectious diseases were negative. An immunemediated anemia with peripheral and splenic erythrophagocytosis was suspected and treatment with prednisolone and cyclosporine was started. Due to the worsening of anemia, splenectomy was performed. Splenic histopathology showed white pulp hyperplasia with a predominant CD3+ T-cell phenotype, diffuse erythrophagocytosis, and extramedullary erythropoiesis, whereas liver biopsies were normal. Interestingly, splenic PARR analysis identified a monoclonal T-cell receptor gene rearrangement on a polyclonal background, consistent with a neoplastic T-cell population. A diagnosis of erythrophagocytic low-grade splenic T-cell lymphoma was assessed. Chlorambucil was started and the anemia progressively resolved.

To our knowledge, this is the first case of feline low grade erythrophagocytic splenic T-cell lymphoma; only rare cases of low grade hepato-splenic T-cell lymphomas in cats are described. It is important to consider this neoplasm in the differential diagnostic in case of nonregenerative anaemia and splenomegaly associated with peripheral and splenic mononuclear erythrophagocytosis. These findings alone are unspecific and histological examination and flow cytometry are inconclusive for neoplasm, due to the reactive underlying process and the presence of small lymphocytes resembling mature ones. PARR analysis was the only exam capable of achieving a definitive diagnosis allowing the differentiation of monoclonal proliferation of mature T-cell lineage within a larger underlying reactive process, which is often present in low-grade feline lymphomas.

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Oral Communication(s)

IMPACT OF CHEMOTHERAPY-INDUCED NEUTROPENIA ON SURVIVAL OUTCOMES IN DOGS WITH HIGH-GRADE MULTICENTRIC LYMPHOMA TREATED WITH A 19-WEEK CHOP PROTOCOL

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In human oncology, treatment-related neutropenia has been associated with improved outcome, possibly reflecting adequate drug exposure and tumour chemosensitivity [1]. In veterinary medicine, limited data are available, with only one previous study suggesting a similar association [2]. The study aimed to evaluate whether chemotherapy-induced neutropenia influences survival outcomes in dogs with high-grade multicentric lymphoma treated with a 19-week CHOP protocol.

Clinical records of dogs diagnosed with high-grade multicentric lymphoma and treated with a CHOP-19 protocol were retrospectively reviewed. Dogs that received at least one cycle of chemotherapy were included. Patients were classified based on the occurrence of chemotherapy-induced neutropenia, according to VCOG criteria. Survival analysis was performed using Kaplan–Meier curves, evaluating overall survival (OS), disease-free survival (DFS), and time to progression (TTP).

Thirty-seven dogs met the inclusion criteria. Fifteen dogs developed neutropenia (GN), while twenty-two did not (GC). Dogs developing neutropenia showed longer survival outcomes compared to non-neutropenic patients. Median OS was 427 days (range 186–476) in GN and 215 days (range 6–500) in GC ($p = 0.14$). Median DFS was 290 days (range 260–362) in GN and 229 days (range 95–471) in GC ($p = 0.11$). Median TTP was 267.5 days (range 116–475) in GN and 170 days (range 5–471) in GC ($p = 0.041$). Cox regression analysis showed an increased risk of death (HR 1.84; $p = 0.150$), recurrence (HR 3.14; $p = 0.125$), and disease progression (HR 2.32; $p = 0.048$) in non-neutropenic dogs compared to neutropenic patients, with a statistically significant effect observed for progression. These findings suggest a more consistent association between neutropenia and improved disease control rather than overall survival.

Additionally, treatment delays secondary to neutropenia did not appear to negatively affect survival outcomes. In contrast with previous reports, vincristine was the drug most frequently associated with neutropenia in the present cohort, being involved in 93% of cases, compared with 33% for both cyclophosphamide and doxorubicin.

Traditionally considered a dose-limiting adverse event, neutropenia in this cohort was predominantly mild to moderate (VCOG grade II in 73% and grade III in 27% of cases) and clinically manageable. When not severe, chemotherapy-induced neutropenia may reflect effective drug exposure and be associated with treatment efficacy, rather than indicating a detrimental event.

These results are consistent with those reported by Wang et al. (2015) [2] and suggest that chemotherapy-induced neutropenia may be associated with improved clinical outcome in dogs with multicentric lymphoma. From a clinical perspective, neutropenia occurring during CHOP chemotherapy should not necessarily be considered an adverse prognostic factor, and treatment delays related to neutropenia may not compromise therapeutic efficacy.

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Oral Communication(s)

RUMEN AND SALIVA MICROBIOME ALTERATIONS IN KETOTIC DAIRY COWS

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Ketosis is a common metabolic disorder in dairy cows which has been associated with altered rumen fermentation and potentially increased CH_4 production [1]. Metabolomic studies on serum of cows affected by ketosis have reported increased levels of methanogenesis-related substrates, suggesting a systemic metabolic shift potentially linked to rumen fermentation patterns and CH_4 production [2,3]. The aim of the study was to assess the alterations of rumen and saliva microbiome in dairy cows affected by ketosis.

Procedures were approved by Ethical Committee for Animal Welfare of University of Padua (n.103549/2024). A total of 60 Holstein-Friesian multiparous dairy cows were enrolled from a single farm. Animals were divided into two groups according to BHB: CTR or control (BHB<1.0 mmol/L; n=43); KET or affected by ketosis (BHB≥1.0 mmol/L; n=17). Rumen fluid sampling via stomach tube and oral swabs were performed in KET cows at the time of diagnosis. The CTR, which remained clinically healthy during the trial, were sampled only once at 21 days in milk to ensure a physiologically comparable control group.

The rumen and saliva microbiota were investigated using an amplicon-based metagenomic analysis amplifying the V3-V4 hypervariable regions of the 16S rRNA gene. Reads were clustered into zOTUs and taxonomically assigned to the latest version of the SILVA database (v.138). Alpha-diversity, beta-diversity (UniFrac), differential abundance, and rumen-saliva distance analyses were performed to evaluate microbiome structure and its association with disease status. A $p \leq 0.05$ was accepted.

Biodiversity and composition analyses revealed significant differences between rumen and saliva, as well as between CTR and KET experimental groups. In rumen samples, phylogenetic diversity was higher in KET animals (Faith's phylogenetic diversity, p=0.027). Differential abundance analysis showed enrichment of *Prevotella, Treponema, Fibrobacter*, and Ruminococcaceae-related taxa in rumen samples from KET, together with a reduction in Firmicutes and Lachnospiraceae groups. In saliva, ketosis was associated with a lower alpha-diversity ($p<0.01$ for all the considered metrics) and a different microbial composition (p=0.008 and p=0.021 for the unweighted and weighted UniFrac distances, respectively), with increased Actinobacteriota, *Staphylococcus, Bibersteinia*, and *Kingella*, and decreased Lachnospiraceae and Succinivibrionaceae.

Distance analyses indicated greater rumen-saliva microbial dissimilarity in KET cows (borderline significance, p=0.05), suggesting disrupted host-microbiome interactions.

Overall, the observed shifts in bacterial communities' support a disease-driven alteration of fermentation-related microbial pathways potentially linked to CH_4 production. These findings highlight the microbiome as a key component of the pathophysiology of ketosis and as a potential target for prevention strategies and mitigation of livestock-related environmental impacts.

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Oral Communication(s)

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 REARING PERIOD

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Mortality in veal calves represents a relevant welfare and economic concern, particularly during the initial post-arrival phase, when calves are subjected to stressors that may increase their susceptibility to disease and death [1;2]. Prompt identification of high-risk calves at arrival could enhance monitoring and management [3]. The objective of this prospective cohort study was to investigate the population-level associations between CLuBS parameters: (C)linical signs, (Lu)ng lesions, (B)ody weight, and (S)erum total protein, recorded 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). The study was conducted at a CC situated in Ghedi, Brescia, Italy, and included 934 veal calves over a six-month period (January–July 2025). Immediately upon arrival at the CC, each calf was weighed. Subsequently, all calves underwent a clinical assessment including rectal temperature, fecal score, evaluation of umbilical structures, joint examination, and thoracic ultrasonography (TUS). A blood sample was also collected from each animal 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 that accounted 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). The overall mortality rate was 9.4% (32/934 early; 88/934 total). In multivariable GEE models, severe lung consolidation (≥3 cm) emerged as the strongest predictor of mortality. Compared with calves showing severe consolidation, those free of 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 negatively associated with mortality, with a 9% reduction in the odds of early mortality (OR = 0.91; 95% CI: 0.84–0.97) and a 4% reduction in the odds of total mortality (OR = 0.96; 95% CI: 0.94–0.99) per additional kilogram. Fecal consistency at arrival was also independently linked to total mortality; calves with normal feces had a lower risk of death compared with those presenting with watery feces (OR = 0.47; 95% CI: 0.25–0.86). In conclusion, severe lung consolidation and low arrival body weight were independently associated with increased mortality risk in veal calves, supporting the implementation of structured arrival screening protocols.

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Oral Communication(s)

INTRAMUSCULAR HEPATOPROTECTIVE SUPPLEMENTATION MODULATES METABOLOMIC PROFILES IN MULTIPAROUS DAIRY COWS DURING THE TRANSITION PERIOD

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The fatty acid oxidation progressively reduces the intermediate of the tricarboxylic acid (TCA) cycle during the transition period in dairy cows. Moreover, the TCA cycle could be comprised by amino acids (AA) deficiency causing an increasing in ketogenesis and hyperketonemia. Additionally, transition period is also characterized by altered oxidative state, inflammatory and immune responses which further use AAs [1]. Supplementation of liver metabolism (hepato-protectors) through AA, vitamins as cyanocobalamin, inositol (insulin-mimetic), and α-lipoic acid (antioxidant) may be used as a preventive protocol for hyperketonemia. The aim of this study was to investigate blood metabolites associated with hepato-protector protocol for hyperketonemia in dairy cows.

The Ethics Statement was approved by the Animal Care and Use Committee of the University of Padua (protocol number 204359/2023). A total of 60 multiparous Holstein-Friesian dairy cows were enrolled during the dry period in one free-stall barn, and randomly divided into two groups. The control group or CTR enrolled 30 multiparous dairy cows that did not receive preventive protocol for hyperketonemia during the study period. The hepato-protectors' group or HEP enrolled 30 multiparous dairy cows that received two treatments, one in pre-calving (12 days before calving) and one in post-calving (6 days after calving) period. Both administrations included two treatments: the first one contained AA (150 mg/mL acetyl-methionine, 15 mg/mL alanine, 15 mg/mL arginine, 15 mg/mL threonine, 15 mg/mL glutamine), 30 mg/mL inositol, and 1 mg/mL cyanocobalamin (IM 2 ml/10 kg of BW); and the second one contained 4.8 mg/mL acetyl-methionine, 0.25 mg/mL α-lipoic acid, and 0.5 mg/mL cyanocobalamin (IM 70 ml/animal). All groups received the same diet. Blood sampling was performed from the coccygeal vein at -21 and -7d pre-partum (pp), and at 7, 14, and 28 days in milk (DIM) for serum metabolomic analysis 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.

Hyperketonemia prevalence was 10.0%, 23.3% and 20.0% in CTR at 7, 14, and 28 DIM, respectively. Instead, the prevalence was 10.0%, 16.7% and 6.67% in HEP in the same time period. A total of 50 metabolites were identified, and 26 out of 50 were significantly different between groups. Thirteen metabolites had greater concentration in HEP compared to CTR (alanine; arginine; asparagine; choline; dimethyl-sulfone; glutamine; glycine; glyoxylate; histidine; methionine; myo-inositol; O-acetyl-carnitine; taurine), while thirteen had lower concentration (2,3-butanediol; 3-hydroxybutyrate; 3-hydroxyisobutyrate; acetate; acetone; dimethylamine; isoleucine; leucine; methanol; methionine sulfoxide; succinate; TMAO; valine). Among different metabolites between groups, a total of 1 and 11 metabolites differed at 21 and 7 pp, respectively (3.84% and 42.31%), and 10, 25 and 21 metabolites differed at 7, 14, and 28 DIM, respectively (38.46%, 96.15%, and 80.77%).

In conclusion, treatments with hepato-protectors improved the level of glucogenic AA, antioxidants, and fatty acid metabolism, with a probable improvement in the functioning of the TCA cycle. In addition, supplementation reduced the level of ketogenic AA and ketone bodies, markers of inflammation and oxidation, and ruminal methanogenic precursors, suggesting better management of the negative energy balance period.

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Oral Communication(s)

PREDISPOSING FACTORS FOR NEONATAL CALF DIARRHEA: AN ANALYSIS IN FAMILY RUN FARMS

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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 [1]. This study aimed to apply a novel checklist designed to identify management-related predisposing factors for NCD in calves.

Thirty bovine farms 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.

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 strong positive correlation was found between D1 and D2 ($rs=0.67$, $p<0.001$), and a moderate 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.

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.

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Oral Communication(s)

ACOUSTIC CHARACTERIZATION OF FREQUENCY- AND AMPLITUDE-RELATED FEATURES OF NORMAL AND PATHOLOGICAL LUNG SOUNDS IN PRE-WEANED CALVES

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Bovine respiratory disease is common in pre-weaned calves and affects welfare, antimicrobial use, and farm sustainability [1]. On-field diagnosis relies on thoracic auscultation, but its accuracy is limited by subjective interpretation [2]. In human medicine, objective analysis of lung sound characteristics, including spectral and amplitude properties, has been used to overcome this limitation and to support the development of artificial intelligence (AI) tools [3].

This study aimed to analyze and compare spectral and amplitude features of normal and pathological lung sounds in calves, hypothesizing that their features differ between lung sound types.

Lung sounds were recorded with an electronic stethoscope, saved as WAV files, and classified as normal (N), increased breath sounds (IBrS), increased bronchial sounds (IBS), crackles (C), or wheeze (W). Recordings were also assessed for quality based on lung sound presence, heartbeat, and environmental noise. The sound database was built using data from previous studies [2] and routine clinical work at three university institutions (Milan, Uppsala, and Montreal). All analyses and retrospective evaluations of the recordings were conducted in an anonymized fashion; therefore, ethical committee approval was not deemed necessary. Acoustic features were extracted using Fast Fourier Transformation and included frequency-related parameters (dominant frequency (DF), minimum frequency (MinF), maximum frequency (MaxF), and frequency range (RangeF)) and amplitude-related parameters (total energy (TotE), mean energy (ME), maximum energy (MaxE), and Root Mean Square in dB (RMS-dB)). Descriptive statistics were calculated, and differences among categories were assessed using the Kruskal–Wallis test followed by Dunn's post hoc test (p-value <0.05).

A total of 994 recordings from 336 calves, mostly Holstein Friesian, were collected. Among the recordings, 249 WAV files were classified as high quality and retained for analysis. Of these, 65 were N, 62 IBrS, 48 IBS, 42 C, and 32 W. Dominant frequency (~1000 Hz) and minimum frequency (~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; MaxE ranged from −36.3±5.0 dB in N to −24.4±7.4 dB in C, and RMS-dB ranged from −53.7 dB (N) to −43.4±6.0 dB (W).

The Kruskal–Wallis test showed significant differences for MinF, MaxF, and all amplitude-related parameters. Post hoc comparisons showed significant differences in MinF between N and IBS, and in MaxF between IBrS and N and between W and N. All amplitude-related parameters differ significantly between normal and pathological sounds.

This study offers a first characterization of lung sounds features in pre-weaned calves, identifying differences in spectral and amplitude parameters. Energy-related parameters showed the most significant variation and could help develop AI tools to improve the diagnostic accuracy of thoracic auscultation.

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Oral Communication(s)

ETIOLOGICAL DIAGNOSIS AND RATIONAL ANTIMICROBICAL USE IN BOVINE MASTITIS: EVIDENCE FROM A MOLECULAR APPROACH

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Bovine mastitis is the primary cause of economic loss in the dairy sector and the condition most frequently associated with antimicrobial use on farms [1]. Often, treatment is initiated without a definitive etiological diagnosis, relying solely on clinical signs or milk alterations, which has serious implications for therapeutic appropriateness and the risk of antimicrobial resistance [2]. The introduction of molecular methods, such as Real-Time PCR, has significantly improved the identification of major pathogens, offering new perspectives for faster and more accurate clinical management compared to traditional culture techniques [3]. This study aims to evaluate the distribution of diagnostic outcomes and compare the effectiveness of Real-Time PCR versus classical microbiological examination in individual milk samples from cows with clinical and subclinical mastitis in intensive dairy herds in the Ragusa province. A total of 384 individual milk samples from various local farms were analyzed. Sampling procedures were performed in a clinical setting during routine farm monitoring activities, with informed consent obtained from the farm owners. As this was a diagnostic investigation conducted on milk samples collected during standard management routine and not an experimental animal study, ethics committee approval was not required. Each sample was subjected in parallel to: Molecular investigation (Real-Time PCR) for the detection of: Staphylococcus aureus, Streptococcus agalactiae, Streptococcus uberis, Mycoplasma bovis, and Prototheca spp.. and a Traditional microbiological examination (culture test and biochemical identification) to compare diagnostic sensitivity. Results Molecular analysis identified pathogens in 27.6% (106/384) of the samples, showing a heterogeneous distribution: S. uberis (11.2%), S. aureus (7.8%), Prototheca spp. (4.7%), S. agalactiae (2.6%), and M. bovis (1.3%). In contrast, microbiological examination showed a significantly lower isolation rate of 13.3% (51/384), highlighting a substantial proportion of silent growth or false negatives in culture. A critical finding is the 72.4% of samples that tested negative by PCR, suggesting that a consistent portion of mastitis cases may be associated with bacterial loads below the detection limit, transient infections, or non-bacterial causes. The superior sensitivity of PCR identifies it not only as a diagnostic tool but as a fundamental decision-support system for the rational selection of cases requiring treatment. The high rate of negative results underscores the risk of antimicrobial overuse in empirical treatments performed without etiological confirmation. The integration of molecular methods into routine diagnostic protocols is therefore a cornerstone of antimicrobial stewardship, essential for improving the economic and health sustainability of dairy farms.

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Oral Communication(s)

NEONATAL CALF DIARRHEA IN THE PROVINCE OF RAGUSA

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Neonatal bovine diarrhea (NBD) is one of the leading causes of mortality and morbidity in calves, regardless of the rearing system or breed[1,2]. It has a multifactorial origin involving factors such as farm management, various etiological agents, and climatic conditions, with repercussions not only on the calf but also on the animal's entire productive life[2,3], making good management essential to limit its occurrence as much as possible. Given the ubiquity of the pathogens involved and their economic impact, an epidemiological study was conducted in the province of Ragusa during 2025 to investigate Cryptosporidium spp. (C. spp.), Rotavirus (RV), Coronavirus (CV), and E. coli K99 (E. coli) in order to assess their distribution over time and space. Fecal or intestinal samples were collected from animals exhibiting clinical signs consistent with DNB, or in the event of the animal's death, and sent to the "A. Mirri" Experimental Zooprophylactic Institute of Sicily in Ragusa for analysis. The 59 samples, each from a single animal 41 fecal samples from live animals and 18 intestinal samples obtained postmortem were analyzed using rapid immunochromatographic tests and RT-PCR, respectively. The most commonly detected pathogens in both matrices were C. spp. and RV, found in 31 (52.5%) and 20 (33.9%) samples, respectively, followed by CV 3 (5.1%) and E. coli 2 (3.4%). A high number of co-infections was observed 17 (28.8%), of which 14 (82.4%) involved C. spp. and RV, and 3 (17.6%) involved C. spp. and CV. Within the province, distribution varies, with the highest number in the municipality of Ragusa (28), followed by Chiaramonte Gulfi 11, Modica 6, Ispica 4, Vittoria 4, Comiso 4, Santa Croce Camerina 1, and Monterosso Almo [1] When examining the temporal distribution of the collected samples and dividing the year into quarters (Q1, 2, 3, 4), the highest incidence is observed in Q1 with 22 cases, primarily caused by C. spp. and RV, often with co-infections. A decrease is seen through Q4, when the presence of C. spp., RV, and CV increases. The data highlight the prevalence of DNB throughout the province, with C. spp. and RV as the primary pathogens in both single infections and co-infections, particularly in intestinal samples a factor that does not, however, necessarily indicate the sole cause of death. The affected farms shared common predisposing conditions: absence of specific vaccination protocols, use of wooden calf crates a porous material difficult to adequately disinfect followed by housing in group pens of 5–10 animals, collectively favoring pathogen persistence and horizontal transmission. These data confirm that NBD remains a significant issue, highlighting the need for targeted biosecurity protocols and surveillance programs. This study serves as a baseline for future regional investigations to strengthen prevention strategies and protect animal health and livestock sustainability.

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Oral Communication(s)

AUTOMATED CLASSIFICATION OF CALF LUNG SOUNDS FROM DIGITAL AUSCULTATION RECORDINGS USING SUPERVISED MACHINE LEARNING: A PILOT STUDY

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Bovine Respiratory Disease (BRD) in pre-weaned calves significantly affects animal health, welfare, and production, representing a major challenge in dairy and beef farming. In human medicine, artificial intelligence (AI) has enabled the development of tools that support the diagnosis of respiratory conditions, helping to overcome the subjectivity of lung sound interpretation and improving accuracy and consistency. Machine-learning (ML) approaches may similarly provide a more objective assessment of calf lung sounds and reduce the subjectivity of field auscultation. Inspired by these advances, this pilot study evaluated the feasibility of automatically classifying digitally recorded respiratory sounds from pre-weaned calves using a Python-based supervised learning workflow. The dataset comprised 296 lung sound WAV recordings, collected on-farm from pre-weaned calves using an electronic stethoscope, and initially labelled according to five categories based on thoracic auscultation: Healthy, Increased Breath Sounds (IBrS), Increased Bronchial Sounds (IBS), Crackles (CR), and Wheezes (WH). The recordings were analysed according to two classification schemes: a binary scheme (healthy vs pathological (IBrS, IBS, CR, WH)) and a five-category scheme (Healthy, Crackles, IBrS, IBS, Wheeze). Audio features were extracted using Librosa (Python) [1] and included Mel-frequency cepstral coefficients (MFCCs) and Mel-scaled spectrograms (MSS), expressed in decibels. Gradient Boosting (GB) and Random Forest (RF) classifiers implemented in scikit-learn [2] were evaluated using cross-validation, grid search hyperparameter tuning, and final assessment on an independent test set. In the binary task, the best configuration used MSS with RF [3], yielding 89.4% training accuracy, 88.3% test accuracy, and an F1 score of 0.8[2] In the five-category task, MFCCs with RF achieved 61.8% training accuracy, 55.0% test accuracy, and an F1 score of 0.55, highlighting the difficulty of discriminating acoustically overlapping patterns. These preliminary findings support the feasibility of ML-based classification of bovine lung sounds and suggest that this approach could enable AI-based tools for early detection of BRD in calves, potentially promoting more judicious antimicrobial use on-farm. Such tools could assist clinicians in screening BRD, although further studies on larger datasets are needed to improve performance and confirm applicability in routine practice.

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Oral Communication(s)

METABOLOMIC CHARACTERIZATION OF BOVINE COLOSTRUM AND CALVES' PLASMA PROFILES AND THEIR INTERACTION IN THE *COLOSTRAL* PHASE

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Besides the pivotal role of bovine colostrum in ensuring passive transfer of immunity in newborn calves, its nutraceutical properties remain poorly understood [1]. Omics approaches, which enable a comprehensive characterization of biological matrices, are therefore essential for gaining deeper insight into the nutraceutical potential of colostrum. This study aims to investigate both bovine colostrum and plasma composition of newborn calves by performing an untargeted metabolomic analysis, to explore their interaction and temporal profile changes. Eighteen healthy Italian Holstein calves were enrolled at birth (T0) and fed colostrum collected directly from their own mothers until the 3rd day of life (approval number 27/2024, University of Pisa). The sample size was calculated based on power analysis made with *G-Power v. 3.1* software. At T0 and at 12, 24 and 72 hours of life, colostrum samples were taken ready before milking; contextually, blood samples were collected from each calf prior feeding. All samples underwent an untargeted metabolomic analysis, using an UHPLC-Q-Exactive Focus system. Colostrum and plasma meta-analyses were processed using *MetaboAnalyst 6.0*. Data were median-centered, $\log_{10}$-transformed, and Pareto-scaled prior to hierarchical clustering and PCA for unsupervised exploration, and sPLS-DA for supervised classification with reference to time. Temporal variations in metabolites were assessed through Volcano Plot analysis ($FC > 1.2$, $p < 0.05$) and visualized using Venn diagrams to identify common and unique features. Colostrum and plasma datasets were then integrated via the DIABLO framework (*mixOmics*, R 4.3.2) to reveal discriminant variables characterizing time-dependent chemical changes. A total of 1274 metabolites were identified across both colostrum and plasma. In colostrum, analyses revealed a time-dependent effect on metabolite composition, with marked differences between T0 and T72 samples. In plasma, most changes were observed at T12, T24 and T72 when compared with the T0. Data integration showed an 80% correlation between metabolites in plasma and colostrum using a single principal component, with the most discriminant metabolites associated with T24 samples in both matrices. When two principal components were considered, the correlation between metabolites decreased to 75% and was influenced by metabolites across all time points, particularly in plasma. Amino acids and lipids were predominant among all the compound classes contributing to the correlation between the two matrices. This study provides a comprehensive overview of colostrum and plasma metabolic profiles. These findings contribute to the characterization of the physiological state of calves at defined time points. Moreover, the metabolomic dataset enhances our understanding of how plasma profiles evolve over and under the influence of colostrum during the early postnatal (colostral) phase in a non-pathological context. Finally, these results represent a valuable resource for expanding existing metabolomic databases [2] and provide a foundation for future studies aimed at exploring the use of colostrum as a nutraceutical in calves.

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Oral Communication(s)

CLINICAL SIGNS AS AN INDICATION OF THE PRESENCE OF GASTRIC ULCERS IN PLEASURE AND BREEDING HORSES HORSES: TRUE OR FALSE?

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Equine Gastric Ulcer Syndrome (EGUS) and its related diseases, Equine Squamous Gastric Disease (ESGD) and Equine Glandular Gastric Disease (EGGD) can be found in horses worldwide, with increased prevalences in racehorses and animals subjected to heavy training loads. Variable percentages have been described also in pleasure and breeding horses, where around 50-60% have been found affected by the disease in different studies. Clinical signs of the disease are vague and nonspecific (weight loss, ill-thrift, recurrent colic, poor performance), but most of the horses are asymptomatic. The gold standard for the diagnosis is gastroscopy with a grading system available only for ESGD. Currently, relationship between clinical signs and presence of ESGD or EGGD are under investigation, given the high number of animals showing no signs [1,2]. Aim of this retrospective study was to determine the percentage of symptoms in a population of pleasure and breeding horses subjected to gastroscopic evaluation, and to correlate them to the presence of ESGD and EGGD. Clinical records of the horses were evaluated, and signalment information collected, together with the main clinical sign reported by the owner and the results of the gastroscopy. Logistic and linear general models were used to determine if the signalment influenced the manifestation of clinical signs and if the symptoms could be related to the presence or severity of ESGD and EGGD. Statistical significance was set at $p<0.05$. In total, 1125 adult horses were enrolled in the study, mostly Warmbloods and Fullbloods (Arabians, Thoroughbreds and their crosses), aged between 1 and 30 years of age, all sexes were present. Only 12% of the horses showed clinical signs, related to either the gastrointestinal tract (GIT, 6%), performance and behavior (PER, 1%) and weight loss (WL, 5%). ESGD was diagnosed in 63% (grade 2/4 22%, 3/4 11% and 4/4 30%) animals, and EGGD in 32%. Based on the results of the analysis, Warmblood horses and geldings were more likely to show clinical signs, compared to the other breeds and sexes. WL was associated with presence and severity of ESGD and presence of EGGD, while GIT only with ESGD, both presence and severity, and PER with severity of ESGD and presence of EGGD. These results confirm the widespread presence of EGUS in horses in Italy, with percentages similar to other papers published in recent years [1-3]. Despite the relatively high prevalence of the disease in horses considered at low risk, the clinical signs were present only a small percentage, but their expression could be related to the presence and severity of gastric ulcers. Further studies are needed to confirm that this relationship, confirming the disappearance of the symptoms after healing of EGUS.

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Oral Communication(s)

APGAR SCORE IN FOALS: ASSOCIATION WITH CLINICAL, SEMIOLOGICAL AND REPRODUCTIVE PARAMETERS

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Apgar score, originally developed for humans, is a validated tool for assessing neonatal vitality in foals [1], but its association with reproductive, clinical, and semiological parameters remains poorly investigated.

This retrospective study analysed 144 foals and their mares using a 4-parameter Apgar score at 5 min. Inclusion criteria required the availability of Apgar score, heart rate (HR), respiratory rate (RR), and body temperature (BT) at birth. Associations were assessed by linear regression ($p < 0.05$).

Median Apgar score was 8/8 (IQR 7–8), with 91.7% of foals scoring ≥7/8. Median HR was 84 bpm (IQR 68.8–108.5 bpm), RR 60 bpm (IQR 48–68 bpm), and BT 37.5 °C (IQR 37.2–37.8 °C). HR was over and below the reference ranges (60-150 bpm) [2] in 4/144 (2.8%) and 1/144 (0.7%) foals, respectively. RR was over and below the reference ranges (40-60 bpm) [2] in 63/144 (43.8%) and 10/144 (6.9%) foals, respectively; BT was below the reference ranges (37-39 °C) [2] in 16/144 (11.1%) of foals.

Head-raising reflex had a median time of 1 min (IQR 1–1 min), occurring after 1 min in 22/144 (15.3%) of foals (reference 1-2 minutes) [3]. Suckling reflex was absent in 1/81 (1.2%) and weak in 1/81 (1.2%) foals (reference <30 min). Median time to sternal recumbency was 3 min (IQR 2–5 min), with 96/142 (67.6%) achieving it within 3 min (reference <5 min). Median time to stand was 78 min (IQR 60–120 min), with 31/144 (21.5%) exceeding 120 min (reference <120 min) [2]. Median time to first suckling was 140 min (IQR 100–200 min), with 17/127 (13.4%) exceeding 240 min (reference <180 min) [3].

Umbilical abnormalities were observed in 8/144 foals (5.6%), with 2 documented cases (twisted and mildly oedematous cords). Median blood glucose concentration at birth was 65.5 mg/dL (IQR 47.8–83.0 mg/dL); glycemia was higher, within or below reference ranges (50.05-63.05 mg/dL) [1] in 57/102 (55.9%), 18/102 (17.6%) and 27/102 (26.5%) foals, respectively. IgG concentration at 24 hours was >800 mg/dL in 80/92 (87.0%), 400–800 mg/dL in 5/92 (5.4%) and <400 mg/dL in 7/92 (7.6%), respectively.

Gestational length (median 338 days, IQR 332–347.8 days) was >360 days in 5/96 (5.2%), within 320–360 days in 87/96 (90.6%), and <320 days in 4/96 mares (4.2%). Placental evaluation showed no macroscopic abnormalities observed in 138/141 (97.9%). Median placental weight was 5.0 kg (IQR 4.18–5.5 kg), with 2.5% above, 68.0% within, and 29.5% below reference range (4.4–7.7 kg). Median placental expulsion time was 65 min (IQR 30–146.3 min), with 7/35 mares (20.6%) showing retention >3 h (reference <3 h).

Higher Apgar scores were significantly associated with increased HR ($B=7.859$; $p=0.017$) and RR ($B=9.011$; $p<0.001$), shorter time to sternal recumbency ($B=-4.301$; $p<0.001$), and lower risk of umbilical abnormalities ($B=-0.077$; $p=0.001$). No associations were found with sex, BT, blood glucose, IgG concentration, time to stand, first suckling, gestational length, or placental weight.

In conclusion, the Apgar score is a reliable tool for early neonatal assessment in foals, reflecting immediate postnatal adaptation but with limited predictive value for longer-term outcomes.

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Oral Communication(s)

IMPACT OF SELF-DIRECTED AUDIO-BASED TRAINING ON VETERINARY STUDENTS' ABILITY TO INTERPRET EQUINE HEART SOUNDS

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Auscultation and interpretation of heart sounds are essential clinical skill in veterinary medicine but remains challenging for students to acquire. Previous studies have shown that digital and remote learning tools, including recorded heart sounds, may improve students' diagnostic abilities through repeated exposure and self-directed practice [1]. However, evidence in equine medicine, particularly using ECG-based audio recordings, is limited. This study aimed to evaluate whether self-directed audio training improves veterinary students' ability to recognize and interpret equine heart sounds compared to theoretical study alone.

Thirty fourth-year veterinary students from the Universities of Milan and Pisa voluntarily participated in the study. The study was approved by the ethics committees of both universities (122/23; 1/2024). Students completed a test at baseline (T0) and again after a 4-week self-directed training period (T1). The investigators were blinded to the identity of the participants and their individual test scores. The test consisted of 18 questions based on audio recordings of equine heart sounds, requiring identification of normal findings, arrhythmias, or heart murmurs and, when applicable, their classification. After the T0 test, participants were randomly divided into two groups. Both groups received the same written materials covering cardiac arrhythmias and murmurs. In addition, Group 2 was provided with audio recordings of sinus rhythm, arrhythmias and murmurs for self-directed practice. At T1, students repeated the test with the same recordings in a different order. Scores were analyzed for total questions (n=18), primary questions (n=10; recognition), and secondary questions (n=8; classification). To ensure the two groups were similarly trained at T0, between-group differences were assessed using the Mann–Whitney U test. To detect training effect between T0 and T1, within-group changes were assessed using the Wilcoxon signed-rank test.

No significant differences were observed between groups at T0 ($p = 0.098$). Group 1 showed no significant improvement between T0 and T1 in total score ($p = 0.347$), primary questions ($p = 0.099$), or secondary questions ($p = 0.964$). In contrast, Group 2 showed significant improvement at T1 in total score ($p = 0.008$), primary questions ($p = 0.049$), and secondary questions ($p = 0.007$).

This study highlights the potential of self-directed audio-based training as an effective educational tool for improving veterinary students' auscultation skills. The significant improvement observed in the group 2 underscores the importance of incorporating active, sensory-based learning methods into veterinary curricula, particularly for skills that rely heavily on auditory perception. Traditional teaching methods, while essential for building foundational knowledge, may not adequately prepare students for real-world clinical scenarios where rapid and accurate interpretation of heart sounds is required. In this context, the use of audio recordings provides a valuable opportunity for repeated, self-paced practice, allowing students to progressively refine their listening skills. In conclusion, although this is just a preliminary study with a limited number of participants, the results suggest that integrating audio-based training into veterinary education may represent an effective strategy to improve competency in cardiac auscultation in equine medicine.

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Oral Communication(s)

FOALING AND EARLY NEONATAL FACTORS ASSOCIATED WITH FAILURE OF PASSIVE TRANSFER IN FOALS

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Neonatal foals rely on passive transfer of maternal immunoglobulins due to an immature adaptive immune system. Inadequate transfer of IgG results in Failure of Passive Transfer (FPT), the most common immunodeficiency in foals, predisposing them to secondary infectious diseases [1,2].

This study, approved by the Ethical Committee of the University of Pisa (27/24), evaluated clinical, foaling-related, perinatal, behavioural and haematological parameters, including IgG levels, in neonatal foals with and without FPT.

Over two foaling seasons (2024-2025), 31 neonatal foals were enrolled and classified as healthy or affected by FPT. Healthy foals met the following criteria: normal gestation time (>320d), eutocic delivery and colostrum quality >50 mg/ml of IgG in the mare; for foals, APGAR score ≥7/8 and neonatal behavioural parameters within reference ranges; good passive transfer of immunity (serum IgG >800 mg/dL at 24h); clinical parameters within reference ranges throughout the study period. Inclusion criterion for FPT foals was serum IgG <800 mg/dL at 24 hours [1].

At birth (T0), foaling-related and perinatal parameters were recorded, including APGAR score, neonatal behavioural parameters, meconium expulsion, umbilical condition, placental evaluation, heart rate (RR), respiratory rate (RR), temperature (T), blood glucose (Glu) and lactate (Lac), and colostral IgG (single radial immunodiffusion, SRID; %Brix). At 12 (T12), 24 (T24) and 72 (T72) hours, HR, RR, T and colostral IgG (SRID and %Brix) were assessed; Glu, Lac and serum IgG (spectrophotometric assay and SRID) were measured at T24.

As data were non-normally distributed (Shapiro–Wilk), categorical variables were expressed as frequencies and percentages, and continuous variables as median and interquartile. Between-group comparisons of single variables were performed using Mann-Whitney and chi-square tests. Repeated measures were analysed with a Generalized Estimating Equations (GEE) model.

Of 31 foals, 18/31 (58.1%) were healthy, and 13/31 (41.9%) were affected by FPT. Among the FPT foals, 8/13 had a serum IgG concentration between 400 and 800 mg/dL (partial FPT), while 5/13 had a serum IgG concentration <400 mg/dL (total FPT) and received hyperimmune plasma (1L, IV) [3] (Plasmalife Srl, Siena, IT). FPT foals were more frequently female (69.2%, p=0.048) and showed longer time to first suckling (p=0.040) and meconium expulsion (p=0.006), and a higher frequency of abnormal amniotic fluid (p=0.028). Colostral IgG concentration was lower in FPT foals (p=0.048), consistent with significantly reduced serum IgG levels (p<0.001). No other significant differences were observed.

FPT incidence was consistent with previously reported rates in hospitalized foals (30-42%) [1]. Only foals with total FPT received hyperimmune plasma. Those with partial FPT, clinically healthy, were monitored and managed without it [2]. The results confirm associations between FPT and selected foaling-related and early neonatal parameters, which may act as either predisposing factors or early indicators of FPT in foals [1,2]. Early recognition is essential to promptly identify at-risk foals and implement timely preventive or therapeutic interventions, thereby reducing the likelihood of secondary neonatal diseases.

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Oral Communication(s)

EFFECT OF ARTIFICIAL COLOSTRUM SUPPLEMENTATION ON CLINICAL PARAMETERS AND GROWTH INDICES IN HEALTHY FOALS AND FOALS WITH FAILURE OF PASSIVE TRANSFER – PRELIMINARY RESULTS

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Foals are highly vulnerable to health disorders. Failure of Passive Transfer (FPT) is one of the most frequent conditions, predisposing foals to secondary diseases such as sepsis, diarrhoea, and respiratory disorders [1]. The use of nutraceuticals, such as artificial colostrum, could represent a strategy to enhance health and welfare, potentially reducing disease incidence and the consequent need for antimicrobial treatments [2,3].

This study, approved by the Ethical Committee of the University of Pisa (27/24), aimed to assess the effect of artificial colostrum supplementation on clinical parameters and growth in healthy foals and foals affected by FPT.

Over two foaling seasons (2024-2025), 29 foals were enrolled at 4 days of age and classified as healthy or affected by FPT. Inclusion criteria for healthy foals: normal gestation time (>320d), eutocic delivery and colostrum quality >50 mg/ml of IgG for mares; for foals, APGAR score ≥7/8 and neonatal behavioural parameters within reference ranges; good passive transfer of immunity (serum IgG >800 mg/dL at 24h); clinical parameters within reference ranges throughout the study period. Foals with serum IgG <800 mg/dL at 24h were classified as FPT.

Artificial colostrum supplementation was administered from day 4 to day 13 of life (10 days). Foals were first classified by health status (healthy vs FPT) and then allocated to treatment (TG) or control (CG) groups. Among healthy foals (n = 9), two subgroups were defined: TG-1 (n=7), receiving 20 g IgG/day; and CG (n=2), receiving no supplementation. Among FPT foals (n=17), two groups were defined: TG-FPT (n=6), receiving 20 g IgG/day, and CG-FPT (n=11), receiving no supplementation.

Data, including heart rate (HR), respiratory rate (RR), body temperature (BT) and body weight (BW), were collected at 4 days (T4), 7 days (T7) and 21 days (T21) of life of foals.

Data resulted in a non-normal distribution (Shapiro–Wilk), and categorical variables were expressed as frequencies and percentages, and continuous variables as median and interquartile. Repeated measures were analysed with a Generalized Estimating Equations (GEE) model.

Higher BW was observed in the TG-1 group compared with the control group. No significant differences in BW were observed between healthy and FPT foals. No significant effects were observed for HR, RR, or BT across groups. During the study period, healthy foals did not show any sign of disease, while in the FPT-NT group, 1 foal developed diarrhoea, and 1 progressed to sepsis.

Colostrum supplementation appears to be associated with an increased BW in supplemented healthy foals, compared to the healthy control group. Additionally, based on very limited observations (n=2), the occurrence of clinical conditions in non-supplemented FPT foals might suggest a potential protective role of colostrum supplementation against early-life disorders, though this association was not specifically investigated. Although preliminary, these findings suggest a potential beneficial role of colostrum as a nutraceutical and warrant further investigation in a larger and more balanced population.

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Oral Communication(s)

METABOLIC AND BIOMECHANICAL PARAMETERS IN SHOW JUMPING HORSES SUBJECTED TO FIELD EXERCISE TESTING

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Exercise testing is an objective tool that allows to evaluate the physical condition of the horse, to monitor the training level and to detect subclinical signs not evident at rest [1]. In literature, several studies reported data concerning exercise test applied on racehorses; conversely, data on metabolic and biomechanical parameters in show jumping horses are still limited [2, 3].

The aim of this study was to describe the metabolic and biomechanical parameters in show jumping horses measured by means of an exercise test on the field, and to evaluate the effect of a three-weeks training period.

Data of 10 sport horses (mean age 9,4 +/- 3,95 y.o), obtained during an incremental field exercise test at baseline (T0) and after three weeks of regular training (T1) were retrospectively reviewed. The protocol included a warm-up phase (Step 1: walk, trot, and canter), followed by two jumping courses of increasing difficulty (Step 2: 60 cm; Step 3: 80 cm), and a cool-down phase at walk. During the test, horses were equipped with a device (Equimetre, Arione, France) that measured speed, heart rate and biomechanical parameters (stride length, stride frequency). At the beginning of the protocol and at the end of every steps, a blood sample was collected to measure blood lactate and hematocrit. Data obtained at T0 and T1 were compared by means of Student t-test or Wilkoxon test and statistical significance was set at $p < 0.05$.

Results showed that lactate values were lower at T1 compared to T0 for baseline lactate ($p=0.02$), lactate at Step 3 ($p=0.008$), and lactate at 5 minutes ($p=0.002$), suggesting improved aerobic capacity. Hematocrit values resulted higher at T1 ($p=0.03$) compared to T0, a possible indication of adaptation to training. Regarding biomechanical parameters, at T1 a significant reduction was observed in Step 2 for mean speed ($p=0.02$), maximum speed ($p=0.004$), and mean ($p=0.02$) and maximum stride length ($p=0.009$), and also in Step 3 for mean speed ($p=0.01$), maximum speed ($p=0.004$), and mean and maximum stride length ($p=0.02$). These results can suggest that horses performed the same level of exercise at lower biomechanical effort, as an indication of improvement in fitness.

In conclusion, the results demonstrated the usefulness of exercise testing for monitoring fitness improvement in sporting horses.

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Oral Communication(s)

COAGULATION PROFILE, PLATELET FUNCTION AND PLATELET AGGREGATION IN THOROUGHBRED RACEHORSES AFFECTED WITH EXERCISE-INDUCED PULMONARY HAEMORRAGE: A PILOT STUDY

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Exercise-induced pulmonary haemorrhage (EIPH) is a common condition among Thoroughbred racehorses. It is characterized by bleeding from lungs during strenuous exercise. The diagnosis of this condition is achieved using tracheoendoscopy (TE) and bronchoalveolar lavage (BAL) fluid analysis. The underlying pathogenetic mechanism is still poorly understood. Recently we studied changes in hemostatic profile in Thoroughbred race horses during their first training period, demonstrating dynamics oh hemostatic system in athletic horses. Since there are no studies about coagulative profiles in horses affected by EIPH, the aim of this study is to evaluate the hemostatic profile in Thoroughbred racehorses with a diagnosis of EIPH and to compare it with healthy animals.Blood samples were obtained from 27 Thoroughbred racehorses of different ages, sexes and race starts, all managed with same feeding and housing schedules and within a month of diagnosis with a distinct grade of EIPH using TE and BAL fluid analysis. Blood samples were obtained also from 29 clinically healthy 2-year-old horses negative for blood presence at TE examination at time of sampling. These equines were tested monthly during their first three months incremental training period (T0, T30 , T60 and T90). Blood samples were collected from the jugular vein into vacutainer tubes with sodium citrate. The parameters evaluated in both groups included coagulation profile (prothrombin time-PT, partial thromboplastin time-PTT and fibrinogen-Fbg levels), platelet function (Platelet Function Analyzer-PFA-100®) using collagen/ADP (C/ADP) and platelet aggregation (Light Transmission Aggregometry-LTA) in response to ADP (1, 5, and 10 µM) and collagen (4 µg/mL). Statistical significance was determined using one-way ANOVA.Compared with healthy ones, horses with EIPH- showed a significant higher PTT values (medium 56.7±1 sec) compared to T90 and lower Fbg concentrations (medium 130.4±4.6md/dL) compared with healthy horses at all time points and these values were also out of reference ranges for the equine specie . On the other side, PT showed no significant differences. Platelet aggregation decrease significantly (ADP 36.5±4.4%; collagen 14.4±3.8%) compared with that observed in healthy horses during training program. Concomitantly, a longer PFA-100®analyzer closure time (C/ADP cartridge 114±9.4 sec) was observed in EIPH-affected horses compared to T30.This paper demonstrates a modification in hemostatic profile in horses with EIPH suggesting a tendency toward hypocoagulability. Moreover, PFA100 and LTA confirmed platelet hypo-reactivity. These findings support the hypothesis of a predisposition to hemorrhages in the etiology of EIPH. As in humans, this condition could be due both to platelet hyporeactivity attributed to an autoimmune mechanism involving platelet inhibitors triggered by intense training or exercise, and to a potential deficit in the coagulation cascade. The aetiology of the condition may be genetic, given the breed-specificity, or it may represent an immune-mediated or idiopathic coagulopathy. While preliminary, and with some limitations (such as low heads number, different ages, sexes) this study suggests that systemic hemostatic impairment may predispose Thoroughbreds to EIPH.

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Oral Communication(s)

OCCURRENCE AND PHENOTYPIC ANTIMICROBIAL RESISTANCE PROFILES OF ZOONOTIC AND COMMENSAL BACTERIA ISOLATED FROM ORNAMENTAL AND COMPANION BACKYARD POULTRY AND WATERFOWL

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In recent years, several birds species traditionally considered production animals have increasingly been kept as companion or ornamental animals. Backyard birds are often raised in private gardens or courtyards, and sometimes even housed indoors, creating close and prolonged contact with humans. Although antimicrobial resistance (AMR) is widely recognized as a global health concern, mainly associated with human medicine and livestock production [1], the potential role of "copmanion poultry" in the epidemiology of resistant bacteria remains poorly investigated [2,3].

The present study aimed to evaluate antimicrobial resistance profiles of bacterial isolates collected from companion poultry living in close proximity to humans. Cloacal swabs were collected from 50 birds belonging to 3 species: domestic chicken (Gallus gallus domesticus) domestic goose (Anser anser domesticus), domestic mallard duck (Anas platyrhynchos domesticus) kept in backyards, private gardens or domestic settings between May and November 2025. Bacterial isolates were obtained using standard culture methods and identified by MALDI-TOF mass spectrometry. Antimicrobial susceptibility testing (AST) was performed by the disc diffusion method on all recovered Gram-positive and Gram-negative isolates using a panel of clinically relevant antimicrobials. In addition all Gram-negative isolates were evaluated for extended-spectrum β-lactamase (ESBL) and AmpC beta-lactamases (AmpC) resistance profiles.

A total of 93 bacterial isolates were recovered from 50 birds. Most belonged to the orders Enterobacterales (46/93, 49.4%) and Lactobacillales (34/93, 36.6%), whereas Bacillales (8/93, 8.6%) and Pseudomonadales (5/93, 5.4%) were less represented. E. coli was the most frequently isolated species (34/93, 36.5%), and Enterococcus spp. were also common (33/93, 35.5%), particularly E. faecium (16/93, 17.2%). Other taxa, including Enterobacter spp., Klebsiella spp., Acinetobacter spp., and Pseudomonas spp., were only sporadically recovered.

For the interpretation of AST, EUCAST v.15.0 (2025) and CLSI M100 (2025) were used as the primary guidelines. In absence of breakpoints, ECOFFs (EUCAST) and/or data reported in the literature were used. When not possible for lack of data no interpretation was performed and the results were left "raw". MDR was defined as resistance to at least one molecule belonging to three or more classes.

AST revealed resistant phenotypes in several isolates, including resistance to antimicrobials primarily or exclusively used in human medicine and considered critically important for human health (e.g. glycyclines, oxazolidinones, penicillins, 3rd and 4th generation cephalosporins, monobactams, fluoroquinolones). Multidrug resistance was observed in Lactobacillales (7/34, 20.6%), Enterobacterales (5/46, 10.9%), and Bacillales (1/8, 12.5%), whereas no relevant resistance patterns were detected among Pseudomonadales (Important: Lactobacillales where defined MDR when phenotypically resistant to molecules to which they normally show susceptibility). Two Enterobacterales isolates (2/46, 4.3%) displayed an ESBL phenotype and four (4/46, 8.7%) showed AmpC-associated profiles (not genetically confirmed), consistent with intrinsic AmpC production.

Our findings suggest that further surveillance would be recommended in companion poultry, considering their increasing popularity and their close contact with humans.

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Oral Communication(s)

INFLUENCE OF PARITY AND LACTATION STAGE ON PFAS EXCRETION IN DAIRY COW MILK UNDER ENVIRONMENTAL EXPOSURE: PRELIMINARY EVALUATION

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Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants of One Health concern due to their potential transfer into the food chain through animal products such as milk [1, 2]. However, limited information exists on PFAS excretion dynamics in dairy cattle across different physiological stages [3]. Therefore, this study investigated the influence of parity and lactation stage on PFAS excretion in dairy cattle.

Forty-nine lactating cows from an area with background environmental contamination were included. Background contamination was defined based on available environmental monitoring data indicating PFAS presence in water and industrial sources in surrounding farm environment, without known point-source exposure at the farm level. Animals were stratified based on parity (primiparous, Group P, n=22; multiparous, Group M, n=27), while lactation stage (days in milk, DIM) was randomly distributed without significant difference between groups. Each cow was sampled once and individual milk samples were analyzed for 20 PFAS (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFBS, PFHxS, PFHpS, PFOS, PFNS, PFDS, FHxSA, FOSA, Gen-X, NaDONA, 4:2 FTS, 6:2 FTS, 8:2 FTS) by validated LC-MS/MS method. Statistical analyses were performed to assess parity effect and association with DIM using the Mann-Whitney test and univariate linear regression for each compound and for the sum of PFAS. Detection frequencies were compared using the chi-square test.

PFOA, FHxSA and 6:2 FTS were detected in all samples, while PFHpA, PFNA, PFDA, 4:2 FTS, 8:2 FTS, PFNS and PFDS were never detected. PFHxS and PFOS were frequently found in both groups (91%-100%), whereas the other PFASs showed heterogeneous occurrence.

Comparing parity groups, Group P exhibited higher milk levels of PFBA, NaDONA, and FHxSA, while Group M had higher PFBS concentrations ($p < 0.05$). Mean total PFAS concentrations were slightly higher in Group P (127.31 ± 32.75 pg/mL) than in Group M (122.95 ± 29.04 pg/mL).

When examining associations with DIM, FOSA concentrations in Group P increased with DIM (p=0.023). In Group M, PFHxA decreased (p=0.042), while PFOS and total PFAS levels increased over time (p=0.049 and p=0.030, respectively).

These preliminary findings indicate that PFAS excretion into milk varies with compound and parity, even under background exposure. Parity and lactation stage should be considered when assessing PFAS contamination in dairy production. Further studies are needed to clarify kinetics and transfer mechanisms into milk.

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Oral Communication(s)

IN VIVO EVALUATION OF THE ONCOLYTIC POTENTIAL OF WILD-TYPE CAPRINE HERPESVIRUS TYPE 1 COMBINED WITH CISPLATIN IN MESOTHELIOMA

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Malignant pleural mesothelioma is an aggressive tumor strongly associated with asbestos exposure and characterized by high chemoresistance, marked tumor heterogeneity, and poor clinical outcomes [1]. Current treatment options, including surgery, chemotherapy, radiotherapy, and immune checkpoint inhibitors, offer limited long-term efficacy, highlighting the urgent need for innovative strategies [1]. Oncolytic virotherapy has emerged as a promising approach capable of inducing selective tumor cell lysis while simultaneously stimulating antitumor immune responses. Caprine herpesvirus type 1 (CpHV 1), a non-human herpesvirus nonpathogenic to humans, has recently demonstrated the ability to selectively infect and kill mesothelioma cells in vitro [2].

In this study, we evaluated the in vivo antitumor efficacy of CpHV 1, alone and in combination with cisplatin, using athymic CD1 mice bearing MSTO 211H mesothelioma xenografts. The study was approved by the appropriate institutional authority (project permit number: 155/2024-PR_August 2, 2024). Animals (n = 10) were assigned to four groups (control, CpHV 1, *cisplatin, and combination therapy—COMBO), and tumor progression was monitored for six weeks.

We assessed tumor growth inhibition, histopathological changes, transcriptomic alterations, and protein-level modifications. Our results show that CpHV 1, particularly when combined with cisplatin, significantly reduced tumor growth ($p < 0.001$), and attenuated 665($p < 0.05$). Alongside phenotypic evaluations, RNA-seq analysis was performed separately on human- and mouse-aligned reads. Consistently, the highest number of differentially expressed genes compared to the control was found in the COMBO condition in both human and mouse datasets.

Functional analysis reveals regulation of immune response and metabolic reprogramming pathways in the xenograft across all treatment conditions, with a greater enrichment of apoptosis-related terms in the COMBO group. In the host, changes in inflammatory response-related terms were also observed.

Moreover, in line with transcriptomic findings, immunoblot analysis of apoptotic markers revealed a significant increase in cleaved Poly ADP-ribose Polymerase in both the CpHV 1 and COMBO groups ($p < 0.01$), indicating enhanced DNA-damage–induced apoptosis. Similarly, cleaved Caspase 3 expression was markedly upregulated ($p < 0.01$), with the highest activation observed in the COMBO group, supporting a synergistic pro-apoptotic effect.

Overall, our findings provide new insights into the therapeutic potential of CpHV 1 and its interaction with chemotherapy, supporting further exploration of CpHV 1 based oncolytic virotherapy as an alternative or complementary strategy for the treatment of mesothelioma.

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Oral Communication(s)

TRANSLATIONAL EQUINE REGENERATIVE MEDICINE: ALPHA-2-MACROGLOBULIN–BASED THERAPY MODULATES INFLAMMATION AND MATRIX DEGRADATION FROM BENCH TO FIELD

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Chronic joint disease is a leading cause of lameness and impaired performance in sport horses and is driven by persistent synovial inflammation, protease activity, and progressive extracellular matrix degradation. Alpha-2-macroglobulin (A2MG), a broad-spectrum inhibitor of proteases and inflammatory mediators, has recently emerged as a promising orthobiologic strategy in equine regenerative medicine. This study integrates experimental and clinical approaches to evaluate the anti-inflammatory, matrix-protective, and clinical effects of A2MG-based therapy in equine joint disease. In vitro, primary equine chondrocytes and synoviocytes were stimulated with interleukin-1β (IL-1β) or lipopolysaccharide (LPS) and treated with A2MG-enriched plasma (A2MG-EP; 10–20% v/v) or matched platelet-poor plasma (PPP). Cytokine production and gene expression were assessed by ELISA and quantitative PCR. In parallel, an equine tendon explant model was used to evaluate extracellular matrix degradation following collagenase challenge. A2MG-EP significantly reduced the production of pro-inflammatory mediators and downregulated the expression of catabolic enzymes compared with PPP in cellular models. In tendon explants, A2MG-EP attenuated collagen degradation and preserved extracellular matrix integrity. In vivo, a prospective pragmatically controlled pilot study was conducted in 20 sport horses affected by chronic joint disease, allocated to a treatment group (n = 10) or control group (n = 10). Clinical outcomes were assessed longitudinally, while synovial fluid was analysed at baseline and 30 days post-treatment. Mixed-effects analysis revealed significant group × time interactions for AAEP lameness score, flexion test response, and joint effusion. All treated horses showed at least a one-grade improvement in lameness at day 7, whereas no improvement occurred in controls. At 30 days, treated horses exhibited coordinated reductions in synovial total protein, total nucleated cell count, polymorphonuclear cell percentage, pro-inflammatory cytokines, matrix metalloproteinases, sulphated glycosaminoglycans, and neurogenic mediators. These findings demonstrate consistent modulation of inflammatory, proteolytic, and nociceptive pathways across experimental and clinical settings, supporting the translational relevance of A2MG-based strategies in equine regenerative medicine. Although the pilot design and limited sample size warrant cautious interpretation, the integration of mechanistic and clinical data provides a strong rationale for further controlled studies.

Oral Communication(s)

INFLUENCE OF POSTBIOTICS FROM PEDIOCOCCUS ACIDILACTICI ON INTESTINAL TISSUE BIOMARKERS

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The integrity of the intestinal mucosa barrier plays a pivotal role in preserving intestinal balance and preventing inflammation, metabolic conditions, and infections. An impairment of this barrier results in increased intestinal permeability and disrupted immune regulation, contributing to the onset of diseases such as inflammatory bowel disease, Crohn's disease, and ulcerative colitis. Due to their stability, safety, and functional properties, postbiotics have recently gained popularity as potential therapeutic agents. Hence, the present study aimed to investigate the impact of a Pediococcus acidilactici-derived postbiotic on intestinal structure and on biomarkers associated with epithelial integrity and fluid transport.

After characterizing the postbiotic, seventy-eight male Wistar albino rats were randomly divided into thirteen groups (n= 6 per group): a control group (group 1), which received MRS broth via gavage for 28 days, and twelve treatment groups (groups 2-13). Each treatment group received the postbiotic via gavage at doses of 250, 500, or 1000 mg/kg for 7, 14, 21, or 28 days. Notably, groups 2-5 received 250 mg/kg, groups 6-9 received 500 mg/kg, and groups 10-13 received 1000 mg/kg. Each group within a dose level corresponded to one of the four aforementioned treatment durations. At the end of each treatment period, the animals were humanely euthanized, and tissue samples obtained from different intestinal segments (duodenum, jejunum, ileum, cecum, colon, and rectum) were used to perform histomorphological and immunoisthochemical examinations.

The postbiotic characterization revealed a complex composition, including organic acids, phenolic compounds, and volatile metabolites, along with strong antioxidant activity, suggesting a potential protective effect on intestinal tissues. Additionally, histopathological analyses showed no structural abnormalities in any of the treated groups compared to the control, confirming the probiotic's safety. In contrast, immunohistochemical examination demonstrated significant modulation of proteins involved in maintaining the intestinal barrier. Notably, claudin-3 (CL3), occludin (OCLN), zonula occludens-1 (ZO1), aquaporin-4 (AQP4), and aquaporin-8 (AQP8) levels were elevated in the treated groups compared with the control. These proteins regulate paracellular permeability and transcellular movement of water. Specifically, tight junction proteins (CL3, OCLN, ZO1) ensure the maintenance of epithelial adhesion and selective permeability, and aquaporins enhance water transport across intestinal cells. Their upregulation can be considered as an index of improved structural and functional integrity of the intestinal barrier. This upregulation was dose-dependent, with the concentration of 1000 mg/mL showing the highest biomarker expression. Conversely, treatment duration did not significantly impact the outcomes, suggesting a more decisive role of dosage rather than treatment duration.

Statistical analysis confirmed the reliability of these findings, showing strong agreement between observers and consistent patterns across all intestinal segments. Overall, the obtained results suggest that postbiotics derived from P. acidilactici enhance intestinal barrier-related biomarkers, without damaging intestinal tissue. However, research is needed to further clarify their mechanism and assess their clinical relevance.

Oral Communication(s)

RESISTANCE TO CRITICALLY IMPORTANT ANTIMICROBIALS FROM LIVESTOCK TO THE ENVIRONMENT: A FIELD STUDY

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The use of antimicrobials on industrial and/or intensive animal farms is often associated with the spread of antimicrobial-resistant bacterial strains, not only among the farmed animals but also into the environment, thereby increasing the risk of infecting other animals and humans.

Therefore, this study was designed and carried out to explore the actual relationship between animal farms and the selection of antimicrobial resistance, especially toward the critically important antibiotics (CIA) such as cefepime (CEP) and enrofloxacin (ENR). In particular, the research focused on the possible association between the use of antimicrobials on industrial animal farms and the selection or co-selection of CIA resistance.

Specimens were collected from two farms each of broilers, bovines, ovines, and swine. Samples consisted of feces, drinking water, soil where manure was typically applied, and plants growing on that soil. In addition to samples, information about the recent use of antibiotics in the farms was collected from the owner and the farm veterinarians. The gathered information revealed that pigs were treated with ampicillin (AMP) during the weaning period, while no antibiotic treatment was administered to the other animals. Strains were isolated on Tryptic Soy Agar supplemented with CEP or ENR, and the identification was carried out by MALDI-TOF. The susceptibility to the other common antibiotic classes was determined by the disk diffusion test according to the CLSI guidelines and interpreted using the CLSI and EUCAST breakpoints.

A total of 212 bacterial isolates were collected. Among these, 156 were resistant to CEP and 56 to ENR. The CEP-resistant strains were uniformly distributed across bovines (43), broilers (34), ovines (33), and swine (46), whereas ENR-resistant strains were mainly found in swine (45) and broilers (8), while they were almost absent from bovines (2) and ovines (1). Similarly, the distribution of CEP-resistant strains was uniform across feces, soil, and plants, whereas ENR-resistant strains were almost exclusively recovered from feces. Considering bacterial genera, the large majority of CEP-resistant strains belonged to the genera *Bacillus*, *Paenibacillus*, and *Kurthia*. Conversely, ENR-resistant strains were mostly Gram-negative, belonging to the genera *Proteus* and *Escherichia*.

Such a different distribution is noteworthy, since most of the CEP-resistant isolates belonged to species intrinsically resistant to third- and fourth-generation cephalosporins, such as CEP, while very few species are known to be intrinsically resistant to ENR, as fluoroquinolone resistance is usually acquired through accumulation of point mutations or horizontal transfer. The susceptibility profiles showed that ENR-resistant strains were also resistant to other antimicrobials, especially AMP, amoxicillin-clavulanate, chloramphenicol, tetracycline, and co-trimoxazole, whereas multidrug resistance was less common in CEP-resistant strains. This suggests that even treatments with first-line antibiotics like AMP could have contributed to the selection of multidrug resistance because the corresponding genetic determinants may be co-located on the same mobile genetic elements as other resistance genes. This might promote the co-selection of CIA-resistant strains, especially Gram-negative ones resistant to fluoroquinolones. which can spread in the environment, as observed with CEP-resistant strains. Further genomic studies, along with molecular epidemiology investigations, may confirm such a hypothesis.

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Oral Communication(s)

A RAPID AND COST-EFFECTIVE SPECTROPHOTOMETRIC METHOD TO ASSESS ETHYLENE GLYCOL INTOXICATION IN ANIMAL TISSUES

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Ethylene glycol (EG) is a colorless, odorless, and sweet-tasting compound widely used in antifreeze and other industrial products, posing significant risks to animals upon intentional or accidental ingestion. EG undergoes rapid absorption and distribution throughout the body, where it is metabolized into more toxic compounds, such as glycolic acid (GA) and oxalic acid, leading to metabolic acidosis, acute kidney injury, and eventually fatal outcomes [1].

In post-mortem analysis, EG detection can be challenging due to its rapid clearance, often requiring more sophisticated techniques, such as mass spectrometry. Moreover, most analytical methods focus on serum or urine, while excluding tissue samples. Histopathological analysis of kidney lesions might support the diagnosis of EG intoxication, but it sometimes lacks specificity. Conversely, GA is considered a much more reliable biomarker of EG poisoning, due to its accumulation in the organism. To date, existing methods for GA determination in tissues are limited and typically involve time-consuming GC-MS or LC-MS approaches [2].

Within this context, the aim of the study was to develop a spectrophotometric method for the determination of GA in paired kidney and liver samples from dogs (n=4) and cats (n=18) suspected of EG intoxication. Additionally, negative control animals (n=17 for both dogs and cats with a total of 33 paired samples and 1 unpaired liver sample) were included in the study based on the absence of renal lesions and fatalities due to road traffic accidents. To the best of our knowledge, this is the first validated spectrophotometric method to quantify GA in post-mortem samples. Tissues were homogenized in an aqueous trichloroacetic acid solution, and the supernatant was subjected to reaction with chromotropic acid under heating conditions. The absorbance of the resulting purple-colored complex was measured at 580 nm. Method validation was performed using matrix-matched samples spiked with GA at known concentrations. The analytical protocol showed satisfactory linearity (R^2=0.99 for both tissues), recovery in the range of 91–117%, and good intra- and inter-day precision (RSD < 7%). Limits of detection were 26 and 28 µg/g for kidney and liver, respectively. Potential interferences due to the presence of EG and its metabolites, glyoxylic and oxalic acid, were also evaluated. The validated method was then employed for blind analysis of real samples. GA concentrations were in the range of 95–1105 µg/g in liver tissue and 89–891 µg/g in kidney tissue, showing a strong correlation (ρ = 0.84) in positive paired samples (n=16). Six suspected cases were below the limit of detection and were excluded from the correlation study. Overall, kidney tissue analysis demonstrated higher diagnostic value for EG intoxication compared to liver samples, which showed false positives (n=11) in control samples. In conclusion, this method provides a practical alternative tool for the screening of EG intoxication when advanced instrumentation is not available.

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Oral Communication(s)

STUDY OF DOPING SUBSTANCES IN COMPETITION ANIMALS: BIOTECHNOLOGICAL AND REGULATORY APPROACHES

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The illicit use of performance-modulating substances in sport animals, particularly racehorses, continues to pose significant challenges in veterinary public health, raising ethical, regulatory, and animal welfare concerns within the framework of the Fédération Equestre Internationale (FEI). Palmitoylethanolamide (PEA) is an endogenous N-acylethanolamine belonging to the ALIAmide family, a class of bioactive lipids that act through the Autacoid Local Injury Antagonism (ALIA) mechanism, down-modulating non-neuronal cell hyperactivity - particularly that of mast cells and microglia - via the endocannabinoid system. Consequently, PEA plays a well-documented key role in the regulation of inflammation and nociceptive pathways. This property has recently attracted regulatory scrutiny due to its potential to influence athletic performance when administered exogenously. These considerations highlight the need for robust physiological reference intervals to support evidence-based anti-doping strategies. The present study aimed to develop and validate a high-sensitivity analytical method for the simultaneous quantification of PEA and its structural analogue oleoylethanolamide (OEA) in equine plasma, enabling discrimination between endogenous baseline levels and concentrations resulting from exogenous administration. A high-performance liquid chromatography-tandem mass spectrometry (HPLC–MS/MS) method was developed, integrating a novel, non-conventional online solid-phase extraction (SPE) system to enhance matrix clean-up efficiency and analytical throughput. Full method validation was performed in accordance with international regulatory guidelines, including the assessment of limits of detection (LOD) and quantification (LOQ), linearity, intra- and inter-day precision, and accuracy. Quantitative analysis of 20 equine plasma samples revealed mean endogenous concentrations of 238.29 ± 70.10 nmol/L for PEA and 6.74 ± 3.77 nmol/L for OEA, highlighting substantial inter-individual variability. These findings represent the first systematically derived reference dataset for PEA and OEA in the equine species and provide a scientifically robust basis to support the future definition of analytical thresholds. The validated HPLC–MS/MS method represents a forensically sound and legally defensible tool for veterinary anti-doping programmes. By defining endogenous baseline levels for PEA and OEA levels in equine athletes, this study addresses a critical regulatory gap and offers a solid scientific framework for the implementation of fair, evidence-based control limits, ultimately contributing to improved equine welfare and competitive integrity in equestrian sport.

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Oral Communication(s)

REAL-WORLD SAFETY PROFILE OF OCLACITINIB AND LOKIVETMAB: APHARMACOVIGILANCE STUDY BASED ON EUDRAVIGILANCE DATA

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Introduction: Dermatological diseases are among the most common conditions in veterinary clinical practice and often require the use of innovative therapies, including monoclonal antibodies (mAbs) and Janus kinase (JAK) inhibitors. However, real-world safety data remain limited, highlighting the importance of pharmacovigilance for monitoring adverse drug reactions (ADRs). This study aimed to evaluate the safety profile of oclacitinib (JAK inhibitor) and lokivetmab (mAb) through the analysis of spontaneous ADR reports.

Materials and Methods: A retrospective pharmacovigilance study was conducted using exclusively data from the veterinary EudraVigilance database (accessed 15 July 2025) to retrieve individual case safety reports (ICSRs) related to oclacitinib and lokivetmab. No duplicate or follow-up reports were identified in the retrieved dataset based on the available information.All reports starting from the date of drug authorization were included. Collected data included animal characteristics (sex, species, and breed), outcomes, reporter type, and geographical origin. Breeds were classified as purebred single, crossbred, multi-breed specified, or unknown/other. ADRs were classified according to the VeDDRA dictionary. Descriptive analyses were performed, and disproportionality was assessed using reporting odds ratios (RORs) with 95% confidence intervals (CI).

Results: A total of 39,997 ICSRs were identified (22,895 oclacitinib; 17,102 lokivetmab), mainly involving dogs (97.3%), with a balanced sex distribution. Most reports were submitted by veterinarians (72.2%) and originated from non-EEA countries (87.0%). Outcomes were predominantly non-fatal (93.9%). Purebred animals accounted for 69.9% of cases, with Labrador Retriever and French Bulldog being the most frequently reported breeds. Over 220,000 ADRs were identified, most commonly digestive tract disorders (18.38%), systemic disorders (18.19%), and neurological disorders (12.02%). Disproportionality analysis showed higher reporting for oclacitinib compared to lokivetmab for metabolic disorders (ROR 5.32; 95% CI 3.09–9.14), neoplasms (ROR 4.76; 95% CI 4.43–5.10), endocrine disorders (ROR 3.03; 95% CI 2.64–3.47), and blood disorders (ROR 2.72; 95% CI 2.50–2.97). Conversely, lower reporting for oclacitinib was recorded for application site (ROR 0.20; 95% CI 0.16–0.25), cardiovascular system disorders (ROR 0.86; 95% CI 0.75–0.98), immune system disorders (ROR 0.22; 95% CI 0.20-0.24).

Conclusions: ADRs associated with oclacitinib and lokivetmab represent a relevant component of their safety profile in veterinary clinical practice. The observed differences between the two drugs highlight the importance of pharmacovigilance and post-marketing surveillance to ensure safe and appropriate use. Moreover, the predominance of reports from non-EEA countries may influence the interpretation of the findings, due to potential differences in reporting practices and drug use patterns.

Oral Communication(s)

PORTUNUS SEGNIS, BLUE CRAB OF MEDITERRANEAN SEA: RESIDUAL METAL AND METALLOIDS LEVELS IN SEAFOOD AND CONSUMERS' SAFETY

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Another alien species of blue crab found in Sicilian waters of Mediterranean Sea is represented by *Portunus segnis*, coming from Red Sea and western Indian Ocean, known as "African blue swimming crab". To protect the Mediterranean marine ecosystem and the biodiveristy of smaller species hunted by this invasive blue crab, *Portunus segnis* could be employed as seafood indeed to human consumption [1]. It is traded in local fisheries and markets of several Mediterranean countries and it appreciated by consumers for its flavourful meat.

The aim of this study was to assess the content of Potentially Toxic Elements (PTE), Hg, Pb, Cd and As in carapace pulp (edible part) of Portunus segnis samples, collected in local fisheries in Sicily, and to analyze the metal and metalloids residual levels according to EU Regulations and specific risk assessment parameters, to guarantee the consumer's safety.

The samples of *Portunus segnis* (n= 15) were omogenized, submitted to acid digestion in a microwave oven system and analyzed in Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) for Cd, Pb and As determination. The quantification of Hg, instead, was carried out using a Direct Mercury Analyzer absorption spectrometer (DMA-80) [2]. All data obtained, expressed as mean values ± S.D., were considered significant with $P<0.05$.

The results obtained showed the presence of all PTE analyzed, with highest concentrations of As, intermediate for Hg and lowest for Pb and Cd. The residual levels of As (range: 0.532-1.022 mg/kg) were significantly higher than the MRL (0.10 mg/kg), established by EU Regulation 2025 [3], differently from other metals [4]. From the toxicological risk assessment, the concentrations of PTE, except As, corresponded to a small percentage of Provisional Tolerable Weekly Intake (PTWI), Estimated Daily Intake (EDI) and Target Hazard Quotient (THQ).

A correlation carried out between metals levels and the size of blue crabs, taking into consideration the Condition factor (K), showed a partially weight-dependent pattern. The metals bioaccumulation could be due to marine environmental pollution where these species lived, as showed by calculation of Average Pollution Load Index (APLI).
The residual levels of As, higher than MRLs, represent a toxicological risk for consumer's safety; however, considering the limited consumption of crabs pulp, "luxury food", the concentrations of other metals correspond to small percentage of PTWI and low values of EDI and THQ (<1) not dangerous for health [5].

Our results are in accordance with those of other authors carried out on African blue swimming crabs from other Mediterranean areas; however, further monitoring studies on metals and metalloids content are needed on a greater number of samples, considering that *Portunus segnis* is becoming a new seafood also in our local fish market.

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Oral Communication(s)

IMPACT OF ENVIRONMENTAL CONDITIONS ON GOATS' IMMUNE RESPONSE TO PFOS AND GLYPHOSATE: A COMPARISON BETWEEN GRAZING AND STALL-FED SYSTEMS

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This study aimed to explore how endocrine disruptors (EDs) interact with different environmental and housing conditions in shaping the immune response of goats raised either on pasture or in stalls. EDs such as PFOS and glyphosate (GLY) are known to enter the food chain and may impair immune function. Goats that graze are typically exposed to a wider range of nutrients and microorganisms compared to stall-fed animals, which can contribute to a more robust immune system.To conduct the experiment, ex vivo approach was applied, where blood samples collected from both groups were treated with EDs to evaluate their effects on immune cell activity and cytokine production.The study was conducted in September during routine official veterinary inspections on two ovine-caprine farms located in Sicily, Italy, which differed in their management systems(intensive farming model vs extensive grazing system).A total of twenty clinically healthy, pluriparous Girgentana goats were selected: ten from each farm and incubated for 24h. All animals were non-pregnant and non-lactating, aged between 3 and 5 years. The whole blood samples were exposed to PFOS (10 µM ; 100 µM) and GLY (10 µM ;100 µM) and their combination.Whole blood samples were firstly subjected to a complete blood count (CBC) and cellular analysis was then performed using a FACS Attune Nxt flow cytometer. To measure oxidative stress biomarkers Myeloperoxidase (MPO) enzymatic activity, was determined spectrophotometrically in plasma and MDA production was measured

using the thiobarbituric acid assay (TBARS).For the investigation of cytokines, TNF-α and IL-6 levels medium samples were prepared following the manufacturer's protocol for ELISA kits. The findings revealed that stall-fed goats showed greater vulnerability to the combined exposure to PFOS and GLY as evidenced by increased oxidative stress levels. In contrast, both grazing and stall-fed animals exhibited comparable responses in terms of inflammatory cytokine production.The granulocyte-to-lymphocyte ratio (G/L), a widely recognized biomarker of chronic stress, was found to be significantly elevated install-fed goats compared with the other group.These results highlight the significant role of housing and feeding systems in influencing how livestock respond to environmental contaminants. This is particularly relevant in a One Health perspective, as EDs present in animal-derived products may pose risks to humans. Further research is needed to better understand the long-term consequences of ED exposure in livestock and to develop strategies aimed at reducing these risks.

Oral Communication(s)

EVALUATION OF THE PHARMACOLOGICAL EFFECTS OF SNAIL SLIME IN THE TREATMENT OF MASTITIS

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Bovine mastitis is the most widespread and costly inflammatory disease in the dairy industry globally, with serious repercussions on animal health, milk quality, and farm profitability. Conventional antibiotic-based treatment is currently closely monitored due to the emergence of antimicrobial resistance and the problem of residues in milk, necessitating the search for sustainable therapeutic alternatives in line with the One Health approach. This thesis aims to evaluate the pharmacological potential of snail secretion filtrate (SSF), derived from Helix aspersa Muller, as an innovative strategy for disease control.

The research was conducted through a multidisciplinary approach, initially involving a systematic literature review, conducted using the PRISMA method, to map the antimicrobial activity of gastropod derivatives against major mammary pathogens. Subsequently, in vitro and ex vivo studies were conducted on bovine mammary cell lines and blood leukocytes to analyze cell viability, oxidative stress, and the expression of inflammatory and antioxidant mediators. Finally, the efficacy of the treatment was verified through a pilot in vivo study on cows with subclinical mastitis, monitoring clinical parameters such as somatic cell count and levels of pro-inflammatory cytokines in the supernatant of MAC-T cells.

The results obtained confirmed the strong antimicrobial, anti-inflammatory, and antioxidant properties of snail slime. Specifically, in vitro data showed a significant reduction in LPS-induced oxidative stress and a modulation of the inflammatory response through the attenuation of key mediators. These findings were supported by in vivo results, which showed improved mammary gland health and a reduction in inflammatory markers following SSF administration. In conclusion, the study demonstrates that snail slime may represent an effective natural and multifunctional agent, capable of reducing dependence on antibiotics and promoting food safety and animal welfare in the livestock sector.

Oral Communication(s)

IMMUNOTOXIC EFFECTS OF IMIDACLOPRID: DISRUPTION OF CYTOKINES BALANCE AND INFLAMMATORY RESPONSE

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Immunotoxicity induced by environmental contaminants is an increasing public health concern, as it may disrupt immune homeostasis and impair the organism's ability to respond to inflammatory stimuli. Among these contaminants, imidacloprid (IMI), a widely used neonicotinoid insecticide, has recently attracted attention due to its potential immunomodulatory effects.

In the present study, we investigated the impact of IMI exposure (10 mg/mL) on immune response in a murine model (Authorization No. 568/2025-PR).

The study analyzed both cytokine production and the activation of key molecular pathways across different immune compartments, including thymus, lymph nodes, and spleen. Levels of pro- and anti-inflammatory cytokines (IL-6, TNF-α, IL-1β, IL-4, IL-10, and IL-33) were quantified using ELISA assays, distinguishing between basal conditions (after 15 days of IMI exposure) and stimulated conditions induced by lipopolysaccharide (LPS), in order to assess the responsiveness of the immune system following prior exposure to the contaminant. In parallel, activation of the NF-κB signaling pathway was evaluated by RT-PCR.

Our results demonstrate that IMI exposure induces a significant modulation of the cytokine profile compared to controls (CTR), affecting both basal and LPS-induced responses. In particular, an imbalance between pro- and anti-inflammatory mediators was observed, suggesting a disruption of immune regulatory mechanisms. Furthermore, analysis across different immune tissues revealed compartment-specific effects, indicating a tissue-dependent susceptibility to IMI exposure.

NF-κB expression was also modulated, supporting a direct involvement of this pathway in the immunotoxic response elicited by the contaminant. Overall, these findings indicate that IMI exposure can alter both the basal immune status and the ability of the immune system to respond to secondary inflammatory stimuli.

These results contribute to a better understanding of the mechanisms by which neonicotinoids may exert immunotoxic effects, highlighting potential implications for human health and emphasizing the need for further studies to assess the risks associated with chronic exposure to these compounds.

Oral Communication(s)

INTEGRATING FUNCTIONAL ASSAYS AND PROTEOMIC PROFILING TO INVESTIGATE INTER-INDIVIDUAL VARIABILITY OF CRUDE VENOM FROM CAPTIVE *VIPERA AMMODYTES*

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Vipera ammodytes is listed by the World Health Organization as a medically important snake species and is considered the most dangerous venomous snake in Europe. Its venom is a complex secretion whose composition may vary within the species. Several studies have examined aspects and applications of *V. ammodytes* venom, including its chemosensitising activity on melanoma cells [1] and its proteomic variability in wild insular and mainland populations [2]. However, the relationship between inter-individual variation in venom composition and its functional effects on human cells remains insufficiently explored.

The aim of this study was to investigate the inter-individual functional variability of crude venom from captive *V. ammodytes* specimens maintained under identical housing conditions and to correlate cytotoxic effects with the proteomic profiles of individual venoms.

Venom was collected by milking from specimens housed at a zoological park*. Eight individual venom samples were analysed. Total protein concentration was determined using the bicinchoninic acid assay. Proteomic analysis was performed by LC-MS/MS on trypsin-digested samples, followed by database searching and label-free quantification to compare protein abundance across individuals. Biological activity was evaluated through in vitro assays on human A375 melanoma cells exposed to increasing venom concentrations (0.0003–0.03% v/v) for 24 hours. Cell viability was assessed by crystal violet staining, and half maximal effective concentration (EC_{50}) values were calculated by nonlinear regression using a sigmoid concentration–response model.

Preliminary results revealed measurable differences in cytotoxic activity among venoms, with EC_{50} values ranging from 0.001292 to 0.003726% (v/v). Total protein concentration was comparable across samples (155.75–185.55 µg/µL), suggesting that the observed differences are unlikely to reflect variations in overall protein amount. Proteomic analysis identified the major toxin families typically found in viper venoms, including metalloproteinases (SVMPs), phospholipases A2 (PLA_2s), serine proteases, disintegrins, cysteine-rich secretory proteins, L-amino acid oxidases, and C-type lectins. Among these, PLA_2s and SVMPs represented the most abundant components, although their relative proportions varied among individuals, with some venoms showing dominance of one family or a more balanced contribution of both. Interestingly, five samples shared the same geographic origin (Montenegro), whereas three derived from specimens of Croatian, Italian and Greek origin, yet the variability observed in cytotoxic activity and proteomic profiles did not appear to follow a clear geographic pattern. Consistently, principal component analysis did not show clustering of samples according to geographic origin.

Overall, these findings suggest that even inter-individual differences in venom composition may translate into detectable functional variability, highlighting the importance of integrating proteomic and functional approaches in venom research.

* *The study was approved by the Animal Welfare Body (OPBA) of the Veterinary Service Center, University of Naples Federico II (protocol n. PG/2024/0138574).*

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Oral Communication(s)

CYTOTOXICITY AND METABOLISM OF OCHRATOXIN A IN CHICKEN LMH CELLS: IDENTIFICATION OF NOVEL METABOLITES

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Ochratoxin-A (OTA) is a secondary metabolite produced by fungi belonging to the genera Aspergillus and Penicillium, frequently detected as a contaminant in food and feed [1]. Exposure to OTA is associated with nephrotoxicity and hepatotoxicity, mainly mediated by oxidative stress (OS) [2]. Information on OTA biotransformation in poultry and the toxicological relevance of its metabolites remains limited [3]. This study aimed to evaluate chicken Leghorn Male Hepatomas (LMH) cells as an in vitro model to investigate hepatic OTA-induced OS and biotransformation, including CYP-mediated metabolism modulation by phenobarbital (PB).

LMH cells were cultured in EMEM medium. For viability and OS assays, cells were exposed to increasing concentrations of OTA (0.625–20 µM) for 24 h under reduced serum conditions (1% FBS). Cell viability, ROS production and GSH levels were assessed using WST-1, ROS-Glo H_2O_2 and GSH/GSSG-Glo assays, respectively. For metabolic and gene expression studies, cells were treated with OTA (3.5 and 7 µM) for 8 and 24 h, with or without 16 h phenobarbital (PB, 1 mM) pretreatment. OTA and its metabolites were quantified in culture media by LC-MS/MS [4], while expression analysis of oxidative stress, biotransformation and transporter-related genes was performed on extracted RNA. Data were obtained from three independent experiments and analyzed using appropriate statistical tests.

OTA decreased cell viability in a concentration-dependent manner, with an IC_{50} of 7.5 µM. Based on these results, OTA concentrations of 3.5 and 7 µM were selected for the remaining investigations. OTA treatment significantly reduced GSH/GSSG ratio ($p < 0.05$) and markedly increased ROS production ($p < 0.01$). After 8h of OTA exposure SOD1 was upregulated, while Nrf2 was downregulated. Conversely, while OTA alone did not affect CYP2A6, CYP3A5, CYP2C45 and CYP2H1 at 8h and 24h; a marked upregulation of these genes was observed in PB-pretreated cells. Different OTA metabolites were detectable in rather low amounts in culturing media: OH-OTA, Tyr-OTA, OP-OTA, OTα, OTHQ. In particular, PB pretreatment increased OH-OTA levels in comparison with OTA-alone groups.

Overall, the results indicate that OTA exposure alters key antioxidant parameters, in line with the expected induction of oxidative stress. The detection of different OTA metabolites, although at low levels, confirms the metabolic competence of LMH cells and expands the current knowledge on OTA biotransformation in chickens, where so far mainly hydroxylated forms (OH-OTA) have been described [3]. Notably, PB pretreatment enhanced OH-OTA formation and markedly upregulated CYP-related genes, suggesting a modulation of phase I metabolism that will be further investigated at the protein level by Western blot analysis.

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Oral Communication(s)

THE POTENTIAL OF PLATELET-RICH PLASMA AND ITS FORMULATIONS IN THE TREATMENT OF BOVINE MASTITIS: A FOCUS ON ANTIBACTERIAL ACTIVITY

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Bovine mastitis is a major cause of economic losses in dairy farms and represents a significant health issue, further complicated by increasing antimicrobial resistance [1]. In this context, platelet-rich plasma (PRP), known for its regenerative, immunomodulatory, and antimicrobial properties, has been proposed as a potential therapeutic alternative [2]. The aim of this in vitro study was to evaluate the antibacterial activity of different PRP formulations obtained from bovine and equine blood against *Streptococcus uberis* and *Staphylococcus aureus*, to assess their potential application in mastitis management. Milk samples were aseptically collected from 13 cows selected based on somatic cell count, and pathogens were isolated and identified. Different PRP formulations (fresh, lysate, and leukocyte-enriched) were prepared from healthy donors. Antibacterial activity was evaluated using broth microdilution and confirmed on solid media at different concentrations (80%, 40%, 20%, 10%, 5%). Efficacy was assessed as minimum inhibitory concentration (MIC), with spectrophotometric readings. Five samples tested positive, and *S. uberis* and *S. aureus* were selected for further analysis. PRP showed antibacterial activity against both pathogens, with a dose-dependent effect. The strongest inhibition was observed against *S. uberis*, particularly at higher concentrations, while *S. aureus* showed a more moderate but consistent response across formulations. Overall, bovine-derived PRP was more effective than equine PRP. Among formulations, fresh PRP showed the highest efficacy, followed by lysate, whereas leukocyte-enriched PRP showed variable results.These findings suggest that PRP, particularly of bovine origin and at higher concentrations, may represent a promising alternative approach for the management of mastitis, especially in the context of antimicrobial resistance. Further in vivo studies are needed to confirm its clinical applicability and define optimal treatment protocols.

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Oral Communication(s)

MOLECULAR DETECTION AND CHARACTERIZATION OF SELECTED VIRUSES IN CAPTIVE PET BIRDS IN SICILY

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Birds are popular household pets and are frequently hosted at bird shows and zoological gardens. Their susceptibility to different viral infections poses a threat to animal health, with possible consequences for the economy and biodiversity. Psittacine beak and feather disease virus (PBFDV) from the Circoviridae family, and Gammapolyomavirus avis (APyV) from the Polyomaviridae family are two highly contagious viruses which can be associated with lethal outcome in young birds or chronic disease characterized by immunosuppression, feather loss, and concurrent infections in older/adult individuals [1]. Adenoviruses (AdV) are other common viruses in birds (as well as in psittacines) [2]. This viral group includes well-recognized pathogens together with a number of species with unclear clinical significance in birds. Despite the relevance of the topic, information on the spread and prevalence of these diseases across Italy remains very limited. Aiming, to partially fill this gap of information, this study investigated the molecular prevalence of PBFDV, APyV, and AdV in Sicily, analyzing individual risk factors associated with higher prevalence and sequencing some of the detected isolates to identify the most common strain or variant. A total of 166 oral-cloacal swabs were collected in captive pet birds from four Sicilian provinces (Messina, Catania, Palermo, Trapani) and belonging to various orders (Psittaciformes, Galliformes, Passeriformes and Musophagiformes). Both clinically healthy animals and those with symptoms were sampled. Symptomatic animals (45%) showed a wide range of clinical manifestations, including infectious-related, aspecific, or secondary signs such as lethargy, anorexia, diarrhea, respiratory and neurological distress, plumage disorders, feather damaging behavior, and alterations in hemato-biochemical profiles and complete blood counts. After DNA extraction, each sample was assessed for the presence of specific nucleic acids using different PCR protocols described in the literature. The most prevalent pathogen was AdV (8.7%), followed by PBFDV (6%), which was identified as well in a Japanese quail for the first time, while APyV showed the lowest prevalence (0.6%). Fisher's exact test revealed a correlation between PBFDV positivity and age (young animals showed higher prevalence) and clinical signs. No risk factors were found to be associated with higher AdV prevalence. The sequencing results revealed a high nucleotide identity between PBFDV, APyV and PsAdV isolates and those described in other countries. The PBFDV and APyV (the first ever described in Italy), revealed a high similarity with genogroup I-c and I, respectively. The PSAdV sequence belonged to PsAdV-12 (the first documented sequences in Europe) from Agapornis roseicollis and PsAdV-2 from several parrot species. This study documented the spread of three different viruses in the bird population from Sicily, revealing risk factors that support virus dissemination and contributing to a better definition of the current epidemiological picture and the genetic features of the circulating strains.

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Oral Communication(s)

ANIMAL VIRUSES AND ENVIRONMENTAL CONTAMINANTS: RISK ANALYSIS ININFECTIOUS DISEASES

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Current evidence suggests that environmental contaminants may significantly increase infectious diseases risk. Recently, in some European countries, including Italy, a remarkable relationship between the level of environmental pollutants with the rate of mortality in COVID-19 pandemic, due to severe acute respiratory syndrome-coronavirus-2, was detected. Among the contaminants, 2,3,7,8-tetrachlorodibenzo-p--dioxin (2,3,7,8-TCDD), known as dioxin, is a widespread environmental contaminant that can suppress humoral and cell-mediated immunity, so making humans and animals more susceptible to infectious agents. Indeed, *in vivo* and *in vitro* investigations indicate that dioxin might interfere with the replication of both human and animal viruses, including influenza A viruses, coxsackie virus B3, cytomegalovirus, herpes simplex II, bovine herpesvirus 1 (BoHV-1) and canine coronavirus (CCoV).

In small animals, CCoV, an alphacoronavirus, is commonly responsible for self-limiting enteric infections but, because of genetic alterations, it can generate new dangerous strains. For instance, outbreaks of lethal infections in dogs were related to the highly virulent CCoV strain CB/05, as well as cases of pneumonia and malaise in humans were associated with CCoV-HuPn-2018 and HuCCoV_Z19Haiti, new canine–feline recombinant strains of CCoV, underlining the cross-species spread capability of CoVs. Specifically, following infection of CCoV in a canine fibrosarcoma (A72) cell line, virus titer and the expression of nucleocapsid N protein were significantly increased by exposure to very small concentrations of 2,3,7,8-TCDD. In addition, *ex vivo* analyses in multiple organs (liver, lungs, spleen, intestine) on cats and dogs from Campania region, revealed the presence of CCoV in 23.5% of canine samples, and feline coronavirus (F-CoV) in 16.3% of feline samples. Interestingly, chemical analyses, performed on the livers of the above subjects, showed that the congener 2,3,7,8-TCDD was present at higher concentrations in CoVs-infected dogs and cats than in non-infected animals, suggesting a positive correlation between coronaviruses infection and dioxin exposure.

In large animals, BoHV-1, the bovine homologue of human herpes simplex virus type 1, causes infectious rhinotracheitis, genital disorders and polymicrobial infections in cattle, provoking considerable economic losses to the cattle industry and trade restrictions. We have also been reported that, in the presence of 2,3,7,8-TCDD, during BoHV-1 infection in bovine kidney cells (MDBK), a significant increase in virus replication was found. Our results showed that dioxin might promote the onset of BoHV-1 infection and render bovine cells more vulnerable to the virus.

In order to further elucidate a potential correlation between viruses and environmental contaminants, based on an International Research Agreement, we are now investigating milk samples from various areas of Chile, presenting different environmental conditions.

Overall, our reported data suggest that the exposure to a toxic environmental contaminant, like 2,3,7,8-TCDD, may interfere with the replication of both small and large animals' pathogens, so acting as an additional risk factor for progression of infections in dogs, cats as well as in cattle.

Oral Communication(s)

FAECAL SHEDDING OF A NOVEL LIVE RECOMBINANT CANINE PARVOVIRUS TYPE 2 VACCINE: NEW CHALLENGES IN DIAGNOSTICS?

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Positive faecal test results for canine parvovirus type 2 (CPV-2) have been reported in dogs for several days after the administration of modified live virus (MLV) vaccines, highlighting the need for a differential laboratory diagnostic strategy [1]. In addition to the historical MLV vaccine formulations, including the original CPV-2 type or the CPV-2b variant, a novel live recombinant vaccine, expressing a capsid-coding insert derived from a currently circulating CPV-2c variant [2], is licensed and has recently become available in the Italian market. To evaluate potential interference with diagnostic tests, a preliminary sequence analysis of this novel vaccine strain and its faecal shedding was performed.

Sequence analysis of the whole coding vaccinal CPV genome sequence was carried out using a classical PCR approach, subject to direct Sanger sequencing, assembled and analysed using BioEdit ver 7.2.5 software and with nBLAST, to search related sequences in public domain databases. Faecal samples, collected from puppies before (day 0) and on days 3, 7, 14, 21 after the administration of the CPV-2c recombinant vaccine, were tested for the detection and sequencing of parvovirus DNA. To elucidate the genetic relationships of the CPV vaccinal strain with already detected field strains, distinctive nucleotide and amino acid substitutions were analysed through sequence alignments, and a phylogenetic tree based on partial VP2 gene sequences was constructed with the IQ-Tree software, using the maximum-likelihood method selecting as substitution model the general time reversible (GTR) with discrete Gamma distribution. Clade reliability was assessed by performing 1000 bootstrap replicates.

Sequence analysis revealed specific amino acid residues (219Val, 386Lys) in the predicted VP2 protein sequence common to those of other vaccinal strains and to a few field (CPV-2b) or likely vaccine-derived (CPV-2) strains, also including CPV-2c strains [3]. Vaccine parvovirus DNA was detected by classical PCR up to 21 days after first vaccination and the VP2 gene sequence was identical to those of the vaccinal strain. A close genetic relationship of the vaccinal strain expressing the CPV-2c capsid-coding insert with other field CPV-2c strains has been observed.
Faecal shedding in healthy puppies of vaccine virus DNA highly related to a current prevalent circulating field CPV-2c variant was proven, and VP2 genomic markers of this novel live recombinant vaccine were described, highlighting the need to update the currently used diagnostic tools to facilitate the differentiation of vaccinal and field CPV-2c virus.

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Oral Communication(s)

NERVOUS NECROSIS VIRUS CIRCULATION AND TRANSMISSION DYNAMICS IN AN OPEN CAGE INTEGRATED MULTI-TROPHIC AQUACULTURE SYSTEM

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Integrated Multi-Trophic Aquaculture (IMTA) systems co--farm species from different trophic levels (i.e. finfish and shellfish) and are increasingly investigated in real world scenarios, providing opportunities to explore system's components interactions otherwise difficult to observe under laboratories settings. Indeed, the transmission dynamics of aquatic pathogens within these marine farming systems, across co-cultured trophic levels, remain largely unexplored. This study aimed to investigate the presence and spread of viral pathogens within an IMTA facility, focusing on nervous necrosis virus (NNV, Nodaviridae, Betanodavirus) and Ostreid herpesvirus 1 (OsHV-1, Malacoherpesviridae, Ostreavirus).

A monitoring activity (2023–2024) was conducted through periodic sampling involving finfish (*Dicentrarchus labrax, Sparus aurata, Argyrosomus regius*) and shellfish (*Ostrea edulis, Crassostrea gigas, Mytilus galloprovincialis*) reared at the farm. OsHV-1 investigation was conducted on oyster mantle and gill tissues [1] while NNV detection was carried out on pooled finfish brain and shellfish digestive gland samples [2]. Positive samples were further characterized sequencing NNV RNA1 and RNA2 [3].

No presence of OsHV-1 was detected in analysed shellfish specimens. On the other hand, NNV was detected in 7 out of 43 samples (16.3%). Among finfish, the only positive species was *D. labrax* (5 samples), with detections from May to September 2023 and in February 2024. Four out of five positive fish samples belonged to on-growing batches and were often associated with reduced survival rates (34–55%). Longitudinal monitoring of selected batches indicated that infection likely occurred at the farm level during on-growing, with evidence of transmission between cages. Sequence analysis showed 100% nucleotide identity among most viral strains detected in *D. labrax*, indicating the persistence of a single strain over time. A minor variant was transiently observed once but did not persist. Identical strains were also detected in *D. labrax* batches of different origin and age, further supporting horizontal transmission within the facility.

NNV was also detected in two oyster species (*O. edulis and C. gigas*) sampled in May 202[3] Viral sequences in the two species were 100% identical. However, this strain showed low nucleotide identity (91.7-92%) compared to viral strains detected in fish, indicating distinct viral populations. Phylogenetic analysis grouped both fish and shellfish viral strains within the RGNNV genotype but in separate clusters, suggesting independent circulation in shellfish and no direct epidemiological link with fish infections.

In conclusion, NNV circulation and persistence were demonstrated within the IMTA system, with evidence of intra-farm transmission among fish batches. The genetic divergence between fish and shellfish viral strains suggests distinct epidemiological patterns in the two hosts indicating that, under the studied site and condition, the co-farming with bivalves has not represented a direct source of infection. The study clearly pointed out the relevance of specific molecular surveillance finalized at establishing biosecurity measures to limit viral spread in IMTA systems.

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Oral Communication(s)

POST-VACCINAL DISTEMPER IN A LITTER OF LABRADOR RETRIEVER FROM SICILY: FROM CLINICAL PRESENTATION TO VIROLOGICAL AND SEROLOGICAL INVESTIGATIONS

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Vaccination against canine distemper virus (CDV) is considered “core” and is being widely applied to reduce the global incidence of CDV. This modified-live vaccine (MLV) has been historically associated with issues regarding residual virulence and the potential for reverse virulence [1]. One of the first MLV, the Rockborn strain, was attenuated in cell cultures and was frequently involved in post-vaccinal distemper (PVD) in both domestic dogs and wild carnivores. As a result, the Onderstepoort strain was used instead, as it was more attenuated, being cultured on chicken embryos. In recent years, PVD has been reported in animals vaccinated with both the Rockborn-like and Onderstepoort strains.

In this study, we described a case of PVD in a litter of Labrador retrievers. Two five-week-old puppies (out of a total of 9) showed clinical signs consistent with canine distemper three days after vaccination with a live attenuated vaccine (licensed for use in 4-6-week-old puppies). These two puppies were underweight at birth and at the time of the first vaccination. Furthermore, the smallest of them was weak, unable to suckle and was artificially fed for two weeks. The puppies showed respiratory and nervous signs, including seizures, fever, and pink mucous membranes. Complete blood count and biochemistry revealed anemia, monocytopenia, and hypoglobulinemia. The treatment included oxygen, midazolam, and fluid therapy. Both puppies survived after two days of intensive care. The presence of the replicating vaccine strain in blood and fecal samples was confirmed by real-time PCR and sequencing of the partial N gene. Blood collected from one of the puppies during hospitalization revealed the absence of specific antibodies, assessed by immunofluorescence and serum neutralization assays. The bitch, although beyond the vaccine window of immunity, had protective titers (1:160 for CPV-2 and 1:320 for CDV).

Previous studies have reported similar outcomes in puppies affected by PVD with more focus on post mortem and/or histological investigations (not carried out in our case due to the survival of the puppies) [2]. The current study describes, for the first time, the findings of PVD in five-week-old puppies, highlighting the clinical features, treatment, and the results of molecular and serological investigations. Furthermore, this case report highlights the importance of maternal immunity in the response to commonly used live attenuated vaccines, which can result in adverse effects under specific conditions.

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Oral Communication(s)

STUDY OF THE VIRAL SPREAD OF BOVINE-ALPHA 1 IN ITALY USING WHOLE GENOME SEQUENCING: PRELIMINARY RESULTS

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Infectious Bovine Rhinotracheitis (IBR) is caused by Varicellovirus bovine alpha 1 (BoAHV-1). This virus is recognized as a major pathogen of cattle worldwide. IBR represents a significant health and economic concern for the livestock industry [1]. The disease affects both beef and dairy production systems. It is associated with reduced productivity, reproductive disorders, and increased use of antimicrobial and chemotherapeutic treatments [1]. Despite the recognized epidemiological relevance of BoAHV-1, data on its circulation and its genomic diversity among Italian viral strains remain limited. Only recently, an Italian BoAHV-1 strain was genetically characterized by Whole Genome Sequencing (WGS), showing > 99% nucleotide identity with the BoAHV-1 subtype 1.1 reference strains previously identified in the United States [2]. The present study aimed to isolate and genetically characterize BoAHV-1 strains originating from different geographical areas of Italy through a WGS-based approach. In addition, commercial IBR marker vaccines carrying gene deletions (gE-; gE-/tk-) were included to allow comparative genomic analyses between field and vaccine-derived viruses. A total of 39 BoAHV-1 strains were selected for the study, including 33 field isolates obtained from confirmed IBR/IPV outbreaks and six commercial vaccine strains. Viral propagation was performed in cell culture, followed by virus concentration by ultracentrifugation and subsequent WGS, according to the protocol described by Petrini et al. [2]. Viral identification was confirmed using real-time PCR assays targeting the gB gene (WOAH, Manual of Diagnostic Test and Vaccines for Terrestrial Animals, Chapter, Cap. 3.4.11, 2024), together with additional real-time PCR assays targeting the gE/gD genes (as previously described by Wernike et al., 2011). BoAHV-1 was successfully isolated from 36 of the 39 investigated samples. Among the propagated isolates, 20 strains have been sequenced to date, yielding near-complete consensus genomes with genome coverage ranging from 95.58% to 99.84%. Comparative genomic analysis using BLAST alignment revealed that 13 field isolates showed high nucleotide identity (99.87–99.97%) with BoAHV-1 subtype 1.1 reference genomes. In contrast, 3 isolates displayed nucleotide identities ranging from 99.18% to 99.28% with BoAHV-1 subtype 1.2 sequences. The four analysed vaccine strains (three gE-marker and one gE-/tk-marker) showed 99.91%-99.96% nucleotide identity with BoAHV-1 subtype 1.1 strains deposited in GenBank. Preliminary findings confirm the circulation of BoAHV-1 strains predominantly belonging to subtype 1.1 in the Italian cattle population, consistent with previous reports describing the global distribution and genetic variability of the virus [1]. The high nucleotide identity observed among the subtype 1.1 field isolates supports the well-documented genomic stability of BoAHV-1, as previously described by Fulton et al. [3]. Conversely, the identification of subtype 1.2 strains indicates the co-circulation of genetically distinct viral lineages within the Italian cattle population, which may contribute to heterogeneous clinical manifestations and more complex epidemiological scenarios. Genomic analysis of live attenuated IBR vaccines (gE-marker and gE-/tk-marker) confirmed their close genetic relationship with the BoAHV-1 Cooper reference strain and the conservation of major genomic regions. Overall, this study provides novel preliminary insights into the genomic diversity of BoAHV-1 circulating in Italy and contributes to addressing the current knowledge gap regarding the epidemiology of the virus at the national level. These findings may support future molecular surveillance activities and the implementation of the Italian National IBR Control Programme currently under development.

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Oral Communication(s)

FROM GENOME TO BIOMARKER: A UNIQUE PEPTIDE FROM *BRUCELLA MELITENSIS* 16M REDEFINES DIVA POTENTIAL

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Brucellosis remains one of the most challenging issues in veterinary public health. It is a disease that can infiltrate production systems, compromising profitability and endangering anyone working with infected animals or contaminated materials. The *Brucella* genus is able to adapt to different hosts while maintaining clear preferences. *Brucella melitensis* is the main pathogen in small ruminants and the main cause of infection in humans. The bacterium's persistence in regions such as the Mediterranean, the Middle East, and sub-Saharan Africa perpetuates an endemic cycle characterised by repeated abortions, infertility, declining reproductive performance, and seropositivity that can persist throughout the animals' productive lives. In these contexts, the disease affects not only health, but also the economic stability of livestock farms and the safety of workers. Controlling the disease in small ruminants primarily relies on mass vaccination with live attenuated strains, particularly Rev.1, combined with test-and-slaughter strategies for unvaccinated seropositive animals. However, a structural limitation of live vaccines is their inability to clearly differentiate between infected and vaccinated animals (DIVA), which hinders epidemiological surveillance, eradication programmes, and international trade dynamics. This highlights the need to identify strain-specific molecular biomarkers that can reliably distinguish between vaccine and field strains. The investigation began with a targeted exploration of genomic segment 824171–824131 on chromosome II of *Brucella melitensis* 16M. This segment was chosen as it is thought to be an area of divergence between vaccine and field strains. This region was subjected to an extensive comparison involving 56 bacterial genomes, complemented by an immunogenicity assessment using state-of-the-art IEDB platforms[1,2]. Interspecific epitope mapping was then performed using bovine MHC alleles as a phylogenetic reference to infer antigenic presentation in small ruminants. This combination of approaches revealed a 13-amino acid peptide that is unique to the field strain *Brucella melitensis* 16M and is completely absent from the Rev.1 vaccine and the other analysed strains. Cross-species evaluation also indicated a strong predicted affinity with the ovine Ovar N*14:01 allele, suggesting particularly relevant potential immune recognition in the host species. This unique peptide appears to be a highly promising biomarker for distinguishing the 16M strain from the Rev.1 vaccine. Beyond immunoinformatic prediction, the peptide's translational value lies in the convergence of three key elements: rigorous genomic exclusivity; robust and consistent MHC-I signalling across overlapping peptide windows; and direct DIVA applicability in vaccinated populations. Together, these aspects make it a highly competitive candidate for improving epidemiological surveillance and supporting more effective control strategies.

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Oral Communication(s)

RAPID MASS VACCINATION EFFECTIVELY CONTROLLED LUMPY SKIN DISEASE IN SARDINIA (ITALY), 2025

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Lumpy Skin Disease (LSD) is a vector-borne viral disease of cattle that was first introduced into Italy in June 2025, marking the re-emergence of the virus within the Mediterranean basin after several years of absence from European borders. The outbreak occurred in Sardinia, a previously disease-free region with extensive grazing systems and frequent inter-herd contacts.

Between June and October 2025, 79 outbreaks were reported in the north-eastern part of the island, mainly in the provinces of Nuoro, Sassari and Oristano. The insular context and rapid implementation of control measures offered a unique opportunity to evaluate vaccination effectiveness under field conditions. Measures included stamping-out of affected farms, strict movement restrictions, protection and surveillance zones, and a mass vaccination campaign initiated soon after the first outbreak. Vaccination with a live attenuated Neethling strain was quickly extended to the entire cattle population, reaching nearly 100% coverage within a few months.

Accurate farm-level vaccination data, supported by a pre-existing traceability system, enabled timely and reliable modeling during this LSD emergency campaign.

To assess vaccination effectiveness at the farm level, a retrospective cohort approach was applied considering farms within a 50 km radius from the initial outbreak. Vaccination was modelled as a time-dependent variable with a 21-day lag to account for the development of protective immunity. A Cox proportional hazards model was used to estimate the association between vaccination status and outbreak occurrence over time. During the study period, farms contributed 3,177.7 farm-years at risk, with 71 outbreaks consistent with local transmission included in the analysis. Crude incidence rates were markedly different between exposure groups, with 4.55 outbreaks per 100 farm-years observed during non-effectively vaccinated periods compared to 0.40 during effectively vaccinated periods.

The time-dependent Cox model showed that effective vaccination was strongly associated with a reduced risk of outbreak (HR = 0.18; 95% CI: 0.06–0.51; p = 0.001), corresponding to an estimated vaccine effectiveness of approximately 82% at the farm level. Model-based cumulative incidence curves confirmed a clear divergence between vaccinated and non-vaccinated farms over time. These findings are consistent with previous field evidence from LSD epidemics in southeastern Europe, confirming the high effectiveness of live attenuated vaccines when rapidly deployed and applied with high coverage. Potential differences in exposure and confounding (herd size, proximity to the main cluster, zoning status) were assessed but not retained due to lack of statistical significance, likely reflecting the low number of outbreaks.

The Sardinian epidemic highlights the critical role of rapid, large-scale vaccination strategies in controlling LSD in extensive production systems, where traditional contact structures may be difficult to capture. Early implementation and high coverage were key to reducing transmission even under high infection pressure. These results support the use of vaccination as the cornerstone of control strategies in newly affected regions and provide quantitative evidence to inform preparedness and response planning for transboundary animal diseases.

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Oral Communication(s)

ASFREE M.E.A.T. (MEET EXPORT AGREEMENT ON TRADING): SAFEGUARDING THE ITALIAN CURED PIG PRODUCTS FROM AFRICAN SWINE FEVER

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African swine fever (ASF) is a non-zoonotic viral disease of suids caused by African swine fever virus (ASFV), recently reclassified as *Asfivirus haemorrhagiae*. It represents one of the most significant threats to the global swine industry, causing both direct and indirect economic losses. In Italy, cured meat products account for approximately 56% of total pork exports. Since the first notification of ASF in the country in 2022, the sector has experienced substantial economic losses due to international trade restrictions on these products. Current estimates indicate losses of approximately €20 million per month due to export limitations and up to €60 million per month during complete export bans.

Available data on the presence and persistence of ASFV in pork products date back to the 1980s–1990s and were generated using genotype I strains, which are no longer circulating in European countries, and detection methods less sensitive than those currently available. This gap highlights the need for updated evidence and complementary mitigation strategies beyond traditional curing processes to reassure importing countries regarding the absence of infectious ASFV in Italian cured products.

The nationally funded project "African Swine Fever Free Meat Export Agreement on Trading" (ASFree M.E.A.T.) aims to evaluate the persistence of ASFV in the most exported Italian cured pork products (i.e., salami and whole muscle cuts) and to assess whether High Pressure Processing (HPP), commonly used to inactivate *Salmonella* spp. and *Listeria monocytogenes* in pork products, can ensure complete viral inactivation thus guaranteeing that these products are safe commodities for international trade.

The project comprises two experimental approaches: an initial phase based on artificial contamination of salami meat mixtures for salami production, followed by an *in vivo* trial using meat from pigs experimentally infected with ASFV genotype II to produce both salami and whole-muscle cured products (e.g. ham, coppa, guanciale, pancetta). Four types of salami representative of major exported products (Milano, Spianata, Napoli, and Felino) were selected. Three batches of each salami type were experimentally contaminated (1% w/w) with a BA71/V ASFV suspension according to International Organization for Standardization (ISO) and Food Safety and Inspection Service (FSIS) guidelines and produced under BSL-3 conditions. At one-third, two-thirds, and the end of the curing period, three salami per batch were collected for ASFV detection by Real Time PCR and virus isolation, as well as for determination of weight loss, water activity (aw), pH, and mesophilic lactic acid bacteria counts.

At the end of curing, all ASFV-contaminated salami were subjected to HPP treatment. Although all samples remained PCR-positive at the end of curing and after HPP treatment, no viable virus was detected by virus isolation on cell culture. These preliminary results suggest that, under the experimental conditions applied, the curing process completely inactivate infectious ASFV in the tested salami.

The second phase is planned to confirm these findings under more realistic conditions, thereby better replicating field scenarios.

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Oral Communication(s)

PERFORMANCE EVALUATION OF SURVEILLANCE SYSTEMS IN VETERINARY PUBLIC HEALTH: A SCOPING REVIEW

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In veterinary public health, surveillance is defined as the systematic measurement, collection, collation, analysis, interpretation, and timely dissemination of animal-health and -welfare data from defined populations. Its purpose is to describe the occurrence of a health hazard, support the planning and implementation of mitigation measures and, ultimately, to protect animal and human health, and facilitate trade [1]. The evaluation of disease surveillance programmes is essential to ensure they provide reliable and useful information in an efficient manner. Surveillance systems' performance can be assessed using measurable characteristics, defined as attributes [2]. This scoping review analyzes the attributes, their assessment methods and evaluation frameworks used to evaluate surveillance for animal infectious diseases and zoonoses at the global level, in the last 15 years. A literature search was conducted on the Pubmed and Scopus databases, identifying 125 eligible studies: 116 describing the evaluation of 157 surveillance systems, and nine illustrating evaluation frameworks. A total of 67 attributes and 18 evaluation frameworks were identified, highlighting both commonly used (such as sensitivity and timeliness) and underutilized measures of system performance. The results of this work illustrate how surveillance priorities are shaped by socio-economic and regulatory contexts and confirm that surveillance evaluation is a relevant topic worldwide, encompassing human, animal health and One Health domains. These findings provide valuable guidance for selecting appropriate attributes when assessing the performance of surveillance systems in future evaluations.

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Oral Communication(s)

ENVIRONMENTAL DISSEMINATION OF ANTIMICROBIAL RESISTANCE IN NORTHERN ALGERIA: PRELIMINARY INSIGHTS FROM WILDLIFE

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Antimicrobial resistance (AMR) represents a growing global health threat. Environmental monitoring of AMR can provide significant insights into diffusion mechanisms of resistant bacteria and resistance genes across ecosystems; however, it is often logistically challenging and resource-consuming, prompting the search for alternative surveillance approaches. In this regard, wildlife, since not directly exposed to antimicrobials, is emerging as potential sentinel or bioindicator of environmental AMR. This study investigated the role of wild birds and reptiles (freshwater turtles) as bioindicators of environmental AMR contamination in Northern Algeria by analysing AMR patterns in coliform bacteria isolated from cloacal swabs. Overall, 60 animals (51 birds and 9 reptiles) were sampled across three ecologically distinct areas (Annaba, Algiers, and Reghaïa lake) between May and June 2025. Coliform isolates were identified using MALDI-TOF MS and tested for antimicrobial susceptibility using agar disk diffusion method. Isolates were classified as wild-type (WT) or non-wild-type (NWT) based on EUCAST epidemiological cut-off values (ECOFFs). Moreover, the absence of inhibition zone was considered indicative of phenotypic resistance when ECOFFs were unavailable. A total of 99 coliform isolates was recovered, of which 34.34% were classified as NWT and 16.16% as multidrug-resistant (MDR), with highest proportions of NWT observed for ampicillin (38.78%; n=19/49), tetracycline (33.33%; n=32/96), and trimethoprim–sulfamethoxazole (19.19%; n=19/99). Distinct phenotypic NWT patterns emerged among the three sampling areas, possibly reflecting specific local antimicrobial pressures. In Annaba tetracycline resistance was particularly high (44.44%), while Algiers exhibited elevated NWT proportions for antibiotics commonly used in clinical settings, such as ampicillin (42.86%) and trimethoprim-sulfamethoxazole (21.74%), and the highest rate of MDR (21.74%). Réghaïa lake also exhibited significant NWT proportions, particularly to ampicillin (40.74%), suggesting potential impacts from industrial and urban wastewater runoff from surrounding areas. Statistical analyses revealed a significant association between sedentary birds and tetracycline NWT at Annaba (p = 0.043) and a borderline association between higher overall NWT prevalence in resident birds (p = 0.055), compared with migratory birds, suggesting that resident species may better mirror the local antimicrobial pressures. The detection of AMR in wildlife with no direct exposure to antimicrobial treatments suggests environmental contamination and potential dissemination of resistant bacteria across Northern Algeria. These preliminary findings support the potential role of wildlife as valuable bioindicator of environmental AMR dissemination and highlight their relevance in providing an early-warning system to track the environmental spread of antimicrobial-resistant bacteria within One Health AMR surveillance frameworks.

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Oral Communication(s)

DEVELOPMENT OF A LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) ASSAY FOR THE DETECTION OF *BORRELIA* SPP. AND ITS APPLICATION TO SCREENING OF BATS IN NORTHERN ITALY

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Bats are one of the most widely distributed mammals, occurring on all continents except in polar regions. Their ability to fly long distances, occupy diverse ecological niches and their longevity make them ideal reservoirs and vectors of pathogens causing zoonotic diseases [1]. Interest in tick-borne diseases has recently increased, partly due to climate change. Over the last decade, *Borrelia* species infection has been reported in bats, suggesting a possible role in the circulation and persistence of these bacteria, with implications for human health [2]. However, limited studies make it unclear whether bats represent a reservoir of *Borrelia* spp. or incidental hosts [3]. This study aimed to develop a field-applicable diagnostic molecular assay for *Borrelia* spp. DNA detection. Furthermore, the assay was applied to spleen samples of 75 bats found dead in Northern Italy (Emilia Romagna region) from June 2023 to November 2025, and detected bacteria were genetically analysed. For these purposes, a loop-mediated isothermal amplification (LAMP) assay targeting the 16S rRNA gene of *Borrelia* spp. was validated. Its sensitivity and limit of detection (LOD) were evaluated using ten-fold serial dilutions of a recombinant plasmid containing a fragment of *B. afzelii* target DNA, and specificity was tested using *B. burgdorferi* ss, *B. afzelii*, *B. garinii*, *B. lusitaniae*, *B. valaisiana* and *B. myiamotoi* isolates. The assay showed a LOD of 10^2 target DNA copies/uL of extract with high sensitivity and specificity for *Borrelia* spp. belonging to both Lyme Disease (LD) and Relapsing Fever (RF) groups. Two of the 75 bats tested positive. A fragment of 16S rRNA gene was PCR amplified and sequenced from the two identified bacteria and BLAST analysis showed higher nucleotide identity with RF *Borrelia* species retrieved in GenBank database. Further analyses with Next Generation Sequencing are ongoing to correctly identify the species of the detected *Borrelia* and clarify their zoonotic relevance. These preliminary findings represent the first evidence of these bacteria in bats in Italy, highlighting the importance of future studies to better understand the role of bats in the epidemiology of *Borrelia spp.*

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Oral Communication(s)

FROM SCREENING TO NANOLIPOSOME ENCAPSULATION: ANTIMICROBIAL ACTIVITY AND CYTOTOXICITY ASSESSMENT FOR THE DEVELOPMENT OF A NOVEL FEED ADDITIVE

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Following the ban on antimicrobial growth promoters in veterinary medicine, plant-derived compounds—particularly Nature-Identical Compounds (NICs)—have gained attention as promising alternatives in animal nutrition due to their antimicrobial properties; even if, their direct use as zootechnical feed additives is often limited by early gastrointestinal degradation and potential cytotoxicity. For these reasons, encapsulation strategies, such as nanoliposome-based delivery systems, have been proposed. In this context, the present study, conducted in two steps, aimed to characterize a novel feed additive based on NICs encapsulated in a nanoliposome delivery system.

In the first step, nerolidol, geraniol, D-carvone, β-caryophyllene, α-pinene, p-cymene, eucalyptol, β-pinene, R-limonene, curcumin, and quercetin were screened for antimicrobial activity by determining the minimum inhibitory concentration (MIC) against a panel of livestock pathogens (MRSA, E. coli-EC, S. Typhimurium-ST, and E. faecium-EF) as well as a commensal bacterium (L. acidophilus-LA). Their cytotoxicity was also evaluated on Madin-Darby Bovine Kidney (MDBK) cells using the MTT assay.

In the second step, the most promising NIC was encapsulated into nanoliposomes, and the resulting formulation was evaluated by Time–kill (TK) assays after 2, 4, 6, and 24 hours of contact. MTT assay was performed on both MDBK and intestinal porcine enterocyte (IPEC-J2) cell lines. Production of nanoliposome formulation was realized using a simil-microfluidic technique (1). Data were analysed by repeated-measures ANOVA using the General Linear Model procedure in SPSS (version 29.1).

Results of first step indicate as nerolidol was active exclusively against Gram-positive bacteria (EF = 76 ± 27.56 µg/mL; MRSA = 4000 ± 0 µg/mL; LA= 250 ± 0); while geraniol inhibited both Gram-positive (EF and MRSA = 666.7 ± 250 µg/mL; LA = 1000 ± 0 µg/mL) and Gram-negative bacteria (EC = 777.8 ± 263 µg/mL; S. Typhimurium = 1666.7 ± 500 µg/mL). Lastly, curcumin was effective only on MRSA (MIC = 250 ± 0 µg/mL). All effective others NIC were ineffective. However, nerolidol, geraniol and curcumin reported high cytotoxicity (cell survival ≤ 45%). Considering these results nanoliposome formulations were prepared at a concentration of 5000 µg/mL containing 1% of nerolidol (1).

In second step, TK and MTT assays were performed on unloaded nanoliposomes (UN), nerolidol-loaded nanoliposomes (LN), and free nerolidol (NER) as control. Free NER inhabited only Gram-positive bacteria, whereas nanoliposome formulations inhibited also Gram-negative. LN showed strongest activity against MRSA up to 6 h, while UN on EF up to 4 h ($P < 0.05$). No significant differences were observed for ST (up to 24 h) or E. coli (up to 6 h), while both LN and UN formulations were effective against LA, up to 6 hours. Lastly, LN and UN showed lower cytotoxicity in MDBK than in IPEC-J2 cells, maintaining ≥ 80% viability at 1250 and 625 µg/mL, respectively.

In conclusion, nanoliposomes formulations exert dose-dependent antimicrobial and cytotoxic effects, however in vivo studies are needed to evaluate their potential application as novel feed additives.

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Oral Communication(s)

HRC-DERIVED PEPTIDE BLOCKS CANINE CORONAVIRUS ENTRY BY INHIBITING VIRAL MEMBRANE FUSION

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Canine coronavirus (CCoV) is an *alphacoronavirus* generally associated with enteric and/or respiratory disease. Canine enteric coronavirus type 2 (CECoV-II) usually causes mild enteritis; however, in some cases, especially when combined with other pathogens, it can lead to more severe forms in puppies, including hemorrhagic enteritis and vomiting. Genetic recombination and the high plasticity of CCoV mutations drive the emergence of more virulent strains, increasing pathogenicity and enabling cross-species transmission and spillover events [1]. In this scenario, attractive drug targets for developing antiviral treatments include internal, conserved regions of viral surface proteins that facilitate the initial membrane fusion required for entry into host cells, such as the spike (S) protein. During viral infection, the S protein is cleaved into S1 and S2 subunits, with S1 binding to the cellular receptor aminopeptidase N (APN) and S2 containing two heptad repeat regions (HRN and HRC). These regions are crucial for viral fusion and the conformational change from a compact pre-fusion state to an elongated post-fusion state [2]. In this study, we designed, synthesized, and tested a peptide derived from the HRC domain of the CCoV S glycoprotein for antiviral activity. We conducted antiviral, cell-cell fusion, and real-time molecular assays to assess the efficacy of CCoV-HRC peptide, complemented by *in silico* docking and molecular dynamics simulations to analyze the interaction with the S protein fusion core. Cytotoxicity assays showed that the concentration causing 50% cytotoxicity (CC_{50}) in HEK293T (human embryonic kidney cells) and A72 (canine fibrosarcoma cells) exceeded 100 µM. Non-toxic doses effectively blocked CCoV S glycoprotein-mediated fusion and significantly lowered live-virus infection in *vitro*, with the concentrations causing 50% inhibition (IC_{50}) values of 35 and 25 µM, respectively. Molecular analyses investigated the expression of the gene encoding the viral S protein and detected a significant reduction in S gene expression at the highest peptide concentrations. These results were supported by in *silico* docking and molecular dynamics simulations, which indicated that CCoV-HRC stably binds to the HR1 regions and mimics the native HR2 region by hindering fusion [3]. These findings provide a solid basis for developing peptide-based therapies to treat or prevent CCoV infections. Further studies are necessary to evaluate pharmacokinetics and stability, and to assess safety in animal models.

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Oral Communication(s)

DEVELOPMENT OF PROPOLIS-BASED TEAT SEALANTS FOR PREVENTING BOVINE MASTITIS

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Rising concerns about antimicrobial resistance in both human and veterinary medicine have encouraged the exploration of alternative treatment options, particularly for addressing prevalent infections in livestock, such as bovine mastitis. Among the most promising natural candidates are beehive products, especially honey and propolis. In our previous study, we assessed the antimicrobial activity of honey and propolis collected from apiaries in three different areas of the Umbria region against *Staphylococcus aureus* and *Escherichia coli* isolates from bovine clinical mastitis grown in planktonic form. Building on those findings, we subsequently aimed to: (1) evaluate the effectiveness of these bee products against *S. aureus* and *E. coli* biofilms, and (2) develop new formulations to be used as teat sealants at dry-off for the prevention of intramammary infections. The Microtiter Crystal Violet Biofilm assay was carried out to assess the ability of honey and propolis to eradicate *S. aureus* and *E. coli* isolates grown in a sessile state [1]. Propolis exhibited the highest antibiofilm activity, with greater efficacy against Gram-positive microbial communities. Specifically, propolis collected in Perugia and Terni, at a concentration of 0.28%, reduced *S. aureus* biofilm by 45–75%, depending on the isolate tested, whereas samples collected in Gubbio achieved reductions ranging from 50% to 80%. Drawing upon prior experimental data, novel propolis-based teat sealants were formulated. An ointment based on lipophilic excipients was selected as the most suitable formulation for intramammary administration of propolis to ensure an appropriate melting and softening point, allowing easy administration with a needle-free syringe while maintaining stability at the cow's body temperature and preventing leakage. Beewax obtained from the same hive as the propolis was incorporated in the ointment after characterization by differential scanning calorimetry (DSC) showing a peculiar thermal behavior associated with two main components: a minor fraction with a melting peak between 40–60 °C (free acids and hydrocarbons) and a major fraction with a peak between 60–70 °C (fatty acid esters). Subsequently, two semi-solid ointments with identical qualitative composition but different quantitative ratios were prepared using the fusion method. Based on their consistency, the most suitable formulation consisted of 0.5 g purified beeswax, 8.5 g vaseline oil, 7.5 g vaseline and 0.5 g silica. This selected ointment was further characterized by DSC to determine its thermal properties, confirming its appropriate melting behavior. Finally, two propolis-containing formulations were developed, with concentrations of 1% and 2%, based on previously determined minimum inhibitory concentration (MIC) values. The propolis extract was first adsorbed onto silica, dried, and then incorporated into the melted excipients at a controlled temperature. This formulation showed suitable spreadability for the intended experimental purposes. We are currently investigating the antimicrobial activity and cytotoxicity of this formulation as a basis for subsequent ex vivo/in vivo efficacy studies. The developed teat sealant is proposed as a preventive strategy against bovine mastitis, with the aim of reducing reliance on conventional antibiotics.

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Oral Communication(s)

PREVALENCE AND ANTIBODY MAGNITUDE OF *BRUCELLA CANIS* IN SHELTER DOGS IN SOUTHERN ITALY

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Brucella canis is an emerging zoonotic pathogen responsible for reproductive disorders in dogs and representing an increasing concern for public health, yet it remains a neglected in Italian legislation, with no mandatory kennels screening. This study investigated the seroprevalence and antibody magnitude of B. canis infection in shelter dogs from the Campania region (Southern Italy) over a four-year period (2022–2025). A total of 400 serum samples from 49 shelters were collected during routine veterinary surveillance in municipal kennels across the five provinces of the region (Avellino, Benevento, Caserta, Naples, and Salerno) and were retrospectively analysed. Samples were screened for anti-B. canis IgG antibodies using an indirect immunofluorescence antibody test (IFAT), with a screening cut-off of 1:40 followed by serial dilutions to determine endpoint titres. Associations between seropositivity and epidemiological variables (year, province, and gender) were evaluated through Chi-square tests and multivariable logistic regression models. Additionally, antibody magnitude in seropositive dogs was analysed using a general linear model (GLM) applied to $\log_2$-transformed endpoint titres. The overall seroprevalence was 17.3% (69/400; 95% CI: 13.6–21.0%). None of the investigated predictors showed a statistically significant association with infection status in either univariate or multivariable models ($p > 0.05$), suggesting a widespread and homogeneous distribution of exposure across the regional territory. However, analysis of antibody magnitude revealed significant effects of sampling year ($p = 0.0024$) and province ($p = 0.0490$). In particular, a significant increase in antibody magnitude was observed over time, with higher titres between 2022 and the reference year 2025 ($p = 0.037$), suggesting an intensification of infection pressure and active pathogen circulation in multi-dog environments. No influence of gender on antibody levels was observed. The relatively high seroprevalence detected in this population considerably exceeds values recently reported in other European contexts and highlights the potential endemic circulation of B. canis in shelter environments in Southern Italy. Our results confirm that Brucella canis is an endemic pathogen in the shelter dog population of southern Italy. The high seroprevalence and progressive increase in antibody magnitude (GMT) (55.4 in 2022; 71.3 in 2023; 73.1 in 2024; 88.5 in 2025) over the study period indicate rising infection pressure, highlighting the urgent need for mandatory screening and a coordinated One Health surveillance strategy to manage zoonotic risk. From a One Health perspective, these findings reveal the need for systematic surveillance strategies in canine shelters, including routine serological screening and confirmatory diagnostics. Strengthening epidemiological monitoring is essential to reduce the risk of pathogen dissemination and reduce zoonotic risk to shelter workers and adopters. Close contact between stray dogs and shelter personnel, combined with the absence of mandatory screening, represents a tangible zoonotic risk. Systematic serological and molecular surveillance programmes are urgently needed in Italian kennels. Mandatory pre-movement screening should also be introduced to prevent the introduction of B. canis into non-endemic households and regions. The implementation of comprehensive surveillance programmes, including routine testing of dogs upon shelter entry, is strongly recommended to mitigate the risk of transmission to both animals and humans.

Oral Communication(s)

EXPLORING THE ANTIBIOTIC RESISTANCE EVOLVABILITY IN *STAPHYLOCOCCUS PSEUDINTERMEDIUS* CLINICAL ISOLATES

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The increasing prevalence of antibiotic-resistant infections underscores the urgent need for novel antimicrobial strategies. While most research has focused on the development of alternative therapeutics targeting resistant pathogens, an alternative approach is to address the evolutionary mechanisms that drive resistance in initially susceptible bacteria. By limiting the emergence of resistance, this strategy may help preserve the efficacy of existing antibiotics. However, the evolvability of antibiotic resistance in many pathogenic bacteria remains poorly understood. In this study, we aimed to evaluate the resistance evolvability of *Staphylococcus pseudintermedius*, a major canine pathogen responsible for over 90% of bacterial pyoderma cases and an occasional zoonotic agent. Specifically, we investigated its capacity to develop resistance to ciprofloxacin, as a representative fluoroquinolone, and to clindamycin, a lincosamide antibiotic commonly used in the treatment of canine pyoderma.

We evaluated the resistance evolvability of 68 fully susceptible *S. pseudintermedius* isolates to clindamycin and 20 isolates to ciprofloxacin, all derived from canine clinical samples. Our methodology was adapted from Papkou et al. [1], with fluctuation tests used to ensure that bacterial inoculum was free from preexisting resistance mutations. Fluctuation assays were performed using rifampicin and clindamycin as selective agents, whereas mutation rates for ciprofloxacin resistance in staphylococcal cells had been previously determined [1]. The experimental setup was designed to mimic in vivo treatment conditions by serially exposing bacterial populations (12 replicate populations for each parental strain) into fresh culture medium supplemented with the clinical breakpoint concentration of clindamycin (0.5 µg/ml) and ciprofloxacin (1 µg/ml) [2]. After four days of antibiotic exposure, evolved populations were identified based on optical density measurements (OD_{595}) relative to blank controls. Fluctuation test results supported the use of a bacterial inoculum of 3×10^{5} cells/well (mutation rate 6.7×10^{-9} per cell division), aligning with previous findings [1]. Clindamycin resistance emerged in 17 out of 68 strains (25.0%), with varying degrees of evolvability: one strain exhibited 9/12 evolved populations, one 5/12, one 4/12, two 3/12, seven 2/12, and five 1/1[2] No resistance evolution was observed for ciprofloxacin. In contrast, prior studies reported significant resistance evolution in *Staphylococcus aureus* at the ciprofloxacin clinical breakpoint concentration [1]. This suggests that *S. pseudintermedius* has a lower intrinsic capacity to evolve resistance to ciprofloxacin compared to *S. aureus*. Ongoing investigations focus on the differential gene expression profiles of high- and low-evolvability *S. pseudintermedius* strains following clindamycin exposure, with the aim of identifying key drivers of resistance evolution. Building on these findings, the development of anti-evolution strategies targeting the mechanisms underlying resistance acquisition will be pursued.

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Oral Communication(s)

SALMONELLA INFANTIS' OUTER MEMBRANE VESICLES AS CARRIERS OF ANTIBIOTICS RESISTANCE GENES: EXPLORING THEIR POTENTIAL ROLE IN HORIZONTAL GENE TRANSFER

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Outer membrane vesicles (OMVs) are spherically bilayered nanoparticles, ranging in size from 10 up to 300 nm, released into the extracellular milieu Gram-negative bacteria. OMVs enclose in their lumen molecules of different nature and they are involved in multiple processes, including intracellular and extracellular communication, horizontal gene transfer, inter and intra-species delivery of molecules [1]. Recent studies have shown that OMVs are involved in antibiotic resistance mechanisms by including β-lactamase enzymes, which degrade β-lactam antibiotics, in their lumen [2]. Since in our previous work we investigated and confirmed the presence of β-lactamase enzymes in Salmonella Infantis' OMVs [3], and given that no studies have yet examined nucleic acids and β-lactam resistance genes in these vesicles, we aimed to determine whether such genetic determinants are OMV-associated and to evaluate their potential role in the horizontal gene transfer of β-lactam resistance to susceptible strains.

OMVs were isolated from β-lactam resistance S. Infantis strains through several steps of filtration and ultrafiltration of culture supernatants. Transmission electron microscopy (TEM) and Nanoparticle Tracking Analysis (NTA) were used to confirm the presence, investigate the morphology and to count OMVs.

To evaluate the presence of β-lactam resistance genes in OMVs, the vesicles were first treated with DNase to eliminate any extracellular DNA associated with their surface, and subsequently mechanically lysed. The lysed OMV content was then subjected to PCR screening for β-lactam resistance genes, including bla CTX-M-[1]

Therefore, a preliminary vesicle-mediated HGT experiment was conducted using Salmonella Enteritidis ATCC 13076 as the recipient strains. The OMVs were pre-treated with DNase to remove any externally associated DNA, then incubated in liquid co-culture with the recipient strain under static conditions, followed by overnight shaking. Subsequently, colonies of the recipient strains were screened by PCR for β-lactam resistance genes. TEM images showed round shaped vesicles, mainly organized in clusters, while NTA indicated a relatively homogenous particle population, with a mean size of 170 nm and a concentration of 1.04 x 109 particles.
PCR analysis did not reveal the presence of β-lactam resistance genes within the OMVs. The preliminary experiment on vesicle-mediated HGT using S. Infantis OMVs did not show the transfer of nucleic acids to the recipient strain, as no β-lactam resistance genes were detected by PCR in recipient colonies.

These preliminary results suggest that, unlike β-lactamase enzymes which are loaded into OMVs, β-lactam resistance genes do not appear to be packaged within vesicles. However, further experiments are ongoing to analyze the nucleic acid content of OMVs and to definitely determine whether they are involved in HGT.

The possibility of investigating and understanding if OMVs play a role in the HGT opens the way towards the chance of developing new therapeutic strategies to fight antibiotic resistance in the future.

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Oral Communication(s)

ASSOCIATION BETWEEN PLASTIC INGESTION AND INTESTINAL ANTIBIOTIC RESISTANCE GENES IN THE LOGGERHEAD SEA TURTLE *CARETTA CARETTA*

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Antimicrobial resistance (AMR) is increasingly recognized as a significant threat to human and animal health as well as environmental integrity. Marine ecosystems have emerged as important reservoirs and transmission pathways for antibiotic resistance genes (ARGs). The loggerhead sea turtle, *Caretta caretta*, represents a valuable sentinel species due to its longevity, broad trophic niche, and chronic exposure to anthropogenic contaminants, including marine plastic debris [1]. This study analyzed ARG occurrence and distribution in fecal samples from *C. caretta* turtles admitted to the Italian National Reference Centre for the welfare, monitoring, and diagnosis of sea turtle diseases (C.Re.Ta.M.). The aim was to assess the relationship between ARG patterns, host age class, and ingested plastic. PCR analysis of 31 fecal samples (n. 14 from juveniles and n. 17 from subadults) was conducted to identify ARGs associated with major antibiotic classes, while simultaneously assessing the presence of plastic in each specimen. ARGs were widely detected in both juvenile and subadult individuals. Although no statistically significant association was found between age class and the prevalence of individual ARG classes ($p > 0.05$), differences were noted. Specifically, subadults exhibited higher positivity than juveniles for β-lactam (77.8% vs 69.2%), quinolone (55.6% vs 38.5%), and tetracycline resistance genes (72.2% vs 46.2%). In contrast, juveniles showed higher positivity than subadults for phenicol (53.8% vs 38.9%) and sulfonamides resistance genes (38.5% vs 16.7%). These patterns suggest that age-related differences in exposure history, trophic ecology, and cumulative exposure to contaminated environments may influence the composition of the intestinal resistome [2]. A more distinct pattern was observed concerning plastic ingestion. Samples containing plastic exhibited a broader resistance profile than those without plastic. ARG prevalence was higher in plastic-positive samples across all antibiotic classes, specifically for β-lactams (77.3% vs 66.7%), sulfonamides (27.3% vs 22.2%), quinolones (54.5% vs 33.3%), and tetracyclines (63.6% vs 55.6%). Notably, phenicol resistance showed a significant association with the presence of plastic ($p = 0.021$; 59.1% vs 11.1%), contributing to a significantly greater overall ARG burden in plastic-positive specimens ($p = 0.0468$). Overall, these findings indicate that plastic ingestion may contribute not only to physical injury but also to the enrichment and diversification of the intestinal resistome in *C. caretta*, potentially acting as a vector for resistant bacteria and associated genetic determinants. Thus, sea turtles serve as valuable bioindicators of AMR dissemination in marine environments, underscoring the need to integrate plastic pollution into a comprehensive One Health framework to elucidate the environmental dimension of antimicrobial resistance [3]. This study was financially supported by the Italian Health Ministry (RC IZSSI 08/23)

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Oral Communication(s)

EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF NAJA CARDIOTOXIN PEPTIDE-3 AGAINST CLINICAL ISOLATES FROM CANINE OTITIS

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Canine otitis externa is a common multifactorial inflammation of the external ear, often complicated by bacteria and fungi. Common bacterial isolates include Staphylococcus, Pseudomonas, Proteus, Enterococcus, Streptococcus, and Corynebacterium, while Malassezia pachydermatis is the most frequent yeast, causing pruritus and discomfort. Although antibiotics is used for acute cases, the ongoing rise in antimicrobial resistance is reducing their effectiveness, highlighting the need for alternative treatments. Among these alternatives to antibiotics, antimicrobial peptides can represent a promising option due to their broad-spectrum activity, low toxicity, and low propensity to induce resistance. Naja Cardiotoxin Peptide-3 (NCP-3), derived from cardiotoxin 1 of the Chinese cobra (Naja atra subsp. atra), has demonstrated potent bactericidal and fungicidal activity with minimal cytotoxicity [1].

The present study aimed to evaluate the in vitro antimicrobial activity of NCP-3 on clinical isolates (n=67) obtained from dogs with otitis externa admitted to the Veterinary Teaching Hospital of the University of Parma. Specifically, *B. cepacia* (n = 3), *E. coli* (n = 9), *E.cloacae* (n = 2), *P. mirabilis* (n = 4), and *P. aeruginosa* (n = 18), and *S. pseudintermedius* (n = 17). Also, reference strains were included in the study.

Antimicrobial activity of NCP-3 peptide was evaluated through Minimum Bactericidal Concentration (MBC) and Time-Kill (TK) assay. The latter was performed using 2x MBC concentration and CFU were counted at fixed time points (5, 10, 15, 30, 60 and 120 minutes). Data were analysed in SPSS 29.1 (IBM Corp., Armonk, NY, USA). Since MBC values were not normally distributed, group differences were evaluated using the Kruskal–Wallis test with Bonferroni-adjusted post hoc comparisons.

NCP-3 exhibited antimicrobial activity against all reference strains except Proteus mirabilis, showing the lowest MBC value against S. pseudintermedius (0.8 µg/mL). Among clinical isolates, the highest bactericidal effect ($P < 0.001$) was observed on S. pseudintermedius group (MBC_{50} = 3.1 µg/mL; MBC_{90} = 6.3 µg/mL). Higher concentrations were required for Pseudomonadaceae (MBC_{50} = 12.5 µg/mL) and neither MBC_{50} nor MBC_{90} were determined for Enterobacteriaceae and M. pachydermatis.
Result of TK assays on reference strains demonstrated as S. aureus, MRSA, S. pseudintermedius, and M. pachydermatis were fully inhibited within 5 minutes of NCP-3 exposure. Conversely, P. aeruginosa was nearly completely inhibited after 15 minutes, while E. coli and P. mirabilis were fully inhibited at 120 minutes. On clinical isolates, NCP-3 achieved complete inhibition of S. pseudintermedius, while Pseudomonadaceae reached > 96% inhibition at 60 minutes. Greater variability was observed among Enterobacteriaceae isolates, since E. coli and E. cloacae showed inhibition ≥ 96% at 120 minutes, while only few strains of P. mirabilis showed inhibition ≥ 50%. Lastly, M. pachydermatis showed the greatest resistance.

Overall, these findings indicate that NCP-3 exhibits strong antimicrobial activity against Gram-positive bacteria, including MDR bacteria, while having less efficacy against Gram-negative bacteria and M. pachydermatis clinical isolates. NCP-3 may therefore represent a promising candidate as topical treatment for canine otitis externa caused by Gram-positive pathogens.

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Oral Communication(s)

SECRETOME SIGNATURES OF PROBIOTIC LACTIC ACID BACTERIA WITH RAPID BACTERICIDAL ACTIVITY AGAINST *E. COLI* STEC

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Shiga toxin-producing *Escherichia coli* (STEC) are major foodborne zoonotic pathogens, and innovative strategies are needed to limit their spread along the food chain. This pilot study evaluated the in vitro antibacterial activity of cell-free supernatants (CFS) from four commercially available probiotic lactic acid bacteria (LAB) -*Lacticaseibacillus rhamnosus* GG, *Lacticaseibacillus paracasei* CNCM I-1572, *Lactiplantibacillus plantarum* Lp-115 and *Ligilactobacillus salivarius* W58-against eight STEC serotypes (O26, O45H2, O103H11, O104H4, O111, O121H19, O145NM, O157H7). Antibacterial activity was assessed by time-kill assay, well diffusion assay and MIC/MBC determination, and integrated with secretome profiling by label-free LC–MS/MS. In time-kill assays, all CFS rapidly reduced STEC viable counts, achieving ≥90% killing within 6 h for every serotype and complete eradication by 4 h in most probiotic–serotype combinations. *L. paracasei* CNCM I-1572 and *L. salivarius* W58 consistently drove counts to the detection limit already at 2–4 h across nearly all serotypes, while Lp-115 reached complete killing slightly later and *L. rhamnosus* GG showed a more strain-dependent profile, with excellent activity against O26, O121, and O157 but incomplete killing of O145. MIC values were predominantly 1/8 of the original CFS and MBC values 1/4, indicating that low supernatant concentrations are sufficient for bactericidal activity. Whole-genome sequencing produced high-quality assemblies (≈2.0–3.4 Mbp, ≈100% completeness, low contamination) and revealed marked genomic diversity among strains. Comparative analysis across 36 LAB genomes identified an extremely open pan-genome (18,761 gene clusters; 1 core, 8,785 shell, 9,975 cloud genes), highlighting extensive accessory content and niche-adaptive potential. Lp-115 clustered with carbohydrate-versatile *L. plantarum*, CNCM I-1572 with dairy-associated *L. paracasei*, W58 within a highly variable and mobile-element-rich *L. salivarius* clade, and GG carried accessory modules related to adhesion, stress response, and exopolysaccharide synthesis. To explore the molecular basis of the observed phenotypes, secretome samples from the four probiotics were analysed by label-free LC–MS/MS. Overall, 326 protein features were quantified, and one-way ANOVA identified widespread abundance differences among the four secretomes ($p < 0.05$), supporting the presence of marked strain-specific extracellular protein signatures. Functional annotation highlighted proteins involved in cell wall turnover, membrane transport, translation, and stress-related processes. Notably, *L. salivarius* W58 showed enrichment of bacteriolytic candidates, including an M23 family metallopeptidase and an N-acetylmuramoyl-L-alanine amidase, supporting a plausible contribution of secreted lytic effectors to its stronger anti-STEC activity; genome-based screening further indicated that all strains harbour bacteriocin-related determinants, although canonical bacteriocins were not directly detected in the secretome under the tested conditions. Altogether, these results show that CFS from four genomically distinct probiotic LAB exert potent and partly complementary bactericidal effects against a broad STEC panel, and that specific genomic and secretomic traits-particularly in *L. paracasei* CNCM I-1572 and *L. salivarius* W58--may underpin their rapid and consistent anti-STEC performance along the food chain.

Oral Communication(s)

ISOLATION, IDENTIFICATION AND CHARACTERISATION OF NEW LYTIC STAPHYLOPHAGES FROM ENVIRONMENTAL SAMPLES TO CONTROL MULTIDRUG-RESISTANT *STAPHYLOCOCCUS AUREUS*

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Staphylococcus aureus causes a wide range of infections in humans and animals, with significant impacts on healthcare due to its multidrug resistance [1]. Bacteriophages are promising antibiotic alternatives thanks to their host specificity, ability to lyse susceptible and resistant strains, and activity against biofilms without disrupting beneficial microbiota [2]. Currently, in the European Union, veterinary phage therapies are defined as novel therapies falling within the scope of the Regulation (EU) 2019/6 on veterinary medicinal products, which governs the marketing authorisation process [3]. For this purpose, isolating new strictly lytic staphylophages is crucial for phage therapy. This work aimed to isolate, characteris and identify strictly lytic bacteriophages against *S. aureus* from different samples: wastewater (n = 2), fresh lake water (n = 1), and a pool of environmental swabs (n = 1). Swabs were collected from various human-contact surfaces (e.g. PC keyboards, PC mice, and door handles) and pooled into one sample with SM buffer solution. All samples were enriched with two mixtures of seven S. aureus strains as bacterial hosts. To verify the presence of bacteriophages, a spot test was performed on the same bacterial hosts used in the enrichment process. Clear plaques were chosen and propagated three times (double-layer agar method) to reach purity. Three out of four samples were positive to spot test, and 10 different clear plaques were purified and amplified to reach a high titer (10^8-10^9 PFU/mL). For preliminary identification, two primer sets were used: targeting resolvase gene (*Herelleviridae*) and major capsid protein gene (*Rountreeviridae*); all 10 phages belonged to the *Herelleviridae* family. The host-range assessment was performed against 127 strains (S. aureus and non-aureus staphylococci). Based on the host range results, the three best-performing phages were selected for further characterization. Stability assays (pH and temperature) showed that all phages were stable at mildly acidic pH (4–5), with stability decreasing toward more basic pH values; none survived at pH [3] They remained stable up to 50 °C, began to lose viability at 60 °C, and did not survive between 70 °C and 100 °C. The three phages also exhibited distinct lengths of their lytic cycles, ranging from 90 to 160 min, with different burst sizes (8–60 PFU/infected cells). To confirm their lytic lifestyle and assess their genetic similarity, transmission electron microscopy and genome analysis will be performed, together with their ability to disrupt biofilm alone or in combination, which is a key feature for phage therapy.

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Oral Communication(s)

INCREASED CARRIAGE OF ESBL-PRODUCING *E. COLI* IN HOSPITALIZED HORSES: FROM ADMISSION TO POST-SURGERY

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The occurrence of zoonotic and multidrug-resistant pathogens represents an ongoing challenge for biosecurity and infection control in veterinary clinics [1]. Equine hospitals have recently been identified as important environments for the emergence and dissemination of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* [2]. These organisms are resistant to most β-lactam antibiotics and to multiple antimicrobial classes, representing significant causes of nosocomial infections in hospitalized horses and a potential public health concern. The aim of this study was to characterize the *E. coli* population in hospitalized horses and to compare animals undergoing elective surgical procedures with horses hospitalized for non-surgical reasons. A total of 60 horses (43 males, 17 females; age range 6 months–20 years) were enrolled, including 48 horses undergoing elective surgery and receiving antimicrobial therapy (cases) and 12 horses hospitalized for medical management without surgery or antimicrobial treatment (controls). Clinical data collected included signalment, surgical variables, anesthesia type and duration, perioperative management, complications, and administered medications. Horses treated with antimicrobials within three months prior to admission or presenting diarrhea at admission were excluded. A total of 120 fecal samples were collected: at admission (T0) and 48–60 hours after surgery or hospitalization (T1). Samples were processed using standard bacteriological methods, and suspected *E. coli* colonies were identified by MALDI-TOF MS. ESBL and AmpC production was assessed using cefotaxime synergy testing with clavulanic acid and cloxacillin according to CLSI guidelines [3]. Genetic relatedness among isolates was evaluated by ERIC-PCR. At admission, ESBL-producing *E. coli* prevalence was 8.3% (5/60). In surgical horses, McNemar's test demonstrated a significant increase in ESBL positivity after hospitalization ($p < 0.001$), with 24/48 initially negative horses becoming positive at T[1] In controls, only 2/12 horses converted to ESBL positivity, without statistical significance. Among all variables tested, the type of anesthesia procedure (standing sedation vs general anesthesia) showed a non-significant association with post-procedure ESBL positivity, though suggesting a possible trend toward higher odds of ESBL positivity in horses undergoing procedures in standing sedation, although this association did not reach statistical significance. ERIC-PCR revealed marked genetic similarity among isolates, suggesting clonal relatedness and possible nosocomial transmission. Although ESBL prevalence at admission was low, hospitalization—particularly when associated with surgery/antimicrobial therapy—appeared to play a major role in ESBL emergence. Molecular findings support hospital-associated transmission rather than independent selection within individual microbiota. Further whole-genome sequencing studies are warranted; however, strict biosecurity and hygiene measures remain essential to limit dissemination of ESBL-producing *E. coli* in equine hospitals.

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Oral Communication(s)

SILENT VISITORS, POTENTIAL RISKS: WILDLIFE ACTIVITY AT POULTRY FARMS REVEALED BY CAMERA TRAPS

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Direct or indirect interactions at the poultry–wildlife interface represent a potential pathway of pathogens introduction into poultry production systems. Given this background, the present study aimed to characterize the spatiotemporal patterns of wildlife activity in the surroundings of poultry houses. A total of eight camera traps were deployed over a one-year period across three commercial layer farms located in a densely populated poultry area in Northern Italy. Time-series analysis, correspondence analysis, and generalized linear models were used to assess daily visitation patterns and identify key drivers of wildlife presence. Overall, 5,978 videos recorded wild birds, 567 wild mammals, and 1,866 domestic animals (cats and dogs). Wildlife activity showed clear seasonal trends, with increased visitation during spring and winter ($p<0.001$). Among mammals, coypus (*Myocastor coypus*) and domestic cats (*Felis catus*) were the most frequently observed species, while Eurasian magpies (*Pica pica*), ring-necked pheasants (*Phasianus colchicus*), and Eurasian collared doves (*Streptopelia decaocto*) dominated among birds. Birds were primarily engaged in foraging activities near poultry houses, suggesting that farm environments may act as attractants for wildlife. These findings highlight the importance to reduce resource availability and environmental attractiveness around poultry facilities. Targeted mitigation measures, including routine inspection of farm premises, prompt removal of feed spills and fallen eggs, and the application of deterrence strategies, may limit wildlife visitation and associated biosecurity risks. Additionally, visual documentation of wildlife incursions may serve as an effective tool to enhance farmer awareness and strengthen biosecurity compliance.

Oral Communication(s)

PERSISTENCE OR REINTRODUCTION? GENOTYPIC INSIGHTS INTO RECURRENT BOVINE TUBERCULOSIS BREAKDOWNS IN ITALY (2023–2025)

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Bovine tuberculosis (bTB) is a zoonosis caused by members of the Mycobacterium tuberculosis complex (MTBC). Since 1995, MTBC infection in Italy has been subject to an eradication plan that has led to the declaration of disease-free status for most of the national territory. Unfortunately, the high environmental resistance of MTBC and the limited sensitivity of diagnostic tests favour the persistence of this agent in infected herds, and a portion of bTB-affected farms experience recurrences in the years following clearing [1].

The aim of this study was to quantify the proportion of bTB breakdowns with a previous history of disease and to assess whether the new episode was linked to the persistence of MTBC within the herd or to a new introduction. Therefore, a comparison was made between genotypes—defined by spoligotyping and by analysing 12 markers using Mycobacterial Interspersed Repetitive Unit–Variable Number Tandem Repeat (MIRU-VNTR) [2]—from strains isolated in the two episodes.

Data on bTB breakdowns and related epidemiological investigations were downloaded from the Animal Disease Information System (SIMAN) of the Ministry of Health's portal (VETINFO). Genotyping data (spoligotype and MIRU-VNTR markers) were obtained from the database held by the National Reference Centre for M. bovis tuberculosis (TBCentro) at the Istituto Zooprofilattico Sperimentale of Lombardy and Emilia-Romagna, Brescia.

Between 2023 and 2025, a total of 1024 bTB breakdowns were reported in Italy, of which 110 (10.7%) occurred in disease-free areas and 22 (2.1%) in buffalo herds. Breakdowns with a history of disease in the previous 5 years numbered 90 (8.8%), while another 239 (23.3%) had been infected in earlier periods. Genotyping data for both episodes were available for only 39 (43.3%) of the breakdowns recurring within 5 years and 99 (41.4%) of those recurring after more than 5 years.

From the comparison of MTBC strains, 60 cases (43.5%) showed a different genotype, suggesting a new MTBC introduction, whereas 78 cases (56.5%) had an identical genotype, supporting the hypothesis of probable MTBC persistence within the herd. This proportion rose to 71.8% (28/39) for breakdowns that recurred within less than 5 years. Among buffalo farms, 10 (45.4%) bTB breakdowns were recurrent, but only two (20%) had a history of disease in the 5 years preceding onset (no strains for comparison).

The lack of strains available for comparison in many episodes limited the results of our study. Where comparison was possible, MTBC persistence in cleared herds was confirmed in more than half of cases, indicating that a previous history of disease is a significant risk factor for the spread of infection in the national cattle population. It is advisable that herds recently reacquiring disease-free status be subjected to enhanced surveillance to prevent the movement of infected animals, that may have remained in the herd due to false-negative diagnostic test results.

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Oral Communication(s)

ANTIMICROBIAL SUSCEPTIBILITY PROFILES OF COMMENSAL AND PATHOGENIC BACTERIAL POPULATIONS FROM ORAL SWABS AND DENTAL CALCULUS IN DOGS WITH ODONTOLITHIASIS

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Periodontal disease represents one of the most prevalent conditions in dogs, with significant local and systemic implications. Indeed, systemic inflammation, possible bacterial implantation in distant organ and even an association with decline in cognitive function have been reported in dogs affected by odontolithiasis [1,2]. The present study aimed to characterize the bacterial populations of the oral cavity and dental calculus in canine patients affected by odontolithiasis and to evaluate their antimicrobial susceptibility profiles. Additionally, the study assessed the reliability of a clinical visual periodontal disease grading system applied in conscious patients compared with the evaluation under general anesthesia.

Dogs affected by odontolithiasis underwent complete clinical examination and dental treatment. Samples for bacterial isolation were collected from two distinct oral niches: the oral cavity, via oral swab, and dental calculus. Bacterial isolates were identified using the MBT Microflex LT/SH system based on MALDI-TOF mass spectrometry, followed by antimicrobial susceptibility testing using the disk diffusion method. Periodontal disease was graded in each patient, always by the same operator, first in consciousness during the clinical evaluation and then during the dental treatment under general anesthesia, applying a previously published visual assessment scale [3]. The Cohen's kappa statistic was applied to evaluate the agreement between the two evaluations.

A total of 33 dogs were included; oral swab and tartar sample were analyzed for each dog. Overall, 94 bacterial isolates belonging to 41 different species were identified. A marked difference between oral and dental calculus microbiota was observed, with only 34% of species shared between the two niches. Among the isolates, 68% were classified as opportunistic, 28% as commensal, and 4% as pathogenic. High levels of antimicrobial resistance were detected, with 90% of isolates resistant to metronidazole, 59% to spiramycin, and 56% showing resistance to both antibiotics. A significant association between periodontal disease grading in conscious and anesthetized patients was identified ($\kappa = 0.37$; $P < 0.0001$), indicating moderate agreement between the two evaluations.

These findings highlight the high diversity of the canine oral microbiome in the presence of odontolithiasis and demonstrate substantial differences between bacterial populations inhabiting the two oral niches investigated. The widespread resistance observed suggests that the empirical use of metronidazole and spiramycin, commonly employed in veterinary medicine to treat periodontal disease, may have reduced their efficacy and contribute to the selection of resistant strains, posing potential zoonotic risks. This novel finding might elucidate the relationships between the oral microbiome and dental calculus formation, as well as guide the development of new potential therapeutic approaches in diseases secondary to oral infections in dogs. Finally, the clinical visual assessment in conscious patients, when performed by experienced clinicians, may provide a reasonably accurate estimation of periodontal disease severity and support appropriate treatment planning.

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Oral Communication(s)

SURVEILLANCE OF ANTIMICROBIAL RESISTANCE (AMR) IN COMPANION ANIMALS: AN EXAMPLE OF COLLABORATION AMONG VETERINARY DEPARTMENTS

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Antimicrobial resistance (AMR) is a major global health threat affecting both humans and animals representing an important One Health challenge. The emergence and spread of AMR in companion animals, particularly dogs and cats, have been driven primarily by antimicrobial use and scarce policies and procedures on the prevention and infection control in veterinary healthcare facilities. Healthcare-associated infections are frequently linked to multidrug-resistant (MDR) bacteria, and contribute to the dissemination of AMR due to the increasing number of susceptible patients and the growing use of invasive procedures in veterinary hospitals. Despite these concerns, European surveillance programmes currently lack coordinated and standardized monitoring systems for AMR in companion animals, representing a significant gap in the European AMR surveillance framework [1]. At the end of 2020, the Department of Veterinary Medical Sciences of the University of Bologna (UNIBO) implemented an AMR surveillance programme at its Veterinary University Hospital (VUH), including passive surveillance on pathogenic bacteria and active surveillance targeting patients (commensal bacteria) and the hospital environment (hospital-acquired bacteria). The aim of the present project is to implement a shared surveillance system, based on a simplified version of the UNIBO programme, across the majority of Italian VUHs to generate standardized data on AMR in companion animals in Italy; to support infection control measures in VUHs to reduce the spread of MDR bacteria; and to promote inter-institutional collaboration through the sharing of approaches and experiences. This collaboration builds on the experience gained at UNIBO and on the pilot testing of the EARS-Vet surveillance network [2]. Several online meetings were held and two questionnaires were distributed to collect information on bacteriological analyses and environmental surveillance activities performed by each VUH. The project will span two years and it will consist of a passive surveillance targeting four bacterial species (*Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Staphylococcus pseudintermedius*). Antimicrobial susceptibility will be assessed against six and seven antibiotic classes for Gram-negative and Gram-positive bacteria, respectively, with epidemiological cut-off values (ECOFFs) setting. Three antibiotic classes (penicillins with β-lactamase inhibitors, fluoroquinolones and third-generation cephalosporins) were selected for antimicrobial consumption surveillance. Active environmental surveillance will include six sampling sessions using a standardized protocol, collecting 25 environmental samples per session. Three Excel templates were developed to standardize data collection, and an agreement document is currently being prepared to formalize the collaboration among participating departments. Data collection has recently started and is still insufficient for meaningful analysis. Nevertheless, this initiative aims to inform the scientific community and highlight how AMR has fostered the creation of a collaborative network among veterinary medicine departments, with the expectation that this network will further expand and promote coordinated action and knowledge exchaning.

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Oral Communication(s)

BLUETONGUE IN SARDINIA: FROM AN EXOTIC TO AN ENDEMIC DISEASE

Ciriaco Ligios

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Invited lecture.

Bluetongue (BT) is a non-contagious infectious disease of ruminants caused by Bluetongue virus (BTV), a double-stranded RNA virus belonging to the genus Orbivirus within the family Reoviridae.

The primary mode of transmission of BTV is via hematophagous midges of the genus Culicoides. To date, 24 "classical" serotypes of BTV have been identified. In recent years, several "atypical" serotypes have emerged, characterized by distinct biological properties, including apparent vector-independent transmission mechanisms.

The first documented outbreak of Bluetongue in Sardinia occurred on August 18, 2000, in the municipality of Pula (Province of Cagliari) and was caused by serotype 2 (BTV-2). Among the various hypotheses regarding the vital introduction, the most widely accepted is that infected Culicoides midges were passively transported to the island by sirocco winds originating from North Africa, particularly Tunisia. This epidemiological pattern highlights the challenges in preventing and controlling the introduction and spread of the disease. As such, traditional control measures—such as restrictions on the movement of susceptible animals—have proven only partially effective.

Indeed, in 2000, despite the immediate implementation of movement bans, BT rapidly disseminated across the island. Within three months, nearly the entire territory was affected, except for some eastern coastal areas. The lack of both direct (e.g., vaccination) and indirect (e.g., vector control) strategies facilitated the recrudescence of the disease in 2001, originating from the eastern regions that had been relatively spared during the previous year.

Following the implementation of vaccination campaigns targeting BTV-2, the disease did not reoccur in epidemic form for several years. However, in 2004, the emergence of serotype 4 (BTV-4) nquired a new vaccination effort in 2005, which included both BTV-2 and BTV-4. Thanks to these immunization campaigns, no major epidemics occurred until 201[3] Additionally, in 2007, the vaccination protocol was extended to include serotype 1 (BTV-1), following the detection of outbreaks in late autumn 2006.

Nonetheless, the lack of consistent and sustained control strategies, combined with reluctance among some livestock producers to participate in vaccination programs, enabled the continued circulation of BTV during the second decade of the 2000s. This culminated in a major epidemic in 2013, primarily driven by BTV-[1] In subsequent years, new serotypes emerged, including BTV-3, initially confined to southern Sardinia.

In 2024, a severe epidemic involving the simultaneous circulation of BTV-3, BTV-4, and BTV-8 confirmed that Bluetongue has become endemic to the region. This scenario underscores the urgent need for annual planning and implementation of integrated control strategies, guided by comprehensive and evidence-based risk assessments.

Decades of Bluetongue endemicity in Sardinia have generated a substantial body of scientific research and epidemiological data that has significantly contributed to the understanding of this disease. Notable findings include:

• the elucidation of infertility mechanisms in rams infected with BTV;

• the demonstration of transplacental transmission of BTV-8 in sheep;

• the identification of pathogenic determinants responsible for the wide range of clinical manifestations observed in infected ovine hosts;

• the experimental sheep-to-sheep BTV transmission using a reproducible wild Culicoides-based model.

Oral Communication(s)

EXPERIMENTAL INDUCTION OF CLINICAL BLUETONGUE DISEASE FOLLOWING SHEEP-TO-SHEEP VIRUS TRANSMISSION USING A WILD *CULICOIDES*-BASED SYSTEM

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Bluetongue (BT) is an infectious but non-contagious disease affecting domestic and wild ruminants, with the most severe clinical manifestations observed in sheep. In this species, BT represents a major health and economic concern. The etiological agent, Bluetongue virus (BTV), is a segmented, double-stranded RNA virus. Transmission occurs via hematophagous midges of the genus *Culicoides*. In Sardinia, 45 *Culicoides* species have been recorded, of which only a subset—-namely *C. imicola, C. obsoletus, C. scoticus, C. pulicaris, C. punctatus,* and two distinct species within the Newsteadi complex—are considered competent BTV vectors.

Here we established and standardized an experimental model that faithfully reproduces natural vector-borne transmission using, for the first time, wild *Culicoides* captured in the field and experimentally infected with BTV by feeding directly on viremic sheep under controlled conditions (Italian Ministry of Health authorization n.o 709/2018-PR and n.o 673/2023- PR).

Preliminary feeding trials identified feeding on the dorsal thoracic region of the sheep,combined with an overnight exposure period, as the most suitable conditions for obtain blood-fed midges.

In transmission experiments, wild-caught nulliparous *C. imicola* midges fed on viremic donor sheep, acquired BTV-1 infection as confirmed by RT-PCR, and were subsequently allowed to feed on naïve recipient sheep at different post-infection intervals. Naïve sheep exposed to BTV-infected *Culicoides* exhibited clinical, virological and pathological features consistent with those observed under field conditions. Specifically, infection was detected in all sheep exposed to infected midges 13 days after the blood meal (6/6), whereas no transmission occurred at shorter post-feeding times.

This versatile model enables future studies on transmission, pathogenesis, epidemiology, and interventions, and represents a significant methodological advance for risk assessment, vaccine testing, and understanding outbreak dynamics in natural host–vector systems. By bridging the gap between artificial laboratory systems and field conditions, it also provides a robust and ecologically relevant platform for investigating arbovirus transmission in natural host–vector interactions.

Oral Communication(s)

CAN THE BODY CONDITION SCORE BE ADOPTED AS A PARAMETER FOR A TARGET SELECTIVE TREATMENT FOR THE CONTROL OF SUBCLINICAL GASTROINTESTINAL NEMATODE INFECTIONS IN SARDA DAIRY SHEEP?

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Targeted Selective Treatments (TST) represent a key strategy to delay the development of anthelmintic resistance (AR) in gastrointestinal nematodes (GIN) of sheep. In Italy, AR has been reported in small ruminants against levamisole, ivermectin, and benzimidazoles, with recent evidence indicating that resistance is continuing to spread [1]. The Body Condition Score (BCS) is a wellestablished criterion for implementing TST in natural infection by non-hematophagous nematodes but the optimal thresholds for different animal categories and production systems remain poorly defined. This study aims to evaluate BCS as a practical TST indicator in Sarda dairy sheep naturally infected with non-bloodsucking nematodes species, by assessing its relationship with fecal egg count (FEC) in sheep across reproductive stages and in replacement ewe lambs.

A total of 2,162 Sarda dairy sheep were enrolled, including ewes at different physiological stages (mid-pregnancy, late pregnancy, mid-lactation) and replacement ewe lambs (5-7 months). Faecal samples were collected for FEC analysis (McMaster technique using a supersaturated NaCl solution (1.2 sg) with an analytical sensitivity of 15 eggs/oocysts per gram – EPG), for the implementation of coprocultures to detect the presence of parasitic genera and BCS was recorded for each animal.

The preliminary parasitological examinations exclusively found GIN eggs, a few *Moniezia* spp. eggs and *Eimeria* spp. oocysts (always less than 100 OPG).

GIN prevalence ranged from 88.5% in ewes at mid-pregnancy to 99.8% in replacement ewe lambs, with intermediate values of 92.2% in ewes at late pregnancy and 93.0% in ewes during mid-lactation. The highest mean FEC was observed in replacement ewe lambs (524.6 EPG). The results of the coprocultures in all animal groups showed *Trichostrongylus* spp. and *Teladorsagia* spp. as the most prevalent nematodes. In all groups, an inverse relationship was observed between BCS and EPG classes, with lower BCS corresponding to higher infection levels.

Based on our results, the threshold for Sarda dairy sheep could be set at ≤ 2.5 for adult ewes during lactation or early pregnancy and at ≤ 3.00 for replacement ewe lambs. No threshold could be established for ewes in late pregnancy, likely due to the increased energy demands of the developing fetus, which may mask the effects of subclinical GIN infections on BCS.

In conclusion, based on the results of our study, according to the literature, we can state that in Sarda dairy sheep, the application of TST based on the BCS, especially in the case of subclinical GIN infections by the genera Trichostrongylus and/or Teladorsagia, can be considered an important approach for the prevention of the appearance/limitation of the AR, leading to a reduced use of the anthelmintic and the increase in the refugia. However, to achieve definitive validation, further field testing is required to accurately assess the practical effects of this TST scheme based on BCSs.

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Oral Communication(s)

EVALUATION OF THE ANTHELMINTIC EFFICACY OF *OPUNTIA FICUS-INDICA* PEEL SILAGE SUPPLEMENTATION IN SHEEP NATURALLY INFECTED WITH GASTROINTESTINAL NEMATODES: A PRELIMINARY IN VIVO STUDY

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Gastrointestinal nematodes (GINs) represent a major constraint to small ruminant production worldwide, causing economic losses through impaired growth, reduced milk yield, increased infection susceptibility and mortality. The global spread of anthelmintic resistance has compromised conventional chemotherapeutic control, prompting interest in complementary management strategies [1]. Agro-industrial by-products rich in bioactive compounds are increasingly investigated as nutraceutical tools within integrated parasite management programmes. Among these, *Opuntia ficus-indica* peel silage is a promising candidate due to its content of phenolic acids, flavonoids and condensed tannins, associated with antiparasitic, antioxidant and immunomodulatory properties [2].

This preliminary study evaluated the effects of dietary supplementation with *O. ficus-indica* peel silage on faecal egg count reduction (FECR) and hematological parameters in lactating sheep naturally infected with GINs. Sixteen animals were allocated to four groups: a control group receiving 450 g/day/head of concentrate (CTR) and three experimental groups fed increasing amounts of silage: Group A (300 g concentrate + 500 g silage), Group B (150 g + 1,000 g), and Group C (1,500 g silage only). Animals were monitored for six weeks, with faecal and blood samples collected every 15 days at baseline (T0) and at three subsequent time points (T1, T2, T3).

Bayesian paired analysis revealed modest progressive reductions in faecal egg shedding in silage-supplemented groups. Between T0 and T1, Group A showed an estimated FECR of 9% (95% uncertainty interval [UI]: 0–19%), Group B 13% (2–21%), and Group C 15% (5–24%). From T0 to T2, FECRs reached 12% (1–22%) for Group A, 14% (4–23%) for Group B, and 15% (5–25%) for Group C. From T0 to T3, FECRs were 18% (6–30%) for Group A, 18% (9–28%) for Group B, and 21% (11–29%) for Group C. The CTR group showed a reduction of 13% at T3, indicating that natural variability in egg excretion may partly account for observed changes. Credible intervals were wide and largely overlapping across groups; however, slightly higher reductions in group C suggest a possible dose- and time-dependent trend. Hematological parameters (RBC, hemoglobin, hematocrit) remained stable throughout, indicating no adverse systemic effects.
Overall, dietary supplementation with *O. ficus-indica* peel silage was associated with modest reductions in faecal egg shedding over time, although these effects remained modest and below thresholds generally associated with meaningful anthelmintic activity. The temporal pattern observed, together with slightly higher reductions in groups receiving larger amounts of silage, may suggest a mild nutraceutical modulation of host–parasite interactions rather than a direct anthelmintic effect. Further studies with larger sample sizes, longer monitoring and additional parasitological indicators are needed to clarify the consistency, dose–response relationships and practical relevance of these effects [3].

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Oral Communication(s)

EPIDEMIOLOGICAL UPDATE ON GASTROINTESTINAL NEMATODES OF DAIRY GOATS IN NORTHERN ITALY

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Gastrointestinal nematodes (GINs) represent a major constraint to goat farming in Italy, affecting animal health, productivity, and farm sustainability due to their widespread occurrence and increasing anthelmintic resistance. To update current knowledge on GINs in goats from northern Italy, an epidemiological survey was conducted on 23 grazing farms in Lombardy between May 2023 and April 2024 (provinces of Bergamo, Brescia, Lecco, Como, Sondrio, Milan, Pavia, and Varese). One pooled faecal sample per productive category was collected on each farm across three seasons (spring, summer, autumn), yielding 313 samples. All samples were analysed using the FLOTAC technique [1] to detect parasitic elements and quantify strongyle eggs (EPG). Coproculture and Baermann techniques were performed to obtain and identify infective larvae (L3) [2-3], allowing the characterization of nematode genera and species. Data on herd management and animal-level parameters (BCS, milk production, coat condition, faecal score, breed, productive category) were recorded. A composite biosecurity score was developed and evaluated alongside animal variables to assess associations with parasite burden. For statistical analyses, herds were considered individually, whereas for descriptive purposes the provinces of origin were grouped into three macro-areas: Area 1 (Northern: Sondrio, Como, and Lecco; n = 5 farms), Area 2 (Western: Varese, Milan, and Pavia; n = 9 farms), and Area 3 (Eastern: Bergamo and Brescia; n = 9 farms). Mean strongyle egg count was 983 ± 1247 EPG (range: 0–8744), with an overall prevalence of 91.69% (287/313 samples). Area 2 showed the highest mean EPG (1248 ± 1325), while Areas 1 and 3 had lower but comparable values (890 ± 1083 and 957 ± 1260 EPG). *Haemonchus contortus* was the most prevalent species (33.4%), followed by *Teladorsagia circumcincta* (22.04%), *Trichostrongylus* spp. (19.85%), and *Oesophagostomum* spp. (19.25%); *Cooperia* spp. (3.74%) and *Chabertia ovina* (1.74%) were less frequent. *H. contortus* was the predominant species in both Areas 2 and 3, whereas *T. circumcincta* was most common in Area [1] Seasonally, parasite burden was lowest in spring (786 ± 1145 EPG), increased in summer (987 ± 1021), and peaked in autumn (1467 ± 1567). Analysis of variance performed on log-transformed EPG counts showed that biosecurity score ($p < 0.0001$), production category ($p = 0.0079$), daily milk yield ($p < 0.0001$), and season ($p < 0.0001$) had a significant effect. These findings confirm the high prevalence of GINs in goat farms in northern Italy and highlight the importance of improved management and biosecurity strategies for effective parasite control.

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Oral Communication(s)

ADAPTED TODAY, VULNERABLE TOMORROW? GENOMIC LANDSCAPES OF ITALIAN GOAT BREEDS

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Italian goat breeds represent a unique reservoir of livestock biodiversity shaped by environmental heterogeneity, historical trajectories, and long-standing management practices. Predominantly raised in extensive and semi-extensive systems, these populations are directly exposed to local conditions, favouring the development of adaptive traits while simultaneously increasing their sensitivity to ongoing climate change.

We analysed genome-wide SNP data from 685 goats across 31 Italian populations, integrating temporal comparisons with samples collected approximately two decades ago (Italian Goat Consortium [1]) and a landscape genomics framework. This approach allowed us to jointly investigate how genomic diversity reflects geography and management, how it has changed over time, and how it relates to environmental adaptation and future vulnerability.

Genomic diversity and structure closely mirrored geographic distribution, with clear differentiation between northern and central–southern populations, the latter displaying higher admixture levels. Temporal analyses showed overall stability of populations' genomic composition but also revealed contrasting trajectories among breeds, including increased genetic homogenisation alongside rising inbreeding or, on the opposite side, shifts towards more admixed genomic backgrounds. These patterns highlight the impact of recent management and breeding practices on the genomic integrity of local populations.

To investigate adaptation, we applied complementary genotype–environment association approaches (LFMM and pRDA), identifying hundreds of candidate loci associated with environmental gradients. The overlap between methods supported the robustness of the signals and the polygenic nature of adaptation and highlighted pathways potentially linked to adaptation and fitness, including immune response, oxidative stress, metabolism, and morphology.

Future vulnerability was assessed through genomic offset under alternative climate scenarios. High offset values were heterogeneously distributed across Italy, and mainly concentrated along the northern Alpine fringe, the eastern Po Valley, and the Gargano area in Apulia. These findings support that some of the regions projected to undergo the most rapid environmental change correspond to areas where populations may be less genomically prepared to cope with these shifts, pointing to potential mismatches between current adaptive variation and future conditions [2].

Comparing past and present genomic patterns helps disentangle processes that maintain diversity from those that may erode it, while landscape genomics provides a forward-looking perspective on adaptive capacity. This integrated view provides a framework to distinguish resilient from potentially vulnerable populations and to guide conservation and breeding strategies aimed at preserving biodiversity while sustaining resilience under climate change [3].

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Oral Communication(s)

SHIFTING GENOMES, CHANGING FLOCKS: RECENT DYNAMICS OF ITALIAN SHEEP BIODIVERSITY

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Understanding how livestock biodiversity is changing over short timeframes is essential to support effective conservation. Italian sheep provide an excellent case study, combining high breed diversity with a wide range of environments, farming systems and breeding histories. Here, we integrated demographic records and genomic data to investigate recent dynamics of Italian sheep breeds.

Demographic data from the national herd book (2010–2024) indicate that most breeds are experiencing contraction in population size and/or number of farms, while only a minority show stable or positive trends; accordingly, the majority of populations are currently classified as at risk under FAO criteria. This heterogeneity points to multiple underlying drivers, including management, economic and policy factors, rather than a single common pattern.

To investigate how these trends are reflected at the genomic level, we analysed SNP data from 816 individuals belonging to 34 Italian populations and compared them with 8 foreign breeds. Despite a clear geographic structuring consistent with historical differentiation, breeds show marked differences in genetic diversity, inbreeding levels, and effective population size, with several populations displaying signals of genetic erosion.

A temporal comparison with populations sampled about 20 years ago (Biovita project [1]) reveals that, although breed identity is largely maintained, many populations have undergone detectable genomic changes. These include increased inbreeding and/or shifts in ancestry composition, often associated with recent admixture. In particular, the current genomic background of Nera di Arbus and Gentile di Puglia shows substantial contributions from Sarda and Merinizzata Italiana, respectively. Local ancestry inference in these breeds indicated that introgression often affects genomic regions associated with breed-specific traits, suggesting that it may alter breed typicality while introducing advantageous alleles.

To further investigate temporal changes, we performed Fst-based comparisons between past and present populations, identifying genomic regions showing the highest differentiation over time. These include candidate genes and QTLs related to production, fitness, and morphological traits, suggesting that both management and selection have contributed to recent genomic shifts.

At the same time, environmental changes appear to play a role in shaping recent genomic patterns. Associations between genomic differentiation and variation in climatic variables across breeding ranges - particularly precipitation- support the presence of ongoing adaptive processes acting over relatively short timescales.

Overall, these results indicate that Italian sheep biodiversity is currently undergoing rapid and heterogeneous change. Rather than representing static genetic resources, these breeds should be viewed as dynamic populations, whose future will depend on the balance between demographic trends, management decisions, introgression, and environmental pressures [2].

Acknowledgements: Associazione Nazionale della Pastorizia (CHEESR and SHEEP&GOAT projects), especially Alessio Negro and Silverio Grande; Viviana Floridia (Messina University); Francesca Maria Sarti (Perugia University).

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Oral Communication(s)

CAN BREED CHOICE CONTRIBUTE TO SUSTAINABLE SHEEP FARMING? A COMPARISON BETWEEN SOPRAVISSANA AND COMISANA SHEEP IN CENTRAL ITALY

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Sustainability of pastoral systems relies on the balance between animal performance and efficient use of natural resources. In this context, breed choice may be a key factor influencing the economic, environmental and social sustainability of farming, while ensuring animal welfare. However, information on breed-related differences in adaptability and resilience remains limited. This study aimed to evaluate the contribution of breed choice to sustainable sheep farming by comparing two different sheep breeds under the same management conditions. The study was conducted in Central Italy during one grazing season (June–October 2023) on 40 adult ewes (20 Comisana and 20 Sopravissana, 10 lactating and 10 dry each) managed in an extensive system. Sample size was determined a priori through power analysis ($\alpha=0.05$; power=0.9), indicating adequacy for both LMM and ANOVA models. The protocols agreed with Legislative Decree No. 146. Animals were equipped with GPS data loggers to assess movement and foraging behavior. Body Condition Score (BCS) and milk production (MP) were recorded every two weeks. Behavioral data were segmented to only include movements during foraging times [1]. Movements were classified into four categories based on speed (S) and turning angle (A): high S-high A, low S-high A, low S-low A and high S-low A that was interpreted as traveling, while foraging was defined as the interval between the two main traveling phases. Foraging tracks were analyzed using generalized additive mixed models (GAMs) with logtransformed distance as the dependent variable, breed and physiological state as fixed effects, Individual ID as a random effect, and time as smooth terms. BCS differences were assessed using a linear mixed-effects model (LMM) with breed and physiological state as fixed factors. Two one-way ANOVAs tested breed effects on MP and on the decline of milk yield over time. The two breeds showed similar movement patterns during foraging, indicating comparable spatial use of pasture and resources. MP did not differ between breeds ($p=0.05$), nor did its seasonal decline during the season ($p=0.53$), suggesting comparable productive performance under the studied conditions. Instead, marked differences emerged in body condition: Sopravissana ewes maintained significantly higher BCS values than Comisana ewes throughout the season ($p<0.001$). Although Comisana is a productive breed and higher MP is expected, the observed differences are not fully explained by MP alone but may reflect breed-related differences in robustness and resource-use efficiency, as Sopravissana lactating ewes maintained significantly higher BCS than Comisana dry ones. Further studies are needed to better understand the mechanisms underlying these differences. Maintaining adequate body reserves under extensive conditions is a key indicator of resilience, linked to animal welfare, reproductive efficiency, and reduced need for external inputs such as supplementation and veterinary care. The higher BCS observed in Sopravissana sheep may reflects a greater adaptability to local environmental constraints. These findings suggest that breed choice can represent a relevant factor in the sustainability of sheep farming systems, supporting low-input management, enhancing animal robustness, and contributing to the long-term viability of pastoral systems in Mediterranean environments.

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Oral Communication(s)

MORPHOLOGICAL CHARACTERIZATION AND PRELIMINARY ASSESSMENT OF GENOMIC ASSOCIATIONS OF THE NEWLY RECOGNIZED MASCARUNA GOAT BREED

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Goats are a key livestock resource in Mediterranean agricultural systems, particularly in marginal areas where local breeds play a key role in economic, environmental, and cultural sustainability. In this context, the recently recognized Mascaruna (or Comune di Sicilia) goat represents an emblematic example of a local genetic resource that is still poorly characterized. The studied breed is a small local population with an estimated census size of approximately 500 animals, and the 107 studied individuals represent about 21% of the entire living population. In-depth studies are needed to describe its phenotypic and genetic characteristics, thereby supporting conservation and enhancement programs. In this context, increasing the sample size was constrained by the limited availability of animals and by the conservation status of the breed. The aim of this study was not to provide definitive causal evidence, but to perform an exploratory genome-wide association analysis (GWAS) to identify genomic regions potentially associated with the morphometric measurements under investigation. The results should therefore be interpreted as preliminary signals requiring validation in future studies or in additional related populations when available.

A total of 107 goats from 12 farms were analysed for quantitative morphological traits such as Height at Withers (HW), Rump Height (RH), Heart Girth (HG), Chest Depth (CD) and two derived indices: the Thoracic Conformation Index (TCI) and Anamorphosis Index (AI). The same animals were genotyped using the Illumina GoatSNP60 BeadChip. Descriptive statistics revealed that HG, CD, TCI and AI were normally distributed, whereas HW and RH required log-transformation due to significant deviations from normality. A clear sexual dimorphism emerged, with males significantly ($p < 0.0001$) larger than females for almost all analyzed traits, except TCI. Heritability estimates highlighted a strong genetic component for some indices, particularly thoracic conformation, and moderate values for other traits, suggesting a variable influence of genetic factors.

Following quality control procedures, 50,245 SNPs and 107 animals passed the filtering criteria and were retained for downstream analyses. Population structure assessed through MDS highlighted clustering by farm. GWAS was performed using the mixed linear regression model implemented in GCTA software, incorporating the genomic relationship matrix (GRM) to account for relationships among animals and adjusting for sex. Moreover, multiple testing correction was applied using Bonferroni thresholds. The GWAS identified 13 SNPs significantly or suggestively associated, especially with HW and TCI, while no significant associations emerged for other traits. The genomic regions identified in this study harbor 17 candidate genes, including DVL2, PRDM4, and GAS7, involved in growth, muscle development, metabolism, and adipogenesis, further supporting the complex genetic architecture underlying morphological traits. Overall, this work highlights how the phenotypic variability of the Mascaruna goat derives from the interaction between genetic heritage and traditional breeding practices, providing the first integrated information on the morphology and genomics of this population. The results represent a fundamental knowledge base for future conservation and selection strategies, contributing to the protection and value of a local genetic resource.

Oral Communication(s)

LANDSCAPE SCALE RESPONSES OF WOODY AND HERBACEOUS VEGETATION TO VARIABLE GOAT STOCKING RATES IN A MEDITERRANEAN MOSAIC

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Grazing by domestic herbivores is a major ecological driver in Mediterranean marginal landscapes, yet quantitative evidence on how goat stocking rates influence both diet composition and vegetation dynamics remains limited. Where shrub–grassland mosaics are increasingly threatened by land abandonment, goats may serve as an effective biotic tool for maintaining structural heterogeneity and controlling woody encroachment. This study aimed to determine how different stocking rates of goats affect seasonal diet selection and the structural and functional responses of woody and herbaceous vegetation.

The experiment was conducted over three consecutive years in a 30 ha hilly area composed of deciduous shrubland/woodland and grassland. Thirty nine goats were assigned to continuous set stocking treatments at low (1.7 head/ha), medium (3.3 head/ha), and high (4.5 head/ha) stocking rates. Diet composition was quantified using n alkane analysis and faecal cuticle microhistology. Vegetation responses were monitored seasonally through measurements of sward biomass, botanical composition, green:dead ratio, sward height, woody plant height, shoot height, browse line, browsing intensity, and basal sprout density. Data were analysed using General Linear Models including month, area, and their interaction as fixed effects.

The two diet estimation techniques produced consistent results at the plant category level. Goats exhibited pronounced seasonal shifts: woody species dominated winter diets (45–56% of identified fragments), whereas grasses were preferentially selected in spring due to increased availability and superior nutritive value. *Hedera helix*, *Rubus fruticosus*, *Quercus* spp. and *Ulmus minor* were the most consistently consumed woody species. More than 90% of woody plants were browsed in all grazed areas. Medium and high stocking rates significantly increased browse line height and browsing intensity below 150 cm, while browsing above this threshold remained largely unaffected. Despite sustained defoliation, spring regrowth compensated for biomass removal, preventing long term suppression of woody strata. Herbaceous vegetation displayed the typical bimodal productivity of Mediterranean climates; sward biomass increased over the study period but was inversely related to stocking rate. Botanical composition remained largely stable, with significant season × area interactions restricted to Geraniaceae, Labiatae and Leguminosae.
Under the tested conditions, goat grazing exerted substantial yet non degradative pressure on woody vegetation and did not reduce herbaceous biodiversity. The selective feeding behaviour of goats — particularly their preference for *H. helix* and *R. fruticosus* — indicates that calibrated stocking rates can be strategically employed to modulate vegetation structure and enhance landscape heterogeneity in Mediterranean marginal environments. These findings support the integration of goats into targeted grazing frameworks for landscape management and biodiversity conservation.

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Oral Communication(s)

IMPACT OF MICROPLASTICS AND BISPHENOL AF ON RUMEN ECOLOGY: INVITRO STUDY USING CONTAMINATED FORAGE

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Microplastics (MPs) and plasticizers are emerging pollutants, very damaging to human and animal health. The soil pollution by plastics is worsened by the presence of plasticizers, such as bisphenols (BPs). These additives are used in the plastic industry to improve the performance of the synthetic polymers, providing elasticity, strength, or resistance, and are colorless and odorless. The plasticizers are not linked to plastics by covalent bonds and, hence, their release in the environment easily occurs when plastics are broken down into MPs. This study aimed to investigate the effect of a forage contaminated with MPs and Bisphenol AF (BPAF) on rumen ecology using an in vitro trial in a thermostatic chamber for 72h, using rumen fluid collected post mortem from a slaughtered ewe as the inoculum. The experiment was conducted using a controlled pot-trial approach in which Medicago sativa (seeds were sourced from the same seed pool) was grown in pots using soil contaminated with MPs (polyethylene terephthalate, PET) and BPAF according to the following design: control (no MPs and no BPAF); presence of MPs and no BPAF; no MPs and presence of BPAF; presence of MPs and BPAF. Plants grown under these conditions were then incubated with rumen fluid in separated tubes to simulate the rumen fermentation.

Analysis of the volatile fatty acid profile indicates that BPAF treatment leads to an increase in acetic acid concentration. Regarding propionic acid, an increase is observed in the presence of MPs. Biohydrogenation processes were slowed in the presence of MPs and BPAF. Iso-acids (C13:0, C14:0, and C15:0) are unaffected by the presence of pollutants, confirming substantial stability. Beta diversity, calculated based on the Bray-Curtis dissimilarity index, showed a separation between the control group and the groups containing pollutants. The core microbiome remained unaffected. Methanobrevibacter, Methanomassiliicoccus Methanosphaera increased with the presence of pollutants.

Oral Communication(s)

ASSOCIATION BETWEEN SOMATIC CELL COUNT, MILK QUALITY AND TECHNOLOGICAL TRAITS IN MID-LACTATION DAIRY EWES

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Somatic cell count (SCC) is a key indicator of udder health and milk quality in dairy small ruminants [1]. In sheep, SCC is influenced by several physiological and management factors, including stage of lactation, milking practices, and farm conditions, and is associated with changes in milk composition, quality traits, and not only local mammary inflammation but also systemic immune responsessystemic immune responses [2]. The aim of this study was to evaluate the association between SCC levels and hematological, biochemical, and milk coagulation traits in mid-lactation dairy ewes. The aim of this study was to evaluate the association between SCC levels and hematological, biochemical, and milk coagulation traits in mid-lactation dairy ewes. The study included 80 crossbred dairy ewes (Valle del Belice × Pinzirita) in mid-lactation (90–150 days in milk), with an average milk yield of approximately 1.0 kg/day. Animals were classified into SCC classes (low, medium, high), defined as follows: low (90.4 ± 25.7 ×10³ cells/mL), medium (258.8 ± 90.7 ×10³ cells/mL), and high (5520.2 ± 8307.8 ×10³ cells/mL). Milk samples were collected during the morning milking. Hematological analyses were performed by a specialized laboratory (Biogene). Milk analyses were carried out at the DaliLab laboratories (Department of Veterinary Sciences) using MilkoScan (milk composition), Fossomatic (somatic cell count), and Formagraph (coagulation properties). Recorded variables included milk composition (fat, protein, casein, lactose, urea), acidity, coagulation properties (r, K20, A30), and hematological parameters (total white blood cells and differential counts). The effect of SCC class was assessed using one-way ANOVA ($P < 0.05$). SCC significantly affected several parameters. Increasing SCC was associated with higher white blood cell counts (low: 9.8 ± 2.8; medium: 10.7 ± 3.4; high: 11.9 ± 4.6 ×10³/µL), lymphocyte absolute counts (low: 5.3 ± 1.7; medium: 5.9 ± 2.1; high: 6.8 ± 2.7 ×10³/µL), and basophil percentage (low: 0.36 ± 0.31; medium: 0.42 ± 0.35; high: 0.58 ± 0.52 %). Milk acidity increased with SCC (low: 3.55 ± 0.45; medium: 3.72 ± 0.51; high: 3.94 ± 0.63 °SH/50 mL). Coagulation properties worsened as increasing SCC, with a longer r time (low: 12.8 ± 3.3; medium: 14.3 ± 3.6; high: 17.3 ± 6.5 min), while a30 (curd consistency measured 30 minutes after rennet addition) decreased (low: 56.4 ± 7.0; medium: 58.5 ± 8.1; high: 52.0 ± 25.7 mm). No clear differences were observed for k20 (curd firming time). Milk composition had moderate variation; notably, urea displayed a non-linear pattern, increasing from low (28.36 ± 27.19 mg/dL) to medium SCC classes (43.00 ± 42.80 mg/dL) and decreasing in the high SCC group (23.83 ± 21.35 mg/dL), while protein, casein, and fat did not show consistent trends. In mid-lactation dairy ewes, SCC is associated with systemic immune activation and measurable deterioration of milk technological properties, particularly coagulation traits. These findings support the interpretation of SCC as an integrated indicator of both mammary and systemic status, with potential impact on cheese yield and processing efficiency in dairy sheep systems.

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Oral Communication(s)

POMEGRANATE CO-PRODUCTS AS A SUSTAINABLE STRATEGY TO CONTROL GASTROINTESTINAL NEMATODES IN SHEEP: EVALUATION OF *IN VITRO* AND *IN VIVO* EFFICACY

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Gastrointestinal nematode (GIN) infections represent one of the main health constraints affecting small ruminant production systems worldwide, particularly under grazing conditions. Control of these parasitic infections has traditionally relied on the repeated use of synthetic anthelmintics; however, the increasing spread of anthelmintic resistance (AR) is progressively undermining the long-term efficacy and sustainability of this approach. Consequently, there is growing interest in complementary and sustainable control strategies, including the use of plant-derived bioactive compounds and agro-industrial co-products rich in plant specialised metabolites.

The present study aimed to evaluate the anthelmintic potential of pomegranate (*Punica granatum*) co-products in sheep through a combined *in vitro* and *in vivo* approac in southern Italy. Initially, aqueous extracts obtained from pomegranate peels were chemically characterised and tested against key developmental stages of GINs, namely eggs and infective third-stage larvae (L3) of the nematodes *Trichostrongylus colubriformis* and *Haemonchus contortus*. The Egg Hatch Assay (EHA) and the Larval Exsheathment Inhibition Assay (LEIA) were performed to assess biological activity.

Subsequently, a field trial was conducted on a naturally infected sheep farm to evaluate the in vivo efficacy of pelleted feed containing pomegranate co-products. Thirty adult sheep were allocated into three homogeneous groups (n = 10 each): untreated control, eprinomectin-treated group (positive control) and pomegranate pellet group. Faecal egg counts (FEC) were monitored over time using the Mini-FLOTAC technique (detection limit: 5 EPG), and treatment efficacy was assessed through the faecal egg count reduction test (FECRT).

In vitro results demonstrated a clear inhibitory effect of pomegranate extracts, particularly on larval exsheathment, suggesting strong interference with parasite establishment mechanisms. *In vivo*, sheep receiving pomegranate pellets showed a progressive reduction in egg excretion compared with controls, although the effect was less rapid and pronounced than that observed with eprinomectin.

Overall, the findings support the potential role of pomegranate co-products as nutraceutical tools within integrated and sustainable parasite control programs. While not intended to replace conventional anthelmintics, their use may contribute to reducing parasite transmission and lowering drug selection pressure.

Oral Communication(s)

CENTRES OF EXCELLENCE SUPPORTING THE SHEEP AND GOAT INDUSTRIES IN ITALY

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The Istituti Zooprofilattici Sperimentali (IIZZSS) host the Reference Centres supporting the sheep and goat sector. These include National Reference Centres (NRCs) and Regional Reference Centres, which provide high-level technical and scientific support on specific diseases and/or production chains.

Among the NRCs, the National Reference Centre for Sheep and Goat Mastitis (C.Re.N.M.O.C.), based at the IZS della Sardegna "G. Pegreffi," coordinates the surveillance of mastitis, developing advanced diagnostic protocols for the control of key pathogens and the monitoring of antimicrobial resistance.

The National Reference Center for the Study of Retroviruses Related to Infectious Diseases in Ruminants (CEREL) is based at the IZS dell'Umbria e delle Marche "Togo Rosati" and is specialised in small ruminant lentivirus infections (SRLV). It achieves this through molecular and serological diagnosis, as well as genetic characterisation of the viruses. CEREL collaborates with the Specialised Centre for Small Ruminant Pathology (CeSPaPiRu), which acts as a multidisciplinary synthesis hub, providing specialised diagnostic support and coordinating field investigations on emerging and complex diseases.

The National Reference Centre for the Quality of Sheep and Goat Milk and Dairy Products (C.Re.L.D.O.C.), based at the IZS Lazio e Toscana "M. Aleandri", standardises technological and hygiene parameters for sheep and goat milk to ensure dairy product safety. By integrating research and technical assistance, the centre promotes high-quality standards and consumer health protection across the entire production chain.

The technical excellence framework supporting the sector is complemented by the academic contribution of the Parasitosis Monitoring Centre (CREMOPAR), which is part of the Università degli Studi di Napoli Federico II. Working closely with the National Reference Centres, CREMOPAR serves as the national hub for the surveillance and control of parasitic diseases in sheep and goat farms. Through advanced diagnostics and monitoring programmes for anthelmintic resistance, the centre provides crucial technical support to farmers, promoting innovative and sustainable control strategies that integrate animal health and production safety activities coordinated by the IIZZSS.

The activities carried out by the NRCs and the CREMOPAR are synergistic and aim to optimize the production chain. Other key activities include research, the organization of specific "Proficiency Tests" to standardize laboratory analytical performances, scientific publishing (particularly in high-impact factor journals), and continuous technical outreach specifically targeting livestock farmers and technicians.

The integration of udder health, lentivirus infections monitoring, parasitosis management, and the qualitative improvement of milk and dairy production promotes animal welfare and veterinary public health and sectoral sustainability. This approach contributes to strengthening the competitiveness of the sheep and goat supply chain in both national and international markets.

Oral Communication(s)

CLINICAL AND SIMULATION LAB FOR THE DEVELOPMENT OF CLINICAL SKILLS IN VETERINARY REPRODUCTION COURSES: WHERE DO WE STAND?

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The opportunity to perform hands-on procedures on clinical tranining simulators or mannequins allows students to understand all the necessary steps, make mistakes, and repeat procedures without the risk of causing harm to live animals. The reduction of stress associated to the principle “never the first time on a live animal” enhances student confidence during the learning process, particularly in disciplines where the effective acquisition of clinical manual skills, ranging from minimally to highly invasive, is essential. The introduction of simulated clinical activities is intended to gradually prepare students to face situations they will encounter first during clinical rotations and subsequently in professional practice.

The aim of this presentation is to share the process that led to the development of a clinical simulation laboratory (Skills and Simulation Lab) for students enrolled in the Veterinary Medicine degree program and other undergraduate programs at the Department of Animal Medicine, Production and Health of the University of Padova.

The facility has been organized into two areas. One replicates a consultation room and a surgical room, while the other, dedicated to group work and peer-observation, is separated from the previous environment by a movable curtain and a one-way mirror. Additionally, there are two microscopy stations and a computer workstation, with the possibility of projecting onto a monitor. Numerous mannequins specifically designed for veterinary medicine have been acquired. The project was funded between 2019 and 2024 through a University grant specific for teaching activities.

The laboratory became operational in the 2022/23 academic year and has enabled the implementation of training activities without the use of live animals. Specific activities include clinical case discussions, simulation of veterinarian–-client communication, suturing exercises through suture pads, venous access procedures, cardiopulmonary resuscitation, and tracheal intubation. Obstetric and gynecological skills training on breeding models focuses on rectal palpation, cervical and deep insemination, uterine biopsy, and the management of common dystocia. In bovine models, in addition to the aforementioned procedures, embryo collection is also practiced. In canine models, the focus is on vaginal and transcervical artificial insemination, as well as parturition and obstetrical maneuvers.

The main challenges encountered concern restrictions on access to the laboratory for safety reasons (limited number of students and restricted access for animals) and the integration of these activities within the formal curriculum. Identifying appropriate curricular placement for these activities requires ongoing discussion at both local and national levels. Furthermore, it is essential to establish strict protocols and learning objectives to be shared with students, in order to ensure the correct acquisition of procedures, with careful attention at each step to potential risks and safety measures.

Oral Communication(s)

ENHANCING CLINICAL COMPETENCE IN VETERINARY EDUCATION: INTEGRATION OF OSCE-BASED LEARNING AND INTERACTIVE TOOLS IN CLINICAL REPRODUCTION TRAINING

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This study aimed to develop and implement an innovative, student-centered educational approach for veterinary intern and clinical-year students by integrating Objective Structured Clinical Examination (OSCE)-based learning with interactive and feedback-driven teaching methods. The system was designed to move beyond traditional lecture-based education and to promote active learning, clinical reasoning, and self-assessment. Three core components were introduced: QR code–supported educational posters, an Artificial Insemination (AI) key concept TABOO-based activity, and structured student feedback. Twelve clinical topics in veterinary reproduction were covered, including andrological examination, vaginal cytology, artificial insemination techniques in different species, sperm evaluation, ultrasonographic reproductive assessment, cryptorchidism, castration indications, and veterinarian-client communication. Posters were prepared bilingually with concise content and QR codes linking to instructional videos, enabling students to access practical demonstrations and reinforce learning outside the classroom. Students were evaluated through OSCE stations under time constraints using simulated clinical scenarios requiring decision-making across examination, diagnosis, and treatment planning. In addition, students were expected to interpret estrous cycle stages, perform and evaluate vaginal cytology, and identify relevant cellular components. In addition, a TABOO-based activity, inspired by the word-association game “Taboo,” was used to reinforce key reproductive concepts through peer interaction. In this activity, students explained clinical or reproductive terms without using predefined restricted keywords, encouraging active recall, communication, and conceptual understanding. Written and verbal feedback collected from students indicated increased engagement, improved understanding of clinical procedures, and enhanced ability to interpret and discuss clinical cases. The iterative feedback process also contributed to refinement of educational materials. Overall, the implementation of an OSCE-based and interactive educational model significantly improved students’ clinical competence, communication skills, and confidence in veterinary reproductive practice, highlighting the value of structured, student-centered learning environments in clinical veterinary education.

Oral Communication(s)

TRAINING SIMULATOR FOR LEARNING ASSISTED REPRODUCTION TECHNIQUES, OBSTETRIC AND GYNAECOLOGICAL SURGERY IN VETERINARY MEDICINE

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Simulators for surgery, laparoscopy, microsurgery and gynaecology recreate conditions that facilitate the learning and practice of various techniques whilst respecting animal welfare and adhering to the 3Rs principle: Replacement, Reduction and Refinement also improving the conditions of the animals used for educational purposes. Simulators enable students to learn various surgical skills and techniques increasing the quality of procedures they will perform once graduate [1].

Simulators for bovine and equine gynaecology currently available on the market are devices consisting of stands containing rubber or silicone replicas of the genital systems of cows and mares. Their price could range from € 5.000,00 up to € 40.000,00 they require appropriate space and the replacement of damaged or worn components. However, they are not entirely realistic, and cannot guarantee practicing of most of the clinical procedures and surgical techniques usually carried out on live animals [2,3].

In the present study a patented support/simulator (cost-effective, foldable, washable, easily transportable) for the practice of procedures related to assisted reproduction techniques and surgical techniques in the field of veterinary obstetrics and gynaecology in horses and cattle is described. The invention consists of a series of metal tubes which, by means of movable connectors, are arranged to form a 'framework' on which, using special clamps (Backhaus surgical forceps or wood clamps), the female genital organs of a cow or mare (ovaries, uterus, cervix, vagina, external genitalia) can be held in situ in a position similar to the natural one.

The device enables the practice of the clinical procedures and surgical techniques performed by the veterinary gynaecologist in the stable (clinical examination, ultrasound, uterine biopsies, artificial insemination, uterine lavage, episiotomy, genital tract surgery).

Whilst a rubber simulator can be used to perform transrectal palpation of the genital tract and artificial insemination, the proposed system allows for a range of other clinical and, above all, surgical procedures: transvaginal palpation and assessment of the cervix, ultrasound of the genital tract, bladder catheterization, deep uterine insemination, uterine biopsy, uterine lavage, catheter placement for embryo retrieval, embryo transfer, buhner suture, caslick suture, perineoplasty, repair of first-, second- and third-degree post-partum lacerations, fetal assessment and sexing, swabs for the diagnosis of equine contagious metritis in mares, surgical urethral dilation.

The practical skills and techniques that can be learnt and performed are significantly superior to those that can be practiced using simulators currently available on the market. The ability to perform these techniques using the proposed device represents a huge advantage in terms of training graduates and acquiring 'day-one skills', as well as for post graduate students, who could significantly improve their clinical and surgical skills.

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Oral Communication(s)

ARTIFICIAL INSEMINATION IN VETERINARY EDUCATION: BEYOND DAY ONE COMPETENCES

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According to the Day One Competences of the European Association of Establishments for Veterinary Education, undergraduate veterinary training should ensure that new graduates achieve the minimum standard required for entry into the profession and acquire adequate knowledge in obstetrics, reproduction, and reproductive disorders [1]. Within this framework, artificial insemination (AI) is not explicitly identified as a stand-alone Day One Competence. Nevertheless, it remains highly relevant in veterinary education due to its practical, clinical, and legal implications. In Italy, AI is regulated as a professional activity performed by veterinarians or qualified operators, but only for domestic ruminants, horses, and pigs [2]. In horses, non-veterinary operators may work only within authorized centres under veterinary supervision. For other species (e.g., rabbits, poultry, bees), AI is generally considered a farmer-managed practice, although ovulation induction is commonly used in rabbits. In companion animals, only soft laws are available. The Fédération Cynologique Internationale states that only veterinarians are authorized to collect semen and perform AI in dogs. The French Kennel Club requires that insemination be performed by trained veterinarians for litter registration. Recently, The Italian Kennel Club has restricted the use of frozen semen, requiring certification by an accredited veterinarian [3]. AI in dogs, and even more in cats, involves evaluation of breeders, handling of biological material, and potentially invasive procedures; therefore, it can be considered a veterinary medical act. AI represents a valuable topic in undergraduate teaching, not because it should be classified as a formal Day One Competence, but because it allows students to reflect on the relationship between competency-based education, professional responsibilities, and regulatory frameworks. Students should approach AI not only as a reproductive technique, but as a professional activity involving technical skills, legal awareness, traceability, biosafety, and animal health. Pedagogically, it links basic reproductive sciences with clinical practice and introduces the broader ethical and regulatory context. This is relevant in both livestock and companion animals, despite differences in professional application. A progressive teaching approach is recommended. Training should begin with simulators to develop basic skills in a safe environment, followed by practice on reproductive organs from abattoirs to support anatomical understanding. These activities must take place in appropriate facilities, such as dissection rooms, ensuring biosafety and respect for students' ethical sensitivity. The final stage may involve live animals for advanced or species-specific techniques, but this raises ethical concerns. When not clinically indicated, animals should not undergo actual insemination during training. Instead, teaching should emphasize simulation and minimally invasive handling, ensuring animal welfare and professional responsibility.

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Oral Communication(s)

FROM SIMULATION TO PRACTICE: THE EDUCATIONAL VALUE OF A COST-EFFECTIVE CAT ORCHIECTOMY MODEL

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Recently, there has been a growing demand for the use of models in students' training before performing practical activities on live animals [1]. Many studies demonstrated the utility of the "never first time on live animals" approach, which allows students to perform initial clinical procedures on a patient with reduced apprehension after prior practice on models or simulators [1]. This approach also promotes greater respect for animal welfare. Although a variety of highly realistic models are available on the market, they are often costly and frequently single-use, as in the case of some models designed for canine orchiectomy procedures. Day One Competences require newly graduated veterinarians to perform a dog or cat orchiectomy independently [2]. During the obstetric-gynecological, andrology, and neonatology clinical course for small animals in the Veterinary Medicine program at the University of Milan, a preliminary activity is scheduled on the same day as the students' live orchiectomy training sessions. This activity consists of a 2-hour session in the skills lab, in which students perform a cat orchiectomy on a disposable, homemade, easy-to-construct, and low-cost model. At the end of the first year of the practical exercises, students were anonymously surveyed to assess their satisfaction with model use. Participation was voluntary, and the survey consisted of four main questions, each including six additional sub-questions; students responded using a 5-point scale value, with 1 indicating "little," 2 "moderate," 3 "sufficient," 4 "high," and 5 "very high." Briefly, the four main questions addressed the key steps of the feline orchiectomy: skin incision, vaginal tunic incision, spermatic cord ligation, and testicle removal. For each step, specific questions assessed the usefulness for understanding anatomical topography, structure, and dimensions; the perceived realism; ease of use; tactile experience; support in acquiring surgical skills; and overall utility for practicing before live orchiectomy. An additional question requested a global evaluation of the skills lab experience before live surgery, and an open comment field was also provided for further feedback. Survey results, obtained from 35 students, showed median scores of 4/5 for all four steps regarding usefulness in understanding anatomical topography, structure, and dimensions; 3/5 for realism; 4-5/5 for ease of use, with slight variation among the different steps; 3.5-4.5/5 for tactile experience; 4-4.5/5 for support in acquiring surgical manual skills; and 4-5/5 for practical utility in preparation for live orchiectomy. The global evaluation of the skills lab experience before live surgery was rated 4/5. Open-ended comments confirmed the value of pre-surgical experience, suggesting an increase in practice hours on models considered realistic, and recommended constructing models using materials that more closely mimic the texture and fragility of real tissues. Regarding time and cost, assembling the model required no more than 10 minutes and incurred an expense of less than 1 euro. In conclusion, while improvement in realism would be beneficial, homemade models—simple, low-cost, and easy to assemble—were appreciated by students as effective tools for practicing feline orchiectomy before performing live surgery.

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[2] Hunt et al. Development and Validation of a Canine Castration Model and Rubric, J Vet Med Educ 47:1–13, 2019.

Oral Communication(s)

USE OF A PHANTOM MODEL FOR ORCHIECTOMY TRAINING IN VETERINARY MEDICINE STUDENTS

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Veterinary students approaching the end of their academic training often show difficulties in performing basic surgical techniques [1]. The aim of this study was to evaluate if the students found useful the introduction of a neuter phantom during their practical activities of the Andrology course, held in the first semester of the fourth year of the Veterinary Medicine degree. Between 2021 and 2023, a total of 121 students attended the 2 hours practical sessions in groups of 4–8, and performed for the first time a canine orchiectomy procedure, using two/three phantom models (Neuter Units, Thales & Co., LLC dba Rescue Critters, Simi Valley, CA) for each session. These activities took place before any Surgery courses. The instructor, assisted by a junior colleague, had thus to provide also basic training on the use of surgical instruments, as well as surgical knots and suturing techniques. At the end of each session, students completed an anonymous questionnaire consisting of eight affirmations related to orchiectomy skills: [1] I learned to tie a surgeon's knot by hand; [2] I understood how to perform an orchiectomy; [3] I understood the difference between "closed" and "open" testicle technique; 4. I learned to make a prescrotal incision; 5. I learned to tie a ligature on the spermatic cord; 6. I learned to apply a continuous suture; 7. I learned to apply a simple stitch on the skin; 8. I learned to tie a surgeon's knot with a needle holder. The scores (ranging 1 to 10) given by the students were analyzed by ANOVA, including in the model the year and the question. The overall mean self-assessment scores showed a statistically significant increase over the three years, with values of 8.82±1.31 in 2021, 9.03±1.24 in 2022, and 9.22±0.85 in 202[3] The 8 questions affected significantly the scores. The highest-rated competencies were those in question 3 (median 10, mean 9.62), question 7 (median 10, mean 9.12) and question 2 (median 9, mean 9.12). The lowest mean scores were observed for question 5 (median 9, mean 8.77), followed by question 8 (median 9, mean 8.84). Out of 968 scores given, 12 (1.24%) were below 6, with the lowest scores being a 3 (question 5) and two 4s (questions 6 and 7). Self-assessment results indicate that the vast majority of students considered the phantom model useful for understanding the orchiectomy procedure and for acquiring the necessary surgical skills. These findings are consistent with previous studies reporting that 91% of students found such models helpful for training in canine castration [2]. The progressive improvement in scores over the years may reflect differences in student cohorts, as well as increased instructor experience and familiarity with the teaching tools. Critical elements of the study were the lack of previous surgical training, that may have influenced the evaluation shifting the score upward, and the lack of comparison with the same activity on a patient, cadavers or anatomical parts. Finally, these phantoms, although can be fixed and used 2-3 times, have very high costs and are made with non-environmental materials. The use of simulators provides, however, a safe training environment, eliminating risks to animals and reducing student stress.

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Oral Communication(s)

CONGENITAL MALFORMATIONS IN FOALS: A 10-YEAR RETROSPECTIVE STUDY

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Congenital malformations (CMs) are inherited or acquired defects of prenatal origin impacting the foal's development and/or survival [1, 2]. Although several case reports describe CMs in foals, few large-scale retrospective studies comprehensively assess CMs of multiple organ systems [1, 2]. Similarly to bovine obstetrics [3], prenatal diagnostic tools are limited in mares and most CMs are diagnosed at birth, although few CMs present pathognomonic clinical signs [1, 2]. The present 10-year retrospective cohort study aims to describe CMs in foals, their incidence in different breeds and outcome in an Equine Perinatology Unit (EPU) of a Veterinary Teaching Hospital. Medical records of 952 foals born at the EPU or admitted after birth from 2010 to 2025 were reviewed and foals diagnosed with CMs included. Statistical analyses of 21 clinicopathological variables in a dataset were conducted using R. CMs were diagnosed in 179 foals, of which 166 suspected upon admission (or at birth in EPU in 30 cases) (95%) and 9 during hospitalization (5%). Age groups included 146 (82%) newborns (NB) 0-3 days (d), 19 (10%) neonates (N) 4–14 d, and 14 (8%) juvenile(J) foals >14 d; 73 filly (41%) and 105 colt (59%). Breeds with the higher frequency of CMs were Quarter Horse 40/121 (33%), Italian Draft Horse 14/46 (33%), Anglo-Arabian 3/9 (33%), Arabian Purebred 14/46 (30%), Thoroughbred 8/27 (29%), English Thoroughbred 8/27 (29%), Standardbreds 74/482 (15%). Mares were 134 pluriparous (75%) and 45 primiparous (25%); 161 bred by artificial insemination (90%), 14 by natural service (8%) and 2 with advanced reproductive technology (2%); median gestational age was 335 (IQR 326-349). CMs were diagnosed based on clinical examination (141; 78%), radiography (19; 11%), ultrasound (6; 3%), upper airway endoscopy (7; 3%), advanced diagnostic imaging (2; 2%), postmortem examination (2; 2%), and laparotomy (2; 1%). Foals presented single (135; 75%) or multiple (44; 25%) CMs, and 36 (20%) were born from dystocia. Body systems affected by CMs included musculoskeletal (123; 69%), gastroenteric (32; 18%), urogenital (4; 3%), neurological (8; 5%), ophthalmic (4; 2%), respiratory (4; 2%), and cardiovascular (2; 1%). Flexural limb deformities were present in 114 foals (63%) and angular deformities in 23 (13%). Other defects included: inguinal (20; 11%), umbilical (13; 7%), and scrotal (1; 1%) hernia, wry nose (6; 3%), and cleft palate (3; 2%). Median length of hospitalization was 10 d (IQR 5-18.5, min 1-max 66 d). Outcome included 138 survivors (77%), 16 non-survivors (9%) and 25 euthanized (14%), of which 19 (23%) underwent postmortem examination. Mortality rate by age was 22% NB, 32% N and 21% J. The present study highlights the relatively high prevalence of CMs (19%) in an EPU. Careful physical examination of NB foals is a cornerstone for detecting CMs, as 78% were diagnosed by clinicians. Similarly to calves [3], the musculoskeletal system was the most affected by CMs, especially in Quarter Horses and pure breeds, probably due to the high level of inbreeding, and this highlights the importance of genetic testing in prevention. These findings support CMs surveillance in Italy and highlight the role of veterinarians in early diagnosis and prompt intervention.

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Oral Communication(s)

THE VALUE OF SURVEILLANCE ON EQUINE PREGNANCY AND PERINATAL LOSS: STAKEHOLDERS' PERSPECTIVE IN NORTHERN ITALY

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Equine pregnancy and perinatal losses (EPPL) have a significant impact on the equine industry, including international trade, animal welfare, and public health [1]. In Italy, data on the etiology and risk factors of EPPL are limited [2], with no effective up-to-date reporting system in contrast with other European countries. This study introduces EQUIPER, a community-based participatory surveillance system of EPPL in Italy, for breeders (B) and veterinarians (V), to monitor data on incidence and risk factors of EPPL. EQUIPER services will actively support stakeholders to manage EPPL, through continuous educational programs, laboratory diagnostic support and integrating epidemiological data with machine learning predictive tools. During a voluntary EPPL training organized at a University in Northern Italy by the EQUIPER multidisciplinary team, a survey on EPPL was conducted. Two anonymous on-line multiple-choice questionnaires were designed for B (34 questions) and V (31 questions) to assess the EPPL knowledge and practices adopted in the last 3 years. The knowledge domain categories were assigned to participants as follows: "very knowledgeable" (>75% correct answers), "knowledgeable" (>50%) and "not knowledgeable" (<50%). Statistical analyses were conducted using R. All participants completed the questionnaire real time (23 V and 37 B). Most respondents (96% V and 84% B) would like to actively participate in EQUIPER, however 16% B and 4% V expressed the need for more information to decide, and none disagreed. Regarding EQUIPER services, about half of V (55%) expressed the need for economical and logistic facilitation to dispatch samples to laboratory, while B (51%) expressed the need for regular training courses about EPPL. Participants acknowledged the need for an app/web dashboard (37% B and 34% V) to access data on EPPL and for educational activities on the epidemiology of infectious diseases (34% V and 8% B). Very knowledgeable (16% B and 8% V), knowledgeable (32% B and 56% V) and not knowledgeable (51% B and 34% V) stakeholders were identified. Although aware of the principal causes of abortion, knowledge gap included also the capacity to identify signs of high-risk pregnancy and abortion, a particularly important gap for passive surveillance components. Abortions, placentitis, and dystocia are reported as main problems in mares over the past 3 years, with less than one-third of respondents having a management plan for these issues. This study lays the groundwork for the development and implementation of EQUIPER, a participatory surveillance system for EPPL in Italy. B and V expressed clear interest in EQUIPER services which are recognized as highly relevant by stakeholders. Considering their crucial role as sentinels of EPPL, targeted continuing education programs are needed with programs including risk factors for EPPL and related prevention strategies.

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Oral Communication(s)

MATERNAL AND NEONATAL FACTORS FOR SHORT-TERM SURVIVAL PROGNOSIS IN PUREBRED LARGE-SIZED NEWBORN PUPPIES, BORN BY ELECTIVE CESAREAN SECTION

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In canine neonatology, the availability of prognostic factors that are easily assessable for survival prediction is particularly valuable. Many studies have demonstrated the prognostic significance of the Apgar score [1], immediately after birth, and birth weight (BW) [2] in predicting short- and long-term survival outcomes. Other factors may influence neonatal survival: breed size, maternal age (MA) and parity, and litter size [3]. This retrospective study on puppies born by elective cesarean section (ECS) aimed to evaluate these factors in relation to neonatal outcomes: stillbirth, puppies born alive but dead in 24 h of age, and puppies born alive and surviving at 24 h of age. Data were collected from 759 puppies from 116 different large-sized (≥25 kg) purebred bitches, all delivered via ECS to prevent dystocia. ECSs were scheduled based on cumulative information provided by progesterone plasma concentrations (P4) at mating, ultrasonographic measurements of the inner chorionic cavity and biparietal diameters, and by the late pregnancy P4 and ultrasonographic monitoring of mothers and fetuses well-being. Within 5 minutes of extraction, each puppy was identified, examined for malformations and weighed; live-born puppies were assessed by Apgar score, addressing individual assistance as needed, assessed for malformations, weighed, and warmed. Data recorded included Apgar score, BW, litter size, MA, and parity and statistically analysed by MANCOVA and ANOVA, followed by a post hoc Games-Howell test to explore potential associations with short-term survival. Results showed that out of 759 puppies, 13 (1.7%) were stillborn, 16 (2.1%) died in the first 24 h, and 7 (0.9%) were affected by severe malformations and euthanized. Significant differences were found between stillbirth and puppies died within 24 h for MA (mean 2.77, 2-6 vs 4.31, 3-6 $p<0.01$), parity (mean 1.15, 1-3 vs 2.0, 1-3 $p<0.05$), and litter size (mean 9.92, 8-12 vs 5.5, 1-12 $p<0.01$). Stillbirths were also significantly different in comparison to puppies alive at 24 h for MA (mean 2.77, 2-6 vs 3.93, 2-9 $p<0.01$), parity (mean 1.15, 1-3 vs 1.94, 1-4 $p<0.01$), and litter size (mean 9.92, 8-12 vs 8.35, 2-15 $p<0.05$). A lower Apgar score (mean 2.94, 2-4 vs 8.30,1-10 $p < 0.001$) was observed between puppies that died and those alive at 24 h after birth. BW was not significantly associated with either stillbirth or neonatal mortality, perhaps because of the many different breeds considered. Based on the findings of this study, stillbirth is associated with younger bitches, with lower parity and larger litter sizes. The Apgar score has been confirmed as a short-term prognostic factor, with lower-scoring puppies at higher risk of death within 24 h after birth. In conclusion, besides the Apgar score, other simple-to-measure parameters, such as MA, parity, and litter size, seem to be useful as short-term prognostic factors. Conversely, BW maybe because the analysis did not consider it related to maternal weight, does not appear to be a reliable prognostic factor for short-term survival when multiple breeds are considered.

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Oral Communication(s)

IN UTERO PARVOVIRUS INFECTION OF AN ENTIRE DOG LITTER

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Canine parvovirus (CPV) is still a leading infectious cause of canine mortality and weaning-age puppies are particularly at risk as maternally derived antibodies (ab) can block active immunization. Early neonatal mortality due to CPV is rare, as is in utero infection, which has been reported to cause cerebellar hypoplasia. The aim of this case report is to describe the clinical presentation of prenatal CPV infection in a litter. Postmortem examination was performed on 5 puppies of an English Staffordshire Bull Terrier bitch, aged 3.5 years, at her second parturition. After a healthy pregnancy, a caesarean section was performed at the Veterinary Teaching Hospital of Turin (Italy) because of uterine inertia (the previous parturition had been natural). At presentation, blood parameters were normal and foetal heart rate was >210 bpm. Surgery was performed under inhalational anaesthesia (sevoflurane in O_2), without complications. Five term puppies (3 females, 2 males) were extracted, but none could be resuscitated; none started breathing after airway clearance. O_2 was administered with a tight-fitting mask. Heart rate was around 200 bpm at birth but progressively decreased until cardiac arrest, despite chest compressions and epinephrine administration (0.2 mg/kg IO). No drug had been administered to the dam that could be reversed in the newborns. At necropsy, puppies weighed 240–265 g, had abundant haemorrhagic fluid in the abdomen, and the docimasia test was negative in all of them. No macroscopic lesions were observed in the organs. The intestine showed moderate mucosal lymphocyte infiltration, while pancreas, liver, kidneys, and lungs were free of lesions. The spleen showed signs of lymphocyte depletion. Microbiological exams yielded unremarkable results, and testing for Salmonella spp. and Neospora caninum was negative. Virologic investigations using PCR on a pool of intestinal tissue detected CPV-2 DNA, and sequencing of the VP2 gene identified the antigenic variant CPV-2a.

This report shows how CPV is still a risk in dog breeding and how it can present with unusual manifestations. The bitch had been vaccinated yearly with modified live virus vaccines; unfortunately, the CPV antibody titre at parturition was not available, but it was measured approximately 90 days later and was very high (>1:640, IMB-VacciCheck Canine, Agrolabo, Italy). The high titre may have followed infection. We can hypothesize that the bitch had not responded adequately to CPV vaccination and was infected during late pregnancy: her antibody titre may have protected her from developing clinical signs, but the virus could have crossed the placenta and infected the foetuses. Foetal heart rates suggest the absence of cardiac lesions, but histological confirmation is lacking: macroscopically, the organs were normal, and the unexpected diagnosis did not prompt further histological investigation. Further studies on maternal immunity and placental transmission of CPV would be valuable in enhancing preventive strategies to protect against such infections in the future.

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Oral Communication(s)

SYSTEMATIC REVIEW AND META-ANALYSIS OF CANINE NEONATAL VIABILITY AND MORTALITY: COMPARING NATURAL BIRTH, ELECTIVE CESAREAN AND EMERGENCY CESAREAN SECTION

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Perinatal mortality still represents a significant ethical and professional challenge for veterinarians [1]. One of the key factors in reducing perinatal losses is the type and management of delivery. Elective cesarean section can be planned to prevent adverse outcomes, particularly in breeds predisposed to dystocia. However, comprehensive data comparing neonatal viability and mortality across different mode of parturition are still lacking.

This study aimed to evaluate the quality of literature through a systematic review and to compare, via meta-analysis, the effects of natural birth (NB), elective cesarean section (ELC), and emergency cesarean section (EMC) on neonatal mortality and vitality. The systematic review was conducted according to PRISMA guidelines, and the meta-analysis was performed using RevMan 5.4.1 software. A dichotomous meta-analysis was conducted using a fixed-effect (Mantel–Haenszel) model for low heterogeneity ($I^2 < 50\%$), and a random-effects (DerSimonian–Laird) model for higher I^2; odds ratios (OR) and 95% confidence intervals (CI) were calculated. In addition, a single-arm proportional meta-analysis was performed using the generic inverse variance method with a random-effects model to estimate pooled neonatal mortality for each delivery mode.

Risk of bias assessment of the 39 studies (2000-2026) included in the systematic review revealed a high risk of selection and performance bias, mainly due to the lack of randomization and operator blinding. In contrast, attrition and reporting bias were generally low, indicating good completeness and transparency of outcomes. Comparative meta-analysis showed a difference only between ELC and EMC (8 studies; I2= 62%), with a 76% risk reduction in ELC (OR: 0.24; CI: 0.11-0.51; p=0.0002). NB was associated with markedly lower risk of low-to-moderate APGAR scores compared C-section overall (4 studies, I2=0%; OR: 0.03; 95% CI: 0.01-0.07; p < 0.00001) and to EMC (4 studies, I2= 44%; OR 0.03; CI 0.01-0.09; p < 0.0000), indicating a 97% reduction in neonatal stress compared to surgical delivery. However, no direct comparison between NB and ELC was possible due to the limited number of studies. Similarly, no difference emerged between ELC and EMC in a separate analysis(5 studies, I2= 86%; OR 0.22; CI 0.02-2.69; p = 0.23). Pooled neonatal mortality rates demonstrated an overall effect (P<0.00001) within each delivery mode: 25% in EMC (CI: 18% to 32%; I2 =92%; 10 studies, 1604 neonates), 14% in NB (CI: 11% to 18%; I2 =100%; 10 studies, 60171 neonates) and 6% in ELC (CI: 4% to 8%; I2 =90%; 19 studies, 2743 neonates).

Our results demonstrate a marked divergence in neonatal survivals and viability across different delivery modes. ELC offers the highest probability of neonatal survival, whereas EMC carries the greatest risk, with a 12-fold increase in mortality risk and 76% risk reduction in favor of ELC. Although NB shows an intermediate mortality rate compared to the surgical methods, it remains the gold standard for immediate neonatal vitality. In conclusion, clinicians should consider ELC exclusively in high-risk deliveries (breed, maternal age) to avoid the increased mortality associated with EMC. However, while ELC improves survival, they may require more intensive neonatal resuscitation and monitoring immediately after birth due to lower initial vitality scores.

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Oral Communication(s)

EVALUATION OF NEONATAL ORAL AND RECTAL MICROBIOTA IN DOGS DELIVERED BY CESAREAN SECTION FROM BIRTH TO WEANING

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In recent years, the importance of microbiota has become increasingly evident. However, the studies focusing on the initial phases of microbial colonization in dogs are limited [1]. The present study provides novel insights into the early microbial colonization dynamics of canine neonates born by cesarean section, with a particular focus on the temporal evolution of rectal and oral microbiota from birth to weaning.

This study involved the enrollment of 4 French Bulldog breeding females. All dams came from the same breeding facility and were raised under uniform management and feeding conditions. Maternal oral, teat skin, and rectal swabs, as well as puppy oral and rectal swabs, were subsequently collected at 0, 15, 30, 45, and 60 days postpartum, following the same sampling procedures. Extracted DNA was used as a template for amplification of the V3-V4 hypervariable regions of the 16S rRNA gene. The 16S library was prepared according to the Illumina 16S Metagenomic sequencing Library Preparation protocol.

A total of 18 puppies were delivered. All neonates were delivered alive, no congenital anomalies were observed, and all puppies presented APGAR scores equal to or greater than 7. A total of 270 samples were successfully processed from four dams and their litter at five time points (T0, T15, T30, T45, T60). The longitudinal follow-up allowed us to document a progressive increase in microbial richness and alpha diversity in both rectal and oral samples. This reflects the gradual ecological succession from pioneer taxa to more complex, stable communities. The dietary transition from maternal milk to solid food during weaning appeared to act as a pivotal ecological shift, promoting diversification and convergence toward the adult-like microbial profile observed in the dams [2]. The microbial taxa identified in both neonatal and maternal samples reveal a clear ecological succession that reflects the functional maturation of the oral and gastrointestinal environments during early life [3]. At birth, the microbiota of puppies was dominated by facultative anaerobes and early colonizers such as *Streptococcus, Enterococcus, Staphylococcus, Escherichia–Shigella,* and *Lactobacillus*. As puppies aged, their microbial communities underwent a shift toward taxa typical of a mature gut environment, including *Faecalibacterium, Bacteroides, Ruminococcus, Blautia, Allobaculum,* and *Collinsella*, confirming the data present in the literature.

This study demonstrates that microbial colonization in dogs begins before birth. It evolves dynamically through the neonatal period and is influenced by multiple maternal sources, shifting from teat-skin microbiota early in life to oral and rectal sources as puppies mature.

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Oral Communication(s)

IS A SPECIFIC EQUATION FOR PREDICTING PARTURITION DATE IN THE STAFFORDSHIRE BULL TERRIER BREED MORE ACCURATE THAN THE EXISTING GENERAL FORMULAS?

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Predicting the parturition date allows the organisation of obstetric care and improves the management of elective c-section planning. In veterinary medicine, ultrasonography is the method of choice for assessing embryo-foetal biometric parameters, including the inner chorionic cavity (ICC) and the biparietal diameter (BPD). Based on these parameters, equations have been developed to estimate the days before parturition (DBP), which are generally categorised by maternal size or weight; for some breeds, breed-specific equations are also available, developed to improve predictive accuracy.

The objectives of this study are as follows: i) to develop two predictive models for estimating DBP in English Staffordshire Bull Terriers, a canine breed characterized by very pronounced morphological traits, based on the biometric parameters ICC and BPD, and ii) to compare the predictions obtained with those derived from equations already available in the literature [1]. Ultrasonographic measurements were performed on 5 English Staffordshire Bull Terrier bitches, for a total of 6 monitored pregnancies. ICC was measured in the first half of gestation, while BPD was measured in the second half. For each parameter, linear regression models were developed to predict DBP: the potential influence of litter size was also explored by including the number of foetuses both as an additive term and as an interaction. The internal predictive ability of the models was estimated using Leave-One-Out Cross-Validation (LOOCV), calculating Mean Absolute Error (MAE) and Root Mean Squared Error (RMSE). The developed models were also applied to an independent dataset (dated 2023) obtained from the monitoring of pregnancies in 4 bitches of the same breed, comparing the predictions with the actual DBP values and with the equations of Luvoni & Grioni [1].

The ICC- and BPD-based models showed a strong linear correlation with DBP (ICC: $r = 0.963$, $R^2 = 0.925$; BPD: $r = 0.993$, $R^2 = 0.986$). LOOCV validation yielded a MAE of 1.27 days and an RMSE of 1.47 days for the ICC model (9/11 predictions within ±2 days) and a MAE of 0.81 days and an RMSE of 1.15 days for the BPD model (15/16 predictions within ±2 days). Including the number of foetuses did not improve the models' performance for either parameter; this finding warrants further investigation in larger datasets with greater variability in litter size. In the external dataset, the BPD-based m0 model showed overall performance comparable to the Luvoni & Grioni [1] equation for medium-sized dogs (MAE 1.62 vs 1.67 days; 8/11 predictions within ±2 days for both), with a higher proportion of predictions within ±1 day (4/11 vs 2/11), while the formula for small-sized dogs showed lower accuracy. In conclusion, the equations developed in this study provide a supportive tool for reproductive management in the English Staffordshire Bull Terrier breed; future studies on larger samples may strengthen the external validity of the models and clarify the role of litter size in predicting parturition date. Nevertheless, the comparable accuracy observed with the existing equation for medium-sized dogs calls into question the actual advantage of developing a specific formula for this breed, possibly because its characteristic broad skull, jaw, and prominent shoulders are not yet evident in the newborn.

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Oral Communication(s)

BACTERIAL FLORA OF THE CANINE REPRODUCTIVE TRACT IN BITCHES: ASSOCIATIONS WITH HEALTH, PREGNANCY, AND UTERINE DISEASE

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The bacterial flora of the reproductive system plays an important role in women's health and in domestic animals of veterinary interest. It provides protection against urogenital infections, influences fertility, and affects pregnancy, neonatal development, and microbiota formation. Dysbiosis has been linked to the development of diseases. Several studies have examined the vaginal and uterine bacterial flora in dogs, assessing how it varies throughout the oestrous cycle in healthy, pregnant, and diseased animals.

This report studied the genital bacterial flora in 28 intact female dogs of different breeds, ages, and weights, considering the phase of the oestrous cycle (luteal vs anoestrus phase), whether the cervix was open or closed, and health status, including reproductive system diseases (e.g., pyometra and endometritis) and pregnancy. The goal was to analyse the bacterial populations of the vagina and uterus, comparing vaginal, uterine, and urinary bacterial flora. Additionally, the reliability of coated vaginal swabs for representing bacterial flora near the external cervical os and in the uterus was evaluated. Bacterial cultures from urine, uterine swabs, intraoperative pre-cervical vaginal swabs (VPI), and coated vaginal swabs taken during ovariohysterectomy or caesarean section were compared. The most common bacteria found were Escherichia coli, Canicola haemoglobinophilus, Streptococcus canis, and Staphylococcus spp., mainly at the vaginal level. Bacteria such as Citrobacter koseri, Klebsiella oxytoca, Pseudomonas aeruginosa, and Aerococcus viridans were identified only during pathological conditions, while Corynebacterium mastitidis and Actinomyces urogenitalis were found only in healthy pregnant dogs. The highest bacterial counts were recorded during the luteal phase compared to anoestrus, and in dogs with disease compared to healthy or pregnant animals. The coated vaginal swab detected the same bacterial flora in both the uterine and cranial vaginal environments in only about half of the cases, suggesting it may not always reliably reflect bacterial flora at the external cervical os and in the uterus.

This raises questions about its usefulness for diagnosing and treating reproductive diseases. No link was found between uterine infections and urinary tract infections, indicating these conditions do not always occur together.

In conclusion, despite its limitations (such as a small number of patients, only two oestrus phases, and no bacterial count), the study suggests that coated vaginal swabs do not always accurately represent the bacterial flora of the cranial vagina and uterus. During reproductive infections, the urinary tract is not necessarily involved. Overall, the findings support existing literature, highlighting that reproductive bacterial flora is influenced by sex hormones, health, and reproductive status.

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Oral Communication(s)

UNDETECTABLE CANINE PROSTATIC SPECIFIC ESTERASE IN AN INTACT DOG WITH HISTOLOGICALLY CONFIRMED PROSTATIC DISEASE

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Canine prostatic specific esterase (CPSE) is an arginine esterase produced by prostatic epithelial cells and released into the bloodstream in association with prostatic activity and disease. It is measured in serum using immunoassays, typically enzyme-linked immunosorbent assays (ELISA). CPSE can be used as a non-invasive biomarker for the screening and clinical assessment of prostatic disorders in intact male dogs [1].

An 8-year-old intact male dog was presented due to marked weight loss and muscle wasting, along with hindlimb lameness. In the last 24 hours, the dog had developed multiple tonic–clonic epileptic seizures and had become non-ambulatory. The patient had a known history of refractory epilepsy. On clinical examination, the dog was markedly hyperthermic and mildly hypertensive. Mentation was severely depressed, with a near-stuporous state and minimal responsiveness to external stimuli. Both testes were markedly enlarged, hyperemic, and painful on palpation. Bloodwork revealed severe neutrophilic leukocytosis with a left shift along with increased C-reactive protein and fibrinogen concentrations. Urinalysis showed inappropriately low urine specific gravity, moderate proteinuria, and active sediment. Abdominal ultrasonography revealed a heterogeneous, predominantly hypoechoic prostate with focal hyperechoic areas. The left epididymis appeared thickened and hyperemic, consistent with inflammation, with concurrent involvement of the left spermatic cord and regional lymphadenopathy. Fine-needle aspiration of the epididymal lesion yielded Escherichia coli, which was also isolated on urine culture. Cytological evaluation of both epididymal aspirates and prostatic parenchyma revealed high cellularity, predominantly composed of degenerate neutrophils. Rare macrophages were observed. Occasional pleomorphic bacteria were present intra- and extracellularly. CPSE was <5 ng/mL. After 48 h of intensive care hospitalization, no clinical improvement was observed. Euthanasia was elected. At necropsy, the scrotum with testis and prostate were collected. Bilaterally, the testis and epididymis were almost completely replaced by a multifocal to coalescing pyogranulomas with Splendore-Hoeppli material and moderate numbers of coccoid bacterial aggregates. Similarly, prostate gland parenchyma was severely affected with some foci of fibrosis, granulation tissue and glandular regeneration.
This case describes an intact male dog with severe prostatic disease in which histopathology revealed end-stage prostatic pathology, characterized by extensive architectural disruption and replacement of functional glandular epithelium with fibrotic tissue. The undetectable serum CPSE concentration is likely explained by the loss and dysfunction of the prostatic secretory epithelium, which represents the primary source of CPSE production. This finding suggests that undetectable CPSE may be associated with terminal-stage prostatic degeneration. Further studies, including larger case series, are warranted to determine whether undetectable CPSE can be considered a marker of end-stage prostatic disease in intact male dogs.

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Oral Communication(s)

ASSESSMENT OF TESTICULAR STIFFNESS IN HEALTHY CATS USING POINT SHEAR WAVE ELASTOGRAPHY

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The ultrasonographic evaluation of the testes is an important step in assessing the reproductive potential of a male cat. Although conventional B-mode ultrasonography allows morphological and biometric evaluation, the non-invasive characterization of early tissue changes remains limited [1]. Shear wave elastography has demonstrated promising diagnostic potential for several animal species [2]. However, there is still a lack of physiological reference values for testicular stiffness in the cat. The aim of this study was to establish species-specific values using point shear wave elastography (pSWE) in healthy cats. A total of 22 healthy male cats presented to the Camagna Veterinary Clinic–VetPartners for bilateral orchiectomy were enrolled. Prior to surgery, all subjects were premedicated with medetomidine (0.05 mg/kg) and meloxicam (0.2 mg/kg) and underwent a ultrasound examination. Testicular evaluation included B-mode, Color and power-Doppler ultrasonography and a quantitative elastosonographic assessment using pSWE. Examinations were performed with a Mindray DC-80A ultrasound system (Mindray Medical Italy S.R.L., Italy) equipped with a linear probe. For each scan of testis, 18 Shear Wave Speed (SWS) measurements were obtained by placing 1x1 mm Regions Of Interest (ROIs) within the cranial, middle, and caudal testicular region of the parenchyma, both above and below the mediastinum testis, 3 for each region respectively. Median SWS values and acquisition quality indices were recorded for each ROI, and mean stiffness values were calculated for each region. Descriptive statistics were performed, and data distribution was assessed using the Shapiro–Wilk test. Comparisons between right and left testes and between supra- and submediastinal regions were performed using Student's t-test for independent samples, while differences among cranial, medial, and caudal regions were evaluated using the Kruskal–Wallis test. Testicular parenchyma presented medium echogenicity with a homogeneous echotexture, interrupted by the presence of the mediastinum in the central part of the testis, which appeared as a hyperechoic line. Color and power-Doppler examinations of testicles showed the presence of small vessels. A total of 792 SWS measurements were obtained. Mean SWS values were 2.91±0.71 m/s for the right testis and 3.07±0.72 m/s for the left testis. No statistically significant differences were observed between sides. When measurements were grouped according to their position relative to the mediastinum testis, mean SWS values were 3.06±0.71 m/s in supramediastinal regions and 2.92±0.72 m/s in submediastinal regions, with no significant differences detected between group. Regional analysis showed mean SWS values of 3.10±0.81 m/s in the caudal portion, 2.95±0.63 m/s in the cranial portion and 2.93±0.70 m/s in the medial portion. Although slight intra-parenchymal variability was observed, statistical analysis revealed no significant differences among regions. This study provides preliminary reference values for physiological testicular stiffness in the cat assessed by pSWE using a Mindray ultrasound machine.

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Oral Communication(s)

REPRODUCTIVE MANAGEMENT AND CONTRACEPTIVE METHODS IN ITALIAN FELINE BREEDING FACILITIES: AN OBSERVATIONAL STUDY

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This study represents the first large-scale investigation in Italy of feline breeding management, focusing on reproductive strategies and contraceptive use. In a national and international context where systematic, representative data are lacking, it aims to fill an important knowledge gap by providing an updated overview of Italian catteries.

A voluntary, anonymous online questionnaire comprising approximately 118 questions was distributed to members of the Associazione Nazionale Felina Italiana (ANFI), Italy's principal feline association. It collected data on structural management, the number of breeding queens and tomcats, reproductive performance, and contraceptive strategies (mainly in queens, with limited information on males). The survey addressed the reasons for contraceptive use, the molecules used (GnRH agonists, melatonin, and progestins), administration routes (oral, injections, or implants), and protocols. Approximately 20.3% of ANFI members (about 2500 individuals, not all of whom are breeders) participated, yielding a reasonably representative sample of Italian catteries. The findings indicate mostly amateur management systems, primarily in Northern Italy, involving small to medium-sized catteries (average of 3 breeding females and 1 male). These catteries are almost exclusively indoor environments (surfaces larger than 4 m^2 per cat, aligned with EFSA, European Food Safety Authority, guidelines), with one litter per queen per year and natural matings. Catteries use commercial diets, conduct regular vaccinations and genetic testing; breeders report substantial experience. The most prevalent breeds are Maine Coon, Birman, and British Shorthair. Reproductive performance typically begins around 18 months, with an average litter size of four kittens. Reported rates include 4.3% stillbirths, 3.5% pre-weaning mortality, and a 3.4% caesarean section rate. These values are lower than those reported in European literature (e.g., 8–11% mortality), potentially reflecting improvements in selective breeding, genetic, and veterinary care.

Contraceptives, primarily used to allow rest or prevent pregnancies, were employed by 28.9% of breeders (147 out of 508), mainly GnRH agonists (67/147, 45.6%: deslorelin 4.7mg implant, median duration 14 months) and melatonin (62/147, 42.2%: varied oral protocols, standardised implants), with infrequent use of progestins (10/147, 6.8%). High reversibility (post-treatment pregnancy rate of 94%, with normal litter size), normal obstetric outcomes, and few side effects (occasional pyometra) were reported with GnRH agonists and melatonin. Deslorelin was the preferred contraceptive method, particularly due to its reversible effects in males. Protocol variability, particularly for melatonin, highlights the need for standardisation and greater collaboration between breeders and veterinarians.

Limitations comprise self-reported data, incomplete responses, and potential response bias. Nevertheless, the results indicate alignment with EU welfare guidelines and scope for optimising contraceptive practices. This preliminary descriptive study offers novel insights into Italy's feline breeding sector, laying the groundwork for future statistical and multicentric research.

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Oral Communication(s)

CLINIC AND REPRODUCTIVE MONITORING OF CAPTIVE AFRICAN LIONESSES (*PANTHERA LEO*) PRIOR TO ADMINISTRATION OF DESLORELIN ACETATE

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The reproductive management of wild felids kept in captivity is a key veterinary concern. The presence of animals in circuses has long been debated, prompting increasingly strict legal measures governing their care and breeding. Recently, the use of long-acting GnRH agonists, such as deslorelin acetate, has become widespread as a non-invasive alternative to surgical sterilization in carnivores [1]. However, recent studies demonstrated post-implantation ovarian and uterine disorders in cats [2]. This evidence questions the use of these drugs in non-conventional animals, particularly in African lionesses, where Carlson et al. (2022) [3] verified a higher rate of endometrial hyperplasia and a greater predisposition to pyometra. Therefore, the purpose of this study is to describe and validate a pre-administration monitoring protocol to exclude pre-existing reproductive disorders and determine the optimal timing for hormonal suppression. Three healthy female African lions, aged between 7 and 10 years, held in the same traveling circus, were selected. All animals were administered general anaesthesia using validated protocols. Subsequently, a clinical examination of the reproductive tract was performed, blood samples were collected for a complete blood count and oestradiol and progesterone assays. Vaginal cytology was performed using modified Wright-Giemsa stain. A multiparametric ultrasound examination was performed with: B-mode imaging to assess the morphology; colour-Doppler to determine the hemodynamic indices; CEUS (Contrast-Enhanced Ultrasound) to evaluate ovarian and follicular/luteal vascularization by administering 0.03 mL/kg of sulphur-hexafluoride.

The results revealed different reproductive phases among the subjects: oestrus, late dioestrus, and early dioestrus. Ovarian and endometrial cysts were identified in two individuals, in agreement with findings reported in recent literature. Based on CEUS and hormonal monitoring, these alterations were determined to lack functional activity. No clinically relevant signs or pathological conditions associated with these abnormalities were detected. CEUS proved to be a key diagnostic tool in differentiating morphological from functional alterations. The integration of the collected data allowed for an accurate characterization of the oestrous cycle. No contraindications to deslorelin implantation were identified in the examined subjects. The adopted protocol proved to be effective and applicable even in complex settings such as circuses. Pre-implantation monitoring was essential in preventing potential complications. The study confirms the high incidence of uterine alterations in captive lionesses, as previously described in recent studies.

Despite the limited sample size, this work provides a basis for the development of standardized protocols and future research advancements.

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Oral Communication(s)

PRESENCE OF MICROPLASTICS IN THE REPRODUCTIVE SYSTEM, BLOODSTREAM IN BITCHES UNDERGOING OVARIO-HYSTERECTOMY AND IN THEIR WATER AND COMMERCIAL DIET: A PILOT STUDY

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Over recent decades, industrial development has driven a substantial increase in global plastic production, which currently reaches approximately 400 million tons annually and is projected to double by 2050 (XiaoZhi Lim, 2021). Once released into the environment, plastics progressively degrade into microscopic fragments known as microplastics (MPs), which are virtually impossible to recover or remove due to their size, ranging from 0.1 µm to 5 mm (Song et al, 2017). Humans and animals are considered indicators of environmental microplastic contamination, and exposure to these particles has been associated with adverse effects on multiple organs and biological systems. High levels of microplastics have been detected in companion animals in various organs, including the intestine, lungs, liver, kidneys, and blood clots (Prata et al, 2022). However, the presence of microplastics in the female canine reproductive system has not yet been investigated. Therefore, the aim of this study was to detect microplastics in the reproductive system of bitches. For this purpose, were enrolled four healthy female dogs (one German Shepherd, one Jack Russell Terrier, and two mixed-breed dogs) housed indoors and their age and body weight ranged from 5 to 8 years and 5 to 29 kg rispectively. All bitches were subjected to elective ovariohysterectomy, following written informed owner consent, at the Veterinary Hospital of the University of Messina. Samples of ovaries, ovarian bursa, uterus, and blood were collected to investigate the presence of microplastics. Additionally, feed and drinking water sample were also collected and analyzed. All samples were processed using the procedure described in European Patent No. 3788344 (July 20, 2022) and recognized in Canada and South Korea. Microplastics (MPs) extracted from the samples were analysed using a Scanning Electron Microscope (SEM ZEISS LEO 1530) equipped with Energy Dispersive X-ray spectroscopy (SEM-EDX, AZTEC v.5.1), applying theanalytical protocol defined in the patent. Microplastics were detected in all samples collected. In particular, the highest mean concentrations were observed in the ovarian bursa and uterus, with values of 79435,25 and 87728,25 MPs (p/g), respectively. Consistently detectable levels were found also in the ovaries, blood, water and in commercial diet, with mean concentrations of 11600,5 MPs (p/g) in ovaries, 9891,75 MPs (p/g) in blood, 1487 MPs (p/g) in drinking water, and 20127, 11500 and 17001 MPs (p/g) in the commercial diet samples, respectively. This study provides the first evidence of the presence of microplastics in the canine reproductive system and commercial diet. Given the close conctact between humans and animals and the growing number of pets, these findings reveal a previously unrecognized route of microplastic accumulation, positioning dogs as effective sentinel species for human exposure, and raising important concerns about potential reproductive health implications within a One Health framework.

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Oral Communication(s)

PRESERVATION OF STRUCTURAL INTEGRITY AND CELL VIABILITY IN FELINE TESTICULAR TISSUE UNDER PROLONGED ORGANOTYPIC CULTURE CONDITIONS

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Male fertility preservation, especially in prepubertal individuals, remains a major concern in reproductive medicine. Organotypic culture of testicular tissue is a promising tool to maintain germ cells and potentially support in vitro spermatogenesis (IVS). These approaches have been successful in rodents [1,2], but adaptation to other species, particularly felids, remains challenging [3]. Beyond providing physical support, successful culture also relies on optimized media and supplements to maintain tissue and cell viability. This study evaluated the effects of a rodent-IVS base medium (B-M) [2] and its supplementation with retinol (RET: 1 µM) and taurine (TAU: 60 µM)—known for their roles in germ cell differentiation and antioxidant activity—alone or combined (RET+TAU), on the survival and integrity of domestic cat cultured testicular tissue.

Testicular tissue fragments (~1.8 mm^3) from juvenile domestic cats (testes weight <0.30 g; n=10) were fixed immediately (CTR) or cultured on agarose gel blocks in different media at 35° with 5% CO_2 for 10, 16, 19 and 22 days, time points chosen to encompass early phases of spermatogonial and spermatocyte development. Samples were processed for histomorphometric analyses (area of tubular, necrotic and fibrotic interstitial tissue, analyzed relative to total tissue area) and immunohistochemical detection of apoptosis (caspase-3), proliferation (MCM-7), spermatogonial (PGP9.5) and spermatocytes (SYCP3) markers, expressed as the number of positive cells per intratubular area (cells/mm^2). Data (mean±SD) were analyzed using the Kruskal–Wallis test followed by the DSCF post-hoc test ($p<0.05$).

Histomorphometric evaluation indicated that tubular, necrotic and fibrotic areas did not significantly change among culture conditions or over time (ranging from 0.311±0.163 to 0.422±0.126, 0.195±0.135 to 0.303±0.194, and 0.312±0.108 to 0.391±0.113, respectively; $p>0.05$), indicating preserved seminiferous structure.

Consistent with these findings, immunohistochemical analysis revealed that apoptotic cells remained stable throughout the culture period and across media (ranging from 6.78±6.83 to 58.9±87.8 cells/mm^2, $p>0.05$), reflecting limited progression of tissue degeneration. Similarly, spermatogonial and proliferating cells were maintained at comparable levels during the entire culture period and in all experimental conditions (ranging from 763±520 to 1756±1065 cells/mm^2 for PGP9.5, $p>0.05$; from 416±319 to 897±473 cells/mm^2 for MCM-7, $p>0.05$), indicating preservation of the germ cell population and sustained proliferative activity. SYCP3-positive cells were already present in some seminiferous tubules in CTR samples and were occasionally observed at later time points ($p>0.05$), without evidence of further meiotic progression.

All tested media preserved tissue integrity and germ cell populations throughout 22 days of culture, highlighting the system's potential for long-term culture and representing a promising step toward the development of IVS in the feline model.

This work was supported by Università degli Studi di Milano "Piano di Sostegno alla Ricerca (Linea 2) - Progetto DIVAS – CORE.

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Oral Communication(s)

ENRICHED MICROCAPSULES FOR THE CULTURE AND PRESERVATION OF DOMESTIC CAT CUMULUS-OOCYTE COMPLEXES

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Alginate microcapsules have proved to be a suitable microenvironment for the in vitro maturation (IVM) of cumulus-oocyte complexes (COCs) in different species. By more closely replicating the native ovarian milieu, these three-dimensional (3D) systems offer significant advantages for oocyte maturation and developmental competence compared with traditional two-dimensional (2D) approaches. More recently, alginate microcapsules have also successfully been employed for the cryopreservation of COCs. The addition of extracellular matrix components could further increase the biomimicry of these systems. Thus, the aim of this study was to assess the effects of hyaluronic acid (HA) and collagen type I (CI) as functional enrichments for domestic cat COCs IVM (Exp. I) and cryopreservation (Exp. II).

Immature grade I COCs were collected from spaying-derived ovaries. In Exp. I, COCs were cultured in IVM medium for 24 h in 2D or 3D conditions. For the latter, COCs were encapsulated in 1% alginate (3D) or in 1% alginate enriched with 2 mg/mL HA (3D+HA) or 0.04 mg/mL CI (3D+CI) [1]. In Exp. II, encapsulated (3D) or non-encapsulated (2D) COCs were slow-frozen following a standard protocol [2]. After thawing, COCs were in vitro matured as above, using a group of fresh COCs as a control. After IVM, all COCs were denuded, stained with fluorescein diacetate for viability, ReadyProbes™ Reagent for actin distribution and Hoechst for nuclear status. At least 3 replicates were performed for each experiment. Data were analyzed by Fisher's exact test, $p<0.05$.

Results of Exp. I (n=36-41 COCs/group) showed no differences in viability (89-97%, p=0.36) and normal actin distribution (88-92%, p=0.71). Full maturation rates were maintained in 3D+CI (56%), but lowered (p=0.04) by the addition of HA (41%) compared to 2D (63%). Therefore, CI was chosen for Exp. II (n=46-53 COCs/group). No differences were found in maturation rates among cryopreserved groups (2D, 3D and 3D+CI, 31-33%, p=1), with fresh control COCs showing a higher rate (66%, p=0.001). Although a similar trend was observed for viability (p=0.28) and actin distribution (p=0.38) among cryopreserved groups, 3D+CI showed viability (69%) and normal actin distribution (71%) similar to those of control fresh oocytes (83% and 81%, p=0.16 and p=0.35, respectively), while 3D did not (57% and 62%, p=0.007 and p=0.004).
In conclusion, while CI did not influence the outcomes of the IVM of fresh cat COCs, its use during 3D cryopreservation could help mitigate freezing injuries. Further studies will help clarify whether preservation of immature oocytes in enriched-3D matrices could enhance developmental competence and indicate if this strategy can be applied for felid germplasm banking.

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Oral Communication(s)

DEVELOPMENT AND VALIDATION OF A HIGHLY EFFICIENT CLOSED VITRIFICATION SYSTEM FOR BOVINE EMBRYOS

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In recent years, bovine embryo production has expanded significantly worldwide. According to the 2024 statistics of the International Embryo Technology Society [1], approximately 87% of embryos produced derive from in vitro production (IVP) systems. Cryopreservation of bovine embryos is a cornerstone in embryo transfer programs. Innovative vitrification systems, based on the use of minimum volume and direct contact with liquid nitrogen, provide high survival rates by eliminating ice crystal formation and prevents cryo-induced damages [2]. However, commonly used open vitrification systems may expose embryos to the potential danger of disease transmission mediated through liquid nitrogen [3]. The objective of this work was to develop and validate a highly efficient closed-circuit vitrification system that bypass the sanitary limits of open vitrification systems by avoiding direct contact between embryo and liquid nitrogen. A handmade prototype of the vitrification device was created using a high-modulus, medical-grade photopolymeric resin with excellent flexural and tensile properties for the main structure, and stainless steel for the hermetic closure, treated with a thermosetting resin in the area of contact with the main support. The materials were chosen in order to guarantee an adequate cooling rate to the support and thermal and mechanical stability. The specific objective of this work was the validation of the new device by comparing its performances with those of routinely used commercial Cryolock vitrification device. For this trial a total of 121 bovine embryos at different blastocysts stage, were used. Ovarian follicles (3–5 mm) were aspirated from abattoir-derived ovaries, and their COCs were subjected to in vitro maturation, fertilization, and embryo culture. On day 7 of culture, embryos were randomly divided into two groups: CTR (n=58) and TOOL (n=63). CTR embryos were individually vitrified on the Cryolock vitrification device, while TOOL ones on the innovative vitrification device previously described, following standard procedures and stored in liquid nitrogen. After 96 h embryos were thawed, incubated for 48 h and evaluated at 24 and 48 h in terms of survival (SR), advancement stage (ASR) and hatching (HR) rates. Chi-square test was used to compare rates between groups. Significance was set at $p<0.05$. No differences between tools were observed in SR, SAR and HR at both 24 and 48 h points. SR was 100% at 24 h in both groups and 94.4 and 87.3% respectively in CTR and TOOL at 48h. The ASR was 94.4% and 85.7% at 24 h and 61.1% and 49.2% at 48 h, respectively in the CTR and TOOL groups. HR was 33.3% and 23.8% at 24 h and 72.2% and 60.3% respectively in the CTR and Tool groups. In conclusion, the comparable performance of these devices confirms the new device is suitability for commercial embryo production. This technology provides a safer alternative that eliminates the danger of embryo contamination by pathogen from the exposure to liquid nitrogen.

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Oral Communication(s)

IMPACT ON REPRODUCTIVE AND PRODUCTIVE PERFORMANCE IN A DAIRY CATTLE HERD DURING A BLUETONGUE OUTBREAK

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Bluetongue (BTV) is a non-contagious infectious disease affecting ruminants, caused by a virus belonging to the genus *Orbivirus*. It is a vector-borne disease, with insects of the genus *Culicoides* representing the main vectors. Vertical transmission has been demonstrated for some strains [1].

All domestic and wild ruminants are susceptible to infection. Although sheep are the most affected species and typically show the characteristic clinical signs of the disease, certain serotypes, such as BTV-8, BTV-4, and BTV-3, have been associated with evident clinical manifestations in cattle as well. Clinical signs observed in cattle include fever, anorexia, lethargy, reduced milk production, ulcers, necrosis, and scab formation at the muzzle, serous nasal discharge, oral ulcers with hypersalivation, and erythema, ulcers, and necrosis of the teats [2-3].

In Italy, during the period June–December 2024, a total of 6,940 BTV outbreaks were reported by the competent authority within the *Sistema Informativo Malattie Animali* (SIMAN). Of these, 213 outbreaks (3.07%) were recorded in Sicily, 37 of which (17.4%) occurred in the province of Ragusa. Serotyping revealed that BTV-8 was present in 35 outbreaks, while in the remaining two there was co-circulation of BTV-8+BTV-3 and BTV-8+BTV-4.

This study evaluated the impact of a BTV outbreak caused by serotype BTV-8, identified in October 2024 in an intensive dairy cattle farm in Ragusa. The investigation, carried out on the entire herd, focused on reproductive (fertility rates, abortions, and stillbirths) and productive (milk yield) performance index, comparing the data collected during the epidemic period (outbreak period: Aug 2024–Jan 2025) with those from the same time interval of the previous year (control period: Aug 2023–Jan 2024). The parameters considered included conception rate (**CR**), pregnancy rate (**PR**), number of abortions, and average daily milk yield (L/day) in lactating cows.

The results obtained showed significant changes in the parameters considered, with marked %decreases in CR (−21.08%), PR (−9.76%), and average milk yield (L/day) (−5.24%), as well as a %increase in the abortions during the outbreak period compared with the control period (+185,09%).
Following confirmation of the absence of new suspected cases on the farm, the outbreak was declared resolved in February 2025. Subsequently, the farm participated in the vaccination campaign proposed by the Veterinary Prevention Department of the ASP of Ragusa, administering the Syvazul BTV® vaccine to all animals in the herd older than 3 months.

The evidence collected over the study period indicates that, although clinical signs observed in cattle were less severe than those recorded in sheep, the economic impact was nonetheless significant, highlighting a tangible effect of the virus on reproductive efficiency and herd productivity. Therefore, integrated Bluetongue control - based on surveillance and vaccination - plays a crucial role in reducing the economic burden of the disease and in protecting livestock assets at both regional and national levels.

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Oral Communication(s)

EFFECTS OF INTRAMUSCULAR ADMINISTRATION OF ANTIOXIDANTS AT PARTURITION IN ITALIAN MEDITERRANEAN BUFFALOES

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The transition period in Mediterranean buffaloes is characterized by marked metabolic and oxidative challenges that can impair both productive efficiency and reproductive performance [1]. This study evaluated the effects of a single intramuscular administration of a vitamin combination containing α-tocopherol and β-carotene within 24 hours postpartum on milk quality and reproductive indices.

Forty clinically healthy buffaloes were randomly assigned to a treated group (D; n=20) receiving a single intramuscular injection of Dalmavital® (15 mg β-carotene + 20 mg/ml α-tocopherol acetate; 5 mL/100 kg) or a control group (C; n=20) receiving isotonic saline solution at the same dosage and time. Milk samples were collected at 7 and 30 days postpartum to assess retinol, α-tocopherol, standard milk quality parameters (fat, protein, lactose, non-fat dry matter, casein, urea and pH), fatty acid profile (SFA, UFA, MUFA, PUFA) and somatic cell count (SCC). Reproductive performance was evaluated through pregnancy rate and calving-to-conception interval.

Data were analyzed using repeated-measures GLM, t-test, and Chi-square where appropriated. A p-value <0.05 was considered statistically significant.

Retinol concentrations did not differ significantly between groups or across time points. α-tocopherol levels were significantly higher in group D compared to group C at T30 ($p < 0.05$), whereas in group C exhibited a significant decrease over time. Fat, SFA, UFA, MUFA, and PUFA levels were significantly elevated in group D relative to group C ($p<0.05$), while SCC showed a significant reduction in the treated group than the control one ($p<0.05$). No significant differences were observed in pregnancy rates; however, the calving-conception interval was significantly shortened in group D ($p<0.01$).

These findings support the hypothesis that early postpartum antioxidant supplementation may mitigate oxidative stress and positively influence both milk quality and reproductive recovery. The increase in α-tocopherol and improvement in lipid profile align with previous evidence linking antioxidant status to mammary function and milk composition [2]. Moreover, the reduction in calving-to-conception interval suggests a potential role of antioxidants in enhancing ovarian function and embryo viability under oxidative stress conditions [3]. However, the absence of significant differences in pregnancy rate indicates that further studies with larger sample sizes are needed to confirm these trends.

In conclusion, a single postpartum administration of α-tocopherol and β-carotene appears to improve milk quality parameters and shorten the interval to conception, suggesting a promising strategy to support productivity and reproductive performance in Mediterranean buffaloes.

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Oral Communication(s)

SPERM LOWER MITOCHONDRIAL MEMBRANE POTENTIAL AFTER SWIM-UP SELECTION AS LASTING EFFECT OF HEAT STRESS EXPOSURE IN BULL

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Heat stress (HS) is a major hazard to sperm production in cattle, since proper testicular function and spermatogenesis require a temperature 3-5 °C below the body core. Recent climatic changes and warmer summers have led to reports of impaired male fertility. Importantly, in addition to successful breeding programs, high-quality sperm samples are required for reproductive biotechnologies, such as in vitro embryo production (IVEP). To increase IVEP rates and overall success, a sperm selection method, such as swim-up, is crucial to single out the most motile and metabolically active cells to be used in the fertilization process. Therefore, we hypothesized that the impact of HS on sperm cells would not be overcome by swim-up and residual quality issues would remain post-selection. The objective of this study was to compare the quality pre- and post-selection (swim-up) on post-thaw sperm samples from the same individuals (n=5 Holstein bulls) in two different temperature-humidity index (THI) categories: non-heat-stressed (NHS), collected in winter 2016 vs heat-stressed (HS), collected in summer 2016. One ejaculate per bull per season was used for experiments. A total of 5 pooled straws from each bull and season were thawed at 37 °C for 30''. The samples were assessed for morphology (eosin-nigrosine staining) and mitochondrial membrane potential (MMP) via flow cytometry post-staining with JC-[1] The same sperm sample was immediately subjected to sperm selection via swim-up, then reanalyzed. Considering post-thaw sperm morphology, NHS presented higher normality (57.6 ± 2.2 vs 46.4 ± 2.3, P<0.05) and smaller number of midpiece defects (23.7 ± 2.4 vs 31.8 ± 2.9, P<0.05) when compared with HS samples, respectively. No difference was observed regarding MMP. On the other hand, post-selection analyses indicated that the swim-up improved the sperm morphology and reduced the midpiece defects in HS samples significantly (P<0.05), reaching similar levels to NHS samples (P>0.05). One point to highlight is that, although an improvement is observed in post-selection MMP on the HS samples when compared to post-thaw (24.96 ± 2.7 vs 79.99 ± 2.0 P<0.05), the swim-up failed to bring the HS samples to the same level as the NHS samples post-selection (79.99 ± 2.0 vs 85.5 ± 1.6, respectively, P<0.05). Overall, there is a clear influence of the THI on impaired morphology and midpiece defects, which are strongly related to the lower MMP observed even post-selection. In conclusion, swim-up presents an interesting way to improve sperm morphology. However, exposure to HS leads to long-lasting effects, such as reduced MMP, that can be observed in sperm samples even post-selection.

Oral Communication(s)

COMPARISON OF TWO CASA SYSTEMS AND COUNTING CHAMBERS FOR THE EVALUATION OF FROZEN-THAWED STALLION SEMEN

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Semen quality assessment represents a fundamental tool for estimating stallion fertility potential and is based on the evaluation of macroscopic and microscopic parameters, including motility, concentration, and sperm morphology [1]. The introduction of Computer-Assisted Semen Analysis (CASA) systems has improved the objectivity and standardization of these evaluations, although the results may still be influenced by technical factors such as the software used, the type of counting chamber, and operating conditions [2,3]. The aim of this study was to compare two CASA systems, AndroVision® (Minitube) and Ceros II® (Hamilton Thorne), in combination with two different counting chambers, Leja® (IMV Technologies) and SpermVU® (Spermtech), for the assessment of motility, concentration, and kinematic parameters in frozen-thawed stallion semen. Semen samples from 10 stallions were analysed; for each animal, a pool of three 0.5 mL straws was prepared and diluted 1:3 with INRA96®. Evaluations were performed at 5 (T5) and 60 minutes (T60) after thawing. Sperm concentration was determined using both CASA systems and a Thoma chamber. The CASA system significantly affected ($P<0.05$) all evaluated parameters except VCL for total spermatozoa and STR for motile spermatozoa. The counting chamber also had a significant effect ($P<0.05$), with the SpermVU® chamber consistently yielding higher motility values compared to the Leja® chamber, regardless of the CASA system or incubation time. These findings confirm that semen evaluation using CASA systems is highly influenced by technical variables, highlighting the need for strict protocol standardization to ensure reliable results. Furthermore, data obtained from different instruments are not directly comparable, and this should be carefully considered for accurate clinical interpretation of stallion semen quality.

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Oral Communication(s)

BIRAGO PROJECT ITALY – FOUR DECADES OF MACROCYCLIC LACTONES AGAINST DIROFILARIA IMMITIS IN SOME ITALIAN REGIONS: SUCCESS OR A NEW CHALLENGE? PRELIMINARY DATA

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The first observation of *Dirofilaria immitis* in dogs was made by Birago in 1626, a nobleman living in Northern Italy. Since the 1980s, scientific studies have identified Northern Italy, particularly the area around the Po River, as a highly endemic region for heartworm disease (HWD). To date, HWD is present across most of the Italian territory [1]. After the introduction of ivermectin in Europe in 1988 and other macrocyclic lactones in the following years, parasitic pressure gradually decreased, leading to a reduction in the prevalence of heartworm disease in this area. However, in recent years this reduction has led, to some practitioners to pay less attention to the disease, including in terms of diagnosis and prevention. The aim of the study was to assess, approximately 40 years after the introduction of macrocyclic lactones preventives, the prevalence and distribution of *D. immitis* in Italy. The inclusion criteria were stray dogs or those without microchips referred to shelters or veterinary facilities, older than 10 months of any breed, and probably untreated with macrocyclic lactones for heartworm prevention. Approximately 2 ml of blood was collected from each dog. An identification form was completed for each animal (location of recovery, age, breed, sex, weight, clinical signs). Each blood sample was analyzed using Knott's test and an antigen test (Idexx 4DX).

Data from 2060 dogs examined with Knott's test, from Lombardy, Emilia-Romagna, Tuscany, Lazio, Campania, Puglia, Calabria and Sardinia, showed an overal prevalence of *D. immitis* of 14.5%. In particular, 10% were positive for *D. immitis*, 5.2% for *D. repens* and 4.5% mixed infection. The data show that, even 40 years after the introduction of chemoprophylaxis, parasitic pressure and risk remain high in dogs not undergoing chemoprophylaxis, particularly in historically endemic areas such as Lombardy, Emilia-Romagna, and Sardinia. Furthermore, the presence of *D. repens* was recorded in 7 out of 8 regions. Moreover, these dogs represent an important reservoir for dirofilariosis. These data highlight the importance of continuing to make a correct diagnosis and prevention for *D. immitis* and *D. repens*, and how practitioners must not lower their attention towards these parasites.

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Oral Communication(s)

EFFECTS OF DIFFERENT FAECAL SAMPLE PROCESSING PRACTICES ON *TRICHURIS SUIS* AND STRONGYLE (*HYOSTRONGYLUS/OESOPHAGOSTOMUM*) EGG COUNTS IN PIGS

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Faecal flotation techniques are widely used in parasitological diagnostic investigations and research, and the choice of the flotation solution (FS) can affect results[1]. Considering compromicroscopic investigation in swine, detailed indications are lacking[2]. The aim of this study was to provide preliminary indications about flotation solutions yielding the highest recovery of two swine endoparasite eggs, *Trichuris suis* and strongyles (*Hyostrongylus/Oesophagostomum*).

Replicates of faecal samples (S1 and S2) from two Large White pigs positive for *T. suis* and strongyle, respectively, were first processed to obtain replicates for testing by FLOTAC® double technique, both with and without the diethyl ether (DE) treatment (fat faeces protocol [2]). S1 replicates were tested using FS4 (NaNO3, s.g.=1200) and FS7 (ZnSO4, s.g.=1350), and the original faecal suspension obtained in step 4 of the FLOTAC® technique was further used to obtain serial dilutions, until stocks last. Considering the available information on strongyle egg detection in ruminants [3], S2 replicates were tested with FS4, FS7, and FS2 (NaCl, s.g.=1200). From S1, 140 replicates were obtained, with 6 serial dilutions from the original faecal suspension. The 150 replicates obtained from S2 permitted the investigation of 4 serial dilutions. Generalised linear mixed models (GLMMs) were implemented, including the FS, the use or non-use of DE, the dilutions, and their interactions as potential predictors of egg count. In the two evaluations, 4,785 T. suis and 59,611 strongyle eggs were counted. FS7 was significantly better at detecting *T. suis* eggs than FS4. For strongyle egg count, FS was a significant predictor too: higher counts were obtained with FS2, followed by FS7; strongyle egg counts recorded with FS4 appeared severely underestimated compared to both FS2 and FS7. DE significantly improved counts of both S1 and S2; the greater improvement was obtained in replicates of the original faecal suspensions; the benefit appeared reduced in dilutions. FSs with the worst performance were those that benefited most from using DE. GLMMs were also implemented with eggs per gram of faeces (EPG) values as independent variables. EPG increased significantly with dilution; this could represent a bias in studies that equally consider results from undiluted and diluted samples.

Our findings provide preliminary practical indications for defining coprological protocols for swine, suggesting the combined use of FS2 and FS7 with DE treatment to recover *T. suis* and strongyle eggs. Poor performances of certain FSs should be taken into account, especially if anthelmintic treatments are administered with undiagnosed *T. suis* infections: considering different formulations, dose, and duration of treatment, specific indications are often reported in claims for effectiveness against trichuriasis in pigs, and sometimes the treatment could be only an aid in the control of adult whipworms without complete effectiveness (in levamisole and flubendazole premix for medicated feeding stuff; in fenbendazole oral powder and oral suspension; in injectable ivermectin); undiagnosed infections could lead to underdosing and to the spread of anthelmintic resistance.

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Oral Communication(s)

SEROPREVALENCE OF *TOXOPLASMA GONDII*, *NEOSPORA CANINUM* AND *ENCEPHALITOZOON CUNICULI* IN RED FOXES (*VULPES VULPES*) FROM ITALY

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Wild carnivores, due to their ecological role and increasing overlap with peri-urban and urban environments, represent valuable epidemiological sentinels for parasitic pathogens. In particular, the red fox (*Vulpes vulpes*), a highly adaptable carnivore with an opportunistic and scavenging feeding behaviour [1], represent a suitable species for monitoring zoonotic parasites such as *Toxoplasma gondii*, and the microsporidian *Encephalitozoon cuniculi* but also further neurotropic pathogens as *Neospora caninum* [2,3].

The present study aimed to assess the seroprevalence of *T. gondii*, *N. caninum* and *E. cuniculi* in 120 red foxes sampled between November 2023 and June 2025 in Central (Umbria, Marche regions) and Southern Italy (Campania region). Peripheral blood was collected from live foxes rescued by the WildUmbria wildlife rescue center and admitted to the Veterinary Teaching Hospital (OVUD) of the University of Perugia, whereas clotted blood was obtained at necropsy from carcasses submitted to the Experimental Zooprophylactic Institutes of Umbria–Marche and Campania. All sera were analyzed by using commercial IFAT assays (MegaFLUOR®TOXOPLASMA g., MegaFLUOR®NEOSPORA c. and MegaFLUO®ENCEPHALITOZOON c.) (Megacor Diagnostik, Hoerbranz, Austria) specific for the three pathogens. To evaluate the influence of epidemiological factors (i.e. sex, age class, location and animal status), seroprevalence differences were tested through inferential statistical analyses. Mann–Whitney tests were applied to compare antibody titers, while co-infection patterns were explored using an UpSet plot. Pairwise associations between pathogens were assessed by Fisher's exact test, with odds ratios calculated at a significance threshold of $p < 0.05$. All analyses were performed in R v4.3.0.

Overall seroprevalence was highest for *T. gondii* (79/120, 65.8%), with titers ranging from 1:20 to ≥1:2800, followed by *E. cuniculi* (18/120, 15.0%, titers 1:20–1:320) and *N. caninum* (4/120, 3.3%, titers 1:20–1:80). Co-infections were identified in 15% of foxes, and a statistically significant positive association was observed between *T. gondii* and *E. cuniculi*. Stratified analyses showed that adults had a markedly higher *T. gondii* seropositivity (79.3%) compared to juveniles (53.2%), and foxes from Southern Italy exhibited higher prevalence (79.5%) than those from Central Italy (59.3%). Logistic regression confirmed age class and location as significant predictors for *T. gondii* exposure. For *E. cuniculi*, adults showed slightly higher seropositivity than juveniles (20.7% vs 9.7%), although no clear spatial pattern emerged. *Neospora caninum* seropositivity remained low and uniformly distributed across categories, with no significant associations detected.

The obtained results highlight the substantial exposure of red foxes to neurotropic parasites, in particular *T. gondii*, and reinforce the importance of this species as an effective sentinel for pathogens circulating at the wild–domestic interface. The increased seropositivity in adult foxes further supports cumulative exposure over time. Overall, the findings emphasize the need for integrated wildlife surveillance to elucidate parasitic circulation and inform veterinary and public health strategies.

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Oral Communication(s)

DOUBLE TROUBLE! TWO CASES OF LARVAL MESOCESTOIDOSIS (*MESOCESTOIDES SPP.*) IN TWO DOGS FROM THE SAME TOWN

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Mesocestoides spp. are intestinal tapeworms of dogs and cats and other wild carnivores. The life cycle is thought to include coprophagous arthropods as first intermediate hosts and vertebrates including reptiles, amphibians, birds and small mammals as second intermediate hosts, in which the infective larval stages (tetrathyridia) develop in body cavities. Definitive hosts like dogs and cats can also harbor larval stages, leading to larval mesocestoidosis (LM), an uncommon and potentially fatal disease [1]. Severity of infection as well as the host response influences the prognosis significantly. Survival rates in dogs have been reported as 72% at 6 months and 60% at 1-year post-diagnosis and depend on the severity of clinical signs at time of diagnosis and application of an aggressive treatment strategy after diagnosis [2]. Given the uncommon nature of the disease, here the authors describe two cases of LM which were diagnosed in late 2025 in two dogs living in the same small town in the province of Parma, northeastern Italy.

Case [1] A four-year-old, 11.5 kg spayed female mixed-breed dog presented in November 2025 with respiratory signs, including cough and increased respiratory frequency. Blood tests showed only neutrophilia. Thoracic radiographs showed a severe, diffuse, nodular broncho-interstitial pattern with enlarged lymph nodes. Abdominal ultrasound (US) showed several cystic nodules in the peritoneal cavity. Cytology of fluid collected by fine needle aspiration of the cysts showed oval calcareous corpuscles, typical of LM. The dog began treatment with fenbendazole at 50 mg/kg bid. At 30 days follow-up, the clinical picture had improved; abdominal lesions had shrunk, but thoracic lesions persisted. Treatment with fenbendazole was continued, but the dog died one month later for acute respiratory distress after chasing a cat.

Case [2] A fourteen-year-old, 9 kg intact male mix-breed dog presented in December 2025 with a three-month history of chronic enteritis. Blood tests showed neutrophilia and slight hyper-gammaglobulinemia. Abdominal US showed different areas of the peritoneal surface covered with cystic structures up to 2 cm in diameter, arranged in bunches with anechoic or hypoechoic corpusculated content. Similar cysts were observed in the epididymis and right testicle.

Fluid collected by fine needle aspiration of the cysts was turbid, with small flat white spherules in suspension. Microscopically, the spherules were shown to be calcareous corpuscles, typical of LM. The dog began treatment with fenbendazole at 50 mg/kg bid. Follow-up one month later showed cysts had shrunken or had resolved completely. The dog is doing well.

These two cases are interesting because: 1) the dogs presented at approximately the same time and lived in the same small town, suggesting perhaps that the parasite is circulating in intermediate hosts in the area; 2) treatment was started immediately after diagnosis, yet one dog responded while the other did not, suggesting that other factors influence treatment success in LM. Further study is necessary to better elucidate the epidemiology and treatment options for LM.

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Oral Communication(s)

EMERGENCE OF *BABESIA* SPP. IN SOUTHERN ITALY: A ONE HEALTH PERSPECTIVE ON CANINE AND HUMAN INFECTIONS

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Babesiosis is an emerging tick-borne disease of increasing relevance in both veterinary and human medicine. In Europe, the disease is primarily associated with *Babesia canis* and *B. gibsoni* in dogs, and *Babesia divergens*, *B. microti*, and *B. venatorum* in humans. Despite the widespread presence of competent tick vectors and animal reservoirs, data on the epidemiology of babesiosis in southern Italy remain limited. This study provides a comprehensive overview of the occurrence, clinical features, and diagnostic challenges of *Babesia* infections in both canine and human hosts within a One Health framework.

A retrospective study was conducted between April 2021 and December 2025 on dogs clinically suspected of babesiosis and referred to the Veterinary Teaching Hospital of the University of Naples Federico II. A total of 57 dogs were included in the study. Hematological abnormalities were common, with 75.4% of dogs presenting anemia and 24.6% thrombocytopenia, while 29.8% exhibited both conditions. Infection with Babesia spp. was confirmed in 10 dogs (17.5%) through microscopic examination of blood smears and molecular analysis. Sequence analysis revealed a predominance of *B. gibsoni* (9/10 cases), with only one case attributed to *B. canis*. Clinical manifestations included lethargy, anorexia, splenomegaly, proteinuria, and hemoglobinuria, with varying degrees of severity. Notably, co-infections with other vector-borne pathogens, including *Leishmania infantum*, *Ehrlichia* spp., and *Rickettsia* spp., were identified in several cases, complicating both diagnosis and clinical management. Treatment protocols consisted of atovaquone combined with azithromycin for *B. gibsoni*, and imidocarb dipropionate for *B. canis*. While most dogs responded favorably after repeated treatment cycles, one severely affected animal died, highlighting the potential severity of infection.

In parallel, a clinical and molecular investigation was performed on 32 human patients referred to Pellegrini Hospital (Naples) with suspected tick-borne infections. Whole blood samples were analyzed using microscopy and molecular techniques, including HRM-PCR targeting the 18S rRNA gene and digital PCR to enhance sensitivity in low-parasitaemia cases. Three patients (9.4%) were confirmed positive for *B. divergens*. In at least one case, intraerythrocytic parasites were also observed in stained blood smears. Additionally, four patients (12.5%) tested positive for *Borrelia burgdorferi s.l.*, including one case of co-infection with *B. divergens*. Clinical presentation in human cases was non-specific, characterized by fever, malaise, and inflammatory laboratory alterations, underscoring the diagnostic difficulty of babesiosis in clinical practice. Importantly, one confirmed case occurred in a normosplenic patient, suggesting that infection may not be limited to traditionally high-risk individuals.

The predominance of *B. gibsoni* in dogs and the detection of *B. divergens* in humans highlight the complexity of the epidemiological landscape and the potential zoonotic implications. The frequent occurrence of co-infections further complicates clinical interpretation and emphasizes the need for comprehensive diagnostic approaches.

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Oral Communication(s)

SEASONAL DYNAMICS OF HARD TICKS IN NORTHERN ITALY: 12 MONTHS PER YEAR OF ACTIVE QUESTING *IXODES RICINUS* IN A PRE-ALPINE AREA OF LOMBARDY

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The spread of ticks and tick-borne diseases is increasing globally due to social, economic, and climatic factors, posing a growing risk to human and animal health. To investigate these dynamics in a high-risk context, an acarological monitoring was conducted to evaluate the seasonal dynamics of hard ticks in a pre-Alpine area of the province of Como, Lombardy. The study area was selected as a model because it lies within a previously identified high-risk cluster for Lyme Disease in Lombardy[1] and due to prior reports of *Dermacentor marginatus* (Epis and Montagna, personal communication). Four transects (T1, T2, T3, and T4) were selected for monitoring by dragging/flagging from April 2024 to March 2025. Each transect was monitored twice a month, in the morning and afternoon; each sampling of a given transect occurred at least 10 days after the previous one. T1 was at the lowest altitude (480-500 m a.s.l.) while T4 was at the highest (900-950 m a.s.l.). T1, T2, T3, and T4 were 400, 700, 800, and 400 m long, respectively; a total of 55.2 km was monitored over the 12 months.

Overall, 5,308 ticks were collected (5,198 *Ixodes ricinus*; 89 *I. frontalis*; and 21 *D. marginatus*). Considering life stage and sex, 2,356 larvae, 2,381 nymphs, 254 females, and 207 males were identified for *I. ricinus*; only larvae of *I. frontalis* were found; 1 larva, 1 nymph, 10 females, and 9 males of *D. marginatus* were collected. Active questing ticks were collected in all 12 months of sampling. Questing *I. ricinus* were collected in 94 of 96 monitoring sessions across the four transects. Active questing *I. ricinus* peaked in May (1380 ticks: 485 larvae, 769 nymphs, 77 females, 49 males); considering life stage, larvae peaked in August (636). In the two coldest months of the year, T3 and T4 tested negative for *I. ricinus* and other ticks in the January afternoon and January morning sampling sessions, respectively. In December and January, a total of 34 questing *I. ricinus* were collected (3 larvae, 26 nymphs, 1 female, 4 males). Adults of *D. marginatus* were found from April to August 2024 and in March 2025. Overall, 16 of 19 adult ticks were collected in April and March, suggesting that early spring is the peak activity period for this species in northern Italy.

Our survey provided valuable insights into the seasonal dynamics of hard ticks in the study area. Unlike an annual monitoring carried out in Piedmont in 2023, where questing *I. ricinus* were collected from February to November but not in December and January [2], our data confirm year-round activity, highlighting the need to consider prolonged tick prophylaxis for companion animals exposed during winter. In addition, although only a limited number of *D. marginatus* were collected, the seasonal dynamics observed in our study appear to differ from those reported for central and southern Italy [3], possibly reflecting adaptations to distinct local climatic conditions. A second year of monthly sampling in transects T3 and T4 (April 2025–March 2026) supported this hypothesis. These findings underline the importance of surveillance in areas identified as hotspots for tick circulation, to support timely public and veterinary health interventions.

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Oral Communication(s)

DEVELOPMENT OF AN INNOVATIVE *IN VITRO* ASSAY TO ASSESS THE REPELLENT ACTIVITY OF WOOD VINEGAR AGAINST *HAEMATOPINUS TUBERCULATUS*

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Intensive water buffalo farming systems favor the proliferation of lice, particularly *Haematopinus tuberculatus*, causing direct damage characterized by clinical signs such as skin irritation, anemia, anorexia, restlessness, and loss of body condition [1]. In addition, lice infestations (pediculosis) are associated with reduced productivity in buffalo herds, which represent an important livestock resource in several countries [2]. Given the lack of alternative solutions, the aim of this study was to evaluate the repellent effect of a low impact by-product, the wood vinegar (WV), against *H. tuberculatus* using a new *in vitro* bioassay test.

The *in vitro* bioassay was performed in glass Petri dishes (ø 19 cm) divided into 3 separate sections, each covered with a layer of bibulous paper soaked with 3 mL of different solutions. The first (starting) and the third (finishing) area layers were soaked with distilled water. The layer of finishing area was previously vigor- ously scrubbed on the host skin and then covered with buffalo fur after soaking, to increase the attraction to the lice. The central (obstacle) area was impregnated with different concentrations of the WV to be tested (100%, 75%, 50% and 25%). In the positive and negative controls, the central layer was soaked with deltamethrin solution and distilled water, respectively.

Each assay included three replicates, with 10 adult lice per replicate, maintained at 27 °C and 75% relative humidity. Observations were conducted at 5, 15, 30, 60, 90, 120, and 180 minutes post-application to evaluate lice movement, survival, and their ability to cross the treated area, expressed as avoidance percentage. In parallel, *in vitro* cytotoxicity tests were also performed.

The results demonstrated a strong repellent effect, with 100% WV achieving 77% avoidance after 30 minutes of exposure, a value comparable ($P < 0.05$) to that observed with deltamethrin (77%). The bioassay developed in this study proved to be a robust and reproducible method for screening repellent compounds against lice under controlled conditions.

Overall, wood vinegar showed promising potential as an alternative tool for the control of buffalo pediculosis. However, further *in vivo* investigations are required to confirm its efficacy and safety under field conditions.

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Oral Communication(s)

WHEN PARASITES DECEIVE: FROM A “LEECH” IN A BLACK STORK TO A HIDDEN HEPATIC INFECTION IN A DOG

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Parasitic infections are frequently underestimated in routine veterinary practice-how often are they missed simply because they are not suspected? Limited exposure to parasitology and lack of specific expertise may lead to misidentification or complete oversight of parasitic findings. These case reports exemplify how parasites can mislead clinical suspicion and remain undetected until thorough post-mortem investigation is performed.

A Black Stork (*Ciconia nigra*) originating from Poland was found dead in Reggio Emilia (Northern Italy) and submitted to the University of Parma with a presumptive diagnosis of leech infestation, due to the presence of multiple dark, elongated organisms attached to the oral cavity. Post-mortem examination instead revealed numerous trematodes firmly adherent to the oral and oesophageal mucosa, associated with diphtheroid lesions, and multifocal granulomas within the small intestine. Stereomicroscopy identified adult flukes containing operculated eggs with miracidia, and histology confirmed parasitic stages within intestinal lesions. Based on morphology, lesion distribution, and host origin, the parasites were consistent with *Cathaemasia hians* [1]. Although described in Eastern Europe, this parasite has not, to the authors’ knowledge, been previously reported in Italy, likely reflecting the limited necropsy data in this species rather than true absence.

The second case involved a two-year-old mixed-breed dog presenting with severe chronic hepatopathy. Clinical findings, imaging, and liver biopsy supported congenital hepatic fibrosis with portal vein hypoplasia and multiple acquired portosystemic shunts, ultimately resulting in end-stage liver failure and leading to euthanasia.

Post-mortem histological examination of the liver revealed numerous nematode eggs scattered within the hepatic parenchyma, morphologically consistent with *Capillaria hepatica*. This finding was incidental and initially challenging to interpret, as it did not clearly correlate with the clinical presentation. Notably, marked fibrosis was observed surrounding many of the eggs. *C. hepatica* is characterized by a peculiar life cycle, in which eggs are retained within the liver and are released into the environment only after host death and decomposition or following predation and ingestion of infected hepatic tissue. Although capable of infecting a wide range of mammals, it is rarely reported in dogs.

In this case, the presence of *C. hepatica*, together with the associated fibrotic reaction, raises an intriguing question: could this parasite have contributed, at least in part, to the development or progression of such severe hepatic damage and vascular alterations, or is it merely an incidental finding in a liver already affected by a presumed congenital disorder? While a causal relationship cannot be established, *C. hepatica* likely did not initiate the vascular anomaly but may have acted as a “second hit”, exacerbating hepatic fibrosis and portal hypertension.

These reports emphasize how parasites may be overlooked not because they are in unusual locations, but because they are not initially suspected. Integrating morphological expertise with a solid understanding of parasite biology remains essential for accurate diagnosis and interpretation.

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Oral Communication(s)

OCCURRENCE OF *CRYPTOSPORIDIUM* AND *GIARDIA* IN RUMINANT FARMS IN SOUTHERN ITALY

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Cryptosporidium and *Giardia* are protozoan parasites widely recognized as major and ubiquitous enteric pathogens in livestock worldwide [1], primarily affecting neonatal and young animals and causing acute, often severe gastrointestinal disease characterized by diarrhea, dehydration and impaired growth. As a consequence, these infections are associated with reduced weight gain, decreased milk and meat production, and reduced overall farm productivity, resulting in substantial economic losses for the livestock sector [2]. Their persistence in the environment and resistance to common disinfectants further complicate their control and contribute to their widespread distribution.

Beyond their veterinary relevance, *Cryptosporidium* and *Giardia* are of considerable public health importance. Both parasites are recognized zoonotic agents, with transmission to humans occurring mainly through the ingestion of contaminated water and food, as well as through direct or indirect contact with infected animals or contaminated environments [3]. Integrated surveillance and control strategies based on a One Health approach, linking animal health, environmental monitoring, and human health, are essential. Since 2025, a monitoring and control programme targeting these parasitic infections has been implemented in a highly endemic area of southern Italy (Campania region). This programme combines innovative procedures designed to better understand and mitigate transmission dynamics. The main activities include: (i) parasitological analyses in livestock farms (sheep, goats, cattle, and water buffalo) with neonatal diarrhea, including analysis of risk factors associated with *Cryptosporidium* and *Giardia* infections; (ii) analysis of water sources used for animal drinking and milk reconstitution in positive farms; (iii) implementation of biosecurity and pest management measures aimed at reducing environmental contamination and infection pressure; and (iv) outreach activities, including communication and dissemination initiatives, and health education programmes directed at farmers and veterinary practitioners to promote awareness and adoption of best practices.

The present study provides an updated overview of the epidemiological situation in southern Italy, confirming a high prevalence of these infections in livestock, with rates reaching up to 39.5% for *Cryptosporidium* in buffalo farms and 64.7% for *Giardia* in cattle farms. These findings underline the relevance of these parasites in local farming systems and point to a potentially important public health concern in this area. Overall, the results highlight the urgent need for effective control strategies based on comprehensive and transdisciplinary actions, in line with a One Health approach.

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Oral Communication(s)

BESNOITIA BESNOITI ANTIBODIES IN BULK TANK MILK FROM DAIRY CATTLE HERDS IN ITALY

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Bovine besnoitiosis (BB) is a parasitic disease caused by the protozoan *Besnoitia besnoiti*. It is chronic and debilitating, characterized by both cutaneous and systemic manifestations, compromising animal welfare and causing economic losses. In Europe, BB is emerging or re-emerging, with increasing geographical distribution and number of infection cases.

Even though outbreaks of BB have been reported in Italy, updated epidemiological data on the spread of *B. besnoiti* are lacking.

This study aims to update data on the prevalence of *B. besnoiti* and the effect of infection on the performance of dairy herds located in one of the largest dairy production areas in Italy (Lombardy).

Bulk tank milk (BTM) samples collected from 586 herds were analyzed by an indirect ELISA to detect anti-*B. besnoiti* specific antibodies.

Overall, 76 herds resulted positive for *B. besnoiti* antibodies in BTM (P = 12.9%). Lower prevalence values were recorded in the provinces of Cremona and Bergamo (P = 8.2 and 8.3, respectively), than in Milano (P = 11.1%), Pavia (P = 13.7%), Brescia (P = 14.1%), and Mantova (P = 16.7%), with the highest value recorded in the province of Lodi (P = 20.2%).

A higher prevalence was detected in large herds (> 400 animals) (P = 19.5%) compared to medium (200-400 animals) and small (< 200 animals) herds (P = 11.7 and 8.5%, respectively). Overall, the medium consistency of positive herds was higher than that of negative ones (483.9 and 330.5 animals, respectively).

Herds with younger animals (≤ 3 years old) resulted more susceptible to parasite infection (P = 14.8%) than herds with cows > 3 years old (P = 6.6%). However, the medium age of positive herds was similar to negative ones (3.1 and 3.2 years old, respectively).

In terms of herd performance, the number of inseminations required for conception was almost similar for positive (2.6) and negative herds (2.5). The prevalence of *B. besnoiti* was 12.4% for herds with repeat breeder cows that required more than three services to conceive and 13.2% with < 3 inseminations. Besides, the period from calving to conception was similar between positive and negative herds (152.8 and 164.8 days, respectively). The prevalence of the infection was lower in herds with a days open period of over 150 days if compared to those with a period of 150 days or less (P = 9.3% and 18.5%, respectively).

The daily milk yield and 305ME were higher for positive herds than for negative ones (28.4 kg and 24.8 kg, 10389 and 9896, respectively). Seropositivity to *B. besnoiti* infection was more frequently detected in herds with a daily milk production higher than 25 kg (P = 18.8%), whereas herds producing less than 25 kg of milk (P = 9.6%) were less often positive. Besides, a higher prevalence of *B. besnoiti* infection was observed in herds with 305ME greater than 11,000 compared to those with a value of 11,000 or less (P = 16.0% and 10.9%, respectively). No differences were revealed in fat and protein content between positive and negative herds (3.83% and 3.78%, and 3.38% and 3.36%, respectively). Somatic cell count was similar for positive (245,246) and negative (244,160) herds; herds with more than or equal to 300,000 cells (P = 14.5) were more frequently tested positive than those with less than 300,000 (P = 12.2%).

This study confirms the circulation of antibodies against *B. besnoiti* in Italian dairy herds. Bulk tank milk serology is a useful tool to estimate and monitor its prevalence in cattle farms. The impact of the parasite on herd performance could be hypothesised.

Oral Communication(s)

ZOONOTIC HELMINTHS AND PROTOZOA IN CAPTIVE NON-HUMAN PRIMATES: IMPLICATIONS FROM A TWO-YEAR STUDY IN A NORTHERN ITALIAN ZOOLOGICAL PARK

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Non-human primates (NHPs) under human care are frequently exposed to intestinal parasites that may affect animal health and represent a potential source of zoonotic infections. Surveillance of parasitic infections in captive NHPs is therefore essential for assessing animal welfare, evaluating disease risk, and understanding zoonotic potential [1]. In this study, we longitudinally investigated the presence, diversity, and zoonotic potential of intestinal parasites in NHPs housed in a zoological garden in Ravenna, Northern Italy, with particular focus on *Lemur catta*. From April 2024 to March 2026, five fecal samplings were performed on lemurs housed in two net-covered enclosures with vegetation and wooden shelters used as refuges. A total of 103 samples were examined. All fecal samples were subjected to microscopic examination following sedimentation with Lugol staining and Ziehl–Neelsen staining; qualitative and quantitative flotation techniques were also performed. Molecular confirmation was also carried out on positive samples. Microscopic examination revealed the presence of *Trichostrongylus* sp., *Strongyloides* sp., and *Trichuris* sp. eggs, as well as *Entamoeba* spp., *Blastocystis* spp., *Chilomastix* sp., *Giardia duodenalis*, and acid-fast apicomplexan oocysts, with frequencies varying across the different sampling events. The detection of *Trichostrongylus* sp. eggs can be confidently attributed to *Trichostrongylus colubriformis*, based on previous molecular characterization conducted on the same colony two years earlier [2]. *Strongyloides* sp. eggs were molecularly identified as *Strongyloides fuelleborni* in lemurs, representing, to our knowledge, the first report of this clinically relevant zoonotic species in Italy. *Chilomastix* sp. was molecularly assigned to *Chilomastix mesnili*, clustering phylogenetically with isolates from other non-human primates. In addition, *Giardia duodenalis* was characterized as zoonotic assemblage B1 based on TPI gene sequencing. *Entamoeba* spp. are currently under molecular characterization. Overall, these findings demonstrate the circulation of both zoonotic helminths and protozoa within the studied facility, highlighting a tangible risk at the human–animal interface. The implementation of targeted biosecurity measures, particularly focusing on animal keepers and visitors, is therefore essential to mitigate zoonotic transmission risks.

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Oral Communication(s)

AN INNOVATIVE DIGITAL PCR ASSAY FOR THE DETECTION OF BENZIMIDAZOLE RESISTANCE IN *HAEMOCHUS CONTORTUS*

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Gastrointestinal nematodes, and particularly *Haemonchus contortus*, cause substantial economic losses in small ruminant production systems worldwide due to reduced weight gain, decreased milk and wool yield, anemia, and increased mortality [1]. The increasing prevalence of benzimidazole resistance in *H. contortus* represents a major challenge for the sustainable control of this parasite. Resistance is primarily associated with single nucleotide polymorphisms (SNPs) in the β-tubulin isotype-1 gene, particularly at codons 167, 198, and 200 [2] . Early and accurate detection of these mutations is essential for effective parasite management. However, several molecular diagnostic methods, including standard PCR-based assays, real-time PCR, loop-mediated isothermal amplification, next-generation sequencing, droplet digital PCR (ddPCR), and three-colour digital PCR (d-PCR), often lack sufficient sensitivity, quantitative accuracy, or scalability [3].

In this study, we developed an innovative five-colour dPCR assay for sensitive and precise detection of benzimidazole resistance-associated SNPs in *H. contortus*. The assay was designed to target the most relevant mutations in codons 167 and 200 in the β-tubulin gene, enabling absolute quantification of resistant and susceptible alleles without the need for standard curves. Specific primers, probes and amplification conditions were optimized to discriminate between wild-type and mutant alleles, ensuring high specificity. Analytical performance was evaluated through limit of detection (LoD) and limit of blank (LoB), as well as repeatability and reproducibility assessments. Furthermore, field samples collected from naturally infected small ruminants, including animals co-infected with Trichostrongylus spp., were analyzed to assess the applicability of the method under field conditions. Analytical validation demonstrated that the dPCR assay provides high sensitivity, with the ability to detect low-frequency resistance alleles in mixed parasite populations. The assay exhibited high repeatability and reproducibility across replicates, as well as a low limit of detection. LoB was defined as a maximum of three positive partitions. The results confirmed the presence of resistance-associated alleles (F200Y) in three out of eight (37.5%) farms analysed. No post-treatment SNP-data were available. These findings highlight the utility of the assay for monitoring emerging resistance. Overall, the developed dPCR method represents a robust, accurate, and highly sensitive and specific tool for the detection and quantification of benzimidazole resistance in *H. contortus*. Its implementation could significantly improve resistance surveillance programs and support evidence-based anthelmintic treatment strategies, contributing to more sustainable parasite control in livestock. Further studies will be conducted to extend the application of the assay to other parasitic species.

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Oral Communication(s)

FURTHER UPDATES ON STRONGYLE INFECTIONS IN SPORT HORSES IN ITALY

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To update data from our previous survey carried out around 10 years ago [1], this study assessed the current epidemiology of Strongyle infections in Italian horses. In 2025, a total of 3,254 fecal samples from 234 stables were examined. Horses aged 5 months to 35 years were tested using the McMaster technique sensitivity: 15 eggs per gram (EPG) with a saturated sodium chloride solution. Results were analyzed by age class (≤1, >1–≤2, 3–≤4, 5–≤14, 15–≤21, >21 years), sex, season, management type (box, pasture, semi-pasture, box + semi-pasture), and national district of origin.

Parasitic infections by strongyles (SI) were detected in 94% of the examined sample batches (220/234), with an overall prevalence of 47.5% (1,542/3,254) and mean eggs per gram of feces (EPG) of 265 ± 562, corresponding to a mean intensity of 559 EPG. Prevalence and mean EPG values for SI showed a decreasing trend with increasing age of the examined horses (χ^2 trend = 121.16038; $P < 0.001$; Kruskal–Wallis test $H = 144.76$; $P < 0.001$).

When stratified by sex, prevalence was significantly lower in males (47.6%) than in females (55.7%) ($\chi^2 = 18.1159$; $P < 0.001$), whereas mean EPG values were significantly higher in males (261.2 ± 530.7) than in females (225.1 ± 499.5) ($W = 18{,}60716.5$; $P = 0.042$). No significant differences were observed in prevalence rates or mean EPG values among the different seasons ($P > 0.05$).

Significantly higher prevalence rates were recorded in horses managed under mixed systems (box + semi-pasture) (59.4%) compared with other management types (χ^2 trend = 17.824; $P < 0.001$), and this management category also showed significantly higher mean EPG values (535 ± 866) ($H = 56.85$; $P < 0.001$).

Prevalence rates were 48.0%, 48.3%, and 34.2% in horses raised in northern, central, and southern Italy plus the islands, respectively, although these percentages did not differ significantly (χ^2 trend = 2.964; $P = 0.085$), in contrast, mean EPG values were significantly lower in horses from southern Italy and the islands (245.1 ± 669.2) than in those from central (266.3 ± 559.8) and northern Italy (268.3 ± 554.4) (H adjusted for ties = 9.71; $P = 0.008$).

Overall, 27.9% of coprological samples showed GIN EPG values >200, above the threshold commonly used to indicate the need for selective anthelmintic treatment in adult horses. With regard to the “80/20 rule”, 80% of all recorded EPG values (690,080) were shed by 34.9% of the positive horses.

The present survey indicates that, compared with the previous decade, strongyle infections in Italian horses have undergone a significant increase in prevalence, in the proportion of animals with EPG levels >200 (27.9% vs 21.4%) ($\chi^2 = 51.965$; $P < 0.001$), and in the percentage of horses accounting for the “80/20 rule” (34.9% vs 12.8%) ($\chi^2 = 292.053$; $P < 0.001$). Some parameters remained unchanged, such as the declining trend of prevalence and mean EPG with increasing age and the key role of pasture-based management as a risk factor for infection.

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Oral Communication(s)

GASTROINTESTINAL PARASITES IN DAIRY CATTLE FROM NORTHERN ITALY: A COPROMICROSCOPIC AND MOLECULAR SURVEY

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Gastrointestinal parasites represent a major health and economic concern in intensive dairy farming systems; however, data on their circulation in northern Italy remain limited. Accordingly, this study aimed to investigate the prevalence and distribution of gastrointestinal parasites, including helminths, protozoa, and microsporidia, in a dairy farm in Lodi (northern Italy).

The herd consisted of 240 Holstein-Friesian cattle, from which 109 animals were selected to obtain a representative subset. Animals were stratified by age and production category, including calves, heifers, pregnant heifers, primiparous, and multiparous cows.

Fresh feces were collected and evaluated macroscopically, with a fecal score assigned based on consistency and color. Samples were then analyzed using the FLOTAC® dual technique with two flotation solutions: FS2 (NaCl; s.g. = 1.200) and FS7 ($ZnSO_4$; s.g. = 1.350). Coccidian oocysts were identified by adding 1 mL of 2.5% potassium dichromate ($K_2Cr_2O_7$) to positive samples, which were incubated at 25 °C for at least three days to allow sporulation. Additionally, rapid antigen tests were performed to detect *Cryptosporidium* spp. in calves younger than 1 month.

DNA was extracted from all samples, and nested PCR protocols were employed for the detection and molecular characterization of *Cryptosporidium* spp. and *Enterocytozoon bieneusi* [1,2].

Coccidia were the most frequently detected parasites (50/109; P = 45.9%, mean OPG: 38.4, SD: 64.02). Identified *Eimeria* species included *E. alabamensis*, *E. auburnensis*, *E. bovis*, *E. canadensis*, *E. ellipsoidalis*, *E. subspherica*, *E. wyomingensis*, and *E. zuernii*. The epidemiological pattern of coccidian infection varied across production categories: all 30-day-old calves tested negative for *Eimeria*, while calves and heifers aged 90–300 days exhibited the highest infection levels. In older heifers and multiparous cows, parasitic load declined. Differences in prevalence were analyzed in relation to housing type, hygiene and management practices, with box overcrowding identified as a contributing risk factor.
The only other parasite detected by copromicroscopy was *Strongyloides* spp., which showed a low prevalence (6/109, P = 5.5%; mean EPG: 75, SD: 71.4) and was found exclusively in animals under 7 months of age.

All rapid antigen tests for *Cryptosporidium* spp. were negative, whereas molecular analyses detected two positive samples (P = 1.8%), identified as *C. parvum* and *C. bovis*, exclusively in 30-day-old calves. Enterocytozoon bieneusi was detected in three animals (P = 2.7%), two of them under three months of age and one adult cow. All three isolates belonged to Group 2, which includes genotypes with zoonotic potential.

This study provides a comprehensive overview of gastrointestinal parasites circulation in an intensive dairy farm in northern Italy, highlighting age- and production category-specific distribution. The integration of copromicroscopic and molecular approaches enabled the identification of key parasite species, emphasizing the importance of laboratory diagnostics and underscoring the need for further investigation of herd-level risk factors, considering both potential economic losses and zoonotic risks.

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[2] Buckholt et al. Prevalence of *Enterocytozoon bieneusi* in Swine: an 18-month survey at a slaughterhouse in Massachusetts, Appl Environ Microbiol, 68:2595-9, 2002.

Oral Communication(s)

BLASTOCYSTIS SP. AND *GIARDIA DUODENALIS* IN A NORTHERN ITALIAN DAIRY FARM: PRELIMINARY INVESTIGATION IN ANIMAL FAECES AND WATER

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Giardia duodenalis and *Blastocystis* sp. are intestinal protozoan parasites of veterinary and public health relevance. *Giardia duodenalis* is widely distributed in livestock and can be responsible for enteric disease, potentially leading to significant economic losses. Its prevalence in Europe is estimated at around 35 % at the individual level and has also been detected in Italian dairy cattle. *Blastocystis* sp. colonises the intestinal tract of humans and a wide range of animal hosts, with a global prevalence of 24.4 % in cattle. However, data regarding its spread in Italian cattle are limited. Both parasites can persist in environmental matrices, including water, facilitating transmission. Recent studies on waterborne protozoan outbreaks have identified *G. duodenalis* as a frequent aetiological agent, and, although less common, *Blastocystis* sp. has also been detected. This emphasises the importance of monitoring protozoa in water sources.

This study aimed to assess the prevalence of *G. duodenalis* and *Blastocystis* sp. in a dairy herd in northern Italy and to evaluate whether the animals' drinking water could be a potential source of exposure.

For this purpose, 109 faecal samples were collected from September 2024 to March 2025 from cattle belonging to different production categories. DNA was extracted from all samples and PCR analyses targeting the TPI gene for *G. duodenalis* [1] and the SSU rRNA gene for *Blastocystis* sp. [2] were performed.

Giardia duodenalis was detected in three animals (P= 2.75%), while *Blastocystis* sp. in 11 samples (P= 10.09%). Following these findings, water samples (10L) were collected from the two drinking sources used in the farm: a tap connected to the municipal water supply and a well. The water samples were processed by centrifugation to separate the pellet from the supernatant, and the pellets were then subjected to DNA extraction. PCR assays targeting *G. duodenalis* and *Blastocystis* sp. were then performed using the same protocols applied to faecal samples and *G. duodenalis* was detected in both sources.

To investigate the potential contribution of water in the observed infections, a second round of sampling was conducted in March 2026. This includes water samples from a well, a tap connected to the municipal water supply, and a private household served by the same supply, as well as 35 faecal samples. Rapid antigen tests performed on faecal samples identified *G. duodenalis* in two animals. Molecular analyses are currently ongoing on both water and faecal samples to evaluate the presence of *G. duodenalis* and *Blastocystis* sp., and to further characterize isolates previously detected through assemblage and subtype identification.

This preliminary study demonstrates the circulation of *G. duodenalis* and *Blastocystis* sp. in a dairy cattle herd and suggests a potential role of water as a source of exposure. The detection of *G. duodenalis* in both animal and water samples supports the need for integrated fecal and environmental monitoring. Ongoing molecular analyses will help to clarify transmission pathways and assess the relationship between water contamination and the positive results observed.

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Oral Communication(s)

EVALUATION OF A *HETERORHABDITIS SPP.*-BASED FORMULATION FOR THE CONTROL OF *AETHINA TUMIDA*: MOISTURE-DEPENDENT EFFICACY OF A BIOLOGICAL TREATMENT

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Aethina tumida, the small hive beetle (SHB), is an invasive hive beetle of *Apis mellifera* colonies causing significant economic losses. Its life cycle includes a vulnerable soil pupation phase, representing a strategic development stage to target for biological control. This study evaluated the efficacy of a commercial formulation containing entomopathogenic nematodes (EPNs) of the genus *Heterorhabditis* spp. as a biological treatment against migrating SHB larvae. The experiment was conducted under controlled laboratory conditions on 120 migrating larvae distributed across 12 experimental units (10 larvae per unit). A sterilized standardized soil substrate was moistened to baseline conditions of 10% relative humidity (RH) before larval introduction. Twenty-four hours later, a standardized dose of 500 infective juveniles (IJ) per unit (50 IJ per target larva) was applied to nine experimental units, while three units served as untreated controls receiving only distilled water. Treated units were subjected to three moisture regimens: low moisture (25–30% RH) very humid (50–60% RH) representing saturated soil, and flooded conditions (100% RH) with visible surface water. Controls were maintained at baseline moisture. All units were incubated at 25 ± 1°C with 80% ambient RH, and adult emergence was monitored daily over 10 days post-pupation. In untreated controls, mean adult emergence was 9.33 ± 0.58 per unit (93% survival), confirming experimental suitability. Adult emergence showed a strong inverse correlation with substrate moisture (Pearson's r=-0.95; p<0.01). Treatment efficacy increased with moisture: emergence was reduced to 1.67 ± 0.57 (82% efficacy) under low moisture, 0.67 ± 0.57 (93% efficacy) under very humid conditions, and completely suppressed (100% efficacy) under flooded conditions. All treated groups differed significantly from controls (p<0.001). These findings demonstrate that *Heterorhabditis* spp.-based formulations represent an effective biological treatment against *A. tumida*, with biological activity critically enhanced by substrate saturation. The observed moisture-dependent modulation provides a rationale for optimizing application protocols in integrated pest management, particularly in humid climates or with strategic pre-treatment irrigation. Future field studies should validate these laboratory findings to establish practical recommendations for beekeepers.

This research was funded by the project "Control of the pre-imago stages of the beehive pest Aethina tumida (Murray, 1867) through variations in soil moisture and the use of entomopathogenic nematodes, other natural biocides, and interceptor traps" ID number IZS ME 10/23RC.

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Oral Communication(s)

HIGH PREVALENCE OF *CRYPTOSPORIDIUM PARVUM* IN DAIRY CALVES: THE ROLE OF VACCINATION, COLOSTRUM, AND FARM PRACTICES

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Neonatal calf diarrhea represents the leading cause of mortality in newborn calves, accounting in some cases for more than 50% of deaths. It can be caused by *Cryptosporidium parvum*, rotavirus, coronavirus and *Escherichia coli*.

Bovine cryptosporidiosis is a disease widely distributed worldwide and is associated with high rates of neonatal morbidity and mortality in many countries. It can affect calves within the first days after birth and requires prompt and intensive therapeutic intervention to prevent death before weaning. Furthermore, *Cryptosporidium* is of significant public health importance, as it can be transmitted to humans and cause disease.

Cryptosporidium infections primarily affect calves during the first weeks of life; clinical signs generally appear during the second week and include diarrhea, weakness, anorexia, dehydration, and massive shedding of oocysts. For this reason, preventive strategies must be implemented as early as possible after birth. The control of cryptosporidiosis relies mainly on careful calf management. However, maintaining high hygiene standards on farms is particularly challenging, as infected calves shed large quantities of *Cryptosporidium* oocysts, which are immediately infective and highly resistant in the environment, as well as to many disinfectants.

Currently, therapeutic options for the treatment of cryptosporidiosis in calves are limited to halofuginone lactate and paromomycin, with variable efficacy. Since 2023, a vaccine has also become available, and in this study we report data on the presence of *Cryptosporidium* in farms that adopted vaccination compared to non-vaccinating farms in the provinces of Parma and Reggio Emilia.

Between June 16, 2025, and June 27, 2025, a total of 160 fecal samples were collected from calves in 31 farms located in the provinces of Parma and Reggio Emilia. Of these, 108 tested positive using fresh smears stained with carbol-fuchsin, indicating a high prevalence (67.5%) of the parasite in the examined farms. In light of these data and the widespread distribution of the parasite in the region, interest in the new *C. parvum* vaccine was immediately evident among many farmers. Therefore, we conducted sampling in 31 farms, including a total of 43 vaccinated cows and 117 unvaccinated cows, and performed parasite detection in the feces of their calves.
A marked difference was observed according to maternal vaccination status: calves born to vaccinated dams (n=43) showed a substantially lower infection rate compared to those from unvaccinated dams (11.6% vs 60.7%), corresponding to a strong reduction in the odds of infection (OR = 0.085; 95% CI: 0.031–0.232; Fisher's exact test, $p < 0.0001$).

Positivity rates varied greatly between farms, highlighting that vaccination alone is not sufficient to reduce the risk of infection. The use of drugs (either prophylactically or when the number of calves with diarrhea increased) was reported by nine farms; in these farms, the proportion of animals positive for *Cryptosporidium* spp. was 69.8%. Among these, three farms used both vaccination and drug treatment prophylactically, and the proportion of positive animals was 55.7%.

Hygiene, biosecurity, and careful calf-rearing management must not be neglected, even when vaccination is implemented. Colostrum management is a crucial component of the vaccination strategy, as antibodies against *Cryptosporidium* are transferred through the colostrum of vaccinated dams. Differences between farms mainly lie in the quality and quantity of colostrum administered, as well as in the timing of administration.

Oral Communication(s)

OPTIMIZING BAITING STRATEGIES FOR STRAY DOGS IN RURAL AREAS TO REDUCE CYSTIC ECHINOCOCCOSIS

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Cystic echinococcosis (CE), caused by the larval stage of the cestode Echinococcus granulosus, is a major zoonotic disease affecting humans as well as domestic and wild animals. It represents one of the most widespread parasitic diseases in Mediterranean countries, including Italy, leading to significant economic losses in both public health and the livestock sector. In highly endemic areas of southern Italy (Campania region), CE control programs are primarily based on integrated strategies that include the treatment of shepherd dogs. Within this framework, the present study aimed to enhance anthelmintic interventions by extending treatment to stray dogs located near CE-positive sheep farms, using praziquantel-laced baits distributed via drone in peri-pasture areas.

The study evaluated the environmental resistance of three different bait formulations over a period of at least ten days under varying climatic conditions, while maintaining palatability and attractiveness for dogs. Additionally, the structural integrity of the baits after aerial release from different heights was assessed. Grazing areas were mapped using GPS devices applied to sheep and shepherd dogs from a CE-positive farm, allowing the identification of a suitable pilot site for bait distribution based on animal movement patterns. Baits were deployed in different configurations and locations, and their uptake by stray dogs was monitored through camera traps.

Among the tested formulations, double-layer praziquantel-laced baits demonstrated the highest environmental resistance while maintaining attractiveness for up to ten days under different weather conditions. These baits also showed excellent mechanical resistance, remaining intact after being dropped from heights of up to 25 meters onto hard surfaces, making them particularly suitable for drone-based delivery.

Field results indicated that 80% of the baits were consumed within the first day, 15% within two days, and the remaining 5% within three days. The majority of the baits (93%) were consumed by stray dogs, while a small proportion (7%) was taken by non-target wildlife, including foxes, badgers, and wild boars.

In conclusion, the double-layer praziquantel-laced baits proved to be highly reliable and effective for the treatment of stray dogs in peri-pasture areas surrounding CE-positive farms. These findings support their potential inclusion as a valuable tool in integrated control programs for cystic echinococcosis.

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Oral Communication(s)

MONITORING CENTRIOLE DYNAMICS IN ANDROGENETIC SHEEP EMBRYOS

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The centriole is a multifunctional organelle involved in chromosome segregation, cell cycle regulation, cell polarity, signalling and motility. Defects in centriole structure or function are associated with several human diseases, including cancer and male infertility. During early embryonic development centriole behaviour differs from that observed in somatic cells. Gametes must halve both their chromosome content and centriole number, and in most mammals, except for mice, the spermatozoon contributes the functional centriole to the zygote, whereas the oocyte centriole is inactivated. This ensures the presence of a single active centrosome during the earliest stages of embryogenesis. In dispermic fertilization, the entry of two spermatozoa introduces two centrioles, often leading to abnormal tripolar spindle formation and defective cleavage.

Androgenetic embryos produced by fertilizing enucleated oocytes with two spermatozoa defeat this role, because, despite the presence of centrioles in double dose, they still divide and developed to the blastocyst stage, suggesting that one sperm-derived centriole may be repressed. These observations raise important questions regarding the mechanisms regulating centriole activation and inactivation during early embryonic development.

The aim of this study was to investigate the behaviour of sperm-derived centrioles in ovine androgenetic embryos, focusing on the potential existence of functional “ON” and “OFF” states of centriole activity. Androgenetic embryos were produced by injecting two spermatozoa into enucleated metaphase II oocytes followed by in vitro activation. Embryos generated by in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) were used as controls to establish the physiological time course of microtubule organization and aster formation after fertilization.

Centriole activity was assessed by monitoring microtubule aster nucleation through immunofluorescence detection of α-tubulin, while chromatin and pronuclear formation were visualized with Hoechst staining and analysed by confocal microscopy. Analysis of androgenetic embryos approximately 6–7 hours after activation revealed heterogeneous patterns of microtubule aster nucleation. Two sperm asters were observed in 24% of embryos (n=5 on 21), indicating active microtubule nucleation from both sperm-derived centrioles. In 43% of embryos (n=9 on 21) only one detectable aster was present, suggesting asymmetric activation of the sperm centrioles, whereas in the remaining 33% (n=7 on 21) no asters were detected, indicating a possible repression of centriole activity at this stage. Interestingly, the proportion of one aster/androgenetic zygotes overlap with the cleavage rate (46%; n=18 on 39), suggesting that the inactivation of one centriole is a sine qua non condition for development.

These preliminary findings suggest that sperm-derived centrioles in androgenetic embryos may exist in different functional states, supporting the hypothesis that regulatory mechanisms control centriole activation during the earliest phases of embryonic development.

Oral Communication(s)

TGF-B3, A PROMISING FACTOR TO IMPROVE FULL-TERM DEVELOPMENT OF IN VITRO–PRODUCED SHEEP EMBRYOS

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In vitro embryo culture is a critical component of assisted reproductive technologies, enabling the zygote to develop to the blastocyst stage for subsequent uterine transfer [1]. However, embryos generated in vitro are typically of lower quality compared to their in vivo counterparts, as indicated by reduced implantation, birth, and survival rates. In livestock species, the efficiency of blastocyst formation in vitro rarely exceeds 20–40% in species such as sheep and pig [2], with reduced implantation rates compared to in vivo embryos [1]. Inadequate activation of essential signalling pathways under in vitro condition is considered a major cause factor contributing to impaired embryonic development.

In this study, we induced activation of the canonical TGF-β/SMAD signalling pathway at the morula stage through TGF-β3 supplementation. This signalling pathway is a key regulator of early embryonic development and cell differentiation. TGF-β3 may influence blastocyst formation and cell differentiation, thereby confirming the biological validity of the research conducted in this study.

Notably, TGF-β3 treated embryos resulted in an approximately twofold increase in blastocyst development compared to the control group [3]. In addition, those embryos displayed improved quality, as demonstrated by immunostaining showing a higher number of CDX2-positive cells [3], indicative of enhanced trophoblast expansion. These observations were further supported by RNA-seq analysis, which compare TGF-β3-treated and control blastocysts, revealed upregulation of genes associated with cell adhesion in treated blastocysts. This suggests improved implantation competence, which was further supported by in vitro outgrowth assays.

To evaluate the functional relevance and translational potential of these findings, we conducted a full reproductive trial. In vitro–produced embryos, were either treated with TGF-β3 at day 4 or maintained as untreated controls prior to transfer at blastocyst stage into recipient ewes. A total of 160 oocytes were fertilized in vitro; half were treated with TGF-β3 and the other half as a control group, resulting in 16 blastocysts in the treated group compared to 6 in the control group. Following embryo transfer of 4 blastocysts per recipient, pregnancy was achieved in one ewe receiving TGF-β3-treated embryos. Ultrasound analysis confirmed normal fetal at 4 months of gestation.

All Experimental procedures have been approved by Ministero della Salute; sito sperimentale Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise with authorization n.1021/2024-PR.

These preliminary results support the relevance of TGF-β3 in improving in vitro embryo production and suggest promising in vivo developmental outcomes, highlighting its potential for translational applications.

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Oral Communication(s)

METABOLIC STRATEGY OF STALLION SPERMATOZOA BEFORE AND AFTER CAPACITATION

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Recent studies suggest that oxidative phosphorylation is the main metabolism to maintain equine sperm function [1]. During capacitation, spermatozoa undergo membrane remodeling, hyperactivated motility, tail-associated protein tyrosine phosphorylation and acrosome reaction. The metabolic pathways supporting this process in stallion sperm are still not fully understood. This study aims to analyze the effect of different glucose concentrations during in vitro capacitation on flow cytometry parameters. Ejaculates are collected from four stallions of different breeds using an artificial vagina. After collection, each sample was incubated for 22h at 37°C and 5% of CO2 with capacitating Whitten's medium containing different glucose concentration: CAP-HighGLU, (5.56 mM glucose) and CAP-LowGLU (0.5 mM glucose). Calcium ionophore A23187 (A23) was added as a positive control for capacitation. The same samples were analysed immediately (T0) and after 6h (T6) and 22h (T22) of incubation. Flow-cytometry was used to assess viability, cellular membrane hyperpolarization (CMH), acrosomal reaction (AR), intracellular calcium concentration (ICC) and the production of NADH and FAD. The relationships between semen physiological parameters, incubation time and capacitating conditions were statistically analyzed using regression models. A significant increase in ICC ($p < 0.0001$) was observed at T22 in sperm incubated in CAP-LowGLU after A23 addition compared with the other experimental groups. Moreover, AR showed a significant increase ($p < 0.0001$) at T22 in cells incubated with CAP-LowGLU, both before and after A23 addition. Following A23 addition, NADH fluorescence increased in both capacitating media at T6 and T22, with the highest increase detected at T6 in CAP-HighGLU. In contrast, FAD fluorescence increased after A23 addition compared with pre-addition levels. This increase was observed at T6 only in CAP-LowGLU, whereas at T22 it was evident in both media, with a tendency toward higher values in CAP-LowGLU. Unlike other parameters, CMH did not show significant differences among the experimental conditions. Overall, the present results indicate that glucose concentration influences key metabolic and physiological responses in stallion spermatozoa. The significant increase in ICC and AR at T22 in CAP-LowGLU following A23 addition suggests that sperm cells may rely on internal energy reserves under low-glucose conditions to sustain late capacitation events. The increase in NADH fluorescence observed at T6 in CAP-HighGLU suggests a stronger stimulation of glycolytic activity and greater provision of substrates for the Krebs cycle when extracellular glucose is available. In contrast, the increase in FAD fluorescence detected in CAP-LowGLU at T6, with its more pronounced increase at T22 compared with CAP-HighGLU, may indicate an earlier and stronger activation of FAD-dependent mitochondrial pathways under conditions of reduced glucose availability. This pattern supports the hypothesis that, when external glucose is limited, stallion sperm shift more rapidly toward oxidative mitochondrial metabolism, potentially relying on alternative substrates or internal energy reserves to sustain capacitation-related processes. Supported by the Italian Ministry of University and Research under the funding "Department of Excellence" (2023-2027): animalS and ENvironmenT: toward a sustaINablE Life (SENTINEL).

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Oral Communication(s)

THE SILENT BURDEN OF REAL-WORLD PET MICROPLASTICS: REVEALING CELL-TYPE-SPECIFIC IMPAIRMENT IN RUMINANT MODELS

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Ruminants are increasingly exposed to microplastic contamination through forage ingestion, particularly polyethylene terephthalate (PET), which is widely used in agricultural practices. Beyond their ubiquity as environmental pollutants, microplastics may act as carriers of endocrine disruptors, raising concerns for reproductive health. Although PET particles have been detected in human placenta, bovine follicular fluid, and semen, their effects on reproductive and accessory tissues remain poorly understood. To investigate how PET microplastics interact with highly vascularized organs, we assessed their impact on ovine term placenta (oTP) cells and bovine mammary epithelial cells (MAC-T). Virgin PET was mechanically processed by progressive milling and sieving to obtain environmentally relevant size fractions (<20 µm and 20–50 µm) and characterized for morphology, size distribution, and crystallinity. Cells were exposed for 24–48 hours to 1, 5, and 50 µg/mL PET microplastics, and cell viability was first assessed; subsequently, functional endpoints (migration, proliferation, apoptosis) and metabolic endpoints (oxidative-stress response, mitochondrial activity) were evaluated.

Size-dependent effects dominated the cellular responses to PET microplastics, with the <20 µm fraction driving the strongest alterations. oTP cells showed marked susceptibility, with viability reduction already at 1 µg/mL, while MAC-T cells were affected only at higher doses. Placental cells exhibited a pronounced oxidative-stress disequilibrium accompanied by mitochondrial functional impairment, indicating that ROS accumulation disrupts mitochondrial homeostasis and ultimately compromises migratory behaviour. Conversely, mammary epithelial cells showed a shift in the apoptosis–proliferation axis, characterized by decreased mitotic activity and enhanced cell death. At elevated doses, both models displayed clear physical interactions with PET particles, consistent with intensified cell–particle contact.

This study is the first to employ PET particles generated through a degradation process that mimics environmental fragmentation rather than relying on commercial microplastics. By using PET fractions that more closely reflect those ingested by ruminants in contaminated forage, the observed effects gain greater ecological and biological relevance. Moreover, considering the chronic exposure of livestock to microplastic-contaminated feed, these results highlight the need to investigate environmentally aged particles and to clarify long-term impacts on reproductive and mammary tissue functionality.

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Oral Communication(s)

PROGESTERONE-DEPENDENT MODULATION OF PLATELET AGGREGATION AND SERUM SEROTONIN IN DIESTRUS MARES

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In several species, during phases of progesterone (P4) dominance, platelet activation and aggregation contribute to a stable uterine environment, supporting tissue repair [1]. Upon activation, platelets (PLTs) release serotonin (5 HT), a mediator involved in vasoregulation and inflammatory modulation, along with other PLT derived mediators involved in peri conceptional communication. Based on these mechanisms, platelet rich plasma (PRP) has been used in mares to improve uterine conditions [2]. However, data regarding how platelet (PLT) indices and PLT related mediators behave during diestrus in mares are limited, restricting understanding of endocrine modulation of PLT physiology. We hypothesized that platelet aggregation (AGREG), indirectly represented by serum 5 HT, is associated with P4 dominance. The objective was to assess P4 dependent AGREG and 5 HT patterns during diestrus in mares. All procedures were approved by the Ethics Committee of Universidad CEU Cardenal Herrera (CEEA 21/012). Twenty healthy Spanish Purebred mares (4–9 years) were monitored to confirm a functional corpus luteum. Blood sampling was performed on Day 14 of diestrus. The study followed a cross-sectional design with a single sampling time point per animal. Systemic 5 HT was quantified by enzyme immunoassay, P4 by ^125I radioimmunoassay, and AGREG using an ADVIA 2120i analyzer. Data normality and homoscedasticity were verified prior to parametric analysis, and Pearson correlations were applied. Mean values were 385.35 ± 116.96 ng/mL for 5 HT, 20.24 ± 1.41 ng/mL for P4, and 84.55 ± 44.45 counts for AGREG. Significant positive correlations were observed between 5 HT and P4, 5 HT and AGREG, and P4 and AGREG ($P < 0.05$). These findings support a functional endocrine–-platelet axis during diestrus in mares. Future studies should explore additional platelet related parameters to further characterize endocrine modulation of platelet activity.

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Oral Communication(s)

IDENTIFYING THE OPTIMAL DIESTRUS SAMPLING DAY FOR THE ASSESSMENT OF PLATELET RELATED BIOMARKERS IN MARES

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Reliable interpretation of platelet related biomarkers requires strict control of physiological variables influenced by the estrous cycle [1]. However, data regarding how platelet (PLT) indices and PLT related mediators behave during diestrus in mares are limited, which restricts our understanding of endocrine modulation of PLT physiology. We hypothesized that the most stable moment of diestrus for detecting increased PLT aggregation coincides with peak luteal activity, when progesterone (P4) concentrations are highest and the release of PLT derived mediators such as serotonin (5 HT) is expected to increase. The objective was to identify the most reproducible day of diestrus for evaluating platelet aggregates (AGREG) and 5 HT in relation to circulating P4 in healthy mares. Twenty clinically healthy Spanish Purebred mares (4–9 years) were monitored by transrectal ultrasonography to confirm a functional corpus luteum. Blood sampling was performed on days 5, 14, and 16 of diestrus. Serum 5 HT was quantified using a competitive enzyme immunoassay, P4 by solid phase ^125I radioimmunoassay, and AGREG using an ADVIA 2120i hematology analyzer. Differences among days were analyzed using ANOVA, and Pearson correlations were calculated after confirming data normality and linearity. All procedures were approved by the Ethics Committee of Universidad CEU Cardenal Herrera (CEEA 21/012). P4 concentrations were significantly higher on day 14 than on day 5 ($P < 0.05$). Similarly, 5 HT and AGREG were elevated on day 14 compared with days 5 and 16 ($P < 0.05$). Correlation analysis revealed positive associations between AGREG and both P4 ($r = 0.630$; $P<0.05$) and 5 HT ($r = 0.363$). Day 14 therefore emerges as a stable framework for platelet biomarker evaluation during diestrus. Future studies should explore additional platelet related parameters and refined temporal sampling to further elucidate endocrine modulation of platelet function.

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Oral Communication(s)

KISSPEPTINERGIC SYSTEM INSIDE PORCINE TESTICULAR TISSUE DURING AGING: GENE EXPRESSION LEVEL AND ITS CORRELATION WITH TESTICULAR PHYSIOLOGY

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Kisspeptin (Kp) is encoded by the Kiss1 gene and first identified in Kiss neurons within the hypothalamus [1]. Studies highlighted a pivotal role of the Kp system in regulating GnRH secretion, but it was only recently that synthesis within the testis parenchyma was discovered, leading scientists to uncover its involvement in local male physiological regulation of reproduction. Kp, acting through local autocrine and paracrine mechanisms, contributes to testosterone (T) secretion, spermatogenesis regulation, pubertal onset, reproductive seasonality, and semen quality. The expression and distribution of Kiss1 and its receptor differ in testicular cells of different species [2]. There are few studies about Kp in swine testis and the local pathways of action of this molecule are yet to be comprehended [3]. This study aims to investigate gene expressions of Kiss1 and Kiss1R (receptor) during the process of aging in porcine testes and valuate the correlation with the main genes involved in testicular physiology: Anti-Mullerian hormone (AMH), androgen receptor (AR), follicular-stimulate hormone receptor (FSHR), luteinizing hormone receptor (LHR). Sixteen (n=16) boar testis were collected and categorized according to age: pre-puberty (< 4 months), post-puberty (10 - 12 months), and older animals at the end of reproductive career (> 25 months). Testes were isolated from the scrota and, after epididymis ablation, were weighed and measured for min and max circumferences: both gonads were weighted and measured. Parenchyma from the central area was collected and stored for T quantification, and gene expression. RNA was extracted with RNeasy mini kit according to manufacturer's instructions and qRT-PCR was performed with SYBR green for target and housekeeping genes. Testicular T was measured with radioimmunoassay (RIA) analysis. All parametric data were analyzed with ANOVA test, while non-parametric one with Kruskal-Wallis test. To correlate gene expression with morphological data and quantification of testosterone, nonparametric Spearman's rank correlation test was performed; significance was set at $P<0.05$. Morphological results showed the physiological growth of testis during different stages. Results from qRT-PCR reported trends of expression between the groups for Kiss1, LHR and AR; but no statistical differences were observed. On the other hand, statistical differences between pre and post puberty were observed for AMH ($P=0.009$) expression and for FSHR between aged and both pre/post-puberty ($P=0.002$; $P=0.010$). The statistical analysis for T concentrations showed a significant decreasing trend ($p=0.032$), with a strong difference between prepubescent and adult animals ($p=0.039$). A positive correlation was observed between Kiss1 or/and Kiss1R and AR ($p=0.001$), LHR ($p=0.013$) and T ($P=0.016$). The observed correlations are likely driven by developmental changes during the transition from pre-pubertal to adult but highlight the role of Kp in testicular maturation. Further analyses are necessary to understand how Kp is involved in testis physiology, in particular protein level and distribution of Kp and Kiss1R. Our results put in evidence a relationship between Kp and testicular physiology underlighting its synthesis and connection with the regulatory pathways in testicular environment. These data could improve knowledge about local role of Kp and its use in animal reproduction management.

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Oral Communication(s)

CORTISOL AND OXIDATIVE STRESS MARKERS IN RAINBOW TROUT UNDER PHOTO-ELECTROCATALYTIC WATER TREATMENT

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The present study is part of the PRIMA project Fish-Photo-CAT, which aims to evaluate a smart purification system to improve water quality, fish production and environmental sustainability in aquaculture. The system employs an innovative TiO_2-based photo-electrocatalytic (PEC) filter for the remediation of nitrogen compounds in water, combining the purifying action of TiO_2 with the biocidal effect of UV radiation.

A monofactorial experimental design was adopted to evaluate the effects of the PEC filter compared to a conventional UV system (CTRL) on the stress status of rainbow trout reared under different conditions. Four independent trials were conducted, combining two fish developmental stages (juveniles and adults) and two stocking densities (low, LD; high, HD). Juveniles were reared at 5 (IBW: 7±0.6g) and 10 kg/m³ (IBW: 5±0.3g) for 3 weeks, while adults were reared at 15 kg/m³ for 4 weeks (IBW: 156±4.5g) and 30 kg/m³ for 2 weeks (IBW: 159±5.2g). Experimental duration varied among trials due to facility management constraints. The trial was carried out at the Spallanzani Institute (Cremona, Italy), where fish were reared in six 500-L tanks (three per treatment). Fish were fed a commercial diet (Nutria, Skretting) twice daily at 3% body weight. Water temperature (14°C) and dissolved oxygen (8.3 ppm) were monitored daily. Prior to the experiment, all fish were verified to be in good health and free of visible disease. Stress status in juveniles (n=60 LD; n=40 HD) was assessed by measuring cortisol, the primary stress-related hormone in fish, in muscle and fin tissues using a specific microtitre radioimmunoassay [1]. In adults (n=30 LD; n=20 HD), cortisol was measured in plasma, skin mucus, muscle, fins and scales to obtain a comprehensive assessment of both short- and long-term stress responses. Plasma glucose and lactate were determined as secondary stress indicators, while oxidative stress markers including Advanced Oxidation Protein Products (AOPP) and the AOPP/Total Proteins (TP) ratio were also evaluated. Statistical analyses were performed using a linear mixed-effects model (treatment as fixed factor, tank as random factor; $p < 0.05$).

No significant differences in muscle or fin cortisol were observed between CTRL and PEC groups in juveniles at either stocking density. Similarly, in adults, cortisol levels across all analysed matrices as well as plasma glucose and lactate did not differ significantly between the two systems. Regarding oxidative stress, no differences were observed the AOPP/TP ratio; however, higher AOPP levels were detected in the PEC group under HD conditions. Despite this increase, cortisol values remained within physiological ranges, suggesting the absence of severe systemic stress.

Overall, the low cortisol levels observed suggest that rainbow trout did not experience significant stress, exhibiting comparable physiological stress responses in the CTRL and PEC systems across both stocking densities. However, the PEC system may be associated with slightly higher oxidative stress in adults under high-density conditions, which warrants further investigation. The PEC system is integrated into conventional UV filtration units and requires only the addition of a TiO_2-based mesh, representing a potentially cost-effective modification for aquaculture applications.

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Oral Communication(s)

A PRELIMINARY STUDY ON THE USEFULNESS OF INFRARED THERMOGRAPHY TO ASSESS FOAL HEALTH BY EYE TEMPERATURE EVALUATION

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The application of infrared thermography (IRT) has become increasingly widespread in veterinary medicine as it is a rapid, non-invasive method for detecting surface temperature changes associated with health and disease [1]. Among the anatomical sites the ocular region assumes a distinctive role because, unlike many other body areas, it is not covered by hair and possesses specific anatomical and physiological characteristics. A notable example is the area around the lacrimal caruncle, which is richly vascularised due to the eye socket's close proximity to the brain. Consequently, measuring eye temperature (ET) allows for the assessment of local changes in blood flow related to the sympathetic nervous system. The aim of the present study was to assess the ET measurement potential as an alternative method for assessing health status in ten pairs of mares and foals using a thermo-camera [2]. The mares were between 10 and 18 years old, with a body condition score (BCS) of 6.2 ± 0.3 and the foals were between 2 and 4 weeks old and showed a BCS of 5.6 ± 0.[2] Thermal images were taken of three ocular regions (ROIs): medial canthus (EL1), lateral canthus (EL2) and central cornea (EL3). In each area, maximum (ETMax) average (ETAvg) and minimum temperature (ETMin) values were assessed. Two-way ANOVA showed a statistically higher ocular temperature values in foals than in mares ($p<0.05$) and no statistically significant differences among the investigated ROIs, with the exception of ETMax of each ROI which were higher in foals than their mothers ($p<0.05$). The higher ocular temperatures found in foals compared to their mares are likely related to the immaturity of neonatal thermoregulatory mechanisms and the changes occurring during the first days of life. Thermography has been confirmed as a non-invasive tool capable of avoiding physical handling.

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Oral Communication(s)

EFFECTS OF SHEARING ON THERMAL WELFARE OF BARBARESCA SHEEP RAISED IN SICILY: SHORT- AND LONG-TERM HAEMATOLOGICAL AND PHYSIOLOGICAL RESPONSES

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Shearing is a fundamental management practice in sheep farming, particularly in Mediterranean regions where high temperatures and solar radiation can affect animal welfare. Despite its benefits, shearing acts as a stressor that triggers neuroendocrine and inflammatory responses. In Barbaresca sheep, an indigenous and resilient breed, understanding these physiological changes is essential for improving welfare-oriented management strategies.

This study investigated the short- and long-term effects of shearing on physiological and hematological parameters, with a focus on acute phase proteins (APPs) as biomarkers of systemic response and thermal homeostasis. A total of 25 clinically healthy female Barbaresca sheep, homogeneous in age (4yo), lactation stage (6 months), and parity, were reared under semi-extensive conditions in Tusa (Sicily). The shearing process (10 min per animal) and measurements were taken at four time points: before shearing (T0), one hour after (T1), 15 days after (T2), and 30 days after (T3). The experimental protocol was approved by the Ethics Committee of the University of Messina under code 07_202[3] Physiological parameters included rectal temperature (RT), heart rate (HR), and respiratory rate (RR) while blood samples were analyzed for complete blood count, fibrinogen, cortisol, and serum protein fractions (albumin and globulins) concentration by means of automatic blood count analyzer, coagulometer, automated immunoassay analyser and electrohoresis technique respectively.

One-way analysis of variance (ANOVA) for repeated measures was applied on physiological and hematological parameters over time and Pearson correlation coefficient was analysed between them before and after shearing.

Statistical analysis revealed significant time-dependent variations.

Cortisol levels increased significantly at T1 ($p<0.0001$), indicating an acute stress response immediately after shearing. In contrast, at T2 and T3, fibrinogen levels increased ($p<0.0001$), while albumin decreased ($p<0.01$), reflecting a sustained inflammatory and adaptive response. Respiratory rate showed a marked increase at T2 and T3 ($p<0.0001$), suggesting a thermoregulatory adjustment following fleece removal during the warmer season.

Hematological findings included a decrease in WBC ($p<0.001$) and MCV at T2 and T3 ($p<0.0001$), and an increase in PLT and MCHC at T3 ($p<0.01$; $p<0.0001$). Correlation analysis revealed positive relationships between RR and fibrinogen ($r=0.45$; $p<0.0001$) as well as α-globulins ($r=0.35$; 0.001), and negative correlations between RR and albumin ($r=-0.62$; $p<0.0001$) , and between RT and albumin ($r=0.37$; $p<0.001$).

Overall, the results demonstrate that shearing induces both an immediate acute stress response and a prolonged adaptive phase in Barbaresca sheep. The early cortisol peak reflects handling stress, while later changes in respiratory rate and APPs indicate physiological adaptation to thermal challenges. These findings highlight the role of APPs and respiratory parameters as reliable indicators of thermal welfare and support the importance of optimizing shearing timing to minimize prolonged stress in Mediterranean environments.

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Oral Communication(s)

HIGH OR LOW? STOCKING DENSITY SHAPES BEHAVIOURAL AND CORTISOL RESPONSES TO AN ACUTE STRESS IN CATTLE

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Animal welfare in farming systems is a multidimensional concept central to both societal expectations and livestock management. Stocking density is a key structural factor in cattle housing and may influence welfare through behavioural dynamics and physiological stress responses [1]. Higher densities have been associated with increased agonistic interactions in cattle [2], although its role in modulating responses to acute stressors remains unclear. Routine litter replacement, although standard practice, may act as an acute stressor depending on animals' coping capacity.

This study evaluated whether stocking density modulates behavioural and physiological responses to an acute stressor represented by litter replacement. Experimental procedures were approved by the Ethical Committee for Animal Welfare-University of Padua (protocol n° 11/2024). Twenty-five Charolaise heifers (≈12 months) were randomly allocated to three pens (6 × 12 m): two low density (LD, n=6) and one high density (HD, n=13). Animals were monitored on Days 0, 30, 60, 90 and 120. Faecal samples were collected for faecal cortisol metabolite (FCM) analysis using ELISA, and behavioural data were obtained from video recordings. Considering the delay between adrenal activation and FCM excretion, samples collected at Day 60 reflected the physiological response to the stressor applied the previous day.

Behavioural observations were classified into four agonistic components to distinguish low- and high-intensity interactions. On Day 60, bedding replacement temporarily reduced available space, representing the acute stressor. Behavioural data were analysed using repeated-measures regression models at pen level, and FCM concentrations were analysed using linear mixed-effects models.

FCM concentrations showed no overall temporal trend but increased significantly on Day 60 in HD animals compared with LD (60.2 ± 3.74 vs 34.8 ± 3.73 ng/g; $p < 0.0001$), while no differences were observed between groups in the absence of the event (37.7 ± 1.88 vs 38.6 ± 1.87 ng/g). Low-intensity agonistic interactions did not vary over time overall; however, at Day 60 HD animals showed a lower interaction rate compared with LD (IRR = 0.34; $p < 0.05$).

Fighting behaviours showed a significant temporal decrease (−36.5% every 30 days; $p < 0.001$), but were strongly modulated by the acute stressor. Specifically, the event induced a marked increase in HD animals, reaching a tenfold rise relative to baseline. Under the stress condition, HD animals exhibited 4.5 times higher fighting rates compared with LD (IRR = 4.5), indicating a density-dependent modulation of behavioural responses.

These findings suggest that higher stocking density amplifies both physiological activation and behavioural responses to acute management-related stressors. Under such conditions, space may acquire greater competitive value, being perceived as a limited resource [3]. Stocking density therefore appears to modulate stress reactivity rather than only baseline welfare conditions.

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Oral Communication(s)

ENDOCRINE PROFILES IN DEBILITATED *CARETTA CARETTA*: A PRELIMINARY STUDY

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Anthropogenic and infectious pressures threatened *Caretta caretta*, therefore, many individuals are admitted rescuing centers for long-term rehabilitation [1]. Hormone levels are reliable indicators of individual and population health, as well as of environmental impact and stress. Integrative assessment of corticosterone, thyroid and sex hormones is essential to define the health status in sea turtles. Corticosterone (CORT) is the primary glucocorticoid hormone produced by sea turtles in response to stressors. Thyroid hormones (T3, T4, TSH) also respond to stress and are studied as a complement to CORT. These are essential for normal development and physiological functions in vertebrates and, in sea turtles, regulate energy-demanding processes such as growth, metabolism, development, and reproduction [2]. Sex hormones, Testosterone (T) and Progesterone (P), represented a biomarker to evaluate how debilitation alters Hypothalamic-Pituitary-gonadal (HPG) axis regulation and associated endocrine dynamics [2]. The aim of this study was to define the hormonal profile in debilitated *C. caretta* and understand whether CORT can influence thyroid and sex hormones. For these reasons, thirty-five loggerhead sea turtles hospitalized at the C.Re.Ta.M. rescue center were included in the study. On each animal, curved carapace length (CCL) and body weight were recorded and divided into two groups: juveniles (n.17; CCL 31.5±5.6 cm) and sub-adults (n.18; CCL 51±10 cm). Blood samples were collected from the dorsal cervical sinus using lithium-heparin tubes. Plasma concentrations of CORT, T3, T4, TSH, T and P were measured by enzyme-linked immunosorbent assay (ELISA) using commercial kits (Assay Genie). Statistical analyses were performed using GraphPad Prism 5. Data were not normally distributed, and Spearman's correlation analysis revealed no significant associations among the measured hormones. Comparisons between juveniles and subadults (MWU) showed a significantly higher CORT (p=0.027) level in juveniles' group and significantly higher P (p=0.006) levels in sub-adult group. Overall, turtles hospitalized at C.Re.Ta.M. exhibited higher CORT levels than those reported in previous studies [2,3], likely due to stressors such as fin injuries, ingestion of hooks, fishing lines, or plastic debris. In sub-adult group, P, showed higher levels, which may reflect the onset of ovarian steroidogenesis associated with reaching a critical size (CCL 51±10 cm), marking the physiological transition toward sexual maturity. The lack of correlation with CORT suggests HPG axis resilience, implying that maturation processes in *C. caretta* may persist despite elevated glucocorticoids from anthropogenic stress and rehabilitation as previously reported in other studies [3]. Although chronic stress is known to alter endocrine regulation and potentially suppress thyroid axis activity, our results showed no differences in the thyroid hormones concentration between the two groups, contrary to previous findings. Variability in thyroid hormones may be influenced by metabolic conditions, environmental temperature and life-history stage. [2,3]. Despite the scarce endocrine-sexual profiling of *C. caretta*, further studies are needed to better characterize stress responses and the drivers of hormonal variability in this species.

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Oral Communication(s)

CORTICOSTERONE LEVELS IN DIFFERENT FEATHERS TYPE OF WILD EURASIAN TEAL (*ANAS CRECCA*) AND NORTHERN SHOVELER (*SPATULA CLYPEATA*)

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In birds, corticosterone is the main glucocorticoid released by the hypothalamic–pituitary–adrenal (HPA) axis [1]. While the “fight-or-flight” response is mediated by the sympathetic nervous system, activation of the HPA axis contributes to slower and integrated hormonal adjustments supporting physiological variations associated with reproduction, nest defence and migration [2]. The difficulty of tracking individuals across different environments and life stages is known, so that the selection of appropriate biological matrices for physiological study is essential. Recently, increasing attention has been given to “depot” matrices such as feathers, which allow non-invasive sampling and reflect circulating hormone levels without being affected by capture-induced stress [3]. However, the lack of species-specific data and the complexity of the keratin matrix require the optimization of rigorous analytical protocols. The aim of this study was to investigate corticosterone (CORT) content in two wild duck species. A total of 54 specimens were analysed, including: 39 Eurasian Teal (Anas crecca) and 15 Northern Shoveler (Spatula clypeata), sampled in Northern Italy during hunting activities in 202[1] For each individual, secondary remiges (RII) and, where available, scapular feathers (SCP) were collected with the aim to provide baseline data on the physiological status of these species and to evaluate the influence of sex, age, and feather type on CORT levels.

Briefly, feathers were treated with water and isopropanol to remove secretions and organic materials. After the calamus removal, samples were pulverized in liquid nitrogen and subjected to overnight methanol extraction. Corticosterone concentrations were then quantified using an ELISA assay, a method widely employed in the literature across different species. Results showed a statistically significant difference in Anas crecca related to sex ($p = 0.0039$), with males exhibiting higher CORT levels than females. This pattern was consistent in adults ($p = 0.0021$), suggesting sex-related physiological or behavioural differences, possibly linked to activity levels, energy expenditure, or seasonal ecological pressures. In contrast, no significant differences related to sex or age were detected in Spatula clypeata, likely due to the limited number of male samples ($n = 4$). A combined analysis of both species revealed that scapular feathers had significantly higher CORT concentrations than secondary remiges ($p = 0.0056$). This difference may reflect variation in feather growth timing or structural characteristics influencing hormone deposition during feather development.

These preliminary results demonstrate that corticosterone can be reliably quantified in feathers and provide insight into species- and sex-related physiological variation. However, matrix standardization remains essential to ensure data comparability, as highlighted by differences observed between remiges and scapular samples.

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Oral Communication(s)

PRELIMINARY INVESTIGATION ON GLYCAEMIA AND GLP-1 LEVELS IN GÖTTINGEN MINIPIGS UPON CHRONIC METFORMIN ADMINISTRATION

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Nowadays, the use of antidiabetic medications during gestation and lactation is rapidly increasing [1]. In the non-pregnant population, metformin is considered the standard treatment for type 2 diabetes mellitus, while insulin is preferred in the pregnant one due to lack of data. As for neonatal outcomes, the few available studies suggest metformin as relatively safe, but no clear information is available [2]. Such lack of data may also be imputable to the peculiarity of metformin in terms of pharmaco-dynamics and kinetics, partially unknown. As an example, it is still to be determined whether some beneficial effect may be mediated by the glucagon-like peptide-1 (GLP-1), which seems to be upregulated by metformin, alongside its receptor [3]. Gaining deeper knowledge regarding the effects on metformin on glycaemia and GLP-1 levels during lactation may support safer administration in breastfeeding women. Therefore, the aim of the study was to assess glycaemia and GLP-1 levels in lactating Göttingen Minipigs either administered (group met, 500 mg/die) or not (group non-met) with metformin. Procedures were approved according to D. Lgs 26/2014 (n. 35/2023-PR). Blood samples were collected from nine lactating Göttingen Minipigs (group met n=4; group non-met n=5) on four days during lactation week II and III, at 4 timepoints: before drug administration, 1, 3 and 6 h post administration. Animals were fed immediately after drug administration. Glycaemia was assessed for all timepoints using a FreeStyle70 Optium Neo® glucometer (Abbott Diabetes Care Inc., Witney, UK). GLP-1 was quantified only in samples collected before drug administration and 3h after, using a commercial ELISA kit (Human GLP-1 72 ELISA Kit; Cat. No. BMS2194, Invitrogen™, Thermo Fisher Scientific, Waltham, MA, USA) already validated in pigs. Mean glycaemia, expressed in mg/dL, was 66 before administration, 103 after 1h, 91 after 3h and 92 after 6h in met group, while 67, 97, 93 and 85 at the corresponding timepoints in non-met group. As for GLP-1, plasmatic levels before drug administration were 6.7 pg/mL in met group and 6 in non-met; 3h after administration levels were 14.5 and 8.7 respectively. To assess differences imputable to treatment and/or timepoints, 2-way repeated measures ANOVAs were set up. For GLP-1, a significative influence of time ($p<0.0001$) and time*treatment interaction ($p=0.0008$) was highlighted, with the strongest difference shown by met group upon timepoints comparison ($p<0.0001$), indicative of a stronger post-prandial GLP-1 increase. At the 3h timepoint, a difference was also recorded when comparing the 2 groups ($p=0.0016$). The analysis of glycaemia only highlighted a statistical effect of time ($p<0.0001$), with met group showing differences between the pre-admin timepoint and all the other time points; the non-met group only showed differences between the pre-admin timepoint and 1 and 3h after. The latter may be indicative of prolonged post-prandial glycaemic peak mediated by metformin, yet this hypothesis should be further investigated. Overall, the results seem to be indicative of a modulatory effect of metformin on both glycaemia and GLP-1 plasmatic levels in this model, despite the lack of clear mechanism of action. These preliminary data may contribute to better understanding the potential implications of metformin during lactation.

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Oral Communication(s)

DESCRIBING PLASMA TARGETED AND UNTARGETED METABOLOMIC PROFILES IN VARIOUS PIG BREEDS TO INFER METABOLIC ADAPTATIONS AND ANIMAL WELFARE CONDITIONS

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Metabolomics is the study of the metabolome, which refers to the complete set of small molecules within a biological system. These metabolites represent intermediate or final products of metabolic pathways and reflect molecular phenotypes that link an organism's genetic background to complex traits, by providing a general physiological picture of an entire animal. Therefore, metabolomics is a valuable tool for identifying biomarkers associated with biological perturbations associated to intrinsic conditions like the breed and sex of the animals, as well as external factors like stressing conditions and animal welfare issues. Particularly, differences in metabolite profiles between breeds primarily reflect genetic variation helping linking genotypes to metabolic patterns and their adaptation to production systems. Similarly, the investigation of sex-related metabolic differences is valuable, especially for understanding the physiological effects of practices such as castration and their implications for animal welfare. In this study, we focused on the identification of breed-related and sex-related metabolites by implementing a comparative metabolomic approach. We applied targeted and untargeted metabolomic platforms to analyse over 1,000 metabolites in the plasma of approximately 950 performance tested pigs from three breeds: Italian Large White (ILW), Italian Landrace (ILA) and Italian Duroc (IDU). The pigs were slaughtered when they reached at least nine months of age and a live weight of approximately 170 kg. Samples were collected just after jugulation in an authorized slaughterhouse and plasma was prepared within two hours of collection. The animals were not raised or treated specifically for this study, so no ethical evaluation was necessary. Plasma metabolomic profiles were compared between breeds and sexes using a machine learning approach (Random Forest). This analysis identified metabolites that could distinguish between pairs of breeds and also the sex of the animals within each breed, that when tested in an unsupervised manner clearly showed their discrimination and classification ability, reflecting the different genetic backgrounds and biological characteristics. In particular, we identified 100, 59, and 40 metabolites discriminating IDU from ILW, ILA from ILW, and castrated males from intact females, respectively. For example, we observed a higher content of lipids in IDU pigs, while ILW pigs had a higher plasma content of molecules belonging to the amino acid super-pathway. We also observed that more than half of the sex-influenced metabolites were from the amino-acid super-pathway. Overall, this study provided a detailed overview of circulating metabolites that can be used to establish a baseline physiological profile and identify differences between pig breeds and sexes, potentially aiding in the discrimination of different welfare states in animals. Acknowledgments: This study has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No. 01059609 (Re-Livestock project).

Oral Communication(s)

SERUM miRNAS REVEAL METABOLIC CHANGES INDUCED BY GRAPE POMACE IN DAIRY SHEEP

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Grape pomace (GP) is increasingly being explored as a sustainable feed ingredient for ruminants due to its fiber and polyphenol content; however, its systemic molecular effects under practical farming conditions remain unclear. This study tested whether 5% dried grape pomace (GP5) modulates serum circulating miRNAs (cmiRNAs) in dairy sheep. Twelve Sarda ewes (n = 12) were enrolled in a 30-day trial and assigned to either a control (CTRL) or GP5 diet. Small RNA sequencing (NGS) was initially performed in GP5 animals in a paired design, T30 vs T0 (day 30 vs day 0). The differentially expressed (DE) cmiRNAs were validated by RT-qPCR in both the GP5 and CTRL groups. RT-qPCR confirmed the NGS-derived miRNA signature, identifying miR-143, miR-382-5p, miR-409-3p, and miR-194 as DE cmiRNAs. These miRNAs showed consistent and significant downregulation ($p < 0.05$) in GP5 animals. Ingenuity Pathway Analysis (IPA) revealed that these DE miRNAs target shared regulatory hubs, identifying PPAR signaling as one of the most significantly enriched pathways ($P = 6.53 \times 10^{-11}$; adjusted $P = 1.35 \times 10^{-9}$), with a negative activation Z-score ($Z = -2.646$), suggestive of pathway attenuation. Cholecystokinin/gastrin-mediated signaling was also strongly enriched ($P = 2.36 \times 10^{-12}$; BH-adjusted $P = 7.08 \times 10^{-11}$) and predicted to be activated ($Z = +2.828$). The S100 family pathway was likewise significantly enriched ($P = 3.69 \times 10^{-8}$; adjusted $P = 3.21 \times 10^{-8}$; $Z = 3.162$). These pathway-level patterns emerged without enrichment of oxidative stress, cellular damage, or acute inflammation signatures and were consistent with preserved systemic health previously documented in the same cohort [1]. Preliminary functional analyses are ongoing to assess the biological relevance of this regulatory axis at the tissue level, including immunohistochemical (IHC) evaluation of phosphorylated PPARA in liver sections derived from the same animals (CTRL vs. GP5 at Day 30) to investigate whether DE cmiRNAs are associated with post-translational modulation of PPARA activity. Overall, GP5 elicited a selective cmiRNA signature consistent with targeted post-transcriptional regulation, supporting GP as a nutritional modulator promoting controlled metabolic adaptation and physiological immune–metabolic integration in dairy sheep.

Oral Communication(s)

NANOPLASTIC EXPOSURE DISRUPTS REDOX HOMEOSTASIS IN CUMULUS CELLS

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Nanoplastics have emerged as a significant environmental and public health concern due to their ability to accumulate in mammalian tissues and induce adverse effects, including oxidative stress and inflammation. Their presence has also been reported in reproductive organs such as ovaries and testes, raising concerns about potential impacts on fertility in both humans and animals (1). Recent evidence indicates that polystyrene nanoplastics (NPs) can be internalized by porcine oocytes, leading to increased levels of reactive oxygen species (ROS) and reduced developmental competence (2,3). Oocyte maturation is tightly dependent on the surrounding cumulus cells, which regulate key processes such as gene expression, protein synthesis, and energy metabolism, and are increasingly recognized as indicators of oocyte quality. For this reason, the present study aimed to investigate the uptake of NPs and their effects on cumulus cell, given their critical role in supporting oocyte development and embryo production. Porcine cumulus-oocytes complexes were collected and exposed to increasing concentrations of 30 nm NPs (0 or control (CTR), 5, 25, 50, and 100 µg/mL): green-fluorescent NPs to assess uptake, or non-fluorescent NPs to evaluate effects on cumulus cell during in vitro maturation (IVM) for 44 hours. Following maturation, oocytes were denuded, and cumulus cell viability (DRAQ7 staining), ROS production (H_2DCFDA and dihydroethidium staining), glutathione (GSH) levels (monochlorobimane staining), and green median fluorescence intensity (MFI, in arbitrary fluorescence units; afu) as a measure of NPs internalization in live cells were assessed by flow cytometry. Results are presented as mean ± SD, and green MFI was corrected for background fluorescence by subtracting the autofluorescence of CTR samples. A significant increase in green MFI was observed in the 50 and 100 µg/mL samples compared with the CTR (CTR: 0 afu; 50 µg/mL: 77,844 ± 29,238 afu; 100 µg/mL: 119,545 ± 36,640 afu; $p<0.05$). Moreover, the 100 µg/mL group showed a significant decrease in GSH levels in live cells compared with CTR (89.38 ± 16.00 afu versus 112.4 ± 22.81 afu, $p< 0.05$). However, no significant differences were observed among samples in hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-) levels. The findings demonstrate that nanoplastics can impair cumulus cells, mainly affecting redox homeostasis. This suggests the possibility of potential downstream effects on oocyte quality and subsequent embryo development. The present results provide further evidence to support concerns about the impact of plastic pollution on animal reproductive health.

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Oral Communication(s)

DISRUPTION OF THE TUBULIN TYROSINATION-DETYROSINATION CYCLE IN CANINE NEUROGENIC MUSCLE ATROPHY IS ASSOCIATED WITH Δ2-TUBULIN ACCUMULATION AND AXONAL DYSFUNCTION

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Neurogenic muscle atrophy is a clinically relevant condition in dogs resulting from peripheral nerve dysfunction and characterized by rapid muscle degeneration due to loss of neuronal input. Despite its impact on motor performance and neuromuscular function, the physiological mechanisms linking axonal dysfunction to muscle atrophy remain poorly defined [1]. We hypothesized that neurogenic atrophy is associated with altered axonal homeostasis driven by maladaptive remodeling of the microtubule cytoskeleton through changes in tubulin post-translational modifications (PTMs), ultimately impairing axonal transport and neuromuscular transmission [2]. Microtubules are central to axonal physiology, ensuring structural stability and supporting the bidirectional transport of organelles and signaling complexes. These functions are tightly regulated by tubulin PTMs. Among these, detyrosinated tubulin (deTyr) and Δ2-tubulin are associated with long-lived microtubules but differ in reversibility. Detyrosination marks stable yet dynamic microtubules that can re-enter the tyrosination cycle, whereas Δ2-tubulin, generated by irreversible cleavage of the C-terminal residues, accumulates in hyperstable microtubules and has been linked to reduced axonal plasticity and transport efficiency [3]. Muscle biopsies from dogs with neurogenic atrophy, stratified by disease severity and compared with healthy controls, were analyzed to investigate cytoskeletal organization in intramuscular nerve branches. Histological evaluation confirmed denervation features, including fiber size variability and angular atrophic fibers. Immunofluorescence analysis of distal axons revealed a progressive increase in detyrosinated tubulin levels with disease severity ($p < 0.0001$), alongside a marked accumulation of Δ2-tubulin in severely affected animals, indicating a shift toward hyperstable microtubule populations in nerve fibers innervating skeletal muscle. Consistent with these structural changes, electrophysiological assessment (electromyography and nerve conduction studies) demonstrated reduced compound muscle action potential amplitudes, increased latency, and decreased conduction velocity in severe cases, reflecting impaired axonal conduction and altered neuromuscular transmission. Overall, these findings support the hypothesis that neurogenic muscle atrophy in dogs involves a disruption of microtubule-dependent axonal physiology, characterized by a shift toward hyperstable and less plastic microtubule networks. The accumulation of deTyr and Δ2-tubulin may interfere with axonal transport dynamics and synaptic maintenance, contributing to progressive denervation. Tubulin PTMs emerge as key modulators of axonal function and represent potential biomarkers and therapeutic targets in veterinary neuromuscular disorders.

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Oral Communication(s)

PATHOGENIC mRNA ACTIVATION PROFILES AND mIRNA COMPENSATORY RESPONSES CHARACTERIZE THE OSTEOARTHRITIC TISSUE MICROENVIRONMENT

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Osteoarthritis (OA) is a multifactorial joint disorder characterized by cartilage degeneration, extracellular matrix remodeling, and local inflammatory processes [1]. MicroRNAs (miRNAs) have emerged as key post-transcriptional regulators involved in OA-related molecular pathways [2]. A selection strategy integrating experimentally validated miRNA–-target interactions (miRTarBase), network-based interaction mapping (miRNet), and functional enrichment analyses (miEAA) identified the miR-30 family as a highly interconnected regulatory cluster targeting multiple OA-relevant pathways [2]. This study evaluated the expression of the miR-30 family in canine OA using formalin-fixed, paraffin-embedded (FFPE) joint tissues. Nineteen samples were selected (7 OA, 12 CTRL). Expression profiling revealed significant upregulation of miR-30a ($p < 0.05$), miR-30c ($p < 0.05$), miR-30d ($p < 0.001$), and miR-30e ($p < 0.05$) in OA, whereas miR-30b was downregulated ($p < 0.05$). OA tissues also showed increased expression of extracellular matrix remodelling genes, including ADAMTS5, MMP2, MMP9, MMP13, and COL2A1 (all $p < 0.05$, except MMP13 $p < 0.01$). In the chronic inflammatory signaling domain, IL1B ($p < 0.05$) and IL1RAP ($p < 0.01$) were significantly increased, whereas IL1R1, JAK2, and TYK2 showed no significant differences ($p > 0.05$). Genes associated with immune–stromal interaction were also elevated, including SPARC and VEGFA ($p < 0.05$), whereas HP and IGFBP6 showed no significant differences ($p > 0.05$). In the neuroinflammatory and pain-related domain, ASIC3 was significantly increased ($p < 0.05$), whereas NGFB and NTRK1 showed no significant differences ($p > 0.05$). These findings indicate a heterogeneous regulation within the miR-30 family in OA-affected tissues and support their involvement in disease-associated regulatory processes. The integration of mRNA and miRNA profiles suggests that OA may reflect a tissue-level imbalance between transcriptional activation of pathogenic pathways and miRNA-mediated compensatory responses. As these findings derive from a preliminary cohort and reflect the limited availability of pharmacologically untreated canine OA tissues, they should be interpreted as preliminary evidence. Ongoing sample acquisition will allow progressive consolidation and validation of the observed molecular signatures.

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Oral Communication(s)

EXTRACELLULAR VESICLES DERIVED FROM HORSE MESENCHYMAL STROMAL CELLS: MICRORNA PROFILING IN THE CONTEXT OF EQUINE OSTEOARTHRITIS

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Osteoarthritis (OA) represents the most common cause of retirement from athletic activities in horses [1]. Although current pharmacological treatments can be used to alleviate symptoms, the restoration of damaged cartilage is not achieved [2]. Therefore, mesenchymal stromal cells (MSCs) have emerged as a promising alternative in regenerative medicine owing to their paracrine effects, largely mediated by extracellular vesicles (EVs), used as key means of communication between cells. A relevant EVs' cargo is represented by microRNAs (miRNAs), significant post-transcriptional gene regulators whose gene expression can be modulated by the surrounding extracellular microenvironment [3].

As further research is needed to explore their role in EVs and how MSC preconditioning influences them, the aim of this study was to characterize EVs secreted by horse MSCs, investigating miRNA expression changes under different extracellular conditions mimicking equine OA environment.

In this study, primary horse MSCs derived from bone marrow and fat tissue collected from carcasses of slaughtered horses, were used to produce pools of 3 individuals for each tissue. Three adipose tissue-derived MSC pools were cultured under standard conditions and exposed to pro-inflammatory stimuli (IL-1β and TNF-α). EVs were isolated via ultracentrifugation and characterized for size distribution and concentration through Nanoparticle Tracking Analysis. In parallel, a literature analysis was performed to identify a panel of miRNAs linked to the inflammatory response of OA. MiRNAs were therefore extracted from isolated EVs and amplified through RT-qPCR, and the expression of miRNAs selected from literature was evaluated.

MSC-derived EVs were successfully isolated, with a concentration mean of 2.57 x 10^{10} particles/mL and a size mean of 139.40 nm. Extracted miRNAs showed a concentration ranging from 0.705 to 4.48 ng/µL. Nine out of the 23 miRNAs linked to OA from literature were detected through RT-qPCR in untreated MSCs. A preliminary assessment of these selected miRNAs was carried out on the 3 adipose-derived MSC pools to determine whether exposure to pro-inflammatory cytokines affects EVs content.

These preliminary results confirm the presence of miRNAs associated with the inflammatory process of OA within extracellular vesicles derived from equine MSCs. Expanding the analyses by increasing sample size will allow to understand how EV miRNAs are differentially expressed according to changes in MSCs milieu.

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Oral Communication(s)

ZEBRAFISH UNGA IS REQUIRED FOR GENOMIC MAINTENANCE UPON GENOTOXIC STRESS AND MALE FERTILITY

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In the past decade, zebrafish emerged as a popular model organism to characterise vertebrate DNA. Repair mechanisms involve multiple pathways to counter different types of damage the genome encounters. The base-excision repair (BER) pathway repairs spontaneous base deamination, during which cytosine (C) is converted to uracil (U), but the same base deamination does not always occur spontaneously as it is one of the few mechanisms capable of actively erasing transcription-repressive methyl groups from the DNA. From four unique uracil-DNA glycosylases in the BER pathway, two isoforms of Uracil-N-glycosylase (UNG) were accounted as the major rate-limiting factor in human cancer cell-lines [1]. The zebrafish orthologue of UNG2 is the uracil-DNA glycosylase A (Unga). Previous study demonstrated the involvement of the maternally deposited Unga in active DNA demethylation [2]. However, the technique used receive increasing criticism due to production of off-target and nonspecific artifacts, complicating results interpretation. Hence, the aim of the study was to create and characterise a CRISPR/Cas9 unga deficient mutant line for the validation of reported results. Bioinformatic analysis was carried out for target identification and genome editing design, after which targeted CRISPR/Cas9-based mutagenesis was used to introduce a 7 bp deletion in the second exon creating a heterozygous novel allele controlled by sanger sequencing and relative quantification with qPCR. Homozygous unga deficient adult zebrafish was obtained by crossing the heterozygous carriers. Mutagenic Diepoxybutane treatment followed by Acridine Orange staining of 2 days post-fertilisation embryo was performed, controlled by Haematoxylin-Eosin staining of retinal sections. Furthermore, toluidine blue staining of testis sections was conducted together with functional analysis of the spermatozoa. Unlike in previously reported Unga morphant animals, no delayed development and embryonic lethality was observed. Homozygous mutant animals can be raised to adulthood. However, adult males are smaller in size, while females present more dominant abdomen. Quantification of Acridine Orange positive cells demonstrates Unga mutant embryo to be significantly more sensible to mutagens, and Unga is involved in DNA repair. Impairment of Unga also resulted in 50% reduction of male fertility. Toluidine blue staining showed no difference in presence of maturing and mature spermatozoa between mutant and wild-type males. Comparative statistics of sperm analysis demonstrated similar median spermatozoa concentration, non-significant reduction of total motility and progressive motility parameters for mutant fish, and not significantly greater median percentile of immotile spermatozoa [3]. These results do not account for the cause of fertility reduction; hence four biological replicas of spermatozoa and 256 cell stage embryo pool were collected from wild-type and heterozygous mutant fish respectively for DNA extraction and subsequent methylome analysis by reduced representational bisulphite sequencing.

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FELINE HERPESVIRUS-1 ASSOCIATED MENINGOENCEPHALOMYELITIS IN A JUVENILE CAT: PATHOLOGICAL AND VIROLOGICAL FINDINGS

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Feline herpesvirus type 1 (FHV-1) is a well-recognized pathogen primarily associated with respiratory tract disease and ocular lesions in cats, particularly keratitis and conjunctivitis, while involvement of the central nervous system (CNS) is very rarely reported. This study describes a case of meningoencephalomyelitis associated with FHV-1 infection in a 9-month-old male European shorthair cat with a prior clinical history of herpetic keratitis.

The cat was presented with progressive neurological signs, initially characterized by mild locomotor deficits that rapidly evolved into severe tetraparesis. Despite supportive therapy, the clinical condition deteriorated, and euthanasia was elected due to poor prognosis and worsening neurological status.

A complete post-mortem examination was performed. Gross findings in the CNS were unremarkable, but histopathology demonstrated severe multifocal lymphocytic perivascular cuffing throughout the brain and spinal cord, associated with moderate gliosis and occasional neuronal degeneration. These findings were consistent with a diagnosis of non-suppurative meningoencephalomyelitis of likely viral origin.

Given the cat's history and the histological pattern, further virological investigations were conducted. FHV-1 DNA was successfully detected from CNS tissue samples, supporting viral neuroinvasion. To strengthen the etiological diagnosis and exclude other potential viral causes of encephalitis, additional testing was performed for feline immunodeficiency virus (FIV), feline leukemia virus (FeLV), and type A avian influenza virus. All these tests yielded negative results.

To further confirm the role of FHV-1 in the observed lesions, immunohistochemistry (IHC) was performed on formalin-fixed, paraffin-embedded CNS sections. Viral antigen was detected within neurons and glial cells in affected areas, demonstrating a direct association between viral presence and histopathological lesions. Molecular characterization by sequencing further confirmed the identity of the virus, showing high similarity to known FHV-1 strains.

This case provides evidence that FHV-1, although classically considered a respiratory and ocular pathogen, can be associated with severe CNS disease in young cats. The route of viral entry into the CNS remains speculative but may involve neuroinvasion via the trigeminal nerve, a known site of FHV-1 latency, particularly in animals with a history of ocular infection. Reactivation and subsequent spread to the CNS could represent a possible pathogenic mechanism.

The presence of lymphocytic perivascular cuffing and non-suppurative inflammation is consistent with viral encephalitis; however, ancillary diagnostic techniques are necessary for definitive etiological identification. In this case, the combination of molecular detection, immunohistochemistry, nucleotide sequencing, and targeted exclusion of other viral pathogens provided strong evidence supporting FHV-1 as the causative agent of the meningoencephalomyelitis.

In conclusion, this report highlights the importance of considering FHV-1 as a differential diagnosis in young cats presenting with acute or progressive neurological signs, especially in the presence of a history of herpetic ocular disease. It also underscores the value of integrating histopathology with virological and molecular techniques to achieve an accurate diagnosis. Further studies are warranted to better understand the neuropathogenic potential of FHV-1 and its clinical implications in feline medicine.

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MONITORING SUSPECTED AND CONFIRMED ANIMAL POISONING CASES AND TOXIC SUBSTANCES USED IN PUGLIA AND BASILICATA FROM 2023 TO 2025: A STATISTICAL ANALYSIS

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In our study, corpses and baits were analyzed at the Istituto Zooprofilattico della Puglia e della Basilicata (IZSPB) over the three years from 2023 to 2025. Following the standard procedure outlined in Ministerial Ordinance 2025 [1] and previously in 2019, if the necropsy of the cadaver or the inspection of the bait does not exclude the suspicion of poisoning, toxicological examinations follow. All the data are included in the national online portal "Portale Nazionale degli Avvelenamenti Dolosi degli Animali "[2] and used to address the current emergency affecting animals, humans and the environment.

Geolocalised reports extracted from the "Portale Nazionale degli Avvelenamenti Dolosi degli Animali" for the 2023-2025 period show that the most affected species in the Puglia Region are dogs, cats, and pigeons. Out of the total species sampled, the percentage of positive specimens has decreased over time, from 14% to 10.8% for dogs, while that for cats has increased sharply from 7.60% to 18%. Remarkably, there is a significant increase in pigeon poisoning cases, from 2.3% in 2023 to 24% in 2025.

Dogs and cats are the only affected species in the Basilicata Region, with increases in cases from 19% to 38% and from 5% to 17%, respectively.

Chemical-toxicological analyses on suspicious cadavers and baits have shown that metaldehyde is the commonly used molecule in each region. In Puglia, the most commonly used toxic substances are metaldehyde (46%), carbamates (18%), anticoagulants such as bromadiolone (11%) and brodifacoum (9%), and pesticides (13%). Note the significant reduction in pesticide use, from 25% to 12%.

In Basilicata, the most commonly used toxic substances are metaldehyde (51%), pesticides (21%), and carbamates (12%). From 2023 to 2025, there is a notable decrease in the use of metaldehyde (from 73% in 2023 to 42% in 2025) and an increase in carbamates and other pesticides.

Furthermore, in both regions, the use of anticoagulants such as bromadiolone and brodifacoum has increased significantly, from 8% to 25% in Puglia and from 0% to 23% in Basilicata, perhaps due to their easy availability.
Following inspection and toxicological assessment, the baits received by the IZSPB from the Puglia region have shown an increase in positive toxicology results. In Basilicata, instead, positive results have remained stable during the three years.

Suspected poisoning cases, both on cadavers and bait, have shown an increase in positive results on domestic and synanthropic animals, which could pose a risk to public health. Despite the actual regulation, the development of an app "Bocconi Avvelenati" [3] (by "Centro di Referenza Nazionale per la Medicina Forense Veterinaria dell'IZS Lazio e Toscana" and "Ministero della Salute") to report suspect baits for citizens, the poisoning trend seems to have increased. We expect a stable and immediate legislative measure to provide support to all stakeholders that preserve planetary health to stop this phenomenon.

[1] https://www.gazzettaufficiale.it/eli/gu/2025/09/12/212/sg/pdf

[2] https://avvelenamenti.izslt.it/ Source Portale Nazionale degli Avvelenamenti Dolosi degli Animali Ministero della Salute and Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri"

[3] https://www.izslt.it/comunicati-stampa-2022/bocconi-avvelenati-scarica-dal-qr-la-nuova-applicazione.html.

FATAL ACUTE PULMONARY ATELECTASIS DURING LAPAROSCOPIC SURGERY IN A DOG: A CASE REPORT AND FORENSIC CONSIDERATIONS

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Animals that die during surgical procedures represent a relatively small proportion of cases submitted for post-mortem examination. However, these cases are a significant source of stress for both veterinarians and owners, may potentially lead to disputes and medico-legal claims, and are also particularly challenging for entrusted pathologists, as determining the exact cause of death is not always straightforward. Intraoperative deaths may be related to the underlying condition prompting surgery, to pre-existing diseases (most commonly cardiovascular or respiratory), or to anaesthetic and surgical complications.

Among surgical techniques, laparoscopic surgery is widely used in veterinary medicine due to its well-recognized advantages, including reduced invasiveness, decreased postoperative pain, and faster recovery. When properly performed, it is considered a safe procedure. Nevertheless, specific complications may occur, particularly those related to carbon dioxide (CO_2) insufflation and its mechanical and physiological effects.

This report describes an intraoperative death in a 2-year-old female Maremma Sheepdog in good body condition undergoing laparoscopic ovariectomy combined with prophylactic gastropexy. Complete post-mortem examination revealed severe, diffuse pulmonary atelectasis as the predominant lesion, associated with marked parenchymal hyperemia. No thoracic effusion, airway obstruction, or space-occupying pulmonary lesions were identified. The heart showed signs of acute right-sided overload, likely secondary to increased pulmonary vascular resistance. Additional findings included multiorgan hyperemia and features consistent with terminal shock. Findings consistent with recent laparoscopic surgery were also observed, including multiple trocar access sites and a gastropexy performed with four sutures, one of which penetrated the diaphragm full thickness and extended into the thoracic cavity. Histopathology revealed concurrent chronic conditions, including bilateral valvular endocardiosis (moderate on the right, mild on the left), chronic proliferative epicarditis, and chronic enterocolitis compatible with inflammatory bowel disease.

During laparoscopic surgery, CO_2 insufflation is required to establish pneumoperitoneum and may induce cardiorespiratory effects, usually mild, related to reduced diaphragmatic excursion and systemic CO_2 absorption. In the present case, the transdiaphragmatic suture likely altered thoraco-abdominal pressure relationships, allowing CO_2 to enter the thoracic cavity, resulting in pneumothorax and contributing to acute respiratory failure.

This case highlights that, although laparoscopic procedures are generally safe, complications related to gas insufflation and pressure dynamics may occur, particularly in association with specific procedures.

From a medico-legal perspective, intraoperative death represents a frequent source of dispute and a significant challenge in veterinary forensic pathology. The pneumothorax was not promptly detected during surgery and in this case, a thorough necropsy was essential to reconstruct the sequence of events and clarify the causal mechanisms leading to death.

In conclusion, accurate post-mortem investigation plays a crucial role in the interpretation of adverse surgical outcomes, allowing differentiation between procedure-related complications and other contributing factors.

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Posters

CORNEAL NEOPLASIA: FIRST DOCUMENTED CASE IN HARBOUR SEAL (*PHOCA VITULINA*)

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Oncology in marine mammals is still in its early stages, although reports of neoplasia have been increasing in recent years. Within this context, neoplasms in harbour seals (*Phoca vitulina*) are relatively rare, and, more broadly, ocular neoplasms in marine mammals are exceedingly uncommon. To the authors' knowledge, no ocular neoplasms have been reported in this species.

A 23-year-old female harbour seal, housed at Zoomarine, underwent enucleation of the right globe following the detection of a large, fungating, exophytic mass on the corneal surface. The eye was fixed in 10% buffered formalin and sent to Pathological Anatomy Department of the University of Milan where it was subjected to ex vivo optical coherence tomography (OCT) preliminary screening and then routinely processed for histology. The histological neoplasm assessment included: i) haematoxylin and eosin staining (HE); ii) Periodic acid–Schiff (PAS) special staining, and further immunohistochemical (IHC) investigation thought: i) vimentin; ii) Ionized calcium-binding adaptor molecule (Iba-1); iii) Cluster of Differentiation 31 (CD31); and iiii) Factor VIII.

While the high density of the neoplasm didn't allow a complete scanning by OCT, the histology characterized it as well-demarcated, non-encapsulated, infiltrating and expanding the corneal stroma and covered by multifocally ulcerated corneal epithelium. Neoplastic cells were pleomorphic, with a spindle-shaped, stellate component arranged in short, dense, irregularly interwoven bundles, and a round one arranged in short, loosely distributed streams embedded in scant mucinous extracellular matrix. The latter emphasized by the PAS, which also revealed particularly thick basal membranes. Vimentin marked positivity staining confirmed mesenchymal origin, while Iba-1 undelighted a delicate scaffold of cells with dendritic morphology. Additionally, IHC showed CD31 negative staining and strong positivity for Factor VIII.

Based on these results, the lesion was characterized as an undifferentiated pleomorphic stromal corneal neoplasm. Histopathologically, the main differential diagnoses included origin from stromal fibroblasts, vascular endothelium, or histiocytes; however, in light of the strong positivity for Factor VIII, the findings support a diagnosis of hemangiosarcoma.
After the surgery, the animal recovered quickly: she was kept in the dry area of the exhibit for 10 days, when she eventually returned to the main pool and started eating regularly.

The relevance of the present case lies not only in its contribution to marine mammal pathology but also more broadly to ocular pathology, as corneal neoplasms are generally rare and those primarily affecting the stroma are exceptionally uncommon across all species.

Their development is often preceded by traumatic or infectious events leading to activation of stromal repair processes and/or neovascularization.

Posters

COMPARISON BETWEEN MOBILE AND CONVENTIONAL SLAUGHTER: EFFECTS ON ANIMAL WELFARE, MUSCLE INTEGRITY AND TERRITORIAL SUSTAINABILITY

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Pre-slaughter phases, particularly transport, handling and lairage, are important predisposing factors for traumatic lesions in cattle, detectable during post mortem inspection and frequently associated with physiological stress conditions. Mobile slaughter, performed directly on farm through mobile units authorized under current European legislation, represents an innovative operational model aimed at reducing live animal transport, limiting pre-slaughter stress and decreasing logistical impact in rural areas, integrating animal welfare, food safety and sustainability within a One Health framework.

The study compared mobile and conventional slaughter in cattle from local farms in the Province of Bolzano, evaluating physiological stress indicators, muscle integrity, meat quality and microbiological aspects under field conditions. Sixty animals were included and divided into two groups according to slaughter system. Blood samples collected immediately after jugulation were analysed for creatine kinase, lactate, cortisol and glucose in order to quantify acute stress response and correlate biochemical findings with muscle damage.

Post mortem inspection included systematic macroscopic evaluation of carcasses with particular attention to subcutaneous haematomas, muscular haemorrhages and soft tissue bruising. In conventionally slaughtered animals, traumatic lesions were mainly localized in dorsal, lumbar, gluteal and scapular regions, whereas in mobile slaughtered animals lesions were absent or minimal.

Samples of longissimus dorsi muscle were collected in all animals as standard tissue for comparison; in animals showing gross traumatic lesions, additional samples from injured areas including subcutaneous tissue, adipose tissue and muscle were also examined. Muscle pH was measured at 24 hours post mortem, while microbiological quality was assessed by carcass surface sponge sampling for total viable count, Enterobacteriaceae and Salmonella detection under the same slaughterhouse processing conditions for both groups. Histological examination showed interstitial haemorrhage, oedema and early degenerative changes of muscle fibres, more frequently observed in conventionally slaughtered animals and associated with higher biochemical stress indicators. Mobile slaughter was associated with reduced traumatic lesions, better preservation of muscle architecture and comparable microbiological parameters, supporting its applicability as a sustainable strategy in mountain livestock systems. These findings support the role of mobile slaughter within a One Health perspective, combining animal welfare, food safety and environmental sustainability.

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Posters

CALCIUM PYROPHOSPHATE DEPOSITION DISEASE IN A DOG: A MULTIMODAL DIAGNOSTIC APPROACH TO A PERIARTICULAR MINERALIZED MASS

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A 12-year-old male Labrador Retriever (42 kg) presented with lameness and a painful swelling of the left metacarpal region. Radiography and computed tomography revealed a mineralized soft tissue mass (4.3 × 3 × 1.8 cm) involving the dorsal metacarpophalangeal region, without evidence of adjacent bone lysis. Cytologic evaluation yielded low cellularity with amorphous mineralized material and rare spindle cells, leading to a suspicion of subcutaneous calcinosis. Incisional biopsies of the subcutis were submitted for histopathology.

Microscopically, multiple poorly demarcated, subcutaneous, non-encapsulated nodular aggregates were observed, composed of variably sized deposits of finely granular to crystalline, lightly basophilic material. These deposits were moderately positive with Von Kossa stain and exhibited weak birefringence under polarized light. They were surrounded by a granulomatous inflammatory reaction characterized by numerous macrophages, occasional multinucleated giant cells, fibrovascular proliferation, fibrosis, and multifocal chondroid metaplasia. The lameness improved following treatment with anti-inflammatory drugs.

Based on the morphologic features, histochemical profile, and birefringence of the crystals, a diagnosis consistent with calcium pyrophosphate deposition disease (CPPD, pseudogout) was considered. CPPD is a well-recognized condition in elderly humans but is rarely reported in dogs, where it typically presents as a periarticular, tumor-like mass that may mimic neoplasia [2,3]. Histologically, it is characterized by weakly birefringent crystalline deposits associated with granulomatous inflammation and fibrous or chondroid stroma, and most cases are idiopathic [1].

This case highlights the diagnostic pitfalls of periarticular mineralized lesions in dogs, as the clinical, imaging, and cytologic findings were initially suggestive of calcinosis. A multimodal diagnostic approach enabled identification of crystalline deposits consistent with CPPD and their distinction from non-crystalline mineral deposition, underscoring the importance of integrating ancillary techniques for accurate characterization of mineralized lesions.

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EVALUATION OF TOTAL AND FREE SIALIC ACIDS AS A POTENTIAL BIOMARKERS IN CANINE NEOPLASIA

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Sialic acids, of which Neu5Ac and Neu5Gc are the most representative, are a subset of α-ketoaldonic acids, often found as terminal residues in glycolipids and membrane glycoproteins. Neu5Gc synthesis is mediated by the enzyme cytidine monophospho-N-acetylneuraminic acid hydroxylase (CMAH), selectively inactivated in humans and other species during evolution. Nevertheless, Neu5Gc can be incorporated into tissues through dietary intake and function as a xeno-autoantigen, eliciting chronic inflammatory responses. This process, referred to as xenosialitis, may contribute to tumour development and progression. It has been displayed that serum levels of total sialic acid (TSA) can be altered in many types of cancer (e.g. ovarian cancer, breast cancer). This indicates that measuring serum levels of TSA and free sialic acids (FSA) is a valuable clinical tool with potential as a tumour marker for diagnostic/prognostic purposes [1]. Conversely, information on this topic in relation to animals is limited [2]. Objective of this study is to determine the levels of circulating sialic acids in dogs with various types of tumours. The study included a total of 28 serum samples from dogs of various breeds and genders, equally divided into an oncological group and a healthy group. Serum samples were collected at the Veterinary Hospital of the University of Camerino, either during oncology consultations or from blood donors at the Veterinary Blood Transfusion Centre. The samples were analysed using an ELISA assay (MAK314, Sigma-Aldrich, St. Louis, MO), to quantify TSA and FSA levels. The data were analysed using the Mann-Whitney test after assessing the type of distribution (MedCalc® v.20.110 software package). The mean concentrations of both TSA (45.45 ± 22.81 vs 29.43 ± 5.71 mg/dL) and FSA (4.03 ± 1.24 vs 3.14 ± 0.60) in oncology patients were significantly higher than those in the control group ($p < 0.05$), regardless of the specific type of neoplasm. The neoplasms observed in this study were predominantly mast cell tumours (n = 5) and lymphomas (n = 4). The analysis revealed an outlier sample for the TSA concentration (129.64 mg/dL) in a case of lymphangiosarcoma, followed by a case of histiocytic sarcoma (63.50 mg/dL). Several studies have indicated that TSA concentration may act as a valuable biochemical marker for the detection of metastases, disease staging or assessment of treatment response, potentially in combination with other markers. In this instance, the data obtained indicate that the TSA and FSA may be regarded as a potential biomarker for the present neoplastic condition in dogs. Nevertheless, the limited sample size and the diversity of tumour types included in the study limit the interpretation of the results. Furthermore, it is not excluded that a significant elevation in TSA/FSA may not be observed during chronic inflammation, which can also be concomitant of a neoplastic condition. Therefore, further characterisation is required to validate this marker, particularly increasing the number of cases for each neoplastic type. Regarding the involvement of Neu5GC specifically, it is essential to direct greater attention towards Neu5Gc levels and Neu5Gc circulating antibodies.

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Posters

REDISCOVERY AND SCIENTIFIC REAPPRAISAL OF ENRICO SERTOLI'S HISTOLOGICAL COLLECTION FROM THE ROYAL ADVANCED SCHOOL OF VETERINARY MEDICINE IN MILAN

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Enrico Sertoli (1842–1910) is universally recognized for the discovery of the supporting cells of the testis. His scientific activity, however, extended widely across histology, experimental physiology, and biological chemistry, in each of which he distinguished himself through brilliance and originality. Throughout his career, 37 years of which were spent at the Advanced Royal School of Veterinary Medicine (ARSVM) in Milan, he promoted an experimental approach to veterinary anatomy and physiology while maintaining a strong commitment to microscopic investigation. The extensive collection of histological preparations he assembled was long considered lost, having disappeared during the many transformations and relocations that reshaped the ARSVM over roughly 120 years into to the current Department of Veterinary Medicine and Animal Sciences (DIVAS) of the University of Milan. During the recent relocation of the DIVAS to the new campus in Lodi, a substantial portion of this collection was rediscovered.

The material consists of 1,623 microscope slides preserved in 116 wooden boxes, many bearing Sertoli's original handwritten labels. Several preparations are of remarkable quality, considering the simplicity of the instruments, materials, and staining methods employed. The collection documents nearly four decades of experimental activity (1866–1903), encompassing a wide range of tissues, animal species, pathological and physiological conditions, as well as fixation and staining protocols, thus providing a comprehensive overview of Sertoli's research and of late nineteenth-century laboratory practices in veterinary histology and physiology.

Analysis of the collection led us to identify many specimens as those used by Sertoli for his publications. We were able to link groups of slides to his works on lymph gland development, testicular structure and spermatogenesis, and nerve endings in tactile hairs. In several cases, his labels explicitly state that they served as figures in specific papers, while microscopic fields in some well-preserved slides show clear correspondence with printed illustrations. For example, six slides of isolated seminiferous tubules are labelled with references to figures from Sertoli's major 1878 paper [1] and correspond closely to the illustrations reproduced in that publication. This approach, combining microscopic analysis with the examination of original labels and archival documentation, allowed us to reconstruct Sertoli's experimental workflows, highlighting the refinement of histological techniques, the adoption of innovative fixation and staining methods, and the integration between laboratory observation and scientific interpretation.

The microscopic anatomy collection of the ARSVM, largely attributable to Enrico Sertoli, is now part of the Museum of Veterinary Sciences at the University of Milan. Housed at the DIVAS in Lodi, it stands as testimony to the genius of a man who, while shunning public attention and devoting his life to research, and armed only with a microscope and a vivid intelligence, made lasting contributions to scientific knowledge. The rediscovery of this collection restores a crucial component of the material heritage of histology and offers a tangible link between nineteenth-century experimentation and the foundations of modern microscopic anatomy, reaffirming its value for both historical inquiry and scientific reflection.

[1] Sertoli E. Sulla struttura dei canalicoli seminiferi dei testicoli: studiata in rapporto allo sviluppo dei nemaspermi. Arch Sci Med 1878;2:267–95.

JENNY BARBIERI: THE UNIVERSITY OF BOLOGNA PRODUCED ITALY'S FIRST FEMALE VETERINARY GRADUATE

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The University of Bologna was the first in Italy to admit a woman to its School of Veterinary Medicine. Jenny (Laura Maria Ada) Barbieri was born in Salsomaggiore (PR) on 1 December 1904. After moving to Bologna with her family, she graduated from high school in June 192[3] In the 1920s, it was no easy feat for a woman to undertake university studies; she was undoubtedly encouraged by the presence of several graduates in her family: her father was a surgeon; her mother's brother, Carlo Laviosa, was an engineer; and her aunt, Pia Zambotti, Carlo's wife, was an archaeologist and a university lecturer in Milan. Jenny graduated with top marks on 14 November 1927, presenting a thesis entitled Diffuse Ossifying Tuberculous Periostitis in Dogs (supervisor: Prof. A. Lanfranchi). Even before completing her studies, she applied to attend the Institute of Normal Veterinary Anatomy at the University of Perugia, which was directed by Prof. G.B. Caradonna [1]. She subsequently served as his assistant there from 1928 to 193[3] During this period, she published several scientific papers and a book on veterinary topographical anatomy. However, her behaviour was deemed excessively independent by Prof. Caradonna, which led to her dismissal. She returned to the University of Bologna to work as an assistant at the veterinary clinic (headed by Prof. Lanfranchi) and then at the Institute of Anatomy and Histology of Domestic Animals (headed by Prof. A. Mannu until 1948 and subsequently by Prof. V. Chiodi). On 31 October 1954, she was dismissed, officially for lack of productivity, but presumably due to clashing personalities with the institute's director. Aged fifty and with an elderly mother to support, she won a competitive position at the tax office, where she worked until retirement age. Dr Barbieri was highly regarded by Lanfranchi and Mannu, yet opposed by Caradonna and Chiodi [2]. Almost 100 years ago, she paved the way for countless women to enter the veterinary profession, and for this reason she deserves to be remembered.

[1] Archivio Storico dell'Università di Bologna, Fascicolo Assistente, 1933-1954.

[2] Archivio Storico dell'Università degli Studi di Perugia, Fascicolo Assistente, 1927-1933.

THE LEADING FIGURES OF THE BOLOGNA SCHOOL OF VETERINARY MEDICINE

Giuseppe Sarli, Arcangelo Gentile, Gaetano Mari, Giovanna Martelli, Alessandro Spadari, Carlo Tamanini, Annamaria Grandis

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The teaching of veterinary medicine at the University of Bologna began in the academic year 1783–84. Giacomo Gandolfi was the first lecturer in veterinary medicine in that year. His son, Gaetano, succeeded him in 1802 immediately proposed the establishment of a proper School of Veterinary Medicine. However, the political upheavals that followed the succession of Austrian and Papal rule prevented this from happening. Following the untimely death of Gaetano Gandolfi in 1819, Antonio Alessandrini was appointed. After establishing a course in both high and low veterinary medicine, he secured the opening of the 'Veterinary Clinic' for practical studies (1827). In 1850, this finally became the School of Veterinary Medicine. Under the leadership of Giovanni Battista Ercolani, in 1876, the Veterinary School became the Higher School of Veterinary Medicine, operating separately from the Faculty of Medicine and Surgery. It was not until 1932 that it became the Faculty of Veterinary Medicine.

The foundations of the modern Bologna School of Veterinary Medicine were laid by the professors active in the post-war period. Each of them established a School within their respective disciplines, and these Schools have continued to the present day through generations of pupils.

Valentino Chiodi (1898–1970) - professor of veterinary anatomy, was an eclectic figure. Expert of the heart and its conduction system, he was also a passionate historian of veterinary medicine and a poet.

Giuseppe Borgatti (1899-1966) - professor of veterinary physiology, he also held a degree in medicine and surgery. He is best known for his research into the neurophysiology of the digestive system in ruminants.

Luigi Montroni (1902–1978) - was a professor of veterinary pathological anatomy who devoted himself to the study of tumors and tuberculosis. The dedication to sharing his knowledge with the students resulted in the publication of a treatise on veterinary pathological anatomy. He also published collections of poetry.

Alessandro Lanfranchi (1877–1958) - professor of veterinary infectious diseases, was the first to hold this position after it was separated from the veterinary clinical medicine. His research focused on various infectious diseases in animals. He edited a widely read scientific journals of the time, La Nuova Veterinaria, for over 30 years.

Albino Messieri (1896–1970) - professor of veterinary clinical medicine, founded the clinical method of the Bolognese school. His research ranged from clinical to forensic medicine. Together with his first pupil, Bruno Moretti, Messieri published the book Semiology and Veterinary Medical Diagnostics.

Attilio Mensa (1888–1960) was a professor of veterinary surgical clinics who focused primarily on podiatry. A brilliant lecturer, he wrote a substantial treatise on surgical pathology.

Ivo Peli (1900–1975) – professor of veterinary obstetrics and gynaecology, he devoted himself to the pathophysiology of reproduction and artificial insemination. He founded the first branch of the National Institute for the Artificial Insemination of Domestic Animals.

Antonio Cugnini (1883–1955) – professor of animal science; his research spanned topics ranging from animal phenotypic evaluation and nutrition to the mechanical and managerial aspects of animal husbandry, as well as the genetic improvement of animals of zootechnical interest.

All these leading figures have contributed to the establishment of schools of veterinary teaching that are still active in Bologna. Their impact is felt daily when we remind our students of them, when we see dedications in their honor, and above all when we reflect on the need to do our very best in our teaching and professional duties to honor these Masters.

THE TRAINING AND USE OF CARRIER PIGEONS IN THE ROYAL ARMY AND IN THE ROYAL NAVY DURING THE FIRST WORLD WAR

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All pigeons have an instinct to return to their home, but homing pigeons possess this instinct to a far greater degree. They grow attached to their lofts and this attachment grows stronger year by year, especially when they breed there and find all the comforts they need.

On their return journeys home, pigeons rarely lose their bearings and if, for various reasons, they struggle to find their way, they do not stop flying but, by constantly exploring all directions, they strive to find the path that leads them back to their nest. Only when tired, unwell or hungry they stop or enter another loft. But as soon as they recover, if given the chance, they fly again to return to their own lofts. The desire to return varies depending on the birds' age and the amount of time they have spent in their lofts [1].

Lieutenant Giuseppe Malagoli of the Engineer Corps, author of the 1887 book "I Colombi" ("The pigeons"), sought to demonstrate that homing pigeons have ancient origins, mentioning as their progenitor the dove Livia, the famous dove that returned to Noah's Ark with an olive branch to announce the end of the Great Flood [2].

It is difficult to determine, even approximately, the origins of pigeons, and their history is lost in the mists of time.

However, it is certain that the use of carrier pigeons has been known since ancient times.

In more recent history, during the nineteenth and twentieth centuries, carrier pigeons were used by almost all the world's Armies and Navies.

In the Royal Army and the Royal Navy, the carrier pigeon service was a key element of military communications before the widespread adoption of radio. This system, inspired by experiences from the Franco-Prussian War (19 July 1870 – 10 May 1871), enabled the exchange of critical information between land units and between naval units and shore-based commands.

During the First World War, carrier pigeons were widely used due to a lack of adequate means of communication [3].

The authors describe the use of carrier pigeons, highlighting aspects relating to training, their deployment by the Royal Army and the Royal Navy, diseases and protection against chemical agents.

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Posters

PRE-ACCEPTANCE TREND IN UMBRIA REGION THROUGH THE SIVA SYSTEM: 2020-2024 DATA INVESTIGATION

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The aim of this study is to illustrate the trend of Pre-acceptance activities in Umbria through the S.I.V.A. system (Veterinary and Food Information System) during the period 2020–2024. In 2009, Umbria Region adopted the SIVA system for the computerized management of control activities related to animal health and food safety under veterinary authority. The system allows data access to the Region, Local Health Authorities, self-employed veterinarians, Istituti Zooprofilattici and other stakeholders [1].

The SIVA System enables sampling technicians to carry out the pre-acceptance phase, defined as an IT-application interoperability procedure, that allows the automatic and real-time transfer of data between the regional IT-system (SIVA) and IZSUM IT-system (SIGLA). This preliminary step ensures that each procedure is properly recorded and that a unique pre-acceptance number is generated. This number is then used by acceptance operators to automatically and consistently transfer all relevant procedure data to the SIGLA system. Over the years, the system enabled the pre-acceptance of samples for Animal Health (since 2012), Piano Nazionale Residui (PNR) (since 2019), Piano di Controllo Regionale Pluriennale (PRCP) and related extra-plan samplings, such as UVAC provisions and alerts (since August 2022). Suspected poisoning cases were also managed through pre-acceptance until January 2023.

Pre-acceptance automates data transfer and digitization, reducing errors and ensuring data quality and synchronization across the National Database (BDN), SIGLA and SIVA. Regarding Animal Health samples, during 2020–2024, 25301 out of 28024 acceptances (90.3%) were pre-accepted. For the PNR, 1500 out of 2290 (65.5%) pre-acceptances were recorded. For PNI and related extra-plan samplings, pre-acceptance started on 25/08/2022, with 1701 pre-acceptances out of 3745 acceptances (45.4%). Throughout the five-year period there were 29081 pre-acceptances of 34937 total acceptances, with a percentage of 83.2%, including 531 suspected poisoning cases and 48 deleted pre-acceptances.

In 2020, 5848 pre-acceptances were performed on a total of 6690 acceptances (87.4%): 5299 out of 5954 for Animal Health, 371 out of 493 for PNR, 178 out of 243 for poisoning cases. In 2021 there were 5941 pre-acceptances out of 6678 acceptances (89%): 5469 out of 6016 for Animal Health, 294 out of 417 for PNR, 178 out 245 for poisoning cases. In 2022, 5391 pre-acceptances were carried out of 6531 acceptances (82.5%): 4831 out of 5375 for Animal Health, 335 out of 456 for PNR, 171 out of 203 for poisoning cases, 54 out of 497 PCRP. In 2023 there were 6037 pre-acceptances out of 7855 acceptances (76.9%): 5036 out of 5568 for Animal Health, 198 out of 483 for PNR, 799 out of 1617 for PCRP, 4 out of 187 for poisoning cases. In 2024, 5816 pre-acceptances were performed out of 7183 acceptances (81%): 4666 out of 5111 for Animal Health, 302 out of 441 for PNR, 848 out of 1631 for PCRP.

Since the introduction of pre-acceptance procedures for each plan, rectifications have significantly decreased: –57.8% for Animal Health, zeroed for PNR, – 42.2% for PNI and –94.9% for poisoning cases.

In conclusion, the gradual increase in the adoption of pre-acceptance procedures has significantly reduced errors and streamlined acceptance

processes, underscoring the value of optimizing pre-acceptance tools to enhance operational efficiency.

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Posters

FROM BULK TANK MILK TO ANTIMICROBIAL STEWARDSHIP: PRELIMINARY DATA ON RISK-BASED CATEGORIZATION OF DAIRY FARMS TO IMPLEMENT SELECTIVE DRY COW THERAPY IN UMBRIA, CENTRAL ITALY

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Antimicrobial resistance (AMR) is a major global health threat, with the dairy sector a key target due to high antibiotic use for diseases such as mastitis. EU and national regulations (Reg. (EU) 2019/6; D. Lgs. 218/2023) promote prudent use and encourage selective dry cow therapy (SDCT) over blanket treatment [1,2]. Thus, the aim of this study was to perform a risk assessment of dairy farms in the Umbria region to support the targeted implementation of SDCT protocols.

Risk classification was conducted according to the criteria defined in the Italian Ministerial Guidelines on the "Prudent Use of Antibiotics in Dairy Cattle Farming" [3]. These criteria integrate three main domains of herd health and epidemiological risk: udder health status, assessed through bulk tank milk somatic cell count (geometric mean bSCC, GMbSCC), with thresholds of 250,000 cells/mL (low vs. medium risk) and 350,000 cells/mL (medium vs. high risk) as a proxy for intramammary infection prevalence; detection of contagious mastitis pathogens (*Staphylococcus aureus*, *Streptococcus agalactiae*, and *Mycoplasma bovis*) through routine microbiological surveillance; and herd-level antimicrobial usage, expressed as annual mastitis treatment rates, with cut-offs of <40% (low to medium risk) and ≥80% (medium to high risk) of lactating cows treated per year. Each farm was assigned a score, and the combined scores were used to classify farms as low-, medium-, or high-risk [3].

In 2025, 53 farms were enrolled, and 168 bulk tank milk samples were collected. Somatic cell counts were measured by flow cytometry, while microbiological analysis was performed using real-time PCR assays targeting 15 mastitis-causing pathogens. To estimate the proportion of clinical mastitis treatments, personnel from the Local Health Authorities reviewed farm treatment records for the entire year. Most farms (56.6%) were classified as high risk, followed by medium (22.6%) and low risk (20.8%). *S. aureus* was the most common contagious pathogen, followed by *S. agalactiae*, often associated with markedly elevated GMbSCC >400,000 cells/mL and high treatment rates, indicating chronic intramammary infections. *M. bovis* was not detected. Moreover, environmental pathogens considered secondary causes of mastitis (including *Enterococcus spp.*, *Escherichia coli*, *Klebsiella spp.*, *Streptococcus uberis*, and *Streptococcus dysgalactiae*) were consistently detected, suggesting deficiencies in hygiene and internal biosecurity. Interestingly, *Prototheca spp.*, a non-bacterial agent causing chronic mastitis unresponsive to antibiotics, was detected at low levels in six herds, emphasizing the importance of early diagnosis to prevent unnecessary antimicrobial treatments.

These findings indicate high infectious pressure in dairy farms in Umbria and highlight the need for targeted control measures. SDCT represents a key strategy to improve antimicrobial stewardship. Continuous monitoring within the Italian National Control Plan on Antimicrobial Resistance (PNCAR) will be essential to evaluate intervention effectiveness.

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Posters

PUBLIC HEALTH IMPLICATIONS OF METHYLMERCURY IN FISHERY PRODUCTS: CONTAMINATION LEVELS AND DIETARY EXPOSURE IN THE ITALIAN POPULATION

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Methylmercury (MeHg) represents one of the most relevant contaminants in fishery products due to its high toxicity and strong bioaccumulation and biomagnification potential. MeHg is frequently detected in seafood and typically accounts for about 80% of total mercury (THg) [1]. However, this proportion varies considerably across species and geographical origins, meaning that reliance on THg alone may lead to either overestimation or underestimation of the risk, highlighting the importance of mercury speciation analysis. Compliance with EU Regulation 2023/915 is also required for public health protection; nevertheless, maximum levels apply to THg, not specifically to MeHg. The study aimed to provide a comprehensive assessment of MeHg and THg contamination levels in commercially relevant fishery products purchased from the Italian market, originating from both the Mediterranean region and other key geographical areas representative of the main national fishery supply chains. A total of 591 fishery products, largely consumed in Italy, including fresh cephalopod mollusks (*Octopus vulgaris*, L.; *Eledone* spp; *Sepia officinalis*, L.; *Loligo vulgaris*, L.; *Todarodes sagittatus*, L.), fresh tuna loins (*Thunnus albacores*. B.), salt-ripened anchovies (*Engraulis encrasicolus*, L.), and smoked salmon (*Salmo salar*, L.), were analyzed for THg and MeHg quantification by thermal decomposition–atomic absorption spectrometry [2]. Based on the available consumption data, risk characterization was performed for Italian adolescents, adults, elderly, toddlers, other children, and infants, following deterministic and probabilistic (Monte Carlo) methods. Among the analyzed products, octopus exhibited similar patterns to tuna for MeHg and THg levels, whereas salmon, squids, cuttlefish, and anchovies showed markedly lower concentrations. Statistically significant ($p<0.05$) differences related to geographical origin were observed in octopus and squid, with Mediterranean specimens showing up to fivefold higher levels than Atlantic samples. Deterministic exposure assessment indicated that average consumption of octopuses and tuna could result in MeHg intakes at or above the Tolerable Weekly Intake (TWI). Considering high consumers and highly contaminated products, Estimated Weekly Intakes (EWIs) exceeded up to six times the TWI. In contrast, squids, cuttlefish, anchovies, and smoked salmon contributed at most 42% to TWI in toddlers consuming flying squid. Probabilistic assessment performed on octopuses and tuna confirmed a cumulative probability of exceeding the TWI up to 81% for toddlers and 74% for other children, confirming the concerns for younger populations. Similarly, for adolescents, adults, and elderly, the probability to exceed the TWI for MeHg resulted up to 45%, 37%, and 45% respectively. The findings of the study reinforce the need for MeHg monitoring in fishery products, extending the focus beyond traditionally contaminated species to include underestimated fishery products like octopus, improving exposure assessment accuracy and strengthening public health protection, especially for vulnerable populations.

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MULTI-RESIDUE PESTICIDE DETERMINATION IN CRICKET FLOUR BY GC-QQQ-MS/MS AND UHPLC-HESI-Q-ORBITRAP-MS

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While edible insects represent a part of the human diet in many countries around the world, especially in Asia, Australia, Africa, and Latin America, this food commodity is mainly considered a 'novel food' in the Western world [1]. In this context, their safety profile appears to be important, particularly with regard to pesticide contamination, since insects can accumulate agrochemical residues through food substrates and the farming environment. This study presents the development of a multi-residue analytical method for the simultaneous determination of 290 pesticides in cricket flour (*Acheta domesticus*), including organophosphates, carbamates, pyrethroids, neonicotinoids, amides (carboxamides and benzamides), strobilurins, triazoles and imidazoles, benzimidazoles, pyridines and pyrimidines, triazines, phenylureas, dicarboximides, phenylamides, diamides (anthranilamides and nicotinamides), morpholines and piperidines, sulfonylureas, macrocyclic lactones (avermectins and spinosyns), and organochlorine compounds. Two complementary analytical platforms were employed: Gas Chromatography coupled to Triple Quadrupole Mass Spectrometry (GC-QqQ-MS/MS) for volatile and semi-volatile pesticides, and Ultra-High-Performance Liquid Chromatography coupled to Heated Electrospray Ionization-Quadrupole Orbitrap Mass Spectrometry (UHPLC-HESI-Q-Orbitrap-MS) for polar and thermolabile compounds, ensuring broad-spectrum coverage and high sensitivity. Sample preparation followed a QuEChERS-based extraction protocol adapted for the complex lipid-rich matrix of insect-derived flour [2]. Both methods were rigorously validated through the determination of key analytical parameters, proving to be specific and sensitive, with limits of detection ranging from 0.008 to 0.015 mg/kg and limits of quantification ranging from 0.004 to 0.032 mg/kg. Methods accuracy of two methods was assessed by evaluating both the recovery percentage values (in the range 70% - 120%) and the relative standard deviations (RSDr), below 20%. Two commercially available cricket flour samples were analyzed. In one sample, three pesticide residues were detected: the carbamate insecticide carbofuran, its metabolite methiocarb-sulfoxide, and the triazine herbicide simazine, at concentrations of 2.27, 24.01, and 27.97 µg/kg, respectively, with RSDr values below 11%. The presence of carbofuran, banned in the European Union since 2008 [3] due to its acute toxicity, together with methiocarb-sulfoxide and simazine, raises food safety concerns for insect-based products, whose regulatory framework remains incomplete, particularly regarding the definition of specific maximum residue limits (MRLs). These findings highlight the need for validated multi-residue analytical methods and matrix-specific MRLs, and confirm that a combined-platform approach is essential to ensure comprehensive pesticide surveillance in such complex food matrices.

The project was carried out thanks to the contribution provided by the Ministry of Health, General Directorate of Hygiene and Food Safety.

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Posters

DEVELOPMENT OF A SYSTEMATIC PROTOCOL FOR EXTRACTING MICROPLASTICS FROM COW'S MILK USING POTASSIUM HYDROXIDE

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The presence of microplastics (MPs) in food is an increasingly important food safety issue with potential implications for human health. While many studies have examined MPs in various food products, research on milk remains limited and lacks a standardised protocol for matrix digestion and MPs extraction. This study aimed to develop a systematic, repeatable protocol based on alkaline digestion using potassium hydroxide (KOH). Whole, semi-skimmed and skimmed UHT cow's milk samples, which had been experimentally contaminated with blue polystyrene MPs (<500 µm), were processed and analysed. The samples were digested with different concentrations of KOH (from 1:12 to 1:1) over varying times (24-72 h), temperatures (22-42°C) and sample mixing (static-mixed). The results showed that the most effective concentrations for digesting whole and semi-skimmed milk were ratios of 1:2 and 1:1, respectively, at a temperature of 42 °C with constant agitation. Skimmed milk, which is low in lipids, was also effectively digested at a ratio of 1:1[2] The protocol ensured high MPs recovery efficiency (99,83%) while preserving particle morphology, colour and surface integrity. This method made it possible to identify an operational combination that is effective, fast, cost-effective and easy to apply. These characteristics make this method readily applicable for future studies on milk contamination by microplastics, given the enormous global daily consumption of milk and its frequent storage in plastic containers or under plastic caps, which may serve as potential sources of contamination.

Posters

ASSESSMENT OF EMERGING MYCOTOXINS ALONG THE PORK SUPPLY CHAIN AND IN WILD BOAR: IMPLICATIONS FOR FOOD SAFETY

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Emerging mycotoxins are fungal secondary metabolites which are neither routinely determined, nor legislatively regulated, but are increasingly detected in food and feed, particularly those associated with *Fusarium* and *Alternaria* species [1]. Their rising occurrence has been linked to environmental and climatic changes that promote their production and co-occurrence with regulated mycotoxins, potentially resulting in synergistic toxic effects that remain poorly understood [2]. Despite growing scientific interest, significant knowledge gaps persist regarding their occurrence in livestock systems and wildlife, as well as their transfer into edible tissues. This lack of information raises concerns for food safety and underscores the need for integrated monitoring strategies.

In this context, the present study aimed to investigate the occurrence of selected emerging mycotoxins along the pork supply chain and in wild boar within a Mediterranean environment (Sicily region). The target analytes included enniatins (ENNA, ENNA1, ENNB, ENNB1), beauvericin (BEA), alternariol (AOH), and alternariol monomethyl ether (AME), with the objective of evaluating feed contamination, potential carry-over into edible tissues, and the role of wildlife as environmental sentinels.

To this end, 6 finisher feed samples, as well as muscle and liver from 27 farmed pigs, and muscle, liver, and kidney from 39 wild boars were collected. Sample preparation involved extraction with an organic solvent mixture, followed by analysis using UHPLC-HRMS. A matrix-specific validation was performed for wild boar kidney in accordance with Commission Implementing Regulation (EU) 2023/278[2] Data were subsequently processed using descriptive statistics to determine occurrence and concentration levels.

The results revealed frequent multi-mycotoxin contamination in pig feed, although at low concentrations (<12.2 ng g^{-1}). ENNA1, ENNB, and ENNB1 were detected in all samples, whereas BEA, AOH, and AME were present with variable frequencies. In contrast, all considered emerging mycotoxins in pig muscle and liver samples were below the limit of quantification, indicating the absence of measurable carry-over under the investigated conditions. Similarly, in wild boar, no analytes were detected in muscle, while AOH and AME were only sporadically identified in liver and kidney at low concentrations (<9.83 ng g^{-1}) and low occurrence rates.

Overall, these findings confirm that pig feed represents a relevant source of exposure to emerging mycotoxins; however, their transfer to edible tissues appears negligible under the studied conditions, likely due to compound-specific toxicokinetics and rapid metabolic processes. The occasional detection of *Alternaria* toxins in wild boar further supports their potential role as environmental sentinels of mycotoxin exposure. This study contributes to the still limited body of evidence on emerging mycotoxins in livestock and game matrices and highlights the importance of adopting integrated monitoring approaches, particularly in Mediterranean agroecosystems.

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DYNAMIC OF COLD-SMOKED SALMON MICROBIOTA DURING THE PRODUCTION PROCESS: FROM RAW MATERIAL TO THE FINISHED PRODUCT

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Cold smoked salmon is a widely consumed ready-to-eat (RTE) product that has recently been implicated in several multi-country listeriosis outbreaks [1]. It has been reported that Listeria monocytogenes contamination occurs primarily during processing, where the salmon undergoes multiple handling and technological steps [2,3]. The aim of this study was to explore the dynamics of food product microbiota along the smoked salmon process in order to identify potential critical control points.

Sampling was performed in July 2024 within an Italian smoked salmon facility located in Emilia Romagna. A total of 24 food samples were collected as independent biological replicates at different processing stages as follows: raw material (n = 6), fish fillet (n = 6), smoked salmon (n = 6), and packaged products (n = 6). From each sample, 10 g were transferred into a sterile bag containing 90 mL of buffered peptone water and homogenized. Afterwards, 1 mL aliquots were used for total microbial DNA extraction using the PowerFood DNA Kit (Qiagen). 16S rRNA gene metabarcoding targeting the V3–V4 regions was performed using the Illumina platform and bioinformatic analyses were conducted in R (version 4.5.1) using DADA[2] Raw paired-end reads were quality-filtered, denoised, merged, and screened for chimeras to infer amplicon sequence variants (ASVs), generating a sequence table of ASV abundances per sample. Taxonomic assignment was performed using the SILVA database (version 138.2). Downstream analyses were conducted using phyloseq, with ASVs aggregated at genus and phylum levels and relative abundances calculated for each sample.

Results showed that microbial diversity in the food product increased along the processing phases. In the early stages of production, the alpha diversity index was low, reflecting a uniform microbial population. Specifically, raw materials and fish fillets were dominated by Pseudomonas (mean = 92.33%). In contrast, in the later stages, including smoked salmon and packaged products, higher microbial richness was observed. In smoked salmon, Pseudomonas remained the most prevalent genus (mean = 52.03%), whereas packaged product was characterized by a high abundance of Photobacterium (mean = 40.76%). Among the minor genera observed in smoked salmon and packaged products, some ASVs were assigned to Listeria.
Overall, these findings suggest that the production process influences the microbial diversity of cold smoked salmon, with shifts in community structure from raw material to the final packaged product. In particular, the increased microbial richness observed in smoked and packaged salmon may reflect a higher potential for cross-contamination during these processing stages.

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Posters

BACTERIOCINS FOR FOOD SAFETY: A SYSTEMATIC REVIEW AND META-ANALYSIS OF NATURAL BIOPRESERVATIVES TARGETING BACTERIAL AND PARASITIC FOODBORNE THREATS

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Foodborne diseases remain a major public health and food-chain sustainability challenge, further aggravated by the rise of antimicrobial resistance (AMR). This has increased interest in natural alternatives to conventional preservatives, particularly bacteriocins as biopreservative agents. This systematic review and meta-analysis aimed to identify and map bacteriocins active against foodborne bacterial and parasitic hazards relevant to food safety, with target selection based on European microbiological criteria and EFSA guidance.

Literature searches were conducted in PubMed, Scopus, and Web of Science using pathogen-specific combinations of "bacteriocin" and target names. Duplicates were removed in Mendeley and records were screened in Rayyan. For antibacterial evidence, only studies on purified bacteriocins reporting quantitative minimum inhibitory concentration (MIC) data were included; MIC values were standardized to mg/mL, log10-transformed, and analyzed using random-effects models. For parasites, due to limited evidence, studies on bacteriocinogenic strains or incompletely characterized bacteriocins were also considered when a bacteriocin-mediated effect was explicitly reported.

The search yielded 5,291 records, of which 5,072 remained after duplicate removal and after rayyan 46 were assessed for eligibility. Descriptive synthesis showed that *Staphylococcus aureus* was associated with the highest number of active bacteriocins, followed by *Listeria monocytogenes*, *Escherichia coli*, *Salmonella* spp., *Clostridium perfringens*, and *Bacillus cereus*. Overall, Gram-positive targets showed a broader and more diverse bacteriocin repertoire than Gram-negative ones. Quantitative synthesis indicated that, among Gram-positive bacteria, Micrococcin showed the lowest mean MIC, followed by Enterocin and Subtilosin, while Plantaricin showed intermediate activity and Lactocin the least favorable profile. For Gram-negative bacteria, evidence was much more limited, and only Plantaricin met the threshold for meta-analysis. Antiparasitic evidence was scarce and restricted to *Trichinella* spp. and *Giardia* spp., with no directly relevant studies for *Toxoplasma gondii*, *Cryptosporidium* spp., or *Anisakis* spp..

Although bacteriocins appear promising as natural food safety tools, nisin (E 234) remains the only bacteriocin currently authorized for food use, highlighting the gap between experimental evidence and practical application. These findings suggest that other bacteriocins may represent promising candidates, especially against Gram-positive foodborne pathogens. However, further studies are needed to assess safety and efficacy in food matrices under realistic conditions before translation into the food sector and future regulatory evaluation.

PRELIMINARY EVALUATION OF THE DYNAMICS OF MICROBIOLOGICAL HYGIENE CRITERIA IN A MULTISTEP WILD BOAR MEAT SUPPLY CHAIN

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The population of wild boar (*Sus scrofa*) has increased markedly across Europe, leading to greater availability of game meat and the development of organised supply chains for its commercialisation. Unlike in livestock production systems, where all procedures are performed at the slaughterhouse, wild boar supply chains are fragmented at a local level and involve multiple operators. The animals are harvested by hunters and taken to collection centres (CCs), where food business operators (FBOs) carry out bleeding, evisceration and chilling. Within four days, the carcasses are transported under refrigerated conditions to a game handling establishment (GHE), where they are skinned and stored in cold chambers by a different FBO. Although large wild game carcasses are not subject to specific EU microbiological process hygiene criteria under EC Regulation 2073/2005, hygiene monitoring is considered essential by downstream operators, including game meat processors and fresh-cut industries. This study aimed to characterise the hygienic status at each stage of a local, multi-step wild boar meat supply chain, assess whether microbial contamination increases throughout the process, and identify the most appropriate sampling points for hygiene assessment. The study was conducted in a game meat supply chain in central Italy. Fifteen wild boars were harvested by selective still hunting during winter. The carcasses were stored in cold chambers at 3 °C at the CC until they reached an internal temperature of 7 °C, in accordance with EC Regulation 853/2004. After three days, the carcasses were transported to the GHE under refrigerated conditions without piling within two hours, and were then immediately skinned. Microbiological sampling was carried out at the following four stages: T0: internal surface after evisceration and before chilling (CC); T1: internal surface after chilling (CC); T2: internal surface upon arrival at the GHE; and T3: external surface after skinning at the GHE. Sampling was performed using a non-destructive swabbing method with a pre-moistened cellulose sponge on four 100 cm ^{2} areas per carcass at each stage. At T0, T1 and T2, samples were taken from the internal costal and lumbar regions bilaterally, and at T3, from the external left side, rump, shoulder, brisket and distal portion of the forelimb. Laboratory analyses included aerobic colony count (ACC), *Enterobacteriaceae* count (EC), and *Salmonella spp.* detection, according to ISO standards, in duplicate. The mean ACC increased significantly from 1.01 log CFU/cm ^{2} at T0 to 1.60 at T1, 2.48 at T2, and 2.44 at T[3] EC values remained relatively stable over time (1.21–1.56 log CFU/cm ^{2}). *Salmonella spp.* were not detected at any stage. One-way ANOVA revealed significant differences ($p<0.05$) in ACC between processing steps. There were no differences between internal and external surfaces at T3, suggesting that contamination mainly increases during refrigerated storage and handling at the GHE. No significant differences were observed for EC, indicating that initial contamination played a predominant role. Overall, microbial contamination levels were lower than those reported in previous studies on hunted wild boar carcasses, although considerable data variability remained, reflecting the intrinsic heterogeneity of game meat hygiene. These findings emphasise the importance of improving hygiene practices among hunters and FBOs at critical stages within their control.

EVALUATING THE CHANGES OF VOCS PROFILE OF X-RAY IRRADIATED HEN EGGS BY MEANS OF HS-SPME/GC-MS

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During last years, the growing demand for high-protein foods contributes to the increase of eggs consumption. However, eggs can be important vehicles for foodborne illnesses mainly due to Salmonella contamination [1]. Food irradiation is a clean, safe, non-thermal technology widely used to mitigate health risk posed by pathogenic microorganisms. Currently, in Europe only the egg white can be irradiated up to 3 kGy, while the irradiation of whole eggs is not permitted [2]. Therefore, analytical methods are essential for official controls to identify possible illegal treatments. The scope of this work was the evaluation of volatile profile modifications of these products as a consequence of X-ray irradiation, and the identification of potential irradiation markers caused by oxygen radical attack on unsaturated fatty acids and proteins [3]. Irradiated (1.0 and 3.0 kGy) and non-irradiated commercial samples (1-2 weeks after deposition) were shelled and the egg white (EW) and yolk (EY) were analyzed separately by headspace solid-phase microextraction with a polydimethylsiloxane fiber coupled to gas chromatography/-mass spectrometry (HS–SPME/GC–MS) [3]. The volatile organic compounds (VOCs) were identified by overlapping the obtained mass spectra with those available in the NIST 17 library, and comparing the experimental linear retention indices with the references. A total of 26 VOCs were identified, belonging to the classes of alkanes, alkenes, alcohols, aldehydes, ketones, esters, carboxylic acids and terpenoid. Regarding the formation of the so-called "irradiation odor", for which volatile sulfur compounds are mainly responsible, these were not detected up to a dose of 3 kGy. From a qualitative point of view, no differences were identified between the VOC profiles of EW and EY, with the exception of benzoic acid ethyl ester which was detected only in both irradiated and non-irradiated egg yolks. Instead, differences were identified between irradiated and non-irradiated samples, and four compounds can be considered as discriminating to identify the irradiation treatment. Specifically, an alcohol (hexanol) and an aldehyde (benzaldehyde), both identified in the non-irradiated EG and EY samples, increased proportionally to the treatment dose. The two other compounds, an unsaturated alcohols (9-tetradecen-1-ol) and an alkane (undecane), can be considered radiolytic products because they were only found in irradiated samples. This behavior is related to the treatment with ionizing radiation, which induces the formation of alkanes, alcohols and aldehydes as products of the oxidation reactions of unsaturated fatty acids and protein cleavage [3]. In conclusion, this preliminary study further explored the effect of X-rays on the volatile profile of hen eggs, which showed no sulfur compound formation up to doses of 3 kGy. Furthermore, four compounds useful to prove the illicit use of x-ray treatment were identified. Future investigations in this area may expand the analytical chemical methods available to support food safety control plans.

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Posters

ENUMERATION OF *CAMPYLOBACTER SPP.* IN ANTIBIOTIC-FREE BROILER CARCASSES COLLECTED AT SLAUGHTERHOUSE

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This preliminary study, conducted within a project investigating the impact of climate on the enumeration and characterization of *Campylobacter spp.* in broilers, assessed the compliance of antibiotic-free broiler carcasses with process hygiene criteria. Five antibiotic-free broiler farms (A–E), representative of different geographic areas of Italy, were included in the study. Between February and March 2026, five pooled neck-skin samples per farm, each obtained from 15 post-chill broiler carcasses at the slaughterhouse, were analysed, for a total of 75 carcasses. Microbiological analyses were performed in accordance with ISO 10272-2:2017 for *Campylobacter spp.* enumeration, ISO 21528-2:2017 for *Enterobacteriaceae* enumeration, and ISO 4833-2:2013 for total colony count. *Campylobacter* counts exceeded the process hygiene criterion of 1000 CFU/g in 15 samples from all tested farms. Farm-related differences were observed for *Enterobacteriaceae* and total colony count, with mean values ranging from 3.2 to 5.1 $\log_{10}$ CFU/g and 5.5 to 7.4 $\log_{10}$ CFU/g, respectively. Farm A exhibited the lowest mean loads for both bacterial groups along with low value of relative humidity.

Posters

BEHAVIOR OF *LISTERIA INNOCUA* AND MICROBIAL DYNAMICS IN ITALIAN FERMENTED SALAMI FOLLOWING HIGH-PRESSURE PROCESSING UNDER DIFFERENT STORAGE CONDITIONS

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The aim of this study was to investigate the behavior of *Listeria innocua* during the period following high-pressure processing (HPP; 6000 bar for 300 s) applied after curing in two Italian fermented salami (Calabrese and Cacciatore). *L. innocua* was used as a surrogate for *L. monocytogenes*. Counts were performed at 7, 21, and 28 days after HPP during storage at 4°C and 20°C in order to better assess post-treatment behavior and potential recovery phenomena.. For each salami type, control (n=6) and inoculated (n=6) batches were produced. Inoculated samples were contaminated with a multi-strain cocktail of *L. innocua* (ATCC 33090 and three field isolates) at approximately 6 log CFU/g. After stuffing, products underwent a 30-day ripening process, were portioned (400 g), vacuum-packaged, and treated with HPP. Microbiological analyses were carried out throughout shelf life at 4°C and 20°C and compared with untreated samples. In Cacciatore salami, HPP resulted in an initial reduction of ~1.25 log CFU/g, followed by further decreases during storage, reaching ~1.92 log CFU/g at 4°C and ~2.84 log CFU/g at 20°C. In Calabrese salami, HPP caused a reduction of ~0.71 log CFU/g; at 4°C, regrowth was observed, whereas at 20°C a progressive decline led to an overall reduction of ~2.29 log CFU/g. A study of the microbiota was performed by analyzing bacterial colonies grown on different culture media using MALDI-TOF MS technology [1]. In Calabrese salami, lactic acid bacteria were mainly composed of *Pediococcus* spp. (44.7%) and *Lactobacillus sakei* (39.3%). After HPP and storage at 20°C, lactobacilli decreased markedly, while *Pediococcus* spp. became predominant, suggesting a strong selective pressure induced by processing and storage conditions. In Cacciatore salami, the microbiota was dominated by *Pediococcus* spp. (86%) and *L. sakei* (8.4%), with *L. sakei* remaining stable throughout shelf life (33.7%) at both temperatures. In addition, *Debaryomyces hansenii* was detected in Cacciatore salami, with an average relative abundance of 21.8%. Overall, HPP effectively reduced *L. innocua*, although its efficacy and post-treatment dynamics depended on product type and storage conditions [2]. The greater reductions observed at 20°C were likely associated with increased microbial competition [3]. These findings highlight the key role of physicochemical properties and microbial ecology in determining HPP effectiveness in fermented meat products, as product-specific physicochemical parameters may significantly influence treatment efficacy, also in relation to the zero-tolerance policy for *L. monocytogenes* in products exported to the U.S.

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Posters

PREDICTIVE TEACHING IN VETERINARY SCIENCES: A LEARNING ANALYTICS DASHBOARD SUPPORTING ADAPTIVE AND INTERDISCIPLINARY TEACHING STRATEGIES

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Veterinary education is increasingly required to prepare professionals able to manage complex challenges related to zoonoses, food safety and public health within the One Health framework. In this context, teaching innovation should not only introduce new educational methods but also support lecturers in selecting the most effective strategies according to the characteristics of each student cohort. This project aims to develop and test a predictive teaching approach based on learning analytics, centred on the creation of a dashboard designed to assist lecturers in adapting teaching strategies to the learning profiles of their classes.

The project involves three closely related veterinary disciplines: inspection of foods of animal origin, veterinary infectious diseases, and veterinary parasitology and parasitic diseases. Learning data will be collected from multiple sources, including online quizzes, formative assessments, laboratory activity checklists, and short questionnaires on learning preferences and self-perceived difficulties. These indicators will be integrated into a learning analytics dashboard capable of identifying patterns of engagement, performance and learning behaviour within the class group.

Based on these patterns, the dashboard will support the categorisation of student cohorts according to their prevailing learning dynamics, helping lecturers select the most appropriate innovative and participatory teaching strategies. Depending on the identified profile, teaching activities may include flipped classroom sessions on key concepts, micro-learning modules, cooperative learning activities, or project-based interdisciplinary tasks.

The approach will be implemented in pilot courses where students will address integrated case studies reflecting real veterinary challenges, such as the management of zoonotic outbreaks requiring the combined interpretation of parasitological identification, clinical implications and food inspection procedures. Educational outcomes will be evaluated through student performance indicators, engagement metrics, questionnaires and focus groups, and compared with cohorts not involved in the project.

The expected impact includes improved alignment between teaching strategies and students' learning needs, increased engagement and participation, and the strengthening of interdisciplinary reasoning in veterinary problem solving. In addition, the project will promote the development of a community of practice among lecturers and produce a transferable toolkit for predictive and adaptive teaching in veterinary education.

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Posters

BOVINE MILK-DERIVED EXTRACELLULAR VESICLES AS NANOCARRIERS FOR NUTRACEUTICALS

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Limited bioavailability remains a major constraint in the therapeutic application of nutraceutical compounds; however, nanocarrier systems may overcome these limitations by enhancing stability, cellular uptake, and intracellular release. Bovine milk-derived small extracellular vesicles (EVs) offer a scalable, cost-effective, and biocompatible platform. These EVs (30–150 nm) demonstrate broad tissue biodistribution, the ability to cross biological barriers, and excellent safety profiles without adverse immune responses [1,2].

Oleuropein (OLE), a phenolic compound from *Olea europaea*, exerts antioxidant and anti-obesity effects, reducing adipose tissue mass and triglyceride accumulation. However, it undergoes metabolic conversion into hydroxytyrosol (HTY), which retains antioxidant properties but may differ in metabolic activity. Whether encapsulation strategies and loading methods influence the biological efficacy of OLE and HTY remains unclear.

This study investigated bovine milk-derived EVs as nanocarriers for OLE and HTY, comparing passive incubation and active loading by ultrasound sonication. Encapsulation efficiency was quantified by UV–VIS spectrophotometry, and vesicle morphology was assessed by transmission electron microscopy. Cellular uptake of EVs labelled with DIL was confirmed in differentiated murine 3T3-L1 adipocytes after 24 hours using confocal microscopy. EVs were enriched using a ratio of 0.2 mg molecule per 8×10^{11} EVs. Passive loading achieved encapsulation efficiencies of approximately 47.2% for OLE and 44.4% for HTY, not significantly different from sonication, indicating that active loading achieved comparable efficiency in a shorter time. TEM observations showed that neither method altered EV integrity or the characteristic cup-shaped morphology, although passive incubation slightly increased vesicle size (120.7 ± 24.7 nm) compared with controls (79.6 ± 13.3 nm).

To evaluate biological activity, differentiated 3T3-L1 adipocytes were treated with encapsulated OLE or HTY (25–200 µM). Both loading strategies preserved cell viability and reduced triglyceride accumulation at higher concentrations. However, EVs loaded via sonication promoted significantly greater lipolysis (2.47 nmol/µg) compared to passive incubation (1.64 nmol/µg), as indicated by increased glycerol release with 200 µM treatments. As lipolysis is highly sensitive to intracellular compound availability and release kinetics, these findings suggest that sonication may enhance intracellular bioavailability and temporal release dynamics of encapsulated nutraceuticals.

Comparative analysis showed that encapsulated OLE was more effective than HTY in reducing lipogenesis (approximately 70% vs 85%, respectively) and in stimulating lipolysis by about 18%, particularly at higher concentrations. This is consistent with evidence that OLE directly modulates lipogenic and lipolytic pathways, whereas HTY primarily exerts indirect antioxidant and mitochondrial effects [3].

In conclusion, bovine milk-derived EVs are safe and effective nanocarriers for OLE and HTY in 3T3-L1 cells, enhancing lipolytic efficacy without compromising vesicle integrity. These findings highlight the importance of the loading strategy in determining biological performance and support the translational potential of milk-derived EVs as nutraceutical delivery systems.

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FROM FRAGILITY TO ACCESSIBILITY: A 1900 EQUINE FLAP ANATOMY ATLAS IN VIRTUAL REALITY

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Veterinary anatomical flap books represent a historically and scientifically significant category of educational materials developed between the late nineteenth and early twentieth centuries, at a time when the need for more effective visual and didactic tools in anatomical teaching was rapidly increasing. These works combine scientific illustration with mechanical ingenuity, relying on a system of superimposed movable paper layers (lift-the-flap) that can be sequentially raised to reveal deeper anatomical structures. This layered construction allows for a progressive, stratigraphic exploration of the body, guiding the user from superficial to internal components in a way that closely mimics dissection. Beyond their pedagogical effectiveness, flap books also embody an important intersection between publishing, craftsmanship, and scientific communication, and can be considered early analog predecessors of modern interactive and immersive visualization technologies. The Museum of Veterinary Anatomy of the University of Naples Federico II houses a valuable equine anatomy atlas published in 1900, characterized by a complex arrangement of movable flaps and accompanied by an explanatory manual. More than a century after its publication, the volume shows significant structural fragility: the paper mechanisms are markedly weakened and, despite a recent conservation treatment, the book is no longer suitable for regular public handling. Within this context, the project was designed with a dual objective: to ensure the long-term preservation of the original artifact by minimizing direct manipulation, and to provide museum visitors with an authentic and engaging consultation experience that faithfully reproduces the original mechanics of the flap book. The digital reconstruction process began with a systematic high-resolution photographic acquisition of all components of the volume, including its external and internal parts, movable elements, and the associated manual. The collected data were then processed and integrated into an immersive three-dimensional environment developed in Unreal Engine 5, where a virtual scene consistent with the museum setting was recreated. Within this environment, users can interact directly with the digital book, access textual content, and explore additional multimedia materials. Particular attention was devoted to system optimization for standalone devices: scene lighting was implemented using static lights, while computational load was reduced through the simplification and compression of environmental textures. At the same time, maximum resolution was preserved for the photographic reproductions of the atlas pages and the manual, ensuring high visual fidelity and full readability. The application supports multiple modes of use, both seated and standing, and incorporates two levels of interaction: a manual mode that simulates the physical act of turning pages and lifting flaps, and an automated mode designed to facilitate sequential consultation and enhance its use as an educational tool. The virtual reality simulation thus enables an accurate replication of the visual and interactive experience of the original flap book, making it accessible to a wide audience without exposing the historical artifact to further risk. The project represents a replicable methodological model for the preservation and enhancement of scientific book heritage, demonstrating how immersive technologies can effectively bridge conservation needs and innovative forms of public engagement.

MULTIMODAL SPATIAL ANALYSIS UNCOVERS PHENOTYPIC HETEROGENEITY IN EXPERIMENTAL PULMONARY FIBROSIS

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The comprehensive characterization of pulmonary fibrosis in the bleomycin (BLM)-induced murine model is significantly enhanced by integrating cutting-edge multimodal technologies with conventional histological scoring systems. In this context, we combined quantitative morphometry with spatially resolved phenotypic profiling to assess lung parenchymal alterations following BLM exposure and nintedanib (NINT) treatment, compared with healthy controls. The experimental protocol was approved by the internal Animal Welfare Body and authorized by the Italian Ministry of Health (Protocol n° 809/2020-PR). We implemented the automated workflow BREATHE (Biomedical Respiratory Analysis Through High-dimensional Evaluation), which has previously demonstrated strong predictive performance for the Ashcroft score (R^2 = 0.9), the gold standard for fibrosis assessment [1]. This workflow was integrated with multiplex immunofluorescence (mIF) data to enable high-dimensional tissue analysis. A panel of 13 biomarkers — including epithelial markers (KRT8, SFTPC, HOP), stromal markers (BGN, αSMA), and inflammatory markers (CD206, CD163, CD68) — enabled the phenotypic characterization of over 1.4 million single cells [2]. Artificial intelligence–based algorithms for dimensionality reduction and clustering (UMAP and HDBSCAN), were applied to quantify cellular remodeling during fibrosis progression. This analysis revealed a marked shift in cellular composition, with epithelial cell decreasing from 76.4% (grade 0) to 25.5% (grade 8), accompanied by a progressive expansion of stromal (from 19.3% to 60.3%) and inflammatory populations (from 4.3% to 14.2%). Importantly, phenotypic heterogeneity was observed within histologically comparable regions. In normal histological appearance lung (Ashcroft grade 1), epithelial cells represent 77.3% in control and 41.5% in BLM-treated animal, while treatment with NINT restored levels toward baseline (72.7%). Stromal cells exhibited an inverse trend, but with the same restoring outcome after NINT treatment (19.7%, 49.8%, and 18.4%, respectively). These findings indicate that similar histological appearance and scores may mask substantial underlying phenotypic differences. At higher clustering resolution, distinct cellular subpopulations with specific spatiotemporal dynamics were identified, including transitional alveolar epithelial cells (ADI, Alveolar Differentiation Intermediate), which are implicated in epithelial repair. Spatial analysis based on single-cell coordinates revealed a progressive reduction in proximity between ADI cells and fibroblasts/myofibroblasts with increasing fibrosis severity. This spatial convergence in advanced fibrotic stages is consistent with the mechanistic link between aberrant ADI states and fibroblast activation reported by Hoffman et al. [3], supporting their role in the formation of fibroproliferative foci. Collectively, this integrated framework enables a high-resolution dissection of pulmonary fibrosis, revealing phenotypic and spatial features that are not captured by conventional histology and providing a basis for the identification of novel cellular interactions and therapeutic targets.

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OPTIMIZATION OF A BIOMIMETIC 3D EQUINE CARTILAGE MODEL BASED ON ISOLATED CHONDRONS UNDER INDUCTIVE CULTURE CONDITIONS

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Articular cartilage is a highly specialized connective tissue in which chondrocytes are embedded within a pericellular matrix (PCM), forming the chondron, the fundamental structural and functional unit of cartilage. Conventional monolayer cultures promote chondrocyte dedifferentiation, whereas three-dimensional (3D) culture systems better preserve the native phenotype (1).

This study aimed to establish a reproducible protocol for the isolation of viable equine chondrons and to evaluate their behavior in scaffold-free 3D culture as a biomimetic model for cartilage research.

Articular cartilage was harvested from four equine metacarpo- or metatarso-phalangeal joints (5–8 years old), obtained from animals slaughtered for human consumption. Joints were carefully examined prior to sample collection, and those presenting macroscopic lesions were excluded.

Equine chondrons were isolated using an optimized enzymatic digestion protocol (2) and characterized by histology (Safranin O-Fast Green, Masson's trichrome) and multiplex immunofluorescence. Histological evaluation confirmed the successfully yield of viable chondrons with a preserved PCM. The molecular integrity of the PCM was confirmed by the presence of type II collagen (COL2), decorin, and biglycan surrounding SRY-box transcription factor 9 (SOX9) positive cells.

The self-aggregation capacity of primary (P0) and monolayer-expanded (P4) chondrons was assessed using the hanging-drop method. P0 and P4 chondrons were cultured in basal and chondrogenic media, respectively, in the presence or absence of synovial fluid (SF). The chondrogenic medium was specifically used to counteract P4 dedifferentiation.

P4 cells exhibited superior performance in 3D culture, forming compact and reproducible spheroids regardless of synovial fluid supplementation. A progressive reduction in P4 spheroid diameter over 14 days (from 450 µm to 350 µm) indicated active matrix remodeling. At day 14, P4 spheroids cultured in chondrogenic medium showed strong and diffuse COL2 expression and homogeneous SOX9 distribution, consistent with chondrogenic commitment (or redifferentation). SF supplementation modulated this pattern, restricting SOX9 expression to peripheral regions. Type I collagen (COL1) was mainly localized in the core, suggesting a transitional matrix, while collagen X at the periphery indicated oxygen-dependent hypertrophic differentiation.

The use of chondrogenic medium was critical to exploit the proliferative plasticity of P4 chondrons, directing their redifferentiation toward a mature phenotype in the 3D system. Moreover, the 3D culture system was further evaluated in a hydrogel-based environment using Silk-Elastin-Like-Protein (SELP) as a potential scaffold. Overall, these findings demonstrate that expanded chondrons retain significant plasticity and can be effectively redirected toward a chondrogenic phenotype in 3D culture. This 3D scaffold-free model represents a reproducible and relevant biomimetic platform for cartilage research and preclinical therapeutic assessment in veterinary medicine.

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EFFECT OF WINE LEES DIETARY INCLUSION ON JEJUNAL MORPHOLOGY AND IMMUNE CELL MARKERS IN BROILER CHICKENS

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To promote environmental and economic sustainability of agricultural practices, a shared objective of European policies is to improve circularity by reducing waste and promoting resource reuse. Within this framework, the valorisation of by-products from different production sectors represents a practical approach, for instance through their use in animal feed. In this context, dried post-distillation lees, a by-product consisting of wine sediments rich in protein (about 18% as fed) and bioactive compounds derived from grapes and spent yeast, may represent a promising feed ingredient for broiler chickens [1,2]. However, limited information is available on how this by-product affect gut morphology and function, raising questions about its potential beneficial or detrimental effects on chicken intestinal health. This study aimed to evaluate the effects of the dietary inclusion of dried post-distillation lees on jejunal morphology in broiler chickens. A total of 450 male Ross 308 chickens were reared from 1 to 35 days of age in 18 pens (25 birds per pen, 6 pens per dietary treatment) and assigned to three dietary treatments: F0 (control diet), F3 (basal diet supplemented with 3% wine lees), and F6 (basal diet supplemented with 6% wine lees). At 35 days of age, birds were slaughtered and jejunal mucosa samples were collected from 6 animals per dietary treatment. Samples were taken at the midpoint between the end of the duodenal loop and Meckel's diverticulum, fixed in paraformaldehyde in PBS (0.1 M; pH 7.4), dehydrated, and embedded in paraffin. Serial sections (4 µm) were stained with haematoxylin and eosin for morphometric evaluation, Alcian-PAS for goblet cell identification, and immunohistochemistry using antibodies against CD3 intraepithelial T lymphocytes and CD45 intraepithelial leukocytes. Image analysis was performed using QuPath software (v0.4.3). Villi height, crypt depth, and goblet cells were manually measured by two independent operators, while CD3 and CD45 positive cells were quantified semi-automatically by a single operator. Data were analysed using a mixed model with diet as a fixed effect and pen as a random effect. Morphometric and immunohistochemical results did not show significant differences among dietary treatments for villi height (on average 996 µm), crypt depth (143 µm), villus-to-crypt ratio (7,67), goblet cell density (21.4 no.), or CD3+ and CD45+ cell counts (4041 and 4102 cells/10,000 µm2, respectively). In conclusions, under the conditions of the present study, the dietary inclusion of wine lees up to 6% did not affect gut structural integrity or local immune status. Acknowledgments: Interconnected Nord-Est Innovation Ecosystem (iNEST), European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.5 – D.D. 1058 23/06/2022, ECS00000043).

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FROM FIBER TO FORK: STRUCTURAL AND OXIDATIVE CHANGES IN CASERTANA PORK DURING REFRIGERATED STORAGE

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The present study investigated postmortem structural and oxidative changes during refrigerated storage in the autochthonous Italian pig breed Casertana (n=4), with a focus on two anatomically and functionally different muscles: the *Longissimus dorsi* (LD) and the psoas (PS). Samples were taken at slaughter (T0), 24 hours after slaughter (T1), 4 days after slaughter (T4), and 7 days after slaughter (T7) and fixed in buffered formalin. On these samples, fiber type evaluation was performed using Hematoxyln and eosin (HE) staining to assess morphology and immunofluorescence (IF) with primary antibodies to study slow oxidative immunofluorescence (IF) with primary antibodies for slow oxidative (SO, MyHC I), fast oxidative glycolytic (FOG, MyHC IIa/x), and fast glycolytic (FG, MyHC IIb) fibers, allowing quantification of their proportion and cross-sectional area. This approach provided information on the metabolic profile of each muscle and its evolution during postmortem changes. In addition, the progression of oxidative processes during storage was evaluated through the assessment of nitrotyrosine (NT) levels, a biomarker of protein oxidation, at each time point, through immunofluorescence and confocal laser scanning microscopy, with quantification based on fluorescence intensity (signal peaks). Structural properties related to meat quality were further examined by calculating the collagen fraction under polarized light microscopy, following histochemical staining with Sirius Red, in order to evaluate collagen organization and its contribution to tenderness over time.

In the LD muscle, there was a predominance of FG fibers, while in the PS, the majority of fibers were SO, which is in accordance with a study by Velotto et al., 2010. As expected, fiber type proportions did not significantly change over time. However, from a morphological point of view, all fiber types showed progressive structural alterations, becoming more irregular and fragmented over time, especially the FG, likely due to a lower oxidative stability, higher glycolytic enzyme activity, and their increased susceptibility to proteolysis (Bhat et al., 2018).

Protein oxidation was evaluated through NT immunofluorescence, revealing a time-dependent increase in both muscles, with significantly higher levels in PS (at T7, the NT intensity was 315.42 in LD vs. 335.57 in PS; $P<0.01$). This finding is consistent with the higher proportion of oxidative fibers in this muscle, which may promote oxidative processes during storage.

In contrast, collagen fraction did not change over time in both muscles, confirming that collagen does not undergo alterations when meat is stored at 0 - 4ºC (Sentandreu et al., 2002).

Overall, this study suggests that muscle-specific metabolic properties may contribute to the progression of structural and oxidative changes during storage, supporting the relevance of muscle fiber composition in meat quality evolution. These preliminary findings provide initial insights into the biological mechanisms underlying meat quality in the Casertana breed and support its valorization. Further studies, including comparisons with commercial breeds, are needed to confirm and expand these observations.

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COLLAGEN-BASED THREE-DIMENSIONAL SCAFFOLDS FROM SEA URCHIN FOOD WASTE FOR SKELETAL MUSCLE TISSUE ENGINEERING

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Skeletal muscle accounts for approximately 40% of total body mass in mammals and is organized into muscle fiber bundles embedded in a collagen-rich extracellular matrix.

Numerous rare genetic disorders affect skeletal muscle, yet effective therapies remain largely unavailable [1]. Preclinical evaluation of novel therapeutic molecules relies on both in vitro and in vivo experimental approaches, with animal models, primarily rodents, representing a mandatory step prior to clinical trials. However, growing ethical concerns surrounding animal experimentation have prompted increasing efforts toward the implementation of the 3R principles, driving the development of advanced in vitro methodologies.

Among these, three-dimensional (3D) cell culture systems have emerged as promising alternatives in the drug discovery pipeline, enabling the recapitulation of native tissue architecture and cell-cell interactions through engineered scaffold matrices. Given that approximately 1.7 billion individuals worldwide are affected by skeletal muscle disorders, the engineering of biologically relevant 3D muscle models represents a valuable strategy for preclinical investigation, potentially reducing animal use while supporting advances in tissue engineering and regenerative medicine.

In the present study, by using tissue engineering techniques, we propose two types of innovative 3D scaffolds composed of collagen derived from sea urchins. The peculiarity of this collagen is that it is obtained and used in its fully native fibrillar form (i.e. preserving the supramolecular organization of collagen), and it closely resembles mammalian type I collagen in chain composition, immunoreactivity, and ultra-structural characteristics [2]. To mimic skeletal muscle architecture, both scaffolds were cellularized with C2C12 myoblasts, which progressively infiltrated and remained viable throughout the constructs, forming a multilayered cellular organization. Notably, the observed expression pattern of Pax7 and MyoD suggested a progression toward myogenic commitment within the 3D culture system.

These findings suggest that sea urchin-derived collagen scaffolds recapitulate the native muscle extracellular matrix and supports cell growth, infiltration, and differentiation. These constructs hold promise for future applications in patient-derived models for precision medicine, contributing to the reduction of animal testing. Furthermore, the use of collagen derived from food industry by-products is consistent with circular economy principles.

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Posters

VASCULAR REMODELING AND MATRIX MATURATION IN THE DEVELOPING PORCINE MENISCUS

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Meniscal vascularization plays a critical role during postnatal development and is tightly regulated by pro- and anti-angiogenic factors. However, the mechanisms governing the transition from a vascularized neonatal meniscus to the largely avascular adult tissue remain incompletely understood. In this study, we investigated the expression and spatial distribution of vascular endothelial growth factor (VEGF) and endostatin, as well as collagen type II (Coll II), in meniscal tissue at three developmental stages: newborn piglets (N, 0 days), weaning piglets (Y, 28 days), and adults (A, 12 months).

Meniscal samples were analysed by immunofluorescence to evaluate the spatial distribution of angiogenic markers in the inner and outer zones and by Western blot analysis to quantify protein expression levels. Immunofluorescence was also performed to study the spatial distribution of Coll II.

Our results demonstrate that VEGF expression is highest in neonatal menisci and progressively decreases in young and adult animals. This pattern suggests that early postnatal meniscal tissue is characterized by a pro-angiogenic microenvironment that supports vascular network establishment. In contrast, endostatin expression was low at birth but increased in young animals, indicating activation of anti-angiogenic mechanisms during the transition toward tissue maturation. In adults, endostatin levels remained relatively sustained, consistent with maintenance of vascular quiescence.

Immunofluorescence analysis further revealed that Coll II expression increased in intensity from neonatal to adult stages. In the inner meniscal zone, Coll II progressively accumulated, consistent with the acquisition of a more cartilaginous phenotype during maturation. An increase in collagen II signal was also observed in the outer zone, particularly within the circumferential and radial fibers region, suggesting progressive matrix remodelling and phenotypic stabilization across structural compartments.

Zone-specific analysis confirmed that the outer meniscal region maintained higher expression of vascular markers compared to the inner region at all time points, reflecting physiological compartmentalization of vascular supply. The inner zone exhibited markedly reduced VEGF expression in adulthood, consistent with the acquisition of an avascular and cartilaginous phenotype associated with increased Coll II deposition.

Collectively, our findings suggest that meniscal vascular remodelling and matrix maturation during postnatal development are regulated by coordinated changes in pro-angiogenic/anti-angiogenic factors and extracellular matrix composition. The temporal increase in endostatin concomitant with the decline in VEGF, together with the progressive enrichment of Coll II, may represent key mechanisms driving vascular regression and tissue differentiation. These data provide insight into the processes underlying meniscal maturation and may have implications for regenerative strategies and tissue engineering approaches aimed at restoring meniscal structure and vascularization after injury.

IMPACT OF HYPERGLYCEMIC METABOLIC STRESS IN HAPPSWE NEURONS: TRANSCRIPTOMIC AND MORPHOLOGICAL INSIGHTS

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Type 2 Diabetes (T2D) and Alzheimer's Disease (AD) are chronic conditions with a huge global impact and growing evidence of correlation [1]. Previous studies indicate that, when T2D coexists with AD, shared mechanisms including brain insulin resistance, chronic inflammation and metabolic dysfunctions are exacerbated, accelerating cognitive decline and increasing the risk of progression from mild cognitive impairment to AD [2].

In this study we investigated the effect of hyperglycemic condition on primary cortical neurons and astrocytes derived from an established model of AD, Tg2576, generated inserting the human APP gene carrying the Swedish APP mutation (hAPPswe).

Primary cortical neurons were isolated from E13.5 hAPPswe and wild type (WT) fetuses. Procedures involving embryonic mouse tissues were prospectively reviewed and approved by the Animal Welfare Body (OPBA) of Fondazione IRET and conducted in accordance with applicable regulations. After 7 days *in vitro*, neuronal cultures were exposed to high-glucose medium (50mM) for 24 hours to mimic the hyperglycemic condition.

In an initial phase, total RNA was extracted from experimental groups including WT, WT under hyperglycemic condition (WT-HG), hAPPswe and hAPPswe under hyperglycemic condition (hAPPswe-HG). Transcriptomic analysis via qPCR array was performed with a focus on genes involved in insulin signaling and resistance and glucose metabolism pathways.

Gene expression results showed dysregulation of genes involved in insulin resistance and glucose metabolism in hAPPswe neuronal cultures, suggesting morphological alterations and activation of AD related molecular pathways under metabolic stress. In particular, several genes that resulted dysregulated in hAPPswe-HG were uniquely altered in this experimental group and not in WT-HG group, indicating that some variations occur specifically in response to hyperglycemic stress in the AD model group. Moreover, most of these genes were not dysregulated under basal pathological conditions but only in response to hyperglycemic stimulus, suggesting a selective vulnerability of insulin resistance, glucose metabolism and insulin signaling pathways triggered by hyperglycemia.

To elucidate molecular mechanisms, future studies will focus on the evaluation of glucose uptake and the AKT signaling pathway, key components of the insulin signaling.

To link metabolic dysfunction to amyloid pathology, the intracellular accumulation of APP/$A\beta_{40/42}$ peptides was investigated by immunofluorescence (6E10 antibody) and confocal microscopy. Using IMARIS voxel-based image analysis software, we reconstructed the volume of 6E10-positive aggregates and tracked the 3D intracellular distribution to compare hAPPswe and hAPPswe-HG neurons and astrocytes.

Morphological analysis showed a higher fluorescence intensity of 6E10 marker in neurons and astrocytes exposed to acute hyperglycemic stimulus compared to hAPPswe cultures in normo-glycemic conditions.

Overall, this study highlights a potential link between the hyperglycemia and AD neuronal dysfunction, supporting the hypothesis that metabolic stress could worse pathologic processes and disease progression.

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IMMUNOFLUORESCENCE-BASED MORPHOLOGICAL READOUTS IN *IN VITRO* MODELS: OPTIMIZING NANOFORMULATED ENZYME REPLACEMENT THERAPY FOR ALPHA-MANNOSIDOSIS

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Lysosomal Storage Diseases (LSDs) comprise a group of over 70 rare inherited metabolic disorders caused by defective enzymes, leading to the accumulation of undegraded substrates within lysosomes [1]. Among these, Alpha-Mannosidosis (aMAN) is autosomal recessive conditions characterized by severe symptoms affecting both the peripheral and central nervous systems (CNS). Enzyme replacement therapies (ERTs) are currently approved as a treatment, with the aim of substituting the lacking or dysfunctional enzyme. However, these therapies cannot cross the blood--brain barrier (BBB), therefore their efficacy is limited to the non-neurological symptoms [2].

This study aims to establish *in vitro* cellular platforms using human primary fibroblasts (hPF) and murine neural cells, both healthy and affected by aMAN, to test efficacy and biodistribution of nanoformulated ERTs. This will be achieved by loading the therapeutic enzyme into poly (lactic-co-glycolic acid) (PLGA) nanoparticles using a microfluidic system. These nanoparticles are designed to protect enzymatic activity and, through functionalization with the g7 peptide—which confers BBB-specific tropism—to facilitate their passage across the blood–brain barrier, thereby enabling treatment of CNS-related symptoms.

Lysosomal defects were initially assessed in hPF and subsequently in neurons and astrocytes isolated from the cerebral cortex of transgenic murine fetuses at embryonic day 13.5. Alterations were quantified via immunofluorescence (anti-LAMP1 antibody for morphology), LysoTracker Red (morpho-functional assessment), and DQ-Red BSA (functional evaluation), followed by 3D voxel-based analysis using IMARIS software. Readouts were optimized for High-Content Screening (HCS) and validated through dose-response curves with approved ERT.

Results indicated that pathological hPF exhibit marked morphological alterations, including increased lysosome number, volume and perinuclear accumulation. Elevated LAMP1--positive structures and intensified LysoTracker Red staining suggested compensatory biogenesis or the accumulation of damaged, acidified lysosomes. Furthermore, significantly elevated DQ-BSA accumulation confirmed impaired proteolytic degradation. Similar alterations were observed in murine cortical neurons and astrocytes, indicating CNS involvement even at the fetal stage.

Exploiting the HCS-optimized platform, the uptake and efficacy of nanoformulated Lamzede® were compared to its free-form counterpart. The nanoformulation showed time- and dose-dependent uptake enhancement in fibroblasts, achieving cellular rescue at lower concentrations (0.2 U/mL) than the free enzyme. These findings demonstrate increased therapeutic efficacy, likely due to improved bioavailability, supporting the advancement of nanoformulations for the treatment of lysosomal storage disorders.

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COMPREHENSIVE HISTOMORPHOLOGICAL AND MOLECULAR MAPPING OF THE NORMAL ADULT MOUSE CEREBELLUM AS A PHYSIOLOGICAL BASELINE FOR ATAXIA-TELANGIECTASIA RESEARCH

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Ataxia-Telangiectasia (A-T) is a progressive neurodegenerative disorder caused by mutations in the ATM gene, primarily affecting the cerebellum and leading to severe motor impairment. While the role of ATM in DNA repair is well established, its influence on the broader histomorphological and molecular landscape of cerebellar homeostasis remains less understood. This study aims to establish a comprehensive physiological map of key signaling, structural, and calcium-binding proteins in the normal adult mouse cerebellum, serving as a critical baseline for future investigations into A-T pathology.

Differential expression patterns of protein markers, including Notch1, Phospho-CREB and Akt, neurotrophins, and their receptors, as well as calcium-binding and structural proteins, were evaluated using indirect immunofluorescence and confocal laser scanning microscopy.

The cerebellar cortex layers exhibited distinct immunoreactivity patterns. Notch 1 was localized in the internal granular layer and in Bergmann glial fibers, but was unexpectedly absent from Purkinje cells. Conversely, Phospho-CREB and neurotrophins (BDNF, NT-3) were highly expressed in Purkinje cells and the granular layer, reflecting their roles in neuronal survival and synaptic plasticity. Calcium-binding proteins showed varied localization. Indeed, calretinin marked both Purkinje and granular layers, whereas calbindin was notably absent from Purkinje cells. Structural proteins such as vimentin and neurofilaments provided a detailed view of cerebellar architecture, highlighting Bergmann glia and axonal integrity.

This study provides detailed molecular records of the physiological mouse cerebellum. These findings highlight both expected and divergent expression patterns compared with the existing literature, particularly for Notch1 and calbindin. This comprehensive mapping is essential for identifying the specific molecular dysregulations in ATM-deficient models, thereby facilitating the identification of potential therapeutic targets for Ataxia-Telangiectasia.

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AMINERGIC PATHWAYS AND THE RISE OF 3D MOLECULAR NEUROANATOMY

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Understanding the brain's functional architecture requires both detailed anatomical maps and molecular specificity. Early neuroanatomical approaches, relying on classical techniques like Nissl staining, provided high-resolution structural descriptions but could not distinguish functionally distinct neuronal populations. Consequently, linking anatomical organization to synaptic function was challenging, particularly in systems with diffuse projection patterns where conventional tracing failed to capture long-range connectivity and quantitative data.

Neurochemical approaches revolutionized this field. Monoaminergic systems are a paradigmatic model for connecting molecular identity to anatomy due to their discrete nuclei and extensive projection fields. A major breakthrough was tyrosine hydroxylase (TH) immunohistochemistry, which allowed selective and reproducible identification of catecholaminergic neurons. This advance marked the transition to molecular neuroanatomy, enabling standardized protocols and quantitative analyses across laboratories.

Despite these innovations, traditional section-based methods remain limited in reconstructing full three-dimensional architectures. Long-range, branched projections are difficult to resolve in 2D samples, leaving gaps in network understanding. Light-sheet fluorescence microscopy (LSFM) addresses these limitations, providing volumetric imaging of intact brain tissue while preserving global anatomical context. Within this framework, TH immunolabeling in cleared tissue enables continuous visualization of catecholaminergic circuits, revealing large-scale organization with unprecedented clarity.

Here, we present (i) 3D TH immunoreactivity atlases of the mouse hemibrain and (ii) targeted high-resolution imaging of TH-positive neurons within A-group nuclei. Datasets were acquired using anti-TH antiserum conjugated with Vio Bright G570 Dye (REAfinity, Miltenyi Biotec), solvent-based tissue clearing, and LSFM. The resulting atlases allow comprehensive visualization across coronal, sagittal, and horizontal planes, preserving both cellular resolution and global topology. By integrating high-resolution imaging with standardized analysis pipelines, this resource provides a reproducible framework for studying aminergic architecture under physiological and pathological conditions.
This study exemplifies the shift toward integrative neuroanatomy, where molecular specificity, volumetric imaging, and computational analysis converge. This platform is compatible with emerging spatial transcriptomics and builds on the pioneering work of Tomas Hökfelt [1], who first established TH as a reliable catecholaminergic marker. By enabling automated registration and quantification, these methodologies transform neuroanatomy from descriptive mapping to quantitative analysis. Collectively, our results provide a framework to investigate the connectivity and functional significance of catecholaminergic circuits, offering new avenues to explore their roles in health and disease.

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ANATOMICAL ORGANIZATION OF THE AXIAL SKELETON IN *LOPHOTES LACEPEDEI* (LAMPRIFORMES), FOCUSING ON THE CEPHALIC CREST

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Lophotes lacepedei, belonging to the order Lampriformes, family Lophotidae, is a cosmopolitan meso-bathypelagic species characterized by an elongated, laterally compressed body and a distinctive cephalic crest [1], whose anatomical organization remains poorly documented. The availability of a stranded specimen from the Sicilian coast (Milazzo, Italy) provided the opportunity for a detailed morphological investigation of the axial skeleton in relation to the dorsal fin, with particular focus on the cephalic crest. The analysis was performed using diagnostic imaging techniques, including computed tomography (Vimago3030, Imaginalis S.r.l., Florence, Italy) and digital radiography (Morpheus, Gieffe Elettromedicali, Catania, Italy; Fujifilm Medical Systems, Milan, Italy), with tomographic datasets processed through multiplanar reconstructions, maximum intensity projection, and volume rendering, allowing a comprehensive assessment of skeletal organization and its integration with the dorsal fin apparatus. The vertebral column consists of tightly articulated vertebrae lacking clear regional differentiation, each displaying a cylindrical centrum. Dorsally, short neural processes contribute to a pyramidal arrangement, while elongated neural arches extend distally to connect with the pterygiophores of the dorsal fin, indicating a continuous structural relationship between the axial skeleton and the fin apparatus along the entire body. The dorsal fin extends uninterrupted from the cephalic to the caudal region and is organized into proximal (pterygiophores), intermediate, and distal (lepidotrichia) elements, consistent with the highly specialized condition described in Lampriformes [2,3]. Anteriorly, the dorsal fin apparatus forms a prominent cephalic protuberance: two modified neural processes from the first vertebrae project forward and, together with an unpaired supraoccipital process, converge into a single apical structure projecting anterior to the jaws, corresponding to the cephalic “horn”. This configuration defines a direct structural continuity between the neurocranium, vertebral axis, and dorsal fin rays. These findings highlight a strong integration between the axial skeleton and dorsal fin system extending to the cephalic region. In line with previous observations in Lampriformes, this organization supports locomotion based on wave propagation along the dorsal fin [4,5], suggesting that the cephalic crest may contribute to hydrodynamic stabilization and force transmission along the body axis, providing new insights into the anatomical organization of this species.

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Posters

EFFECT OF *LIMOSILACTOBACILLUS REUTERI* DSM 32203 ON INTESTINAL HEALTH OF HEALTHY ADULT MAINE COON CATS

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Limosilactobacillus reuteri DSM 32203 has been authorized by EFSA as a food additive for dogs; probiotics isolated from healthy cats are generally considered more effective in colonizing the feline gut and producing beneficial effects. Despite similarities, the intestinal microbiota of dogs and cats presents differences: cats are strict carnivores and their intestinal flora differs from that of dogs (omnivores), requiring specific strains for their digestive needs. Therefore, this study aims to analyze the ability of L. reuteri DSM 32203 to exert beneficial effects on the body condition and fecal parameters of healthy cats, also evaluating its ability to modulate the intestinal microbiota. The research was conducted in accordance with Directive 2010/63/EU; forty-two healthy adult male and non-pregnant female Maine Coon cats were selected for the study and randomly assigned to the control group (CTR; n = 21) and the treated group (LACTO; n = 21). The diet of the control group was supplemented with maltodextrin (used as a placebo), while that of the treated group was supplemented with Limosilactobacillus reuteri DSM 32203 (5 x 109 CFU/kg of feed). Cats were assessed daily by a veterinarian for any health problems throughout the entire experimental period (two weeks of acclimatization + 35 days of study). Body weight (BW) and Body Condition Score (BCS) were recorded on days 0 (T0), 7 (T1), 14 (T2), 21 (T3), 28 (T4), and 35 (T5). The body weight of each animal was measured by the same person at the same time, with the same instrument. To evaluate the effect of the probiotic on fecal quality, fecal score (FS) and fecal moisture (FM) were assessed. Fecal sampling was performed at T0, T1, T2, T3, T4, and T5, and the collected samples were stored at +4°C until arrival in the laboratory. Microbiological analysis was performed at T0, T1, T3, and T5. Fresh feces were diluted in sterile saline and then vortexed until, the suspension was then streaked onto culture media for bacterial counting and identification (total lactobacilli and coliforms). All analyses were performed in duplicate. All cats remained healthy throughout the experiment; BW and BCS did not change during the study in either group. FM was lower at the end of the study in the LACTO group compared to the CTR group ($p<0.001$); FS values ranged between 3.28±0.13 at the start of the study and 3.21±0.13 at the end in the control group, remaining almost stable, while the LACTO group showed values between 3.26±0.13 at the start of the trial and 2.38±0.13 at the end of the experiment. The decrease of approximately one point (recorded in the LACTO group) on a scale of 1 to 7 may have implications for the intestinal health of cats. At the end of the experimental period (T5), a significant increase in Lactobacilli was observed in the treated group (LACTO) compared to the control group, going from 4.79±0.03 log UFC/g ($p=0.08$) at the beginning of the experiment to a final concentration of 5.13±0.03 log UFC/g ($p<0.001$), followed by a slight decrease in the total amount of coliforms ($p<0.001$). The collected data highlight the ability of the probiotic L. reuteri DSM 32203 to improve fecal quality parameters in healthy cats, promoting the formation of firmer stools, an indicator of good intestinal health and proper digestion. Furthermore, the increase in Lactobacilli highlights the probiotic's ability to improve the composition of the intestinal ecosystem of Maine Coon cats, promoting an increase in beneficial species and a slight decrease in potentially pathogenic ones.

Posters

NUTRITIONAL AND MICROBIOLOGICAL EVALUATION OF HYDROLYSED DRY DOG FOOD SUPPLEMENTED WITH *LACTOBACILLUS REUTERI* DSM 32203

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Chronic enteropathies are among the most frequent causes of gastrointestinal disorders in dogs and include food-responsive (FRE) forms [1] and are frequently linked to dysbiosis [2]. Dietary trials in dogs with chronic enteropathies have been shown to positively influence gut microbiota composition and function and hydrolysed protein diets helps reduce intestinal inflammation [3]. Probiotics are increasingly incorporated as functional ingredients in premium pet foods, reflecting the growing interest of pet owners [4].

This study aimed to evaluate the nutritional digestibility, long-term stability and technological feasibility of incorporating live probiotic cells into hydrolysed dry dog food.

The tested diet was a hydrolysed extruded dry dog food. The probiotic strain used was Lactobacillus reuteri DSM 32203 (NBF 1). The probiotic premix was prepared by diluting 2 kg of freeze-dried probiotic with 8 kg of maltodextrins (1:5). A total of 10 kg of premix was incorporated into 2000 kg of feed (0.5%). Diluted mixture was added 1:5 to the kibble at 0.5%, which ultimately corresponds to a final inclusion of 0.1%.

The post-extrusion coating phase was selected to minimize the thermal stress of the probiotic.

Fermentation tests were conducted to measure the growth of lactobacilli and bifidobacteria (log CFU/ml) and the production of SCFA. Microbiological assays assessed probiotic viability in pure batches and in the finished product, followed by accelerated stability trials at 15 °C and 30 °C over 24 months.

The analyses performed also included dry matter, protein, and starch digestibility, glycemic index and load, amino acid profile.

Results showed 92% starch digestibility, glycemic index of 44%, balanced amino acid composition exceeding FEDIAF requirements, progressive increases in lactobacilli and bifidobacteria and SCFA production.

Three pure probiotic batches were analyzed for the microbial concentration, with a median vitality of 9.89×10^{10} CFU/g, and a survival rate of 98.90%. Finished dry pet food contained 9,13x107 CFU/g after the probiotic inclusion with a survival of 91.29%.

Stability trials were performed at 0, 3, 6, 12, 18, 24 months to assess the microbial quantitative composition in the final product at different temperatures, with lower losses at 15 °C.

The nutritional evaluation of the dry dog food supplemented with L. reuteri DSM 32203 demonstrated high digestibility and a well-balanced nutrient composition. The progressive increase in lactobacilli and bifidobacteria during in vitro fermentation, together with a marked rise in SCFA production, indicates a symbiotic effect to enhance fermentative metabolism. A probiotic strain intended for use in commercially processed pet foods must exhibit high resiliency to processing-related stresses such as heat, prolonged shelf life and chemical properties of the product itself. The probiotic stability analyses highlighted the robustness of L. reuteri DSM 32203 during industrial processing, with high initial survival in the final product.

These findings support the functional efficacy of the hydrolysed formula supplemented with L. reuteri DSM 32203.

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Posters

VALORIZATION OF TOMATO BY-PRODUCTS THROUGH FERMENTATION WITH LACTOBACILLUS PLANTARUM FOR THE PRODUCTION OF FOLATES FOR FEED FORTIFICATION

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The valorization of agro-industrial byproducts through microbial fermentation represents a promising strategy for improving sustainability and generating value-added products. In this study, tomato byproducts were evaluated as substrates for folate production via fermentation mediated by Lactobacillus plantarum strains isolated from dairy products. The objective was to evaluate the ability of the selected strains to adapt to a tomato-derived matrix and increase its folate content. Tomato waste, after drying and enzymatic pretreatment, was used as a fermentation substrate and inoculated with three L. plantarum isolates (TP1, TP2, and TP3) under controlled conditions at 37°C for 24 hours. Microbial growth, pH evolution, and folate concentration were monitored and compared to an uninoculated control. Experiments were carried out in triplicate and results were expressed as mean ± standard deviation. Statistical analysis was performed by one-way ANOVA, considering $p < 0.05$ as statistically significant. All inoculated strains showed good adaptation to the substrate, with viable counts increasing from approximately 7.0 to 8.5–8.7 ± 0.13 log CFU/g after fermentation. Significant acidification was also observed, with pH decreasing from approximately 4.5 ± 0.01 to 3.6–3.8 ± 0.01 in the inoculated samples, while the control showed no significant changes. Folate concentration increased in all fermented samples, while the uninoculated control showed no improvement. The initial folate content in the substrate was approximately 43 ± 0.3 µg/100 g, while final values ranged from 67.2 ± 2.8 to 84.6 ± 3.2 µg/100 g in the fermented samples. The highest production was achieved by strain TP2, followed by TP1 and TP3 respectively. The resulting folate-enriched fermented matrix could be further exploited as a fortifier for animal feed.

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AN EXPLORATORY STUDY ON SUSTAINABLE FEEDING MANAGEMENT IN SPORT HORSE STABLES IN SOUTHERN ITALY

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Sustainable feeding strategies are increasingly relevant in equine nutrition due to their economic and environmental implications [1]. In equine nutritional management, forage represents the main component of the diet, but inappropriate feeding practices can lead to feed waste and nutritional inefficiencies [2]. However, limited information is available on forage management in competition horse stables in Southern Italy. This study aimed to explore feed management in sport horse stables in this geographical area, focusing on the observation of forage waste and the identification of mitigation strategies adopted by stable managers. An exploratory survey was conducted in 21 sport horse stables located in Southern Italy. Data were collected through structured questionnaires administered to stable managers. The survey investigated feeding management, forage-to-concentrate (F:C) ratios, forage storage, and delivery practices, with a specific focus on identifying waste reduction strategies. Statistical analysis consisted of descriptive statistics and Pearson correlation coefficients to identify relationships between management variables ($P < 0.05$). The stables were distributed across three size categories as follows: small 22% (fewer than 10 sport horses), medium 31% (between 10 and 20 sport horses), and large 47% (more than 20 sport horses). Hay was the main source of forage in all stables, offered either ad libitum or in multiple daily meals. The forage-to-concentrate (F:C) ratio exceeded 3:1 in 57% of farms, while 19% had balanced ratios (2:1 to 3:1); the remaining 24% fed concentrate-based diets (F:C < 2:1). Forage wastage was classified into three levels: low (<1 kg/horse/day), moderate (1-2 kg/horse/day), and high (>2 kg/horse/day). Moderate wastage was observed in 13 stables, while high wastage occurred in only one. Regarding distribution methods, hay was fed on the ground in 57% of the barns, in racks in 29%, and via slow-feeding nets in 14%. Notably, all the stables that used slow-release nets recorded low levels of waste, suggesting a potential role for this delivery method in containing feed waste. A significant positive correlation was found between the total hay ration and the amount of waste ($r = 0.8380$; $P < 0.0001$), suggesting that increased forage availability without specialized distribution systems may increase hay losses under practical barn conditions. These findings highlight that forage management in Southern Italian sport horse stables is frequently characterized by traditional practices, such as ground distribution, which contribute to appreciable hay wastage. The significant correlation between hay allowance and losses suggests that increasing forage availability must be coupled with improved delivery strategies to enhance farm sustainability. Further research should evaluate the long-term impact of slow-feeding systems on both horse welfare and farm profitability.

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INFLUENCE OF MATERNAL AND POST-WEANING GRAPE POMACE SUPPLEMENTATION ON OXIDATIVE STRESS, SERUM LIPIDOMICS, AND MEATQUALITY IN LAMBS

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Grape pomace is one of the main by-products of the wine industry and represents a promising feed resource for ruminants within circular and sustainable production systems. Due to its fiber and phenolic compound content, grape pomace may influence oxidative balance, lipid metabolism and, consequently, animal performance and meat quality. However, information on the effects of prolonged exposure to grape pomace in lambs, from suckling to the fattening phase, is still limited. Therefore, this study evaluated the effects of maternal and post-weaning grape pomace supplementation on growth performance, serum oxidative stress markers, serum lipidomic profile and selected meat quality traits in lambs. From birth, lambs were assigned to either a control (CON) or treated (TRT) group. Lambs in the TRT group suckled milk from ewes fed grape pomace and, after weaning at 30 days of age, received a diet supplemented with 10% grape pomace during a fattening period of approximately 60 days. Live weight was recorded throughout the trial. At slaughter, hot and cold carcass weights, pH at 45 min and 24 h post mortem, and meat quality traits including color, drip loss and cooking loss were assessed on the Longissimus dorsi muscle. Blood samples were collected at weaning and before slaughter for oxidative stress and lipidomic analyses. Data were analysed using R software; growth performance and meat quality traits were evaluated by one-way ANOVA, whereas serum oxidative stress markers and lipidomic data were analysed using linear mixed models for repeated measures, including treatment, time and their interaction. Differences were considered significant at $P < 0.05$. No significant differences were detected between groups for growth performance, slaughter weight, carcass yield, pH, drip loss or cooking loss. Meat color was also unaffected, except for a slightly lower a value in CON than in TRT lambs (15.7 vs 16.1; $P = 0.029$). Among oxidative stress markers, only advanced oxidation protein products (AOPP) showed a significant treatment × time interaction ($P = 0.033$), decreasing in CON lambs from weaning to pre-slaughter (35.0 ± 7.3 vs 28.9 ± 4.2 mg/mL) and slightly increasing in TRT lambs (31.0 ± 1.8 vs 33.7 ± 1.8 mg/mL). The serum lipidomic profile was mainly affected by sampling time, whereas no significant main treatment effects were observed. However, linoleic acid (18:2 c9,c12), total ω6 and total PUFA showed a tendency for a treatment × time interaction ($P = 0.052$, 0.068 and 0.066, respectively), with a slightly greater increase in TRT lambs than in CON lambs from weaning to pre-slaughter. In conclusion, dietary grape pomace supplementation can be included in lamb diets without detrimental effects on growth performance, meat quality or overall physiological status. Nevertheless, the treatment-related changes observed in AOPP dynamics and the trends detected in serum polyunsaturated fatty acids suggest a possible modulation of redox status and lipid metabolism, warranting further investigation.

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VIRGIN OLIVE POMACE AS A FEED INGREDIENT FOR DAIRY EWES: EFFECTS ON RUMEN FERMENTATION, METHANE EMISSIONS, AND MILK PERFORMANCE

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Virgin olive pomace is a by-product of the olive oil industry and a potential sustainable feed ingredient for ruminants due to its availability and bioactive compounds. Its inclusion in dairy ewe diets may affect rumen fermentation and methane emissions, with possible implications for animal performance and milk traits. This study evaluated the effects of dietary virgin olive pomace supplementation (15% DM) on in vitro rumen digestibility and fermentation, and on in vivo methane emissions and milk production in dairy ewes. For the in vitro trial, rumen digestibility was evaluated using the Ankom Daisy II incubator, while rumen fermentation was assessed by quantifying total gas and methane production using the Gas Endeavour system. Two diets were tested: a control diet (CON) and a treated diet (TRT) supplemented with 15% virgin olive pomace (OP). Each sample was analysed in triplicate. In vitro digestibility was expressed as in vitro true dry matter digestibility (IVTDMD, %), whereas total gas and methane production were expressed as mL/g DM. For the in vivo trial, the same diets were administered to 30 Assaf ewes from about two weeks before lambing until 90 days postpartum. Methane emissions were measured using a Laser Methane Detector (LMD) and expressed as ppm·m. Methane spectra were partitioned into eructation and respiration phases according to Carta et al. (2025). Milk yield was recorded weekly, and milk samples were analysed for fat, protein, casein, lactose, urea and somatic cell count. Data were analysed using R software and linear mixed models. In vitro models included treatment as fixed effect and rumen fluid as random effect, whereas in vivo models for methane and milk traits included treatment, time and their interaction as fixed effects and animal as random effect. Differences were considered significant at $P < 0.05$. In the in vitro trial, OP supplementation significantly reduced rumen digestibility compared with CON (80.7 vs 94.5%; $P < 0.05$). Total gas production was also lower in TRT than in CON (181.6 vs 197.6 mL/g DM; $P < 0.05$), whereas methane production did not differ between diets. In vivo, a tendency towards lower total methane emissions was observed in TRT ewes compared with CON ewes (55.4 vs 57.6 ppm·m; $P = 0.09$), while methane emissions during the respiration phase were significantly reduced by OP supplementation (47.7 vs 50.5 ppm·m; $P < 0.05$). Milk yield and the main milk quality traits were not affected by treatment, whereas milk urea was lower in TRT ewes than in CON ewes (57.7 vs 60.5 mg/100 mL; $P < 0.05$). In conclusion, dietary virgin olive pomace reduced in vitro rumen digestibility and total gas production, while showing potential for methane mitigation in vivo, particularly during the respiration phase, without impairing milk yield or the main milk quality traits. These findings support the use of virgin olive pomace as a sustainable feed ingredient in dairy ewe diets.

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BIOCHEMICAL CHARACTERIZATION OF DAIRY BY-PRODUCTS (WHEY AND SCOTTA) FOR MICROALGAL CULTIVATION

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The dairy industry generates millions of tons of by-products annually, whose management represents an important environmental and economic challenge. Within a circular economy framework, these streams can be valorized for sustainable biotechnological processes such as microalgae cultivation. This study aimed to characterize the biochemical composition of dairy by-products and evaluate their potential for microalgal cultivation.

Whey and scotta samples (n=300) were collected from five dairies located in the Bologna area, with the exception of one site in the Tuscan-Emilian Apennines. Sampling was carried out daily for two weeks in winter and summer, and three times per month from March to June. Total protein concentration was determined using the Lowry assay, followed by the development of a protocol for protein separation and characterization, combining SDS–PAGE and size-exclusion chromatography (SEC), coupled with flame atomic absorption spectrometry for iron and zinc quantification.

Total protein content was higher in whey than in scotta across all five dairies. Protein concentrations showed significant seasonal variations (ANOVA, $p<0.05$), in agreement with previous studies[1], with relatively high values in winter, increasing towards a spring peak in May (β-lactoglobulin: 2.05 µg/µl whey and 0.34 µg/µl scotta), followed by a decline in summer (values: 1.68 µg/µl and 0.18 µg/µl, respectively). SDS–PAGE analysis confirmed the presence of the main whey proteins, β-lactoglobulin and α-lactalbumins, representing approximately 55% and 20% of the total proteins respectively, along with serum albumin and lactoferrin. Similarly, α-lactalbumin and β-lactoglobulin were identified as the most abundant proteins in scotta, with traces of caseins also detected. SEC profiles of proteins in whey revealed multiple peaks likely corresponding to the major proteins identified by SDS–PAGE, including serum albumin (~66 kDa), β-lactoglobulin (~18 kDa) and α-lactalbumin (~14 kDa). The consistency between electrophoretic bands and chromatographic peaks supports the reliability of the analytical approach and confirms the protein composition of whey. In SEC fractions, iron was detected associated with high molecular weight fractions, suggesting its partial binding to proteins and as free ion. In contrast, scotta showed a simpler chromatogram dominated by low molecular weight compounds, reflecting protein loss and thermal denaturation during ricotta production, with reduced iron levels. Overall, these findings demonstrate that dairy by-products represent a valuable source of proteins and iron with potential applications in biotechnology and nutrition [2].

Preliminary experiments have been initiated using whey and scotta as substrates for the cultivation of Galdieria sulphuraria [3]. The developed analytical protocols provide a solid basis for further investigations on microalgal growth under mixotrophic conditions and on the valorization of dairy by-products within a circular economy approach.

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PRODUCTION RESPONSES OF LACTATING HOLSTEIN COWS TO INCREASING DIETARY SUPPLY OF RUMEN-PROTECTED METHIONINE

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The present study was conducted to evaluate the effects of a newly developed rumen-protected methionine supplement (RPMet™, Milk Specialties Global) on metabolizable methionine supply and on productive responses in lactating Holstein cows. The experimental trial lasted 84 days and was carried out at the Cornell University Ruminant Center. A total of 144 lactating cows were enrolled in the study and allocated to replicated pens containing 16 animals each, with three pens assigned to each dietary treatment.

Experimental diets were formulated using the Cornell Net Carbohydrate and Protein System (CNCPS; version 6.5.5). Three dietary treatments were therefore created with progressively increasing concentrations of metabolizable methionine: 0.96, 1.05, and 1.19 g/Mcal of metabolizable energy (ME); metabolizable lysine was included at 3.2 g/Mcal ME of and metabolizable histidine at1.19 g/Mcal ME. All rations were designed to be isocaloric, containing approximately 5% sugars, 28.5% starch, 28.5% ash-corrected neutral detergent fiber, and 4.3% ether extract on a dry matter basis.

Throughout the experimental period, cows were milked three times daily. Individual milk yield was automatically recorded by the herd management software. Milk samples were collected weekly from three consecutive milkings and analyzed to determine fat percentage, fatty acid composition, true protein concentration, milk urea nitrogen, and somatic cell count. Body weight and body condition score were evaluated weekly, whereas dry matter intake was measured daily at the pen level.Data were analyzed using a mixed statistical model implemented in SAS. Diet was included as a fixed effect, while pen nested within diet and cow nested within pen and diet were considered random effects.

Increasing the dietary supply of metabolizable methionine resulted in a significant improvement in energy-corrected milk production (Diets 2 and 3 - 50.1, 50.4 kg/d - vs Diet 1 - 48.7 kg/d; $P = 0.02$). In addition, higher methionine availability was associated with progressive increases in milk true protein concentration (3.09%, 3.24% and 3.34% - $P < 0.01$). Milk fat percentage also tended to increase in cows receiving the highest level of methionine supplementation (Diet 3 - 4.36 % - vs Diets 1 and 2 - 4.21 and 4.25 %; $P < 0.01$). Furthermore, the concentration of de novo synthesized fatty acids in milk increased with greater methionine supply, suggesting a potential relationship between methionine availability and mammary lipid synthesis.

Overall, the findings of this study indicate that supplementation with RPMet™ can effectively improve the balance of metabolizable amino acids in diets for lactating dairy cows, when lysine and histidine are not limiting.

PHENOL ENRICHED EXTRACT AFFECTS LYMPHO-MONOCYTE VIABILITY, APOPTOSIS, AND INTRACELLULAR ROS PRODUCTION IN GROWING LAMBS AND ADULT SHEEP

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In the last decade, agro-industrial by-products have been spread as partial replacer of animal feeds, in particular for ruminants, containing a huge amount of interesting bioactive compounds, such as polyphenols, vitamins, and flavonoids, which can contribute to improve animal welfare and product quality. The present experiment aimed at evaluating the effect of different concentrations of phenol enriched extracts (PEE) on viability, apoptosis, and intracellular ROS production in peripheral blood mononuclear cells (PBMC) from growing lambs and adult sheep in order to determine their potential antioxidant activity at the cellular level. The chemical characterization of PEE revealed the presence of 3,4-DHPEA, p-HPEA, β-hydroxyverbascoside, verbascoside, and 3,4-DHPEA-EDA, with verbascoside and 3,4-DHPEA-EDA being the most abundant compounds. Peripheral blood samples were collected from jugular vein of growing lambs (n=8) and adult sheep (n=8), then PBMCs were isolated by density gradient (Hystopaque, 1077). The tested PEE concentrations were selected based on the potential in vivo phenolic concentrations in the diet. For growing lambs, PEE concentrations of 27.3, 13.65, 6.8, 3.41, and 0 mg/mL were tested, whereas concentrations of 2.20, 1.10, 0.55, and 0 mg/mL were used for sheep. A total of 1x10^6 PBMCs were seeded into 96 well plate for MTT cell proliferation assay, and the evaluation of cells apoptosis LPS-activated by PEE treatment was performed by flow cytometry on PBMC by using the same PEE concentration. Briefly, PI and Annexin-FITC was added to 1x10^6 PBMC, live cells were identified as PI^-/Annexin-$FITC^-$, early apoptotic cells as PI^-/Annexin-$FITC^+$, late apoptotic cells as PI^+/Annexin-$FITC^+$, and necrotic/dead cells as PI^+/Annexin-$FITC^-$. Total ROS production was determined by flow cytometry after PEE treatment in phorbol 12-myristate 13-acetate (PMA)-activated cells. Data on PBMC proliferation and apoptosis were analysed using MIXED model, while data on ROS by using one-way ANOVA with SAS software. PBMC proliferation in growing lambs was affected by PEE treatment, showing a significant decrease when concentrations increased from 3.41 and 6.8 to 13.65 and 27.3 mg/mL. Conversely, in sheep PBMCs proliferation increased in a dose-dependent manner. Apoptosis was influenced by PEE treatment in growing lambs ($P<0.001$) and by the interaction among PEE treatment and apoptosis rate ($P<0.001$), with a reduction in live cells and a consequent increase in dead cells when PBMCs were treated with 6.8 mg/mL compared with 27.3, 13.65, and 3.41 mg/mL. Apoptosis rate in adult sheep PBMC was significantly affected ($P<0.001$) registering the highest percentage of viable cells. Intracellular ROS production significantly increased at all tested PEE concentrations in both PMA-activated lamb and sheep PBMCs. Overall, the results suggest that PEE, depending on the tested concentration, may stimulate PBMC proliferation and modulate apoptosis, while influencing oxidative metabolism through increased ROS production associated with immune cell activation.

PRO-INFLAMMATORY AND ANTIVIRAL EFFECTS OF IL-1β AND ITS RECEPTOR ANTAGONIST ON PORCINE MACROPHAGES

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Macrophages are key players of the innate immune systems and are characterized by remarkable plasticity. These cells can quickly change their function and phenotype in response to external stimuli [1]. Swine are attracting increasing attention as a close-to-human biomedical model, due to many immunological similarities with humans [2], however porcine macrophage polarization has not been extensively analyzed so far. In this study, we investigated for the first time the impact of two key IL-1 family members (IL-1β and its receptor antagonist IL-1Ra) on porcine macrophages.

Four healthy pigs were used as blood donors to generate macrophages (authorization n° 1232/2020-PR). Macrophage were stimulated with recombinant porcine IL-1β or IL-1Ra (both at 100 ng/mL), alongside untreated controls. At 3 and 24 h post-stimulation, expression of 70 anti-viral and pro-inflammatory genes were analysed with RT2 Profiler PCR Array. At 24 h, release of key immune cytokines (IL-1α, IL-1β, IL-1Ra, IL-6, IL-10, IL-12, IL-18) was also monitored using ELISA multiplex.

Our gene expression analyses revealed that stimulation with IL-1β resulted in early (3h) up-regulation of few genes coding for molecules with pro-inflammatory and antiviral activities. In particular, a strong up-regulation of JUN was observed, with fold-change 99.924. Significant increased was also observed for IFNB1, CXCL11, MYD88. At 24 h, stimulation with IL-1β resulted insignificant up-regulation of several genes coding for molecules with pro-inflammatory and antiviral activities. The strongest up-regulation was observed for JUN, with fold-change > 500. At this time point, several genes were significantly up-regulated: AZI2, FOS, MAP2K1, MAP3K1, MAPK14, IFNB1, CXCL11, MYD88, TNF, CXCL9, MEFV, CASP8, CYLD, SPP1, TKFC. No significant up-regulation was observed in cells stimulated with IL-1Ra at both tested time points, whereas a tendency was observed for IL1B ($p = 0.076$), with a fold-change of 27.936 at 24 h. For both cytokines, no genes were significantly down-regulated, with the exception of a down-regulation of CSF2 in response to IL-1β stimulation at 24 h.

Multiplex ELISA revealed that stimulation with IL-1β resulted in release of pro-inflammatory IL-6 and IL-18, but also anti-inflammatory IL-10 and IL-1Ra. Stimulation with IL-1Ra resulted instead in release of IL-10 only.

Overall, we observed that stimulation with IL-1β polarised porcine macrophages toward a pro-inflammatory and anti-viral phenotype, but in parallel anti-inflammatory IL-10 and IL-1Ra were released. Porcine macrophages try to self-block inflammation once it started, with a strong release of anti-inflammatory cytokines, including IL-1Ra, in response to IL-1β stimulation, and higher expression of IL1B in response to IL-1Ra stimulation. It seems that in pigs macrophages try to keep a balance between these two cytokines, as a defence mechanisms to control inflammation. Overall, in this work we provided a better understanding of porcine macrophage polarization, which could benefit translational studies using this large animal model.

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GILTHEAD SEABREAM (*SPARUS AURATA*) WELFARE: DIFFERENCES BETWEEN ORGANIC AND CONVENTIONAL AQUACULTURE SYSTEMS

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Aquaculture growth demands reliable and standardised tools to assess fish welfare. The aim of this study is to evaluate gilthead seabream, Sparus aurata (Linnaeus, 1758), welfare using physiological indicators, comparing organic (Farm A) and conventional farming systems (Farm B). Forty blood samples were collected at the beginning (August 2024) and at the end (November 2024) of the trial and analysed for haematological [haematocrit, red blood cells (RBC), haemoglobin, white blood cells (WBC), platelets] and biochemical parameters [glucose, lactate, cortisol, insulin-like growth factor 1 (IGF1), total proteins].

Most of the haematological parameters, including haematocrit, remained stable across sites and sampling times. However, several biochemical markers showed significant site×time interactions. Glucose and lactate levels differed between sites and over time, with generally higher values in Stintino in August and contrasting trends in November. At both sites haemoglobin levels decreased over time, while RBC showed a similar temporal decline. Cortisol levels were higher in Stintino in August, indicating greater stress, but they decreased over time with no differences observed in November. IGF1 increased over time, suggesting improved growth-related conditions. Total proteins showed site-specific changes, increasing only in Stintino farm. WBC and platelets decreased over time, with no differences between two sites.

Overall, most parameters fell within expected physiological ranges. The results suggest that sea bream from Stintino appear to be raised under better welfare conditions, although some indicators, such as lactate, showed contrasting trends. These results highlight the complexity of welfare assessment and confirm the importance of a multiparametric approach that combines environmental, physiological and behavioral indicators.

This study was approved and founded by Italian Ministry of Health (RC IZS SA 05/21).

Authorization of the experimental procedure n.522/2023PR.

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IMMUNOTHERAPY IN VETERINARY MEDICINE: EVALUATION OF THE IMMUNE RESPONSE INDUCED BY AN AUTOGENOUS VACCINE IN THE TREATMENT OF CUTANEOUS LESIONS CAUSED BY PAPILLOMAVIRUS IN EQUINES AND BOVINES

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Papillomaviruses (PVs) infect a wide range of animal species and are responsible for proliferative cutaneous and mucosal lesions, including papillomas in cattle and sarcoids in equines, with significant health and economic impacts. The immune response in cattle is often suboptimal, likely because the viral replication cycle is confined to the epithelium, thereby limiting antigen exposure (1,2). The aim of this study was to evaluate the efficacy and immunogenicity of an autogenous (farm-derived) vaccine produced from biological material collected from affected farms. The study involved four farms in Lombardy, including a total of 18 cattle with papillomatous lesions and 7 healthy animals as controls. Blood samples were collected at four time points to assess the immune response; peripheral blood mononuclear cells (PBMCs) were isolated from these samples for real-time PCR analysis, and a complete blood count profile was performed. Serum haptoglobin was also measured, and clinical biochemistry analyses were conducted on plasma obtained from blood collected in lithium heparin tubes. Samples were analyzed by electron microscopy, Real-Time PCR for bovine Papillomavirus (BPV) screening and typing, L1 gene sequencing, and histological examination. Vaccination was performed with a booster administered approximately three weeks later. Electron microscopy revealed viral particles consistent with Papillomavirus in all samples, measuring 55–60 nm and lacking an envelope. All samples tested positive for BPV strains following PCR screening on L1 subunit. Histological examination showed findings consistent with fibro papilloma, characterized by dermal fibroblastic proliferation associated with acanthosis, hyperkeratosis, and cytopathic effects in keratinocytes, with chronic lymphocytic inflammation observed in several cases. From a clinical perspective, vaccinated animals showed progressive regression of lesions starting after the second inoculation, with complete remission observed within 25–30 days after the end of the vaccination protocol. Hematological analysis revealed clinically relevant alterations in the main parameters. Lymphocyte counts showed a significant decrease over time ($p < 0.001$), whereas neutrophils exhibited a significant increase ($p < 0.001$). Red blood cell indices, including MCV and MCH, also demonstrated a significant progressive increase over time (both $p < 0.001$). Monocytes showed a modest and transient decrease at time point 1 ($p = 0.014$), while leukocytes displayed a borderline trend without consistent significance across time points. No significant temporal variation was observed for eosinophils, basophils, or platelets.PBMCs gene expression analysis performed on of *IL18*, *IL1B*, *CXCL8* and *TGFB* showed no statistically significant modulation after vaccination. Lesions gradually decreased in size until complete disappearance, with no recurrence during the observation period. One out of seven animals in the control group developed papillomatous lesions on the teats. The results demonstrate that the autogenous vaccine can induce an effective immune response, promoting regression of papillomatous lesions. Moreover, the application of this protocol reduced the need for surgical interventions, particularly critical for lesions located on the udder or in anatomically sensitive areas. This approach represents a sustainable strategy in field conditions, especially in intensive farming systems.

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Posters

CANINE ATOPIC DERMATITIS IN ONE HEALTH CONCEPT LAB: A NOVEL IN VITRO MODEL TO BETTER UNDERSTAND THE IMPACT OF EXPOSOME ON CANINE SKIN

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Atopic dermatitis is a chronic, inflammatory, and pruritic skin disease affecting both humans and animals. Canine atopic dermatitis (CAD) shares clinical and immunological similarities with human atopic dermatitis. Dogs share the same environment as humans, being exposed to the same load of allergens and pollutants, which allows for the investigation of the triggers that induce and/or exacerbate the disease. As in humans, CAD exhibits variability in clinical presentation, severity, and treatment response, which can complicate data interpretation and hinder the identification of consistent biomarkers. It is often difficult to dissect disease patterns, prevent the onset of dermatitis, and control clinical signs; alternative therapeutic targets based on pathogenesis also need to be explored. Here at the University of Perugia, we have established a 'one health' interdisciplinary laboratory called: 'Innovative animal and human-ORGANOid platform to advance understanding of vesicle-induced MICROenvironment, in pathology throughout the LIFE course' (ORGANOMICROLIFE), realising a specialised platform to investigate the tissue microenvironment in cancer and immune-mediated diseases.

The study aimed to develop 3D in vitro models (organoids) of canine epidermis from CAD patients that replicate the architecture, function, and exposome interactions of the target tissue more closely than traditional 2D cell cultures, for use in basic research, disease modelling, drug development, and testing.

Canine skin biopsies obtained from 6 adult dogs diagnosed with CAD by an ECVD board-certified veterinary dermatologist and 6 healthy dogs as controls were collected with the owner's consent. The study protocol was approved by the Bioethics Committee of the University of Perugia (protocol number, 38/2024). Skin punch biopsies of 8 mm were performed under local anaesthesia from the interscapular region. To obtain organoids, biopsies were immediately transported into DMEM-F12 medium and digested in the ORGANOMICROLIFE lab. Upon digestion, the single-cell suspension was cultured in Matrigel and growth factor-enriched medium. Growth was monitored daily through a microscope and the Spark multimode microplate reader (growth rate of pre-differentiation organoids mean:152.93µM, SD±27.36; growth rate of post-differentiation organoids mean:304.69µM, SD±60.76;). Thus, quantitative monitoring of organoid cultures allowed 3D cell imaging with fast, precise screening. Organoids cultured in differentiation medium showed an equivalent structure to the dog epidermis and expressed key marker proteins in similar proportions. In particular, the post-differentiation organoids were positive for Cytokeratin 7. For the first time, we successfully generated organoids from the epidermis of dogs with CAD (success rate 83,4%). Epidermal organoids represent, therefore, a promising in vitro model for the in-depth study of epidermal abnormalities in atopic dermatitis and may offer a potential strategy to stratify patients into subgroups. In addition, this technology may be useful to explore novel diagnostic tools, host-pathogen interaction models, and the role of the exposome in disease pathogenesis.

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AI-POWERED HAEMATOLOGY REVEALS SPURIOUS LEUKOCYTOSIS CAUSED BY NUCLEATED RED BLOOD CELLS IN A NON-ANAEMIC CAT

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Nucleated red blood cells (nRBCs) are occasionally identified in both anaemic and nonanaemic feline blood samples. In physiological erythroid maturation, nRBCs are confined to the bone marrow [1,2]. Their presence in peripheral circulation commonly reflects increased marrow stimulation, with regenerative anaemia, or pathologies causing disruption of the marrow–blood barrier [1]. Low numbers of nRBCs in the absence of anaemia and reticulocytosis have been associated with systemic inflammation, splenic dysfunction, bone marrow stress, glucocorticoid exposure, hypoxic or metabolic disease [2]. nRBCs can be misclassified as leukocytes in automated complete blood count (CBC), leading to a spurious elevated white blood cell (WBC) count [1]. Modern haematology analysers can flag this interference, prompting blood film review.

We report the case of a 10-year-old spayed female Domestic Shorthair cat undergoing routine preanaesthetic screening. A CBC indicated erythroid mass within limits (RBC 10,67 M/µL, reference interval [RI] 6,54-12,20; haematocrit 42,0%, RI 30,3-52,3; haemoglobin 11,9 g/dL, RI 9,8-16,2; reticulocyte count 40,5 K/µL, RI 3,0-50,0) but marked leukocytosis (48,48 K/µL; RI 2,87–17,02), with pronounced neutrophilia (40,84 K/µL; RI 2,3–10,29). The analyser qualified both total and differential WBC results flagging suspected band neutrophils and nRBCs. Scatterplots showed a poor separation between neutrophils and lymphocytes, compatible with significant nRBCs interference.

To investigate the abnormalities, the blood sample was analysed using the IDEXX inVue Dx™ Cellular Analyser. This platform uses multiple wavelengths of light and fluorescence to characterise the cells, enabling an AI-powered morphological classification and enumeration capable of updating the total and differential WBC counts. In this case, the IDEXX inVue Dx identified the nRBCs (83 nRBCs/100 WBCs as extrapolated from the corrected WBC count of 8,30 K/µL), resolving the previously reported neutrophilia.

Cytological evaluation of the peripheral blood smear was performed, which estimated 80 nRBCs/100 WBCs and confirmed the absence of a true leukocytosis. SNAP™ FIV/FeLV Combo Plus Test results were negative, and lead blood levels were extremely low, excluding potential toxicosis. Bone marrow cytology and FeLV PCR were recommended but declined by the owner given the patient's excellent clinical condition; monitoring was elected.

By reliably differentiating nRBCs from leukocytes, the AI-powered analyser corrected the WBC count and avoided misclassification within the leukogram, reinforcing its contribution to refined haematologic evaluation.

This case underscores the value of combining AI-supported assessment of blood morphology with the CBC results, to ensure comprehensive and accurate interpretation.

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AN UNUSUAL CAUSE OF COUGHING IN A WARMBLOOD: THE STRANGE CASE OF THE PULMONARY GRANULAR CELL TUMOR

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Tumors of the respiratory tract are rare in horses, and usually metastatic in origin. Primary neoplasms, on the other hand, have been rarely reported in the literature. Among these, granular cell tumors are the most common ones, with only 1,1% of submitted samples diagnosed as such in an American laboratory. They derive from Schwann cells of the peripheral nervous system. Most of the reports are incidental findings at necropsy, but in some cases, due to their progressive nature, they could be associated with respiratory signs, such as coughing, exercise intolerance, respiratory distress and increased respiratory rate, but also with non-specific signs such as weight loss [1,2,3]. In this case report, a case of pulmonary granular cell tumor will be described, together with its treatment and follow-up. A 14 year-old Italian Saddlebred mare was admitted for further diagnostics after the referring vet visualized a mass at the beginning of the bronchus of the right caudal lobe. The horse had two episodes of pneumonia, treated with antibiotics and anti-inflammatory medications, with only partial response. At admission, the horse was bright and alert, and clinical examination was unremarkable. Examination of the respiratory tract allowed to detect abnormal lung sounds, especially on the right hemithorax. Radiographs of the thorax showed an increased opacity of the caudal aspect of the lung, compatible with the presence of a mass, that was later confirmed with bronchoscopy: a smooth localized mass was observed at the beginning of the bronchus of the right caudal lobe. Pushing the scope after the mass, the anatomy and the respiratory mucosa were normal. Biopsy samples were taken using a uterine biopsy forceps passed through a tracheostomy performed in the distal cervical trachea, with the horse standing and under sedation. At the same time, to reduce the bleeding and try to shrink the mass, a diode laser application was performed. The histology of the biopsy allowed to diagnose a granular cell tumor. The mare was discharged from the hospital in excellent clinical conditions: a prophylactic treatment with marbofloxacin and N-acetylcysteine was prescribed. Two follow up endoscopic laser treatments were performed at the stable, the first eight months after discharge and the second one 45 days later. At both, the horse was clinically healthy, bright and alert, and the owner reported no coughing or signs of respiratory distress also during lounging or while the horse was stabled at pasture and running with the other animals. At the first endoscopy the mass was similar in size to the diagnosis, while at the second, it appeared sensibly reduced in volume. The laser applications did not result in side effects, and follow up treatment was limited to nonsteroidal anti-inflammatory drugs for three days and N-acetylcysteine for one week. Despite being rarely described in horses, granular cell tumors should be included in the differentials of horses with respiratory distress and cough resistant to treatment.

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Posters

FELINE ANAL SAC SQUAMOUS CELL CARCINOMA WITH DOCUMENTED METASTATIC BEHAVIOUR AND LIMITED RESPONSE TO ELECTROCHEMOTHERAPY: A CASE REPORT

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Squamous cell carcinoma (SCC) is a common malignant epithelial tumour in cats, typically affecting the oral cavity and cutaneous sites, whereas involvement of the anal sac region is exceedingly rare, with only two cases previously described in the veterinary literature [1].

This report describes the clinical presentation, diagnostic challenges, and clinical management of a feline anal sac SCC. An 8-year-old neutered male Domestic Shorthair cat was referred for persistent tenesmus, constipation and anorexia. One month earlier, a perianal swelling had been identified, and cytology was consistent with septic anal sacculitis, leading to antimicrobial treatment with only partial clinical improvement. Due to persistence of clinical signs, further investigations were performed. Imaging revealed a circumferential perianal mass infiltrating the perirectal tissues, and histopathology confirmed SCC with cytologically confirmed nodal metastasis.

Therapeutic options were severely limited by the extent of local invasion. Surgical excision was not considered feasible without unacceptable functional consequences, and radiation therapy was declined by the owner. A systemic approach with toceranib phosphate resulted in approximately 4 weeks of clinical disease stabilisation, while subsequent electrochemotherapy provided only transient clinical benefit. However, both treatments failed to achieve durable tumour control, and rapid disease progression ultimately led to death 68 days after diagnosis.

This case underscores several clinically relevant considerations. Inflammatory conditions of the anal sac may mask underlying neoplasia, potentially delaying diagnosis when cytology is used as the sole diagnostic modality. In this patient, histological features suggestive of moderate atypia and low mitotic activity were not predictive of clinical behaviour, as the tumour showed rapid local progression and early metastatic spread. This finding supports previous evidence that histological features alone may be insufficient to predict biological behaviour in feline SCC. From a clinical standpoint, SCC arising in the anal sac region may represent a more aggressive disease entity compared with more common feline SCC localisations, with a higher metastatic potential and rapid progression. From a treatment perspective, currently available options, including tyrosine kinase inhibitors and electrochemotherapy, appear to provide only short-term clinical benefit in advanced cases, suggesting that these approaches may be insufficient once extensive local invasion has occurred. Anal sac SCC, although rare, should therefore be considered among differential diagnoses in cats presenting with persistent perianal disease unresponsive to medical therapy.

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ANALYTICAL AND CLINICAL PERFORMANCE OF A POINT-OF-CARE TEST FOR CANINE C-REACTIVE PROTEIN USING THE FUJIFILM DRI-CHEM NX600V

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Canine C-reactive protein (cCRP) is a key biomarker of inflammation in dogs [1]. The introduction of new analytical methods requires validation and verification of their analytical and clinical performance, particularly in the context of method harmonization within hospital settings [2]. Fujifilm has recently introduced a new dry-chemistry platform (Dri-Chem NX600V), which includes a novel assay for cCRP measurement (vc-CRP-P).

The aim of this study was to evaluate the analytical agreement and clinical performance of the Fuji vc-CRP-P assay on the Dri-Chem NX600V compared with immunoturbidimetric methods commonly used in reference laboratories.

A total of 64 serum samples from 17 healthy dogs and 47 dogs affected by diseases characterized by varying degrees of inflammation were included. cCRP concentrations were measured using the Fuji Dri-Chem NX600V and two immunoturbidimetric assays (Gesan and Futurlab reagents) on an automated clinical chemistry analyzer (BT3500, Biotecnica). Analytical agreement was assessed using Passing-Bablok regression and Bland-Altman analysis. Agreement in detecting inflammatory conditions according to method-specific reference intervals was assessed using Cohen's and Fleiss' kappa.

A strong positive correlation was observed between Gesan and Futurlab assays (r=0.85) and Fuji and Futurlab assays (r=0.84) while a moderate positive correlation was observed between Fuji and Gesan assays (r=0.73). Bland-Altman analysis showed that the Fuji assay slightly overestimated cCRP compared to Futurlab assay (mean bias 1.33±0.50 mg/dL) and slightly underestimated values compared to Gesan assay(0.58±0.58 mg/dL), while Futurlab underestimated cCRP compared to Gesan (−0.94±0.25 mg/dL), confirming limited interchangeability between methods. However, Passing-Bablok regression revealed systematic and/or proportional bias across all comparisons. Fuji vs Futurlab showed both systematic and proportional bias (intercept −1.65, 95% CI: −2.26 to −1.30; slope 5.16, 95% CI: 4.32–6.61), while Fuji vs Gesan showed no systematic bias (intercept 0.10, 95% CI: −0.28 to 0.28) but proportional bias (slope 0.64, 95% CI: 0.50–0.91). Gesan vs Futurlab also showed both systematic and proportional bias (intercept 0.32, 95% CI: 0.28–0.36; slope 0.15, 95% CI: 0.12–0.16). Agreement analysis showed moderate concordance between Gesan and Futurlab assays (κ=0.47; 49/64 concordant cases, 76.6%), lower agreement between Gesan and Fuji assays (κ=0.37; 44/64, 68.8%), and moderate agreement between Futurlab and Fuji assays (κ=0.53; 49/64, 76.6%). Fleiss' kappa confirmed an overall moderate agreement among the methods (κ=0.44). Concordance among all methods was observed in 39/64 cases (60.9%).

The Fuji vc-CRP-P assay demonstrates fair-to-moderate agreement and moderate-to-strong correlation with the other methods. Combined with its practical advantages as a point-of-care test, it may represent a useful screening tool in emergency settings. Of note, our results show that differences between methods are particularly pronounced near decision thresholds and at higher concentrations, indicating that the evaluated methods are not fully interchangeable. Further validation studies and the definition of optimized diagnostic cut-offs are warranted to improve test performance and support harmonization between methods.

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Posters

PLEURAL EFFUSION IN DYSPNEIC CATS: A RETROSPECTIVE STUDY OF CAUSES AND OUTCOMES

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Pleural effusion is a common finding in dyspnoeic feline patients, with an estimated prevalence of approximately 39% in the literature [1] Various conditions may contribute to the accumulation of fluid within the pleural space, including cardiomyopathies, infectious diseases such as Feline Immunodeficiency Virus (FIV), Feline Leukaemia Virus (FeLV), and Feline Infectious Peritonitis (FIP), septic processes, and neoplasia [2] Rapid identification of the underlying cause is critical for appropriate management and prognosis.

The aim of this retrospective study was to characterize dyspnoeic feline patients with pleural effusion, identify the underlying aetiology, and determine the associated mortality rate.

Medical records of cats presenting to the emergency unit of the Veterinary Teaching Hospital of the University of Milan with a history of dyspnoea between January 2021 and December 2025 were reviewed. Data collected included signalment, clinical history, and results of diagnostic tests, such as thoracic radiographs, echocardiography, FIV/FeLV testing, cytological, bacteriological, and molecular diagnostics. Cats with pleural effusion were included, and the aetiology of the effusion was classified accordingly.

A total of 154 dyspnoeic cats were initially identified, of which 53 (34%) had pleural effusion and were included in the study. One cat died during stabilization, and 3 did not receive a diagnosis as owners declined further testing. Consequently, 49 cats were diagnosed: 19 (36%) had thoracic neoplasia (four of which were FeLV+), 17 (32%) cats were diagnosed with cardiomyopathy (one of which was FIV+), 6 (12%) cats had a diagnosis of pyothorax (one FeLV+), 3 (6%) had a diagnosis of FIP, 2 (4%) were both FIV/FeLV co-infected, while 1 (2%) was only FeLV+ and 1 (2%) was only FIV+.

Of the 19 cats with a diagnosis of thoracic neoplasia, 9 did not receive further diagnostic workup, 5 were diagnosed with carcinoma (3 of which were metastases from mammary carcinoma), 3 were diagnosed with lymphoma, one with a mediastinal mass, and one with epithelial neoplasia.

Among the 17 cats with cardiomyopathy, 2 were younger than 10 years, and 15 were older than 10 years. Pleural effusion due to infectious diseases such as FIV, FeLV, and FIP was observed exclusively in cats under 7 years of age. Of the neoplastic cases, only 5 cats were younger than 8 years, two of which were coinfected with FeLV.

Of the 53 cats included in the study, 22 died during hospitalization: 12 (55%) had neoplastic disease (3 of which were FeLV-positive), 4 (18%) had cardiomyopathy, 1 (4.5%) had FIP, 2 (9%) had pyothorax, and 2 (9%) were co-infected with both FIV and FeLV. One cat (4.5%) died during stabilization procedures and did not receive a diagnosis. Of the 22 cats that died during hospitalization, 5 (23%) died of natural causes, while 17 (77%) were euthanized.

In conclusion, pleural effusion is a frequent finding in dyspnoeic feline patients. In cats under 7 years of age, pleural effusion is more commonly associated with infectious diseases such as FIV, FeLV, and FIP, whereas in cats over 7 years of age, it is more frequently related to cardiomyopathies and neoplasia. Notably, cats with neoplastic diseases had a lower discharge rate compared to other groups, reflecting the poor prognosis associated with pleural effusion in these patients.

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DERMATOLOGICAL MANIFESTATIONS IN DOGS WITH FOOD AND MICROBIOTA-RELATED MODULATION RESPONSIVE ENTEROPATHY: IS THERE A GUT-SKIN AXIS?

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The gut-skin axis is a communication system linking the gastrointestinal tract and the skin as components of a shared inflammatory pathway, primarily driven by intestinal dysbiosis and barrier dysfunction. While well recognized in human medicine, its clinical characterization in veterinary medicine remains limited. The aim of this study was to assess both gastrointestinal (GI) and dermatological (DERM) manifestations in dogs with Food-Responsive Enteropathy (FRE) and/or Microbiota-related Modulation-Responsive Enteropathy (MrMRE) and to evaluate their clinical evolution following dietary and microbiota modulation. Dogs were evaluated at three timepoints: baseline (T0), after 4 weeks (T4) and 8 weeks (T8). At T0, all dogs were placed on a dietetic trial with a novel protein source selected based on their dietary history. Signalment (age/sex/breed), diet category administered (hydrolysed/monoproteic highly digestible) and protein source (chicken/fish/pork/other) were recorded. GI and DERM signs were registered and characterized at each timepoints. Enteropathy severity was assessed using the Canine Chronic Enteropathy Clinical Activity Index (CCECAI), whereas pruritus severity was evaluated using a pruritus Visual Analog Scale (pVAS). Clinical improvement outcome was defined as a reduction of ≥25% in CCECAI and ≥50% in pVAS score. If an adequate clinical response was not achieved at T4, an additional dietary modification and/or administration of symbiotic were allowed. No antipruritic or corticosteroid therapies were administered before (up to 1 month) and during the study period. A total of 70 dogs were prospectively included at T0, with follow-up available for 46 and 29 dogs at T4 and T8, respectively. At T0, diarrhea and vomiting were present in 74% and 53% of dogs respectively; diarrhea was predominantly of large intestinal origin. Minor GI manifestations were frequently observed, including grass eating (67%), flatulence (57%), borborygmi (54%), and empty chewing (42%), with 87% of dogs presenting at least two of these signs. Median T0 CCECAI was 5. Pruritus was reported at T0 in 74% of dogs, predominantly mild to moderate in severity, with limbs, ears, and ventral abdomen being the most commonly affected areas. Additional DERM manifestations included history of otitis (30%), erythema (27%), alopecia or hair loss (16%), scaling (11%), dull or coarse coat (10%) and recurrent pyoderma (7%). At T4 GI signs improvement was documented in 80% of dogs and at T8 in 90%. Median CCECAI at both follow-up timepoints was [2] Pruritus improved in parallel, with response rates of 63% at T4 and 72% at T8, and complete resolution in 66% of dogs by the end of follow-up. No significant associations were found between GI/DERM improvement and diet category, protein source, dietary changes, and symbiotic administration. These results with parallel improvement of both GI and DERM manifestations, support the presence of the gut–skin axis. Furthermore, in our population, predominant colonic involvement, frequent minor GI signs, and DERM findings including not only pruritus, but also skin lesions were highlighted.

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TRANSFUSION MEDICINE IN SEA TURTLES (*CARETTA CARETTA*): CROSS-MATCHING TO ASSESS INTRASPECIES BLOOD COMPATIBILITY

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The loggerhead sea turtle (*Caretta caretta* - CC) is the most common marine turtle species in the Mediterranean Sea. A large proportion of individuals admitted to rehabilitation centers are affected by fisheries interactions, particulary bycatch, often associated by trauma, debilitation and secondary clinical conditions. Among these, anemia may occur and can complicate clinical management, expecially in severe cases requiring prolonged hospitalization, and is associated with increased mortality risk [1]. Considering the slow RBCs regeneration in reptiles, blood transfusion may represent a valuable support therapy to improve recovery and reduce rehabilitation time. However, scientific knowledge on blood groups and transfusion compatibility in reptiles remains limited. The present study investigated transfusion compatibility using both rapid and classical cross-match methods for clinical application.

Twenty-three CC admitted to the "Luigi Cagnolaro" Sea Turtle Rescue and Rehabilitation Center (Pescara, Italy) were included in this study. Blood samples were collected from the dorsal cervical sinus into lithium-heparin tubes, stored at 4°C, and analyzed within 24 h. None of turtles had history of prior transfusions. Due to sampling timing and limited blood volume, not all turtles were cross-matched with each other, therefore a total of 122 cross-matches were carried out. Transfusion compatibility was assessed using both rapid and classical cross-match methods. The rapid method consisted of a slide agglutination test, performed by mixing three drops of plasma from one individual with two drops of packed red blood cells (RBCs) from another on a glass slide, followed by gently agitation and microscopic examination after 1 minute under a light microscope at 40× magnification. Positive reactions were defined as macroscopic and/or microscopic RBCs agglutination. Although less sensitive than the rapid tube test, this approach provides a practical and timely option for transfusion decisions [2]. The classical method includes washing RBCs in isotonic saline, followed by incubation of plasma–RBC mixtures for cross-matches in Eppendorf tubes for 30 minutes prior to microscopic evaluation, as described by Donnelly et al. [3]. Samples were evaluated for hemolysis and agglutination as indicators of incompatibility. All procedure were conducted at room temperature slightly higher than the holding tank water temperature, to account for ectothermic physiology of sea turtles. Both major and minor cross-matches were performed with each method. No incompatibility reactions were observed in any of tests.

These results suggest the absence of naturally occurring antibodies against conspecific RBC antigens in the studied population. Both rapid and classical cross-match methods yielded identical results; however, the absence of positive reactions prevented evaluation of sensitivity of rapid tests. Although these findings are encouraging, the limited sample size represents a constraint. Further studies on larger populations are needed to confirm these preliminary results and to assess the sensitivity and specificity of the rapid cross-match.

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Posters

GASTROINTESTINAL INVOLVEMENT IN DOGS WITH MYXOMATOUS MITRAL VALVE DISEASE: A MULTIORGAN PERSPECTIVE

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Myxomatous mitral valve disease (MMVD) represents the most prevalent acquired heart disease in dogs. Gastrointestinal (GI) signs are often observed in affected patients, yet their frequency and relationship with abdominal organ involvement are not fully understood. This study aimed to quantify the occurrence of GI signs in dogs at different stages of MMVD (preclinical ACVIM B1/B2 vs. clinical ACVIM C/D) and to explore potential associations with alterations in abdominal organs.

A retrospective analysis was performed on 160 dogs diagnosed with MMVD: 26 (16%) in ACVIM stage B1, 40 (25%) in B2, 82 (51%) in C, and 12 (8%) in D. Clinical records were reviewed to identify GI signs (nausea, vomiting, diarrhea, anorexia, and dysorexia) alongside hematobiochemical data. Abdominal ultrasonography was available for a subset of 48 dogs [23 preclinical (48%) and 25 clinical (52%)]. Data were analyzed using chi-square tests and multivariable logistic regression.

The proportion of dogs exhibiting GI signs rose in parallel with disease progression (12% in B1, 21% in B2, 29% in C, and 51% in D; p<.0001). Dogs in stages C and D had a significantly greater likelihood of presenting GI signs compared to B1 (odds ratios 4.6 and 7.5; p=.023 and p=.016). Indicators of systemic inflammation, including neutrophil-to-lymphocyte ratio and C-reactive protein, increased with advancing MMVD but showed no correlation with GI signs. Similarly, elevations in liver enzymes (ALP, ALT, GGT) and bilirubin were more common in clinical stages without a direct association with GI signs. In contrast, hypoproteinemia—detected in 15% of stage C and 25% of stage D dogs—was significantly linked to GI signs (p=.04). Although creatinine abnormalities became more frequent with disease severity, no association with GI signs was identified.

Ultrasonographic evaluation revealed that hepatobiliary abnormalities were more prevalent in dogs with clinical MMVD, particularly affecting the liver (68% vs. 26%, p<.0001), and these changes were significantly associated with GI signs (p=.013). Gallbladder alterations were also more frequent but not linked to GI signs. No significant differences were observed in renal or intestinal ultrasonographic findings between groups, nor were these associated with GI signs.
Overall, GI signs become increasingly common as MMVD progresses and appear to be linked to hypoproteinemia and hepatic abnormalities, supporting the concept of an interconnected heart–liver–gut axis in affected dogs.

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Posters

IMMUNOTHERAPY AGAINST CSPG4 PROTEIN IN DOGS WITH VISCERAL HEMANGIOSARCOMA: PRELIMINARY RESULTS

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Canine hemangiosarcoma (HSA) is a highly aggressive tumor with poor prognosis. Distant metastases occur early, and even with standard treatment (surgery plus chemotherapy), median survival is only 6–8 months. Given its high immunogenicity, immunotherapy may improve outcomes. Previous studies showed that HSA expresses chondroitin sulfate proteoglycan-4 (CSPG4), a protein overexpressed in several tumors. A DNA vaccine targeting CSPG4 has already prolonged survival in dogs with malignant melanoma and osteosarcoma.

The primary aim of this study was to assess whether CSPG4-based immunotherapy, delivered using a needle-free device (PharmaJet®), could improve survival time (ST) and disease-free interval (DFI) in dogs with visceral and cardiac HSA. A secondary aim was to evaluate changes in anti-CSPG4 antibody levels and T lymphocytes after each vaccination.

Two groups of dogs with CSPG4-positive HSA were prospectively enrolled and compared for ST and DFI (Kaplan Meier survival curves, Log Rank Test; statistical significancy: $p< 0,05$).

CSPG4 expression was assessed by immunohistochemistry using a semiquantitative score (0–8). The treated group included stage I–II patients receiving standard therapy plus immunotherapy, while the control group received standard therapy alone. After surgery, all dogs underwent five cycles of doxorubicin. In the treated group, vaccination was administered monthly, alternating with chemotherapy, for 12 months via intramuscular PharmaJet® delivery. Blood samples were collected after each vaccination for immunological analysis. All patients were periodically restaged with imaging to monitor disease progression.

Fifty-seven samples were analyzed: most from the spleen (89.5%), with smaller numbers from cardiac, retroperitoneal, and subcutaneous sites. Overall, 72% were CSPG4--positive. Among four metastatic samples, two were positive. To date, 14 dogs were enrolled in the treated group and 13 in the control group. No significant adverse effects were observed, except mild, transient local redness.

In treated dogs, anti-CSPG4 antibody levels remained stable in those with longer survival but decreased with metastatic progression.

The median ST was significantly longer in the treated group (200 days) compared to controls (120 days) ($p= 0,016$).

The high rate of CSPG4 positivity suggests that this vaccine could be widely applicable in HSA patients. The needle-free device showed excellent safety and required no anesthesia. The decline in antibody levels at metastasis may reflect antibody binding to tumor lesions or tumor-induced immunosuppression. These preliminary results indicate that CSPG4 vaccination may improve survival in dogs with HSA, although larger studies are needed to confirm these findings.

THE IMPORTANCE OF COMBINING COMPUTED TOMOGRAPHIC LYMPHANGIOGRAPHY AND METHYLENE BLUE DYING IN DETECTING AND REMOVING SENTINEL VERSUS REGIONAL LYMPHNODE FOR SURGICAL TREATMENT OF MALIGNANT NEOPLASMS: A LITERATURE REVIEW AND CASE SERIES.

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Accurate assessment of lymph node status represents a critical component of oncologic staging in dogs affected by malignant tumors. Lymphatic metastasis is one of the most important prognostic factors in veterinary oncology and plays a major role in determining therapeutic strategies and expected survival outcomes. Traditionally, lymph node evaluation has relied on palpation, ultrasonography, and cytologic examination of anatomically regional lymph nodes. However, these techniques have important limitations because lymphatic drainage patterns are highly variable and the anatomically closest lymph node does not always correspond to the true first draining node, potentially leading to inaccurate staging and misclassification of disease progression.

The regional lymph node is typically defined as the anatomically closest node to the primary tumor, but this approach does not account for individual variability in lymphatic drainage or the presence of aberrant pathways. In contrast, the sentinel lymph node (SLN) represents the functional first node receiving lymphatic drainage from the tumor and is therefore the most likely site of initial metastatic spread. Reliance solely on regional lymph nodes may result in underestimation of metastatic disease, whereas SLN mapping provides a more accurate and individualized assessment of lymphatic dissemination. This distinction is particularly relevant in tumors with unpredictable drainage patterns, where conventional staging may fail to identify clinically significant metastases.

The aim of this study was to evaluate the feasibility and clinical value of sentinel lymph node mapping using computed tomographic lymphography combined with intraoperative dye guidance in dogs with cutaneous or subcutaneous tumors. Dogs underwent CT staging with lymphangiography to identify lymphatic drainage pathways and the SLN. Surgical excision of the primary tumor and sentinel lymphadenectomy were then performed, with intraoperative identification facilitated by peritumoral injection of methylene blue, allowing improved visualization of lymphatic vessels during dissection.

Forty-eight dogs were included. Mast cell tumors were the most common neoplasm, followed by mammary tumors, melanomas, and other tumor types. Sentinel lymph nodes were successfully identified in the majority of cases, with superficial inguinal, axillary, and superficial cervical nodes most frequently involved. In several cases, lymphatic drainage did not correspond to the expected regional lymph node, highlighting the limitations of an anatomy-based approach. Histopathology revealed variable metastatic involvement, ranging from negative nodes to partial or extensive infiltration by neoplastic cells.

Postoperative complications were uncommon and generally mild, consisting mainly of localized swelling, seroma formation, or minor wound complications. Most dogs recovered without significant adverse effects, supporting the safety and reproducibility of the technique in a clinical setting.

In conclusion, sentinel lymph node mapping using CT lymphography combined with intraoperative dye guidance is a feasible and clinically valuable technique for staging canine tumors. This approach enables precise identification of lymphatic drainage pathways and improves detection of nodal metastasis compared with conventional methods. Consequently, incorporation of SLN biopsy into routine oncologic staging protocols may significantly enhance diagnostic accuracy and support more tailored therapeutic decision-making in veterinary oncology.

EARLY SCREENING OF BOVINE OCULAR SQUAMOUS CELL CARCINOMA USING ARTIFICIAL INTELLIGENCE: A STUDY OF THE THIRD EYELID IN SICILIAN CATTLE

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Ocular Squamous Cell Carcinoma in cattle is among the most prevalent type of eye cancer in livestock, with considerable welfare implications and significant economic consequences, including reduced productivity, increased management costs, and potential carcass depreciation at slaughter. This malignancy is characterised by marked local invasiveness, with a tendency to infiltrate the periocular tissues and adjacent structures, although metastatic spread is relatively infrequent. The aetiology is multifactorial, with a strong correlation between prolonged exposure to ultraviolet radiation and a lack of periocular pigmentation, the predominant risk factor in animals with light-coloured or patchy coats [1]. In the Sicilian context, these factors are particularly pronounced on extensive farms or in barns with access to pasture. Although the nictitating membrane is the primary site of onset, early diagnosis remains a clinical challenge, especially during initial phases when the lesions are not macroscopically attributable to neoplasia. This study is a preliminary investigation into Sicilian cattle and aims to evaluate the effectiveness of Artificial Intelligence (AI) as a non-invasive and predictive screening tool. The aim is to assist the veterinarian and potentially also the farmer in the early detection of suspicious ocular lesions, even when these are not clearly neoplastic, and in distinguishing between operable and not operable lesions. In addition to assessing severity, the study focuses on implementation of specific labels to refine the tool's diagnostic capacity, such as lesion localisation, that is the precise identification of the site of origin(e.g. nictitating membrane, upper and lower eyelid, conjunctiva, cornea), and the model's ability to correctly distinguish between papilloma and OSCC, thereby facilitating the differentiation between precancerous lesions and malignant tumours. The work is based on the application of deep learning models, specifically convolutional neural networks (CNNs). The process involves the collection of photographic datasets, the pre-processing of images to reduce noise and enhance contrast, and the training of algorithms for the automatic classification of lesions. These approaches have already been validated in the veterinary field for both OSCC and equine ophthalmic conditions [2]. The "DeepCattle" framework, developed by Manikandan et al.(2025), has demonstrated a diagnostic accuracy of up to 93% in the detection and assessment of ocular squamous cell carcinoma in cattle, suggesting that AI can identify early-stage lesions with a high degree of confidence, even when macroscopic assessment is limited[3]. The hypothesis is that AI could be used as a new diagnostic system for the early screening of OSCC directly on-farm. The integration of this technology would facilitate the identification of at-risk animals before the tumour invades the deep structures of the eye or bone, thereby optimising the timing of intervention and significantly improving clinical prognosis and animal welfare standards in susceptible cattle populations.

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PRELIMINARY STUDY ON THE INCIDENCE OF INCISIONAL INFECTIONS IN HORSES UNDERGOING EXPLORATORY LAPAROTOMY USING AN N-BUTYL-2-CYANOACRYLATE SKIN ADHESIVE

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Preliminary study on the incidence of incisional infections in horses undergoing exploratory laparotomy using an n-butyl-2-cyanoacrylate skin adhesive.

incisional complications in horses undergoing exploratory laparotomy are relatively common, with a highly variable incidence reaching up to 40% or more [1]. Numerous methods have been investigated to reduce the incidence of infections, ranging from the quality of the laparotomy wound closure technique to the use of abdominal bandaging and topical adhesives applied to the surgical incision. Cyanoacrylate-based adhesives are widely used in human medicine and in experimental models for wound closure, as they provide good cosmetic outcomes and cause minimal patient discomfort. This substance has demonstrated clear antibacterial properties against Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Corynebacterium pseudodiphtheriticum, Klebsiella pneumoniae, and Pseudomonas aeruginosa [2]. The present study aims to evaluate the use of an n-butyl-2-cyanoacrylate skin adhesive applied as a protective layer over the incision following exploratory laparotomy. Nine horses undergoing exploratory laparotomy for colic syndrome were included in the study. In seven horses, an n-butyl-2-cyanoacrylate tissue adhesive was applied to the skin following cutaneous suturing, whereas in two horses no adhesive was used. Allocation to treatment groups was randomized. None of the seven horses treated with the adhesive developed signs of incisional infection within 30 days postoperatively. Of the two horses that did not receive the adhesive, one developed an incisional infection caused by Enterobacter spp., which began four days after surgery, while the other showed no evidence of incisional infection at the 30-day follow-up. Although no incisional infections were observed in the cyanoacrylate-treated group (0/7), compared to one case in the control group (1/2; 50%), statistical analysis using Fisher's exact test did not reveal a significant difference ($p = 0.22$). However, the observed effect size was large (odds ratio ≈ 0.07), suggesting a potential protective effect of the adhesive. These findings should be interpreted with caution due to the very small sample size and the consequent low statistical power. Further studies with larger cohorts are warranted to confirm this trend.

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Posters

MULTIMODAL IMAGING OF A CANINE CORTICAL RENAL CYST: ROLE OF CONE-BEAM COMPUTED TOMOGRAPHY IN DIAGNOSIS AND PRE-SURGICAL ASSESSMENT

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Kidney disease is a major cause of morbidity and mortality in dogs [1]. Proper characterisation of renal lesions relies on diagnostic imaging techniques. Two-dimensional modalities, such as radiography (X-rays) and ultrasonography (US), have limited sensitivity and spatial resolution. In contrast, three-dimensional modalities such as computed tomography (CT) offer improved anatomical detail. In recent years, cone-beam CT (CBCT) has become a valuable diagnostic imaging technique, providing high-quality images with excellent spatial resolution [2]. This case report describes a multimodal imaging approach used for an 18 kg female mixed-breed dog with a suspected cortical renal cyst. Abdominal radiography revealed an ovoid radiopaque area partially overlapping the stomach and displacing the colon dorsally and the intestinal loops caudally. Abdominal ultrasonography identified the same lesion as a thin-walled, hyperechoic-rimmed cystic lesion with anechoic particulate content adjacent to the left kidney. A CBCT examination was then performed using a flat panel scanner (Vimago™ GT30, Imaginalis, Epica Animal Health, USA) to better characterise the lesion and assess its origin and local extent. The patient was premedicated with dexmedetomidine and butorphanol. General anaesthesia was induced with propofol and maintained with isoflurane. The patient was positioned in sternal recumbency, with both hindlimbs extended caudally and both forelimbs extended cranially and symmetrically within the CBCT gantry, parallel to the table. Data volume acquisitions were performed using standard resolution algorithms. Studies were performed before and after intravenous administration of a non-ionic iodinated contrast medium using a power injector. Breath-hold methods were applied to minimise motion artifacts. Pre- and post-contrast three-dimensional reconstruction allowed identification of the lesion origin within the cortical region of the upper pole of the left kidney (beak sign), as well as assessment of its extent, vascular supply, and relationships with adjacent structures. These findings suggest that CBCT may represent a valuable diagnostic imaging technique for the evaluation of cortical renal cysts in dogs, enabling detailed lesion characterisation and providing information relevant for pre-surgical assessment. In this case, CBCT provided high-resolution three-dimensional images, allowing precise evaluation of the anatomical relationships between the lesion and surrounding structures. Furthermore, this case highlights the importance of a multimodal diagnostic approach for the accurate assessment of renal lesions.

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Posters

IMPACT OF LAMENESS ON MILK YIELD AND THERMAL PROFILE IN DAIRY COWS: PRELIMINARY EVIDENCE ON ANIMAL WELFARE INDICATORS

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Lameness, often caused by claw alterations (1), is one of the main critical issues in dairy systems: pain and altered locomotion compromise animal welfare and cause significant production losses, estimated between 270 and 574 kg of milk for cow (2,3). Early diagnosis is complex, while direct examination of the foot requires laborious procedures. Infrared thermography (IRT) allows the detection of surface temperature variations related to local inflammatory processes (1), offering an effective non-invasive diagnostic support. The study evaluated the impact of lameness on milk production and animal welfare by thermographic monitoring. 10 cattle affected by different foot pathologies and 5 healthy subjects as controls were analyzed in a specific three-month period, from March to May, classified according to the locomotion score (scale 1-5) and monitored periodically. The thermographic images obtained were analyzed to monitor thermal variations in specific regions of the foot (central R1, interdigital R2, lateral R3, and medial R4). Cows with clinical lameness (LS ≥3) had significantly higher temperatures ($P < 0.01$) in the R1 and R2 regions compared to healthy cows (LS = 1; $P < 0.001$). Subsequent data analysis highlighted a decrease in foot region temperatures in parallel with clinical improvement, although the persistence of environmental and management risk factors can lead to temperature increases even during remission. For milk production, statistical analysis showed a significant group effect ($P = 0.02$), with differences between healthy and diseased subjects, especially in May. Despite a slight recovery in April, linked to a temporary improvement, management and hygiene issues favored relapses, making the production decline more evident in May. This highlights how the persistence of predisposing factors can lead to early culling (20%). In this study, thermography proved to be effective in identifying lesions thanks to the thermal difference between healthy and diseased feet; it was also able to detect thermal variations over time related to the evolution of the pathologies (2). Regarding production, the slight improvements highlighted over time confirm that rapid intervention is essential to guarantee well-being and prevent serious complications (1). Although the results of this study are preliminary, given the small number of animals sampled, they lay the foundations for future investigations extended to a larger number of subjects that can confirm the validity of these indicators. In summary, the study confirms that thermography and milk production represent valid indicators, respectively direct and indirect, of animal well-being. The degree of lameness is immediately reflected in the thermal profile of the foot, while productivity serves as a mirror of the general state of well-being. However, success in controlling the pathology does not only depend on timely diagnosis, but is inextricably linked to environmental management and floor hygiene (3), essential prerequisites to guarantee clinical resolution and productive longevity of the cow.

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CLINICAL AND PATHOGENETIC CHARACTERIZATION OF ACUTE LAMINITIS IN SWINE: THE ROLE OF GENETIC, ENVIRONMENTAL, AND NUTRITIONAL FACTORS

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Laminitis is a debilitating condition affecting ungulates, characterized by inflammation and degeneration of the sensitive laminae within the hoof. While extensively documented in horses and cattle, literature regarding risk factors and pathogenesis in swine remains limited. In pigs, this condition involves a multifactorial etiology linked to traumatic, septic, and nutritional determinants. Traumatic forms are closely related to intensive housing systems: the use of abrasive flooring, slatted surfaces, or inadequate bedding induces biomechanical stress and repeated concussion on the foot apparatus [1, 2]. In this context, genetic selection for rapid weight gain represents a critical predisposing factor, as excessive structural load increases the vulnerability of the sensitive laminae to mechanical insults [2]. Septic etiology is associated with systemic endotoxemia following acute inflammatory processes [3]. Finally, nutritional factors are implicated in "pasture laminitis," a condition related to the massive ingestion of non-structural carbohydrates (fructans) present in young forage, particularly during seasonal growth peaks in spring and autumn.This study characterizes the clinical presentation of acute laminitis in swine to enhance current knowledge of its etiopathogenesis and underlying pathophysiological mechanisms. The research involved six cases from an intensive farm in the Ragusa province (Italy), where anamnestic investigation highlighted a convergence of environmental risk factors (abrasive housing surfaces) and nutritional determinants (ingestion of fresh forage high in fermentable carbohydrates). A multidisciplinary approach was adopted, based on systematic physical examinations, locomotion scoring, and hemato-biochemical profiling to identify biomarkers of systemic inflammation and tissue damage. Clinical examination revealed severe symptoms characterized by high-grade lameness, stiffed movements, and a marked tendency for prolonged recumbency. In advanced cases, significant postural alterations were documented: antalgic kyphosis was associated with a cranial projection of the forelimbs to shift weight away from painful areas. Local hyperthermia and a pronounced pain response to pressure on the coronary band were detected at the hoof level. Pathological involvement was predominant in the lateral claws, confirming the biomechanical susceptibility of these structures in swine. The hematological profile showed marked neutropenia, consistent with massive leukocyte sequestration during the acute phase. Biochemically, significant increases in creatine kinase (CK) and aspartate aminotransferase (AST) suggested secondary muscle damage due to both the mechanical stress of antalgic posturing and recumbency-induced tissue hypoxia. The results highlight strong pathogenetic and clinical analogies between swine laminitis and its counterparts in cattle and horses. The scientific evidence produced suggests that acute laminitis in swine is an under-recognized nosological entity in clinical practice and current literature. It is clear that, on numerous occasions, lameness observed in farms is actually caused by undiagnosed laminitis, the non-identification of which requires a profound revision of diagnostic and management protocols in intensive farming systems.

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Posters

SELF-MUTILATION OF THE TAIL IN ELDERLY CAPTIVE FELIDS: A PAIN-RELATED BEHAVIORAL DISORDER?

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Self-mutilation has been reported both in domestic cats and in large felids kept in captivity. It can manifest as compulsive biting or scratching of the skin or appendages and can later result in mutilation. This behavior is often expression of frustration, nevertheless it can also be displayed to alleviate discomfort deriving from itching.

This report describes two cases of tail self-mutilation in elderly big cats kept in captivity. The first case was a 19-year-old male snow-white tiger, with a history of orthopedic pain and idiopathic epilepsy, which was found with a completely amputated tail. The tiger shared the space with another tiger, but there were no signs of fighting on either animal or within the enclosure. No intervention was needed since the stump was clean and not bleeding. The animal was euthanized one year later for reasons unrelated to the injury, and severe discospondylosis was found at necropsy. The second case was an 18-year-old female puma living alone with a history of orthopedic issues especially in the hindlimbs which was found with deep self-inflicted scratches at the base of the tail, leading to amputation. After the amputation, the animal never displayed self-injury behavior again.

No changes were made to either animals' social life before the mutilation, and neither animal displayed self-harming behaviors before or after the incident. These findings, along with the orthopedic pain associated with the advanced age, suggest that the anomalous behavior may be associated with chronic pain, as previously reported in cats affected by hyperaesthesia syndrome (AMENGUAL BATLE et al., 2019). Moreover, since improvement of both veterinary care and husbandry have led to increased longevity in captive felids compared to their free-ranging counterparts, novel geriatric diseases are being encountered (LONGLEY, 2011). Therefore, careful monitoring of the quality of life of elderly captive felids is essential to avoid prolonging their lives beyond ethically acceptable conditions in terms of welfare.

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ANALYSIS OF HUNTING DOG INJURIES DURING WILD BOAR HUNTS IN CALABRIA, ITALY (2023-2024)

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Wild boar hunting is a widespread practice in many Italian regions. While collaboration between hunters and dogs is essential, it poses severe physical risks to the animals due to the defensive capabilities of wild boars [1].

This study collected, classified, and evaluated cases of dogs injured during the 2023-2024 hunting season in Calabria, Italy. The aim is to analyze the injuries sustained by hunting dogs caused by wild boars.

In literature, the most common damage reported is to muscles, tendons, nerves, internal organs and, more rarely, bones [1,2]. Indeed, a wild boar's tusks can penetrate deeply into tissue, causing damage even far from the point of entry [1].

Prompt assessment and management of the injured dog's condition can make a difference in improving the patient's prognosis and recovery, demonstrating how vital knowledge of first aid practices is for owners and hunters. In severe cases, such as disembowelment with protruding viscera, it is essential to prevent the dog from ingesting its own organs or or worsening the contamination [3].

The study recorded 105 clinical cases, involving specialized breeds such as the Maremmano, French Hound, Italian Hound, and the Italian Spinone. Surgical protocol was adapted based on injury severity. For complete Achilles tendon rupture, a double-block suture was performed using absorbable threads, followed by adequate limb immobilization with compression bandages. Nerve injuries were treated with realignment and epineural suturing when feasible. For abdominal trauma with suspected intestinal involvement, enterotomies were performed to remove foreign bodies or devitalized segments; alternatively, enterectomies with end-to-end anastomosis were used for perforations or extensive necrosis. In patients with pneumothorax, a conservative approach (oxygen therapy and radiographic monitoring) was preferred, reserving chest drainage for severe cases due to challenges in home care compliance.

Analysis revealed 23 abdominal wounds (4 disembowelments, 4 intestinal injuries, and 15 skin/muscle wounds), 33 thoracic wounds (2 rib fractures, 2 hemothoraces, 7 pneumothoraces, 4 pulmonary contusions, 4 diaphragmatic hernias, and 14 skin/muscle wounds), 19 hind limb wounds (1 lesion of the Achilles tendon, 1 fracture of the tibia/femur/-fibula, and 17 skin/muscle wounds), 4 perineal, 14 forelimb, 7 head, and 5 neck wounds. Two deaths were recorded.

In conclusion, thoracic wounds were the most frequent, with an incidence of 31.4% (skin and muscle injuries, pneumothorax), followed by abdominal wounds (21.9%), hind limbs (18.1%), forelimbs (13.3%). Wounds to the head, neck, and perineum were less common. Furthermore, the complication was attributed to inadequate home management or premature resumption of hunting activity, underlining the critical importance of careful observance of post-operative instructions.

In a cultural context where hunting ungulates, and wild boar in particular, is deeply rooted in tradition, as is the case in Italian regions such as Calabria and Sicily, early diagnosis and treatment of injuries sustained by hunting dogs are crucial for a favorable prognosis and optimal follow-up.

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ENDOSCOPICALLY ASSISTED INTRACORPOREAL HOLMIUM:YAG LASER LITHOTRIPSY FOR LOWER URINARY TRACT UROLITHIASIS IN DOGS

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Lower urinary tract urolithiasis is common in dogs and is increasingly managed with minimally invasive techniques when anatomy, stone burden, and equipment allow [1].

Over the last decade, management of canine urolithiasis has progressively shifted from routine open surgery toward patient-specific, minimally invasive approaches whenever equipment, operator expertise, anatomy, and stone burden permit. The ACVIM consensus recommendations emphasize a patient-centered strategy that prioritizes complete stone removal while minimizing tissue trauma, postoperative discomfort, hospitalization, and the need for salvage procedures such as urethrotomy or urethrostomy [2]. In this framework, cystoscopic-guided retrieval, laser lithotripsy, percutaneous cystolithotomy, and other endourologic techniques represent key alternatives to conventional surgery for lower urinary tract stones [3].

To describe the immediate technical outcome, procedure--time profile, and early urethral morbidity of endoscopically assisted intracorporeal holmium:YAG laser lithotripsy in dogs with urethral and/or bladder urolithiasis.

Forty-three client-owned dogs treated between September 2022 and August 2024.

Retrospective case series based on a thesis dataset and the corresponding raw spreadsheet. Approximately 95 dogs with lower urinary tract signs were screened. Dogs were included when urethral and/or bladder urolithiasis had been confirmed by imaging and treated with endoscopically assisted intracorporeal holmium:YAG laser lithotripsy. The primary outcome was complete immediate stone clearance, defined as the absence of radiographically visible residual fragments at the end of the procedure; fragments >= 3 mm were considered treatment failure.

The study population comprised 36/43 (83.7%) males and 7/43 (16.3%) females, with a median age of 7.0 years and a median body weight of 10.0 kg. Provisional anatomical allocation from the cleaned spreadsheet identified 33 urethral, 6 bladder, and 4 combined urethral-bladder cases. Struvite (18/43) and calcium oxalate (9/43) were the most frequent stone types. Immediate complete clearance was recorded in all 43 dogs. Urethral lesions were documented at immediate endoscopic reassessment in 22/43 (51.2%) dogs. Thesis-derived meant procedure time was 48 minutes 41 seconds overall, 34 minutes 26 seconds for urethral cases, 54 minutes 59 seconds for combined cases, and 110 minutes 15 seconds for bladder cases.

This dataset supports the technical feasibility and excellent immediate efficacy of endoscopically assisted intracorporeal holmium:YAG laser lithotripsy in carefully selected dogs, particularly for urethral disease. Interpretation should remain cautious because of the retrospective design, the absence of a control group, limited follow-up, and the need to audit the raw timing field before final submission. With a view to wanting to act in an increasingly less invasive manner on our patients, this is confirmed to be a very valid alternative to conventional surgery for lower urinary tract stones [3].

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MULTIMODAL ANESTHESIA IN RABBIT (*ORYCTOLAGUS CUNICULUS*): PRELIMINARY OBSERVATIONS

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Anesthesia in rabbits presents significant challenges for veterinary practitioners due to the species' high susceptibility to stress, complex respiratory physiology, and a historically higher perioperative mortality rate compared to dogs and cats. This species is particularly susceptible to arrhythmias, which can result in the death of the subject, which can be attributed to its evolutionary status as a prey species. Indeed, handling stimulates the release of endogenous catecholamines, potentially triggering cardiac arrhythmias, especially in an anesthesiological setting [1,2].

The objective of this study is to evaluate the clinical efficacy and safety of a balanced anesthesia protocol. By combining different pharmacological agents, this approach aims to achieve good anesthesia, analgesia, and muscle relaxation while minimizing the dosages and side effects of individual drugs.

The study was conducted on a sample of 5 rabbits undergoing a surgical procedure for odontogenic pathologies. The anesthesiological procedures were performed in a clinical setting, the informed consent was acquired by the animal owners. This is not an experimental study, therefore, ethics committee approval was not required. To achieve sedation and preemptive analgesia, the following premedication protocol was performed: a combination of midazolam (0.3 mg/kg) and methadone (0.3 mg/kg) was administered via the intramuscular (IM) route. After 10 minutes, dexmedetomidine (50 µg/kg) was injected IM. Induction was achieved with propofol (2-5 mg/kg) injected intravenously (IV), while maintenance was obtained with isoflurane (1.5-2%) vaporized in oxygen. Intraoperative analgesia was provided by an initial fentanyl bolus of 10–40 µg/kg administered over 5 minutes, followed by a constant rate infusion (CRI) of 5–10 µg/kg/h [3]. Locoregional anesthesia was also performed using 1% lidocaine diluted 1:10, up to a maximum volume of 0.2 ml/kg. Vital parameters, including heart rate (HR), respiratory rate (RR), oxygen saturation (SpO_2), and end-tidal CO_2 ($EtCO_2$), were monitored throughout the perioperative period. The degree of sedation was measured by observing physiological parameters via monitoring.

The protocol demonstrated good clinical stability. The combination of midazolam, methadone, and dexmedetomidine provided deep and rapid sedation, significantly reducing the dose of propofol required for induction. Continuous fentanyl infusion resulted in patients' stability in both autonomic reflexes and physiological variables (tachycardia or hypertension). Vital parameters, especially heart rate, respiratory rate and body temperature (T), remained almost always within the physiological ranges: HR 120-220, RR 19-49, T 36.5-40°C. Recovery was generally smooth and free of agitation; notably, the majority of subjects resumed voluntary food intake within a few hours following surgery, despite the invasive nature of the odontogenic intervention. This specific data can demonstrate the quality of the recovery.

The data suggest that this protocol ensures hemodynamic stability and effective pain management. However, the study emphasizes that pharmacological success must be paired with adequate monitoring and constant perioperative care to further reduce mortality risks in lagomorphs.

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EVALUATION OF CARBON DIOXIDE FOR THE "HUMANE" KILLING OF RODENTS USED IN SCIENTIFIC RESEARCH

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Rodents are among the most widely used species in scientific research worldwide. One of the main concerns that the scientific community is now facing is the methods of humane killing of mice at the end of experimental procedure. According to Annex IV of Directive 2010/63/EU implemented by Italian law 26/2014, carbon dioxide (CO_2) is a permitted method in "sufficient quantity" [1].

Despite its widespread use and regulatory acceptance, increasing scientific evidence points the fact whether this method can actually be considered humane and under which conditions.

According to some authors pain would be perceived at a percentage higher than 40%, nevertheless, there is evidence of noxius stimuli at concentrations of 25% [2]. Another important aspect to take into consideration is the fear that exposure to CO_2 causes, which translates into the onset of panic and anxiety, that is extremely distressing even for rodents. Several studies have been conducted using CO_2 in mice and rats to study the dynamics of panic attacks. These studies revealed an inevitable activation of the sympathetic-adrenomedullary (SAM) and hypothalamic-pituitary-adrenal (HPA) axes, resulting in increased secretion of catecholamines and cortisol. This physiological response was accompanied by a typical behavioral repertoire characterized by startling, rearing up, labored breathing, and escape attempts [2]. These findings are consistent with a marked aversive response, suggesting that exposure to CO_2 may induce a state of significant distress prior to loss of consciousness.

Here we test the hypothesis to Refine this method of killing through the use of appropriate premedication, including both a sedative and analgesic molecule to minimize pain, discomfort and suffering.

Although several studies have been conducted to evaluate the activity of benzodiazepines in alleviating these behaviors, the analgesic component is certainly missing. Given that the optimal flow concentration for losing consciousness before experiencing pain is not yet well defined and considering the anxiety-inducing effects caused by exposure, it would be appropriate to evaluate the use of a premedication molecule that can act on both fronts.
A future perspective could be to evaluate molecules such as alpha-2 agonists, e.g. dexmedetomidine. This molecule is known for its analgesic, sedative, and cardiorespiratory stabilization properties, even in rodents [3]. This approach may alleviate suffering of laboratory rodents, in fully compliance with the 3Rs Principle, with particular attention to the "Refinement".

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Posters

LAPAROSCOPIC RIGHT PARTIAL PANCREATECTOMY IN TWO DOGS: WITH AND WITHOUT FLUORESCENCE GUIDANCE

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Laparoscopic pancreatic surgery is gaining increasing interest in veterinary minimally invasive surgery; however, accurate intraoperative identification of tumor margins remains a critical challenge, particularly in oncologic cases. Indocyanine green (ICG) fluorescence imaging has been proposed as a tool to enhance tissue differentiation and improve surgical precision.

Two client-owned dogs (8 kg and 10 kg) were referred for evaluation of hypoglycemic episodes. Clinical history and diagnostic work-up, including blood tests and serum insulin measurement, raised suspicion of insulinoma. Computed tomography identified a solitary lesion localized to the right pancreatic limb in both cases, with no evidence of metastatic disease. Patients were medically stabilized prior to surgery.

Both dogs underwent laparoscopic partial pancreatectomy via a flank approach in left lateral recumbency. Port placement allowed appropriate triangulation, with the optical port positioned approximately 2–3 cm lateral to the linea alba and 2–3 cm from the umbilicus, and two additional instrument ports placed cranially to the optical port. Upon laparoscopic exploration, the pancreatic nodules (2–3 cm) were readily identified. One procedure was performed under standard laparoscopy, while in the second case ICG fluorescence imaging was used to assist intraoperative margin assessment. Resection was achieved using a bipolar sealing device, 5 mm endoclips, and laparoscopic scissors.

No intraoperative or postoperative complications were observed in either case. Both patients were hospitalized for 48 hours, with no recorded glycemic alterations during the postoperative period. Histopathological evaluation confirmed complete excision with clean margins in both cases. The use of ICG subjectively improved visualization of the transition between neoplastic and normal pancreatic tissue.

Although limited by the retrospective nature and small sample size, these findings suggest that ICG-assisted laparoscopy may represent a valuable adjunct in minimally invasive pancreatic oncologic surgery. Further studies are warranted to better define its role and clinical impact

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HYPOTENSION IN FOALS: A REVIEW OF THE CURRENT STATE OF THE ART

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Intraoperative hypotension is a common complication in anesthetized foals and is associated with increased mortality. The physiology of equine neonates, often compounded by sepsis, hypovolemia, and systemic inflammation, limits their ability to compensate for anesthetic-induced cardiovascular depression, predisposing them to prolonged hypotension. This critical review aimed to analyse the existing literature on foal anaesthesia to define intraoperative hypotension and intervention threshold values, and to describe current monitoring techniques and treatment strategies [1]. A literature search was conducted using PubMed, Google Scholar and Scopus. Specialized equine anesthesiology textbooks were also included as expert opinions. Publications up to January 2026 were considered. Keywords included “foals”, “hypotension”, “anesthesia”, and anti-hypotensive treatments (“dobutamine”, “dopamine”, “ephedrine”, “norepinephrine”, “vasopressin”). Studies on general anesthesia not addressing hypotension and those investigating hypotension outside anesthetic context were excluded. A total of 36 sources were included: 17 clinical studies, 11 review articles, 1 case report, and 7 textbooks. Among the 17 clinical studies, 16 were conducted under general anesthesia. Seven studies defined hypotension using mean arterial pressure (MAP) thresholds of 50-70 mmHg. Overall, 14/36 studies investigated treatment strategies for intraoperative hypotension. Dobutamine (1–10 µg/kg/min) was evaluated in 6 studies and was associated with increases in cardiac index and cardiac output. Norepinephrine (0.05–1.0 µg/kg/min) was investigated in 6 studies and was associated with increases in MAP and systemic vascular resistance [2]. Oscillometric blood pressure monitoring showed acceptable accuracy in healthy foals. Good agreement between direct and indirect blood pressure measurements has been reported when appropriately sized cuffs are placed over the coccygeal or dorsal metatarsal arteries. However, a negative correlation between pressure and cardiac output has been observed [3].The available evidence highlights a lack of standardization in both definition of hypotension and monitoring techniques used in foals. The reliance on non-invasive methods, which remain largely unvalidated in critically ill patients, may underestimate cardiovascular impairment compared to invasive techniques. In addition, the small sample sizes and the predominance of experimental studies limit the strength and generalizability of the current evidence. The prevalence of hypotension appears to be higher in foals receiving inhalant anesthesia, supporting their marked sensitivity to anesthetic agents. This susceptibility is likely related to their poorly compliant cardiovascular system, heart rate–dependent cardiac output, and immature sympathetic nervous system. Further research is warranted to establish consensus definitions, standardized monitoring approaches, and develop evidence-based guidelines for the treatment of hypotension in foals and to better assess its impact on morbidity and mortality.

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JEJUNAL ADENOCARCINOMA IN A HORSE: CLINICAL PRESENTATION, DIAGNOSIS, TREATMENT AND OUTCOME

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Recurrent colic in older horses represents a diagnostic challenge due to the wide range of possible causes and nonspecific clinical signs. Although intestinal adenocarcinoma is a rare neoplasm in horses, it should be considered in cases with persistent gastrointestinal symptoms and weight loss (Spanton et al., 2020). This malignant tumor, typically affecting the small intestine, can cause progressive obstruction and poor prognosis if undiagnosed (Harvey-Micay, 1999).

While ultrasound (US) and glucose absorption tests are commonly used, exploratory laparoscopy remains crucial for definitive diagnosis and treatment planning.

This case report describes a 20-year-old Arabian mare diagnosed with jejunal adenocarcinoma, highlighting the key role of laparoscopy in diagnosis and surgical management.

A 20-year-old Arabian mare was presented for recurrent colic and weight loss over 4 months, with episodes initially responsive to medical treatment.

Initial abdominal US was unremarkable, while gastroscopy revealed gastric ulcers (ESGD grade 4), treated with omeprazole (4 mg/kg).

Due to persistence of clinical signs, further evaluation revealed small intestinal wall thickening, leading to corticosteroid therapy and dietary adjustments. As colic episodes continued, the mare was referred to the Veterinary Teaching Hospital (VTH).

On admission, physical examination and bloodwork were unremarkable, except for serum creatinine at the upper normal limit (1.59 mg/dL). Transcutaneous and transrectal US confirmed small intestinal wall thickening (5.7 mm).

Gastroscopy and an oral glucose absorption test revealed ESGD grade 4 ulcers and partial malabsorption (+51%).

Based on these findings and previous clinical response, exploratory laparoscopy via the right flank was performed. This revealed haemorrhagic multifocal lesions affecting the jejunum.

Extension of the paralumbar incision allowed exteriorization of the small intestine, revealing a thickened, stenotic segment of the proximal jejunum.

Due to lesion extent, a ventral midline celiotomy was performed, and a 3.5-meter segment of proximal jejunum was resected followed by anastomosis.

Recovery was uneventful, and the mare was discharged after 14 days. At 8-month follow-up, no further colic episodes were reported.

Histopathology confirmed jejunal adenocarcinoma, characterized by neoplastic glandular proliferation infiltrating the submucosa and muscularis, with marked anaplasia and desmoplasia; surgical margins were free.

This case highlights the importance of a comprehensive diagnostic approach in horses with recurrent colic and weight loss. Conventional diagnostics failed to identify the underlying pathology, whereas exploratory laparoscopy was essential for detecting intestinal lesions and guiding surgical intervention. Without advanced diagnostics and timely surgery, the condition would likely have progressed to a fatal outcome due to severe luminal obstruction.

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Posters

CT-GUIDED MICROWAVE ABLATION FOR PALLIATIVE TREATMENT OF INOPERABLE CANINE NASAL CARCINOMAS: A PRELIMINARY CASE SERIES

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Canine nasal tumors are locally aggressive neoplasms for which radiotherapy remains the standard treatment, although its clinical use may be limited by cost, availability, repeated anesthesia, and radiation-associated toxicity. In this context, computed tomography (CT)-guided microwave ablation (MWA) may offer a minimally invasive and repeatable option for local cytoreduction and symptom palliation. This preliminary study evaluated the feasibility, safety, and short-term clinical impact of CT-guided MWA in four dogs with histologically confirmed inoperable nasal carcinoma or adenocarcinoma, staged by CT and without detectable distant metastases. Procedures were performed under general anesthesia using a 2.45 GHz microwave system equipped with 16 G internally cooled antennas. Energy delivery was tailored to tumor volume, with power settings mainly ranging from 40 to 50 W and application times from 10 to 15 min; one dog with advanced disease underwent shorter repeated applications. In all cases, post-procedural CT demonstrated non-enhancing hypodense areas within the treated tissue, consistent with coagulative necrosis, confirming successful intratumoral energy delivery and ablation effect. Clinical benefit was observed in all evaluable dogs, mainly as reduction of epistaxis and hemorrhagic nasal discharge, with improvement in local symptom control and quality of life. Follow-up CT demonstrated variable local responses: one dog showed significant reduction of neoplastic tissue extension, two dogs had persistent necrotic changes with partial local control, and one dog experienced progressive disease despite repeated treatment. Survival from first treatment ranged from 144 to 270 days. These preliminary findings suggest that CT-guided MWA is feasible in dogs with advanced nasal tumors and may provide rapid debulking, vascular coagulation, and meaningful palliation when surgery or radiotherapy are not viable. MWA should be considered a promising component of a multimodal strategy, particularly in combination with metronomic or targeted systemic therapy.

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QUANTITATIVE AND QUALITATIVE OCT ASSESSMENT OF POST-MORTEM CORNEAL CHANGES IN CATS: A PILOT STUDY FOR PMI ESTIMATION

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Accurate estimation of the post-mortem interval (PMI) remains a major challenge in veterinary and forensic pathology, and ocular tissues are increasingly investigated as potential indicators of time since death. The present study aimed to evaluate post-mortem corneal thickness changes in cats using optical coherence tomography (OCT) and to explore their potential application in PMI estimation. Six feline cadavers, including both males and females and ranging in age from 2 to 17 years, were included in a prospective observational study. All animals had died from causes unrelated to the present investigation, and no clinical or morphological evidence of corneal disease was observed at the time of the initial (T0) examination. Following death, animals were maintained in a controlled indoor environment in sternal recumbency, under stable ambient conditions (21–22°C) throughout the observation period. Corneal measurements were obtained using OCT (Heidelberg Engineering) at nine predefined post-mortem time points: immediately after death (T0), and at 3, 6, 9, 12, 18, 24, 36, and 48 hours post-mortem. Both eyes were examined at each available time point.

A total of 70 corneal measurements were obtained. Corneal thickness ranged from 325 to 1174 µm, with an overall mean of 672 ± 174 µm, indicating substantial variability across subjects and time points. Despite this variability, a time-dependent pattern of post-mortem corneal change was observed. In the early post-mortem phase (0–6 hours), corneal thickness showed marked variability, with several eyes exhibiting an initial decrease, likely associated with early post-mortem dehydration. From approximately 9–12 hours post-mortem onward, a progressive increase in corneal thickness became evident in most subjects, with the highest values generally recorded between 36 and 48 hours, consistent with the development of post-mortem stromal edema. In addition to quantitative changes, qualitative OCT findings became more apparent after 24 hours post-mortem, when the corneal profile began to show a mildly undulating (“wavy”) appearance, which became progressively more pronounced at later time points.

Pronounced inter-ocular variability was observed within the same subjects. At identical time points, the right and left eyes frequently showed different absolute thickness values and did not follow identical rates or timing of change, with inter-ocular differences ranging from 0 to 740 µm. However, no overall statistically significant difference was found between eyes (Mann–Whitney $U = 511$, $p = 0.238$), suggesting that, despite local variability, both eyes follow a comparable general pattern. A statistically significant but weak negative correlation was identified between post-mortem time and corneal thickness (Spearman’s $\rho = -0.274$, $p = 0.022$). This apparent negative association reflects the non-linear nature of the process, where an initial decrease in corneal thickness during the early post-mortem period is followed by progressive thickening at later stages.

These findings demonstrate that OCT can detect measurable and dynamic post-mortem corneal changes in cats under controlled environmental conditions. The observed combination of progressive thickening and structural irregularity supports the potential use of OCT-derived corneal parameters as non-invasive adjunct markers for PMI estimation in veterinary forensic practice. Further studies including larger sample sizes and variable environmental conditions are warranted to validate these preliminary findings.

INCIDENTAL FINDING OF A PERFORATING FOREIGN BODY (WOODEN STICK) IN THE JEJUNUM DURING SURGICAL INTERVENTION FOR COLIC IN A HORSE

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Colic is the leading problem affecting equines worldwide. The primary cause of colic is gastrointestinal disease. Intestinal perforation due to a foreign body is relatively rare in horses and may present with signs of colic, with or without signs of endotoxemia (1). When horses fail to respond to medical treatment or show signs of colic that do not respond to analgesia, exploratory laparotomy is indicated. Our case report aimed to describe the clinical presentation, diagnosis, and treatment of a perforating foreign body in the intestine (jejunum) of the horse.

A 13-year-old Italian Saddle mare was brought to the clinic with acute abdominal pain, that did not respond to analgesics. On clinical examination, the subject presented with temperature of 38.8 °C, heart rate of 53 beats per minute, mucosal congestion, capillary refill time (CRT > 3 s), Packed Cell Volume (PCV) of 35%, total protein (TP) of 8 g/dL, white blood cell count of 16,600/mm^3 and lactate levels of 15 mmol/L. Abdominal palpation per rectum revealed distension of the intestinal loops, which could not be attributed to any pathology of the large intestine. An ultrasound scan was therefore performed, revealing several loops of tightly distended small intestine; consequently, the provisional diagnosis oscillated mainly between a strangulating mesenteric lipoma and entrapment of the small bowel in the epiploic foramen. Based on the severity of pain and clinical parameters, an exploratory laparotomy was immediately performed, under general anesthesia. On inspection of the abdominal cavity, it was observed that all the intestinal loops were correctly positioned; however, a mass of approximately 5 cm was identified at the level of the jejunum, involving the intestinal loop, the omentum and the mesentery. A surgical assessment of the affected intestine was performed, and an enterectomy with end-to-end anastomosis was carried out. The decision was supported by intraoperative thermographic monitoring, which highlighted temperature variations between the intestinal stumps (35.5 °C, range 34.6–36.0 °C) and the affected segment (34.1 °C, range 33.1–34.6 °C), with a □ = 1.3 ° Postoperative dissection of the resected mass revealed the presence of a plant-based foreign body (wooden stick) located in the perimesenteric region, associated with an occlusive granulomatous inflammatory reaction in the jejunum and sequestrum formation (3). This case emphasizes that foreign body injury should be included in the differential diagnosis of small intestinal colic, especially when a focal inflammatory mass is encountered during surgery. While resection is the definitive treatment, intraoperative tools like thermography can objectively assist in assessing tissue viability, ensuring a safer surgical outcome.

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CLINICAL ASSESSMENT OF PAIN AND STRESS IN PET RABBITS: CONTRIBUTION OF VALIDATED SCALES AND CANDIDATE FACIAL AND BEHAVIORAL FEATURES

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Pain assessment in pet rabbits is challenging because, as a prey species, rabbits often display subtle facial and behavioural signs that may overlap with stress responses induced by transport, handling and hospitalisation. In clinical practice, this overlap may reduce diagnostic accuracy and complicate analgesic decision-making [1]. This preliminary prospective repeated-measures study evaluated both the performance of validated rabbit pain scales and the contribution of individual facial and behavioural features in distinguishing pain associated with acute disease from stress without pain under standardised clinical conditions.

Client-owned rabbits were assessed in three phases: Pain (emergency admission for potentially painful gastrointestinal disease), High-stress (hospital recheck at least 14 days after recovery) and Low-stress (home assessment). Standardised video recordings were obtained in each phase and scored using the Centro Animali Non Convenzionali Rabbit Scale (CANCRS), Rabbit Grimace Scale (RbtGS), Bristol Rabbit Pain Scale (BRPS) and Rabbit Pain Behaviour Scale (RPBS) [1]. Discrimination between Pain and High-stress was assessed by ROC analysis. In addition, item-level screening was performed to identify facial and behavioural features more strongly associated with pain than with stress alone. Candidate features were prioritised according to their discriminatory performance in Pain versus High-stress and their lower reactivity in High-stress versus Low-stress comparisons, in order to highlight the most promising pain-specific indicators.

In the Pain versus High-stress comparison, all validated scales showed good to excellent diagnostic accuracy: CANCRS AUC=0.906, RbtGS AUC=0.890, RPBS AUC=0.858 and BRPS AUC=0.84[2] Published analgesic cut-offs [2] also suggested different clinical profiles among scales: CANCRS showed the highest specificity in stressed but non-painful rabbits, whereas BRPS and RPBS appeared more sensitive but more susceptible to stress-related positivity. Item-level analyses identified the most promising candidate features mainly among facial expression and behaviour, particularly changes involving nostrils, eyes, ears and orbital tightening, together with posture- and activity-related variables. These findings suggest that a restricted set of facial and behavioural items may retain clinically useful pain discrimination even under stressful conditions.
Overall, validated pain scales, especially CANCRS and RbtGS, provided the most clinically useful discrimination between pain and stress in pet rabbits. At the same time, item-level screening identified a focused group of candidate pain-specific features that may support the future development of simplified, standardised and potentially automated approaches to rabbit pain assessment. These preliminary findings should be confirmed in a larger cohort.

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CHANGES IN PULMONARY ALVEOLI/MICROVESSEL DIMENSIONS AND SUBLINGUAL MICROCIRCULATION IN A PIG MODEL OF ACUTE RESPIRATORY DISTRESS SYNDROME

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In this study, we used Incident Dark Field (IDF) vital microscopy on the lung to characterize changes in pulmonary alveolar and microvessel diameters in an experimental model of ARDS in pigs, and compare this technique with histology. The study was approved by the Italian Ministry of Health (n. 701/2023-PR, protocol nr. E81AC). Six pigs were sedated with 0.04 ml/kg of tiletamine/zolazepam, xylazine (1 mg/kg) and atropine (0.02 mg/kg). Anesthesia was induced with fentanyl (1 mcg/kg), propofol (5 mg/kg) and rocuronium (0.4 mg/kg). Animals were placed under mechanical ventilation, in volume-controlled mode: tidal volume (TV) 8 ml/kg, I:E ratio 1:2, positive end-expiratory pressure (PEEP) of 5 cmH2O and FiO2 0.5. Anaesthesia was maintained with propofol (0.1-0.3 mg/kg/min), dexmedetomidine (1 mcg/kg/h), fentanyl (5-10 mcg/kg/h) and rocuronium (0.5 mg/kg/h) IV. The femoral artery was cannulated and a Swan-Ganz catheter was placed in the right jugular vein for measurement of cardiac output (CO), systolic pulmonary arterial pressure (sPAP), pulmonary arterial occlusion pressure (PAOP) and central venous pressure (CVP). A 5-cm thoracotomy was performed in order to enable in-vivo pulmonary videomicroscopy. Acute lung damage was induced by intratracheal instillation of hydrochloric acid (0.5 N solution; 1.5 mL/kg). Arterial blood gas analyses were performed every 15minutes until the PaO2/FiO2 ratio decreased to <150 mmHg, defining the ARDS. Thereafter, protective ventilation was started with TV of 6 ml/kg, PEEP of 10 cmH2O; the RR and FiO2 were modulated to maintain EtCO2<55 mmHg and SpO2>89%. Measurements including lung mechanics, hemodynamic parameters and bronchoalveolar lavage (BAL), collected at baseline, after induction of ARDS, and after 2 hours of protective ventilation. IDF videomicroscopy was used to evaluate the subpleural alveoli/microvessels and sublingual microcirculation at same times and 3 videos were recorded. Overall, cell counts were performed from BAL fluid in May–Grünwald–Giemsa-stained cytocentrifuged preparations. Briefly, a score was applied to classify the cellularity and type of exudation [absent: score of 0; there were no neutrophils and mononuclear cells or fewer than 5 cells per high-power field (HPF), mild (score of 1) for 5 to 19 per HPF, moderate (score of 2) for 20 to 49 cells per HPF, marked (score of 3) for 50 to 99 cells per HPF, severe (score of 4) for 100 to 200 cells or more per HPF]. All data were analysed and a value of $p<0.05$ was considered statistically significant. The average vessel diameter significantly increased over time in dependent areas and was higher after 2h of protective ventilation compared to non-dependent areas. The static compliance was positively correlated with the average alveolar diameter in the dependent areas ($r = 0.55$, $p=0.019$) but negatively correlated with the average vessel diameter ($r = -0.57$, $p=0.013$). The cellular score showed a significant increase in cellular damage from baseline (1,16±1,6) to the end of the trial (17,5±2,4) at 24h. In this study, ARDS was characterized by a reduction in the average alveolar diameter in the dependent areas with an increase in microvessel diameters, reflecting atelectasis and vascular congestion. Protective ventilation led to more homogeneous alveolar inflation but had no effect on vascular congestion. There were also vasodilation and capillary recruitment in the sublingual microcirculation, likely due to hypoxemia/hypercapnia. IDF video-microscopy may be a valuable tool for the evaluation of lung alveoli/microvessels in animal models of ARDS.

USE OF STABILIZED ANTIMICROBIAL PEPTIDES FOR TREATMENT OF OPEN WOUNDS IN DOGS AND CATS: A PILOT STUDY

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Use of Stabilized Antimicrobial Peptides for Treatment of open Wounds in Dogs and Cats: A Pilot Study

The increase in the number and complexity of traumatic injuries has contributed to making the wounds that surgeons are required to manage increasingly complex. Often, the management of these patients remains challenging due to the high incidence of postoperative wound complications.

Standard treatment of open wounds includes debriding agents, topical antimicrobial dressings, hydrocolloids, topical application of autologous blood products, growth factors, cultured skin, and Negative Pressure Wound Therapy.

Antimicrobial peptides (AMPs) constitute an essential component of the innate immune system of all living organisms and currently represent promising alternatives to traditional antibiotic treatment, particularly in the context of rising antimicrobial resistance.

The mechanism of action of AMPs is physical in nature: they interact with the lipid membrane of bacteria, inducing pore formation or destabilization, resulting in rapid cell lysis.

To date, no published data exist regarding the application of medical devices based on AMPs in dogs and cats.

For this reason, the aim of this pilot study was to evaluate the efficacy of the AMPS patch in the treatment of open wounds in dogs and cats, with particular attention to the granulation tissue formation, local infection control, and systemic antibiotic therapy.

Inclusion criteria were dogs and cats affected by open wounds, confirmed by in-situ microbiological culture. Each animal underwent a complete clinical examination and hematobiochemical profile. Wound management included lavage with sterile solution, enzymatic or surgical debridement, microbiological swab collection and subsequent application of the AMPS patch. The patch was covered with laparotomy swab and a modified Robert Jones bandage or a tie-over dressing in non-appendicular areas.

Systemic antibiotics were administered, based on antibiogram results, as follow: if a sensitive culture was isolated, target antibiotics were administered for five days; in case of multiresistence culture, sistemic antibiotic was not admistrated or suspended.

Dressing changes were performed every 2-3 days depending on exudate production. At each control, the wound was lavaged with saline solution before reapplying the patch and bandages.

Wound healing progression was monitored during the follow-up receck through acquisition of calibrated photographs, in order to measure lesion length and width. The percentage of wound healing and granulation tissue formation was calculated and recorded using a dedicated artificial intelligence (AI) tool for images analysis using ImitoWound. Changes observed on general clinical examination and the potential evidence of abnormal wound secretions were also documented.

Ten patients met the inclusion criteria: they were five cats with a median body weight of 2,7 kg (range 2- 4.5 kg) and median age of 5 year-old (range 1–8 year-old) and five dogs with a median body weight of 14 kg (range 7-32kg) and median age of 4 year-old (range 4 months–12 year-old). All wounds had traumatic origin, except for one case of periprosthetic infection with exposure of an orthopedic implant and bone. The median duration of treatment with the skin patch was 12 days (range 5–36 days), with a median of four dressing changes (range 3- 7). In three cases, the patch was used as preliminary therapy before reconstructive surgery (one cutaneous flap and two free flaps), and treatment was continued with the same device to protect the surgical site.

In dogs the median wound area was 34 cm2 (range from 4 cm2 to 70 cm2), in cats the median wound area was 4 cm2 (range from 2 cm2 to 26 cm2).

The median application time of the AMP 9 days, depending on the area of the wound and the tissues involved.

The median time to achieve the 90% a proper granulation was 7 days.

The results showed a mean reduction in wound area greater than 90% in all cases, reaching values of up to 99.8%. In

one comparative case — an 8-month-old intact male DSH cat weighing 3.5 kg with two wounds located in the tarsal area — one lesion was treated with AMP and the other with honey. The healing rate with AMP was 66.6 mm²/day, while the healing rate with honey was 35.5 mm²/day.

Systemic antibiotics were suspended after five days in all cases except those in which bacterial multiresistance was detected, In these cases, systemic antibiotics was not given, using only application of the skin patch nevertheless allowed wound healing.

No differences of healing times were recorded.

No side effects or adverse reactions to the product were observed.

In the two cases of deep wounds with bone exposure, the estimated healing rates were 35–40 mm²/day and >50 mm²/day, respectively. Both were culture-positive; systemic antibiotic therapy was suspended after the second dressing change because the isolated pathogen was resistant to the prescribed antibiotics. At the third follow-up, bacterial load had decreased from mild to no growth in the first case, and from high to mild in the second case. Both wounds are currently in the phase of complete cicatrization.

In conclusion, the AMPs demonstrated high efficacy in the local control of infection and in accelerating the healing of contaminated or infected wounds in dogs and cats, even in the presence of bone exposure and multiresistant bacteria. The technology enabled a mean wound area reduction of >90% in shorter times compared to historical literature data, with an excellent safety and tolerability profile. In case of infection and bone exposure, healing times are generally even slower (10–30 mm²/day)

The main limitations of this study were:1) the small sample size; 2) dogs and cats with different healing process were included. Further comparative studies are strongly encouraged to validate the outcomes obtained in this preliminary investigation.

HOOF LESION EPIDEMIOLOGY IN SOUTHERN ITALIAN DAIRY COWS: INSIGHTS FROM A NATIONAL CLAW HEALTH FRAMEWORK (2023–2025)

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Hoof lesions are a major cause of lameness in dairy cows, with important implications for animal welfare, productivity, and herd-level economic performance [1;2]. As part of a national initiative to standardise claw health data collection in Italy, this retrospective observational pilot study analysed routinely collected hoof-trimming records to quantify lesion prevalence, lesion-type distribution, and anatomical patterns in Southern Italian dairy herds. Data from 94 farms were included, comprising 24,158 cow-trimming events from 13,782 unique cows across 246 trimming visits conducted between 2023 and 2025. Records included lesion diagnosis and anatomical location, and were analysed at cow-, farm-, and claw-level using harmonised definitions. Lesion prevalence was calculated using clearly defined epidemiological denominators, and differences in claw-level lesion risk were evaluated using mixed-effects logistic regression models with farm and cow included as random effects to account for clustering and repeated observations. Overall cow-level lesion prevalence was 27.4%, while farm-level prevalence reached 37.2%, with substantial between-farm heterogeneity. Cow-level prevalence declined with increasing visit size, from 49.3% in the smallest quartile to 22.5% in the largest, suggesting an important influence of trimming strategy and case selection on observed prevalence estimates. A total of 11,408 lesion episodes were recorded, with digital dermatitis (30.1%), corkscrew claw (21.4%), white line disease (15.6%), and sole haemorrhage (14.4%) being the most prevalent conditions. Lesions were predominantly located in the hind feet (≈71%), particularly affecting the lateral claw. Compared with the front lateral claw (reference), lesion odds were significantly higher in the hind lateral (OR 16.93), front medial (OR 5.85), and hind medial claws (OR 2.53), confirming a strong anatomical predilection. These findings provide robust, region-specific epidemiological evidence on hoof lesion occurrence in Southern Italian dairy systems and highlight pronounced heterogeneity between farms. The high frequency of corkscrew claw further emphasises its potential importance as a routinely recordable trait for inclusion in future genetic evaluation programmes. Overall, routinely collected hoof-trimming data represent a scalable and valuable resource for surveillance, benchmarking, and future analytical studies integrating management and environmental risk factors to support targeted, herd-specific lameness prevention strategies.

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BURNOUT ASSESSMENT AMONG ITALIAN VETERINARIANS WORKING IN ZOOLOGICAL MEDICINE: A CROSS-SECTIONAL STUDY USING THE BURNOUT ASSESSMENT TOOL

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Veterinarians are recognized as a professional group at high risk of burnout, with reported prevalence ranging from 27% to 89% [1]. Veterinarians working in zoological medicine, including the care of exotic companion animals, wildlife, and zoo species, face additional stressors related to highly specialized knowledge requirements across diverse taxa, limited standardized therapeutic protocols, emergency duties, and complex ethical decision-making. Despite this, this professional subgroup remains poorly represented in the burnout literature. This study assessed, for the first time, burnout prevalence among Italian veterinarians working in zoological medicine and explored associated demographic and organizational factors within the Job Demands-Resources framework.

A cross-sectional observational study was conducted using a questionnaire first disseminated in October 2025 at the SIVAE (Società Italiana Veterinari Animali Esotici) Congress through QR code distribution and social media, with subsequent email follow-up until February 2026. A total of 190 completed questionnaires from Italian veterinarians were collected. Ethical approval was obtained from the Bioethics Committee of the University of Turin (protocol no. 0348953/2025). Burnout was measured using the validated Italian version of the 12-item Burnout Assessment Tool (BAT-12), applying pooled European cutoff values [2,3]. Demographic, occupational, and 12 organizational variables were also collected.

Overall, 45% of participants scored within the at-risk or burnout range, with 19.0% at risk and 26.3% above the severe burnout threshold. The mean BAT-12 total score was 2.516, only 0.024 points below the at-risk cutoff. Exhaustion was the most critical dimension (M=3.274), and work-life balance showed the lowest organizational score (M=2.23/5). Veterinarians involved in emergency duties reported significantly higher burnout scores than those not performing emergency work (M=2.61 vs 2.42; p=0.024). Multiple linear regression (R^2=0.360) identified perceived client conflict as the main risk factor (β=+0.236; p<0.001), whereas professional autonomy (β=−0.122; p<0.01), number of children (β=−0.124; p<0.01), and career development opportunities (β=−0.099; p<0.05) emerged as protective factors. Age and sex were not significant predictors.
These findings indicate a substantial burden of burnout in this previously unexplored veterinary subgroup and suggest that organizational variables are more informative than demographic characteristics in explaining burnout risk. Notably, the protective association of having children represents a novel finding in this field. Interventions aimed at reducing client-related conflict, improving autonomy, and strengthening career support may therefore be effective. Longitudinal studies are needed to clarify causal pathways and to define BAT cutoffs specifically for the Italian veterinary profession.

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USE OF PREGABALIN FOR THE PRE- AND POST-OPERATIVE MANAGEMENT OF CATS UNDERGOING OVARIO-HYSTERECTOMY

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Domestic cats frequently experience significant stress and anxiety during veterinary handling, transportation, and the perioperative period. While gabapentin (GBP) is widely used to alleviate these symptoms, pregabalin (PGB) offers a more potent alternative due to its higher affinity for the alpha 2-delta (α2-δ) subunit of voltage-dependent calcium channels (1). This binding modulates the release of excitatory neurotransmitters such as glutamate and substance P, providing both anxiolytic and analgesic effects(2). Despite its favorable pharmacokinetic profile characterized by high bioavailability (94%) and a long half-life in cats clinical data regarding its efficacy in postoperative pain management remain limited. This study aimed to evaluate the sedative and analgesic effects of a single oral dose of pregabalin (PGB) in cats undergoing ovariohysterectomy, and to assess its impact on physiological variables and pain scores. It was hypothesized that PGB, in addition to its sedative effect, would provide a good analgesic effect and could therefore be used to enhance the multimodal perioperative protocol. This study was approved in accordance with the standards recommended by the Guide for the Care and Use of Laboratory Animals and Directive 2010/63/EU for animal experiments (approval number: 074/2022), and informed consent was obtained from the owners prior to the enrollment of all cats.

In this prospective, randomized, blinded clinical trial, 60 healthy female cats (ASA I) were enrolled. Cats were randomly assigned to either Group P (Pregabalin, n=30) or Group C (Control, n=30). Group P received 5 mg/kg of PGB orally 90 minutes before hospital admission, while Group C received empty capsules (placebo) identically. Sedation was assessed after premedication using a Feline Multiparametric Sedation Scale (FMSS) and a Simple Qualitative Scale (SQS). All cats were premedicated with methadone (0.2 mg/kg IM) and alfaxalone (1 mg/kg IM). Anesthesia was induced with propofol intravenously (IV) at a dose of 2 mg/kg and maintained with isoflurane. Physiological parameters (HR, RR, SpO2, EtCO2, Blood Pressure, and Temperature) were monitored throughout surgery. Postoperative pain was evaluated at 0, 15, 30, and 60 minutes after extubation using the Glasgow Feline Composite Measure Pain Scale (CMPS-Feline) and the Feline Grimace Scale (FGS) (3). Statistical analysis included Two-way ANOVA for physiological data and Wilcoxon-Mann-Whitney tests for pain and sedation scores ($p < 0.05$).

There were no significant differences between groups regarding age, weight, or surgical and anesthesia duration. However, five cats from the control group were excluded due to the requirement of rescue analgesia during the procedure. Sedation scores were significantly higher in Group P compared to Group C ($p < 0.001$). Postoperatively, pain scores (CMPS-Feline and FGS) were significantly lower in the pregabalin-treated group at all time points ($p < 0.001$). No adverse effects, such as ataxia or vomiting, were observed in any subject. Results demonstrate that a single oral dose of 5 mg/kg of pregabalin provides effective preoperative sedation and sustained postoperative analgesia in cats. Furthermore, the significantly lower postoperative pain scores in Group P suggest a pre-emptive analgesic effect, likely mediated by the modulation of central sensitization and inhibitory effects on neurotransmitter release. In conclusion, pregabalin is a valuable and safe addition to multimodal anesthetic protocols for feline ovariohysterectomy, offering superior sedation and contributing to a more comfortable recovery compared to traditional protocols.

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Posters

MORTALITY AND TOXICOLOGICAL ANALYSIS OF THE WOLF (*CANIS LUPUS*) INTHE LIGURIA REGION (ITALY) FROM 2012 TO 2025: A RETROSPECTIVE ANALYSIS

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The wolf (Canis lupus), which had reached the brink of extinction in Italy in the early 1970s, has now effectively recolonized almost the entire national and regional territory. The discovery of a dead wolf represents an event of considerable interest, as it can provide valuable information for species monitoring and management. Between 2012 and 2025, a total of 119 dead wolves were recovered in the Liguria Region. The systematic collection of wolf mortality data in Liguria began in 2019 with the launch of the LIFE WOLFALPS EU project. Carcasses were recovered throughout the region, with the highest numbers in Savona, followed by Genoa, and lower numbers in La Spezia and Imperia. All age classes and both sexes were represented among the recovered specimens. Adults aged between 1 and 4 years accounted for 46% of the individuals, while 27% were less than one year old. Age could not be determined in 20% of the cases, and only a very small proportion of individuals were older than four years. The sex ratio of the recovered wolves was nearly balanced, with 57 males, 55 females of the specimens; sex could not be determined in 7 animals. The causes of death were attributed to anthropogenic factors (vehicle collisions, poaching, and poisoning) in 61% of cases, natural causes (diseases and inter- or intraspecific aggression) in 9% of cases, and undetermined causes (skeletal remains, trauma of unknown origin, and other events) in 30% of cases. Among anthropogenic causes, road collisions represented the most frequent cause of mortality. Sixty animals were tested for toxicological substances using two analytical techniques, LC-MS/MS and GC/MS, targeting panels of 12 and 36 compounds, respectively. For each animal were analysed one or more samples. In particular, were analysed: pooled liver and kidney (34), liver (25), kidney (6), and gastric contents (39). Seventeen individuals tested positive: 14 for anticoagulant substances, 2 for insecticides and one for both. The detected compounds included brodifacoum, bromadiolone, difenacoum, alpha-chloralose, chlorophacinone, flocoumafen, warfarin and α-endosulfan, β-endosulfan. In 7 animals multiple compounds were found. The detection of toxic substances indicates exposure and does not necessarily mean that poisoning was the primary cause of death. In fact, the attributed causes of death among wolves that tested positive for rodenticides and/or insecticides were: 8 road collisions, 1 firearm injury, 2 traumatic injuries, 1 case of severe physical deterioratio and 3 undetermined cause (1 due to advanced stage of organic decay). Poisoning was considered the cause of death in only two cases. Of these individuals, 10 were found in the province of Savona, 4 in Genoa, 1 in Imperia, and 2 in the province of La Spezia. These findings highlight that wolves are widely exposed to rodenticides and insecticides, even when poisoning does not appear as the primary cause of death. Temporal trend analysis over the study period shows an overall increase in the number of recorded mortality events, which most likely reflects a concurrent increase in the wolf population within the region. Based on the 2023/24 estimate of 168–259 wolves in Liguria, the recorded annual mortality (14 in 2023 and 22 in 2024) represents a non-negligible share of the population. These data should be considered carefully as the observed wolf mortality in Liguria represents only a fraction of actual deaths, since carcasses are found by chance and mainly in human-frequented areas; this may also bias the estimated impact of anthropogenic causes.

Posters

DELTA-TOCOPHEROL ENHANCES NEURONAL DIFFERENTIATION AND REDUCES LYSOSOMAL ACCUMULATION IN AN IN VITRO MODEL OF MUCOPOLYSACCHARIDOSIS IIIB

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Mucopolysaccharidosis IIIB (MPS IIIB) is a lysosomal storage disorder caused by the deficiency of alpha-N-acetylglucosaminidase (NAGLU), an enzyme involved in heparan sulfate degradation. This defect leads to progressive accumulation of undegraded substrates, resulting in central nervous system impairment, neurodegeneration, skeletal abnormalities, growth retardation, and multi-organ dysfunction. In this context, we investigated the potential therapeutic role of delta-tocopherol, a vitamin E isoform with antioxidant and cytoprotective properties.

An in vitro neuronal model of MPS IIIB was established using the human neuroblastoma SK-NBE cell line stably silenced for NAGLU expression. Three clones were analyzed: SK-NBE CTRL (control, transfected with non-targeting shRNA), and two NAGLU-silenced clones (CL5 and CL8), generated using a pool of three shRNAs targeting NAGLU transcript. These clones display different degrees of enzyme deficiency: CL5 reproduces a severe pathological phenotype, whereas CL8 retains residual NAGLU expression and enzymatic activity [1]. Cell viability was assessed by cell counting after treatment with increasing concentrations of delta--tocopherol (1, 5, 10, and 20 µM) for 24, 48, and 72 hours. Delta-tocopherol was well tolerated at 1 µM in both control and diseased clones, while higher concentrations resulted in a reduction of cell number at 48 and 72 hours, indicating dose-dependent cytotoxicity.To evaluate its effect on neuronal differentiation and lysosomal alterations, cells were treated with retinoic acid (2.5 µM) in combination with delta-tocopherol (1 µM) under low-serum conditions for 4 days. Immunofluorescence analysis was performed using β3-tubulin, a marker of neuronal differentiation and axonal growth [2], and LAMP1, a lysosomal membrane protein. The combined treatment significantly enhanced β3-tubulin staining in all clones and reduced LAMP1 signal in NAGLU-silenced cells compared to untreated controls, indicating improved neuronal differentiation and decreased lysosomal accumulation.

In conclusion, delta-tocopherol is well tolerated at low concentrations and exerts beneficial effects in an in vitro neuronal model of MPS IIIB. Its combination with retinoic acid promotes neuronal differentiation and partially rescues lysosomal defects, suggesting its potential as a supportive therapeutic strategy to ameliorate key pathological features of MPS IIIB. Further studies are needed to validate these findings.

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INNOVATIVE PHYTOTHERAPEUTIC APPROACHES IN BLOOD CANCER MANAGEMENT: INSIGHTS FROM SKELETONEMA MARINOI

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Marine microalgae represent a promising source of bioactive compounds with potential applications in veterinary and human oncology (1). Skeletonema marinoi (SM), a diatom widespread in temperate marine environments, produces numerous secondary metabolites with relevant biological activities; however, its antitumor potential remains largely unexplored (1,2). In this study, we evaluated the cytotoxic activity of SM on two hematopoietic tumor cell lines—the canine lymphoma cell line CLBL 1 and the human promyelocytic leukemia cell line HL 60—while simultaneously assessing its safety profile on healthy cells.

Tumor and healthy cells were incubated with SM extracts at the dose of 0.5 and 0.75 mg/ml for 12, 24 and 48 hours, and cell viability was measured using the MTT assay. To further investigate the mechanisms underlying SM-induced cell death, the expression of apoptotic markers Bax and Bcl 2 was evaluated through protein analysis. Moreover, modulation of the mTOR–AKT-PI3K signaling pathway was assessed via western blot to explore the potential interference of SM with key regulators of cell proliferation, metabolism, and tumor cell survival.

The results revealed that SM exerts a significant cytotoxic effect on both tumor cell lines. After 24 hours, a moderate yet significant reduction in viability was observed ($p < 0.01$), while a marked and highly significant decrease was detected at 48 hours ($p < 0.0001$), indicating substantial cell death in both CLBL 1 and HL 60 cells. By contrast, healthy cells did not show reduced viability or signs of cytotoxicity, suggesting that SM selectively targets tumor cells while maintaining a favorable safety profile in non transformed cells.

Molecular analyses confirmed an increase in Bax expression ($p<0.05$) together with a reduction in Bcl 2 levels ($p<0.05$) in treated cells, supporting the activation of the intrinsic apoptotic pathway as a primary mechanism of action. Additionally, the investigation of the mTOR–AKT-PI3K axis revealed decreased levels of their activated forms, indicating that SM is capable of modulating this key signaling pathway involved in tumor growth, metabolism, and resistance mechanisms. Taken together, these findings demonstrate that SM triggers apoptosis and interferes with proliferative signaling, supporting its role as a potential antitumor agent.

Overall, our results highlight the considerable therapeutic potential of SM, which appears capable of selectively inducing apoptosis in hematopoietic tumor cells while preserving the viability of healthy cells. These observations open new perspectives for the exploitation of microalgal metabolites in veterinary and human oncology. Further in vivo studies are warranted to fully characterize the bioactive molecules responsible for the observed antitumor effects and to evaluate their applicability in future therapeutic strategies.

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EXPLORING ANTIMICROBIAL RESISTANCE AND GENETIC DIVERSITY IN *ACTINOBACILLUS PLEUROPNEUMONIAE* ISOLATES FROM PIGS IN PIEDMONT

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Actinobacillus pleuropneumoniae (APP) is the etiological agent of porcine pleuropneumonia and a major concern for swine production worldwide [1]. Limited cross-protection among available vaccines and the increasing occurrence of antimicrobial resistance (AMR) highlight the need for integrated surveillance approaches to support effective control strategies and informed therapeutic decisions [2]. This study aimed to characterize APP isolates circulating in Piedmont (Italy) by combining phenotypic antimicrobial susceptibility testing with whole-genome sequencing (WGS).

Twelve APP isolates from swine herds in Piedmont were analysed. Antimicrobial susceptibility was assessed by minimum inhibitory concentration (MIC) testing using a broth microdilution method (Sensititre system, Thermo Fisher Scientific, USA) performed by an accredited external laboratory. Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. WGS was performed using Illumina technology, followed by in silico analysis of resistance determinants, virulence-associated genes (apx toxins-related genes) and comparative genomics.

Resistance to macrolides (tilmicosin and tulathromycin) was detected in 2/12 isolates, while resistance to tiamulin was also observed in 2/12 isolates. Among β-lactams, 2/12 isolates were resistant to both amoxicillin/clavulanic acid and ampicillin. Resistance to kanamycin was identified in 1/12 isolate, and resistance to sulfamethoxazole/trimethoprim in 2/12 isolates. Tetracycline resistance was the most frequently observed phenotype, occurring in 3/12 isolates. Genomic analysis identified tetracycline- and phenicol-resistance genes (*tet*, *flor*) in one isolate, while genes associated with macrolide and elfamycin resistance were distributed across all samples. The *apxIB* and *apxID* genes were detected in all 12 isolates (Table S2). The *apxIC* gene was identified in only one isolate. The apxIIC and apxIIIA genes were present in 11/12 isolates. Genes encoding the APX III secretion apparatus components showed greater variability, with *apxIIIB* detected in 6/12 isolates and *apxIIID* in 5/12 isolates. A serovar 9/11 isolate showed a partial deletion within the capsular polysaccharide biosynthesis locus, confirmed by PCR and sequencing. Comparative genomics revealed distinct lineages among serovar 6 isolates, suggesting multiple origins.

Overall, this study provides an integrated phenotypic and genomic characterization of APP isolates circulating in the Piedmont region, contributing to a better understanding of local epidemiology. The relatively low frequency of AMR compared to previous reports suggests a relatively favourable resistance scenario within the isolates included in the present study, although the limited sample size should be taken into account. [3]. The observed genomic variability within the same serovar supports the concept that serovar classification alone may not fully reflect the pathogenicity of APP strains.

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PRELIMINARY INSIGHTS INTO THE ANTIPROLIFERATIVE ACTIVITY OF SALOGRAVIOLIDE A IN CANINE LYMPHOMA CELL LINES

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Canine lymphoma (CL) is one of the most common hematological malignancies in dogs [1]. Although multi-drug chemotherapy protocols induce remission in the majority of cases, relapse frequently occurs due to the development of drug resistance. Furthermore, treatment-related adverse effects often limit therapeutic continuity [2]. In this context, plant-derived compounds may represent a promising strategy to improve treatment efficacy while reducing toxicity [3]. The aim of the present study was to evaluate the effects of Salograviolide A (Sal), a sesquiterpene lactone isolated from Centaurea kotschyi var. persica (Boiss.) Wagenitz, on the canine lymphoma cell line CLBL-[1] MDCK cells were used as a non-tumoral control. To assess the biological effects of Sal, cell viability and apoptosis were evaluated by MTT assay and Western blotting following exposure of CLBL-1 and MDCK cells to increasing concentrations of the compound (5.5, 7 and 8,5 µg/mL) for 24 hours. Sal treatment significantly reduced cell viability ($p < 0,001$) in a dose-dependent manner, with a calculated IC50 of 5.23 ± 0.3 µg/mL. Furthermore, Sal induced a marked increase in apoptotic cell death in CLBL-1 cells ($p < 0,01$), as evidenced by enhanced expression of key apoptotic markers, including cleaved Caspase-3, cleaved PARP, and BAX. At the same time, a significant downregulation ($p<0,05$) of NF-κB, was observed in treated CLBL-1 cells. In contrast, under the same experimental conditions, MDCK cells did not show significant cytotoxic effects, suggesting that Sal exhibits a selective anti-tumor activity toward neoplastic cells. These preliminary findings suggest that Sal exerts antiproliferative and pro apoptotic properties in CLBL-[1] By interfering with key survival signaling pathways and promoting programmed cell death, Sal may represent a promising candidate for the development of alternative therapeutic approaches. Further studies are warranted to elucidate the underlying molecular mechanisms.

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Posters

EFFECTS OF CO-EXPOSURE TO POLYSTYRENE NANOPLASTICS AND SARS-COV-2 ON A549 LUNG CELLS: PRELIMINARY RESULTS

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In the last decade, nanoplastic particles (NPs) have attracted increasing attention due to their well recognised impact on living organisms [1]. Their accumulation, mainly resulting from eating contaminated food, skin penetration and inhalation of NP-containing aerosols [2], has been associated with adverse effects on lung physiology [3], potentially contributing to the onset or exacerbation of respiratory diseases. Emerging evidence also suggests that NPs may modulate viral infection and pathogenicity, triggering biological effects. We aimed to investigate the influence of polystyrene (PS)-NPs on SARS-CoV-2 replication in the human lung adenocarcinoma A549 cell line, as well as their role in shaping the inflammatory response. Cytotoxicity induced by SARS-CoV-2 at a multiplicity of infection of 0.1 and by PS-NPs (1–100 µg/mL; 50 nm), either alone or in combination across different exposure times, was assessed using the MTT assay. In a separate experimental setup, cells were pre-exposed to PS-NPs at concentrations of 1, 10, and 100 µg/mL for 6 hours, followed by SARS-CoV-2 infection. At 24, 48, and 72 hours post-infection, cells were harvested for RT-qPCR quantification of viral load, determination of the 50% tissue culture infectious dose, and microscopic evaluation of virus-induced cytopathic effects. Additionally, all experimental groups were analyzed after 72 hours of co-exposure by RT-qPCR to assess changes in cytokine gene expression. Co-exposure to SARS-CoV-2 and PS-NPs did not significantly affect cell viability compared to virus-treated cells, but increased cytotoxicity compared to PS-NPs alone at 72 hours post-infection (at least $p \leq 0.05$). Cells pre-exposed to PS-NPs showed a slight overall reduction in viral load and infectious titer compared to cells infected with viral suspension alone, while the size of cytopathic effect appeared mild and comparable across conditions. Gene expression analysis revealed upregulation of antiviral and pro-inflammatory cytokines in co-exposed cells compared to virus alone. IL6 exhibited the strongest induction (up to ~4-fold in cells co-exposed to virus and PS-NPs at 1 µg/mL), while IL1β showed a moderate increase (~1.4–1.6-fold). CXCL8, was consistently elevated across all co-exposure groups (~1.4–1.6-fold). In contrast, the anti-inflammatory cytokine IL10 showed no significant changes. Antiviral cytokines IFNβ1 and IFNα2 were markedly upregulated (up to ~3-fold for IFNα2 in cells co-exposed to virus and PS-NPs at 100 µg/mL), indicating activation of interferon-mediated pathways. These preliminary findings suggest that PS-NPs may partially interfere with virus-induced cytotoxicity and SARS-CoV-2 replication. Pre-exposure of cells to PS-NPs, followed by SARS-CoV-2 infection, enhances antiviral and pro-inflammatory cytokine gene expression, which may limit viral replication while influencing cellular responses to the virus. These results highlight a potential dual role of PS-NPs in modulating host-virus interactions and warrant further investigation.

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Posters

NUTRACEUTICAL FORMULATIONS MODULATE ROS PRODUCTION AND EXERCISE RESPONSE IN SPORT HORSES: AN INTEGRATED *EX VIVO* AND PILOT CLINICAL STUDY

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Intense training activity in sport horses is associated with increased oxidative stress and overproduction of reactive oxygen species (ROS), contributing to muscle damage, fatigue, delayed recovery, and impaired performance(1). Nutraceutical strategies based on natural antioxidants may represent an effective approach to counteract exercise-induced oxidative stress and improve athletic condition. The attenuation of oxidative stress may help in protecting horses from ROS-associated damage and, consequently, for promoting optimal athletic condition(2-3).The present study evaluated the effects of selected nutraceutical formulations containing plant-derived bioactive compounds (Punica granatum, Lycium barbarum, Silybum marianum, and Cynara scolymus), rich in polyphenols and antioxidant molecules, using an integrated ex vivo and field-based approach, with a primary focus on ROS modulation. In the first phase, whole blood and peripheral blood mononuclear cells (PBMCs) were exposed to individual compounds and their combinations to assess intracellular ROS production and redox balance in response to inflammatory stimulus (H2O2 and LPS). Based on these results, the most promising formulations were selected for field-based (real-world) study. Trained jumping horses (n=20) underwent a standardized exercise test (jumping course). Blood samples were collected at baseline (T0), and at different time points post-exercise to evaluate ROS production, antioxidant capacity in whole blood and biochemical parameters. Horses received dietary supplementation for 30 days, after which the protocol was repeated (T1). Ex vivo results demonstrated a significant reduction in oxidative stress (ROS, MDA) and inflammation (PGE2, NO, MPO) production in whole blood\PBMCs treated with nutraceutical combinations, particularly those rich in polyphenols. In the field-based (real-world) study, supplementation significantly enhanced antioxidant capacity and attenuated exercise-induced ROS (MDA, GSH) and inflammation (PGE2) increase. The consistency between ex vivo and clinical findings supports a mechanistic role of these nutraceuticals in modulating oxidative stress. In conclusion, the tested nutraceutical formulations effectively reduce exercise-induced oxidative stress in sport horses under real-world conditions, supporting their use as a practical nutritional strategy to optimize performance and recovery.

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Posters

ANALYSIS OF CELLULAR HOMEOSTASIS AND UPTAKE DYNAMICS IN RAW 264.7 MACROPHAGES EXPOSED TO MICROPLASTICS AND ENVIRONMENTAL CONTAMINANTS

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The widespread presence of microplastics (MPs) in aquatic and terrestrial ecosystems represents an emerging global health concern, particularly due to their potential to interact with coexisting environmental pollutants. This study uses a multimodal in vitro approach to investigate the biological responses and functional alterations of the RAW 264.7 murine macrophage cell line under complex co-exposure conditions.

RAW 264.7 macrophages were exposed to fluorescent microplastics of two different sizes (0.2 and 1 µm), both as single treatments and in combination with selected high-impact environmental contaminants, namely perfluorooctanesulfonic acid (PFOS), glyphosate, and fipronil. Experiments were conducted in 96-well microplates to ensure robust statistical replication. Cell viability was assessed using the MTT assay, which evaluates mitochondrial metabolic activity as an indicator of cytotoxicity. In parallel, intracellular oxidative stress was quantified using fluorescent probes capable of detecting reactive oxygen species (ROS), allowing assessment of redox imbalance under different exposure conditions.

Particle internalization dynamics were characterized in detail. The uptake of 0.2 µm microparticles (MPs) was quantified by flow cytometry, revealing variations in fluorescence intensity associated with co-exposure to contaminants. These results were complemented by microscopic observations (EVOS M5000, 20× magnification), which confirmed the accumulation of MPs within cytoplasmic vacuoles, providing morphological evidence of macrophage phagocytic activity according to the phagocytic index.

ROS production was significantly increased under combined exposure conditions. Furthermore, EVOS analysis demonstrated increased particle uptake in co-exposed cells, with increased fluorescence intensity in flow cytometry. Microscopic observations corroborated these findings, revealing intracellular accumulation of MPs in the presence of contaminants.

These results may indicate that co-exposure scenarios significantly modulate both cytotoxic and pro-oxidative responses, while also altering phagocytic dynamics. Although the precise physicochemical interactions at the interface between microplastic surfaces and PFOS, glyphosate, or fipronil remain to be elucidated, the data support the hypothesis that microplastics can act as carriers of environmental contaminants. This interaction could increase the bioavailability of pollutants and amplify inflammatory responses in immune cells. Overall, the findings may underscore the synergistic impact of particulate vectors and xenobiotics, highlighting the need to revise current environmental risk assessment models to better reflect the complexity of real-world exposure conditions.

Posters

SEASONAL IMPACT ON GRAM-NEGATIVE DIVERSITY ISOLATED FROM MILKING PARLORS OF BUFFALO FARMS IN THE CAMPANIA REGION (ITALY)

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Bacterial contamination in milking parlors varies with sampling season, typically peaking during summer and decreasing in autumn; however, some pathogens may increase in autumn due to higher humidity and cooler temperatures that favor their persistence [1, 2]. This study aimed to evaluate seasonal variations and antimicrobial resistance (AMR) profiles of Gram-negative bacteria isolated from samples collected in buffalo milking parlors in the Campania Region (Southern Italy). Ten Mediterranean buffalo farms in the province of Salerno were sampled during summer and autumn 2025. Each farm was visited twice between June and December to collect bulk tank milk, teat skin swabs, milking machine teat cup swabs, and nasal swabs from milking operators. Samples were cultured on MacConkey agar, and bacterial identification was performed using MALDI-TOF MS. Antimicrobial susceptibility testing was carried out by disk diffusion method on Mueller–Hinton agar using 11 antimicrobials from eight classes to determine the phenotypic resistance profiles of the isolates. A total of 220 samples was collected. Gram-negative bacteria were isolated in both seasons, with prevalences of 41.5% in summer and 45.1% in autumn. Greater species diversity was observed in autumn. In summer, teat cups showed the highest contamination rate, with *Escherichia coli* (*E. coli*) as the predominant species, followed by *Citrobacter freundii*, *Serratia nematodiphila*, and *Raoultella ornithinolytica*. Other environmental opportunistic pathogens included *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Aeromonas veronii*, *Enterobacter hormaechei*, and *Pseudomonas putida*. Teat skin and milk samples yielded *E. coli*, *Pantoea agglomerans*, and *Raoultella ornithinolytica*, while nasal swabs from operators revealed *Citrobacter koseri* and *Pantoea agglomerans*. In autumn, microbial diversity increased markedly. Teat cups and teat skin samples were mainly contaminated by *E. coli*, together with *Acinetobacter* spp., *Klebsiella oxytoca*, *Proteus vulgaris*, *Kluyvera ascorbata*, and other *Enterobacterales*. Milk samples yielded *Enterobacter roggenkampii*, *Citrobacter freundii*, *Klebsiella oxytoca*, and *Pseudomonas koreensis*. Nasal swabs from operators showed *Klebsiella aerogenes* and *Pseudomonas* spp.

Preliminary antimicrobial susceptibility testing revealed variable resistance profiles among isolates, suggesting the circulation of antimicrobial-resistant Gram-negative bacteria within the milking environment. Overall, milking parlors showed greater Gram-negative diversity in autumn than in summer, with a similar trend observed in operator nasal samples. Notably, environmental opportunistic pathogens predominated in both seasons across all sample types. This finding suggests that seasonal climatic changes may influence the microbial composition of both the farm environment and of milking operators.

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Posters

ISOLATION FREQUENCY AND ANTIMICROBIAL RESISTANCE PROFILES OF UROPATHOGENS IN PET ANIMALS WITH SIGNS OF URINARY TRACT INFECTIONS

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Bacterial urinary tract infections (UTIs) represent one of the most common reasons for clinical presentations in small animals. The increasing emergence of multidrug-resistant (MDR) bacteria as causative agents of these infections poses a major therapeutic challenge and an important concern for both animal and public health [1]. The aim of this study was to evaluate the frequency of isolation and antimicrobial resistance profiles of the main bacterial species associated with UTIs in dogs and cats. From 2021 to 2025, a total of 102 urine samples were collected by cystocentesis from dogs and cats, showing clinical signs consistent with urinary tract infection and referred to the Veterinary Teaching Hospital of the University of Naples Federico II. Samples were immediately submitted to the Department's Bacteriology Laboratory and cultured on selective and differential media for Gram-negative and Gram-positive bacteria. Bacterial identification was performed using MALDI-TOF MS, followed by antimicrobial susceptibility testing by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar plates. A total of 18 antimicrobial agents belonging to 10 different classes (aminoglycosides, phosphonic antibiotics, carbapenems, third-generation cephalosporins, nitrofuran derivatives, fluoroquinolones, macrolides, penicillins, sulfonamides, and tetracyclines) were tested. Among the analyzed samples, 60.8% (61 out of 102) were culture-positive, of which 68.9% (42 out of 61) were of canine and 31.1% (19 out of 61) of feline origin. A total of 66 bacterial isolates were identified, with a higher frequency of Gram-negative (77.3%; 51/66; 95% CI: 65.3-86.7%) compared to Gram-positive (22.7%; 15/66; 95% CI: 13.3-34.7%) bacteria. In dogs, *Escherichia coli* (*E. coli*) was the most frequently isolated Gram-negative species (53.8%; 21/39; 95% CI: 37.2-69.9%), followed by *Proteus mirabilis* (28.2%; 11/39; 95% CI: 15.0-44.9%) and *Klebsiella pneumoniae* (10.3%; 4/39, 95% CI: 2.9-24.2%). Similarly, in cats, *E. coli* was the most common isolate (33.3%; 4/12; 95% CI: 9.9-65.1%), while *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* each accounted for 16.7% (2/12; 95% CI: 2.1-48.4%). Antimicrobial susceptibility testing revealed high resistance levels among Gram-negative isolates from both dogs and cats. In particular, canine isolates of *E. coli*, *Proteus mirabilis*, and *Klebsiella pneumoniae* showed high resistance rates to ampicillin (83.3%, 81.8%, and 100%, respectively), cefazolin (50%, 62.5%, and 66.7%), and imipenem (53.3%, 77.8%, and 66.7%). The overall frequency of multidrug-resistant strains among these three species was 66.6% (24/36; 95% CI: 49.0-81.4%). In feline isolates, higher resistance levels and a greater frequency of multidrug-resistant strains were observed, reaching 91.6% (11/12; 95% CI: 61.5-99.8%). In conclusion, multidrug-resistant bacteria in dogs and cats with urinary tract infections represent a therapeutic challenge. Routine urine culture and antimicrobial susceptibility testing are essential for appropriate treatment, and the potential spread of these pathogens from companion animals poses a public health concern.

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Posters

HEPATITIS E VIRUS IN WILD RUMINANTS: RESULTS FROM A SURVEILLANCE STUDY IN LIGURIA REGION

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Wildlife reservoirs of zoonotic viruses represent an increasingly recognized public health concern in the 21st century, particularly as environmental changes alter host–pathogen dynamics. Hepatitis E virus (HEV), a positive-sense RNA virus belonging to the family Hepeviridae, has long been identified as an emerging zoonotic pathogen responsible for hepatitis cases in high-income regions, including Europe. Traditionally associated with domestic pigs and wild boar as primary reservoirs, increasing evidence indicates that HEV can infect a broader range of mammalian hosts, including wild ruminants such as deer and chamois across Europe. Seroprevalence studies have documented the presence of HEV antibodies in cervids and other ungulates, with variable prevalence, while molecular detection of viral RNA in roe deer and fallow deer confirms active infection in wildlife populations. This study aimed to investigate the presence of HEV RNA in wild ruminants from the Liguria region between 2023 and 2025, using biomolecular methods, as part of an ongoing project (GR-2021-12374932). A total of 416 liver samples were collected from hunted or found-dead wild animals belonging to three species: 277 roe deer, 136 fallow deer, and 3 Alpine chamois. RNA was extracted using a commercial kit (RNeasy Mini Kit, QIAGEN, Hilden, Germany), according to the manufacturer's instructions, and amplified by real-time RT-PCR using primers and probe previously described [1]. All samples tested negative for HEV RNA. These findings indicate the absence of detectable viral circulation in the examined populations during the study period. In Italy, HEV epidemiology in wildlife has been extensively investigated in wild boar populations, whereas data on its presence in wild ruminants remain limited. The results obtained in this study are consistent with previous investigations conducted in Alpine and pre-Alpine cervids in Italy, which reported extremely low frequencies or absence of HEV RNA in wild cervid tissues, even in the presence of antibody titers [2]. In contrast, studies on deer in central Italy, where environmental characteristics may facilitate interspecific contact between wild ruminants and wild boar, have reported molecular positivity in Capreolus capreolus and Dama dama, with liver lesions associated with the virus, highlighting the zoonotic potential of these species [3]. One potential explanation for our findings could be the reduced environmental exposure of wild ungulates in the studied area to potential sources of HEV, such as infected wild boars. This may be related to the African swine fever (ASF) epidemic, ongoing since early 2022 and affecting wild boar populations in the Liguria region, which has led to a reduction in the density of this species within the study area. Despite the results obtained, knowledge of the health status of wildlife remains essential, not only to minimize risks to human health associated with the consumption of hunted game, but also to better understand the circulation and spread of zoonotic diseases within ecosystems.

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COMPARISON OF SANGER, NANOPORE AND ILLUMINA SEQUENCING FOR THE GENETIC CHARACTERIZATION OF A POTENTIAL NOVEL FIV SUBTYPE

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Feline immunodeficiency virus (FIV) is a member of the family Retroviradae, genus Lentivirus, characterized by its ability to integrate into the host genome as provirus. It establishes lifelong infections that progressively lead to immunodeficiency in the host [1]. FIV exhibits a high mutation rate, particularly in the envelope (env) gene, the main target for phylogenetic analysis. This variability poses significant challenges for both diagnosis and molecular characterization [2]. A previous epidemiological study identified a FIV strain that did not cluster with known subtypes and was hypothesized to represent a novel subtype [3]. However, this classification was based on a short env fragment, limiting its robustness. The primary aim of the present study was to sequence the full-length env gene of this FIV strain to better characterize its genomic features and subtype classification. Given the intrinsic genetic variability of FIV and the associated technical challenges, multiple sequencing approaches were tested and compared. Specifically, Sanger sequencing, Oxford Nanopore (MinION Mk1B platform), and Illumina (MiSeq) sequencing were employed to identify the most suitable strategy for overcoming these limitations. Reads obtained from Nanopore and Illumina platforms were trimmed, filtered, and analysed using different bioinformatics pipelines, applying both de novo assembly and map-to-a-reference approaches. In addition, intra-host viral variability, potential recombination events, and env protein structure were investigated. Both Nanopore and Illumina sequencing enabled the recovery of the complete env gene sequence, whereas Sanger sequencing yielded only a partial sequence. The sequences generated by Nanopore and Illumina were identical and showed some discrepancies compared to the Sanger-derived sequence. Illumina sequencing further allowed the assessment of intra-host variability, identifying nine SNPs distributed across the env gene and indicating the presence of minor viral variants within the same host. No evidence of recombination events was detected. Overall, next-generation sequencing approaches outperformed Sanger, highlighting their suitability for analysing long genomic regions and highly variable viruses such as FIV. Illumina sequencing provided the most comprehensive dataset, while limitations observed with Nanopore sequencing were likely related to protocols and kits employed. Phylogenetic and protein structure analyses showed that the strain has distinct features compared to known subtypes; however, its classification as a novel subtype remains uncertain. Although the strain clustered separately alongside a reference sequence classified as “subtype U”, it showed a nucleotide identity ranging from 79% to 88.5% with subtype B reference sequences, suggesting affiliation to this viral subtype. Further studies are needed to complete the genetic characterization of this strain and to clarify its epidemiological significance.

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INTERSPECIES SHARING OF *STAPHYLOCOCCUS SPP.* BETWEEN DOGS AND OWNERS: A ONE HEALTH STUDY INTEGRATING MICROBIOLOGICAL AND BEHAVIOURAL DATA

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The close interaction between humans and pets may facilitate the exchange of commensal and antimicrobial-resistant bacteria, including *Staphylococcus spp.*, within a One Health framework [1]. This cross-sectional study investigated the occurrence of interspecies sharing between dogs and their owners by integrating microbiological findings with behavioural data. Nasal swabs were collected from dog–owner pairs attending two Veterinary Teaching Hospitals in Northern (Turin, 160 pairs) and Southern (Naples, 120 pairs) Italy. Owners completed a structured questionnaire addressing pet management, antimicrobial use and intensity of close-contact behaviours. *Staphylococcus pseudintermedius* was the most frequently detected species in dogs in both areas. Among owners, *S. epidermidis* predominated in Naples, whereas both *S. epidermidis* and *S. aureus* were most common in Turin. Species concordance between dogs and owners was observed in 19.2% of pairs, exceeding chance expectation based on permutation testing ($p < 0.001$), with a significantly higher proportion in Turin compared to Naples (26.3% vs 10.0%). While this concordance alone does not demonstrate direct transmission, the different distribution across study sites and the subsequent analysis of paired isolates support the hypothesis of interspecies sharing.

A subset of 54 concordant pairs (108 isolates) was further analysed to assess antimicrobial resistance (AMR). Within this subset, *S. aureus* was the species most frequently shared between dogs and owners, followed by *S. epidermidis*, with no significant geographic differences. Methicillin resistance was detected in 16.7% of isolates in Turin and 41.7% in Naples, with concordant positivity observed in 5 pairs in Turin and 4 in Naples, suggesting possible co-carriage of resistant strains. Multidrug resistance (MDR) was observed in 34.3% of isolates, with a significantly higher prevalence in Naples compared to Turin (58.3% vs 27.4%) [2]. No extensively drug-resistant (XDR) or pandrug-resistant (PDR) isolates were identified.

Questionnaire data revealed frequent and prolonged close contact between dogs and owners, including cohabitation and daily physical interaction. Logistic regression analysis, using the dog-owner pairs as the unit of observation, identified associations between species concordance and selected variables, including owner gender, dog sex and hand hygiene practices, although wide confidence intervals suggest cautious interpretation. No association was observed between biofilm production and MDR, in agreement with previous findings [3].

Overall, these findings indicate that *Staphylococcus spp.* concordance occurs in a notable proportion of dog-owner pairs and, when combined with phenotypic AMR similarity and behavioural data, supports the possibility of bacterial sharing. However, the cross-sectional design limits causal inference, and further longitudinal and genomic studies are needed to confirm transmission dynamics at the human–animal interface.

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Posters

STAPHYLOCOCCUS AUREUS IN DOGS AND OWNERS: ANTIMICROBIAL RESISTANCE, CLONAL DIVERSITY AND EVIDENCE OF INTERSPECIES SHARING IN ITALY

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Within the One Health framework, the close relationship between humans and companion animals may facilitate the circulation of antimicrobial-resistant bacteria. Building on a broader investigation of *Staphylococcus spp.* distribution in dog–owner pairs [1], the present study focused specifically on *Staphylococcus aureus* within the same cohort to investigate its nasal carriage, antimicrobial resistance (AMR) and clonal diversity. A total of 564 nasal swabs were collected from 282 dog–owner pairs attending two Veterinary Teaching Hospitals located in Northern (Turin) and Southern (Naples) Italy. Isolation and identification were performed using culture and MALDI-TOF MS, antimicrobial susceptibility was assessed by broth microdilution and interpreted according to EUCAST 2025 and CLSI criteria, methicillin resistance was investigated by PCR detection of mecA and mecC genes, and molecular characterization was performed by spa typing. *S. aureus* was isolated from 27% of owners and 11% of dogs, confirming its higher prevalence in humans. Seventeen dog–owner pairs were positive in both hosts and, among these, 12 shared identical spa types, supporting possible interspecies transmission or shared environmental exposure [2]. A high genetic diversity was observed, with 44 different spa types identified, and the most frequent type (t1451) was detected in both geographic areas, suggesting the circulation of common lineages. Resistance was most frequently observed to penicillin (72.2%) and erythromycin (38%), while no resistance was detected to linezolid, vancomycin, tigecycline or nitrofurantoin. Multidrug resistance (MDR) was identified in 22.2% of isolates and 13.9% were classified as methicillin-resistant *S. aureus* (MRSA), all carrying the mecA gene. A significantly higher prevalence of AMR, MDR and MRSA was observed in the Southern hospital ($p=0.005$), highlighting geographic differences in antimicrobial selective pressure. Dogs showed higher resistance rates than humans for selected antimicrobials, although no overall host-related differences were observed for MDR or MRSA. A significant association between spa types and antimicrobial susceptibility profiles was detected ($p<0.001$), suggesting that clonal background contributes to resistance patterns, as previously reported using molecular typing approaches [3]. These findings, obtained from the same study population, confirm the high clonal diversity of *S. aureus* at the human–animal interface and highlight relevant geographic differences in AMR. The detection of shared spa types between dogs and owners supports the potential for interspecies transmission or common sources of exposure, reinforcing the need for integrated surveillance and antimicrobial stewardship strategies within a One Health framework.

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AUTOGENOUS VACCINES: USE OF *IN VITRO* METHODS AS AN ALTERNATIVE TO ANIMAL MODELS IN ABNORMAL TOXICITY STUDIES

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Autogenous veterinary vaccines are an essential tool for preventing infectious diseases. In particular, this approach aligns with both antimicrobial resistance (AMR) mitigation strategies and the "One Health" perspective, as well as with principles of animal welfare [1]. In recent years, advances in vaccine production have led to stricter quality control measures supported by advanced *in vitro* analytical techniques. However, these approaches are not yet fully implemented across all procedures. The abnormal toxicity test (ATT) still represents the main *in vivo* assay for toxicity screening, despite its well-known limitations, including low specificity, poor reproducibility, and limited scientific relevance. The European Pharmacopoeia has removed ATT as a regulatory requirement, encouraging the adoption of more ethical and scientifically robust alternatives [2]. This study aimed to develop an *in vitro* method to evaluate the toxicity of autogenous vaccines as a replacement for ATT. The proposed assay is based on the ATP Cell Viability Assay, which measures intracellular adenosine triphosphate (ATP), a key indicator of cellular metabolism and viability [3]. Autogenous vaccines produced by the Istituto Zooprofilattico of Brescia and Turin (IZSLER and IZSPLV) were analysed according to the following procedure: L929 cells were seeded in 96-well plates at a density of 5×10^4 cells/mL and incubated for 24 hours (h) at 37°C. The vaccines were tested from undiluted (ND) original material to a 1:64 dilution and incubated with the cell cultures for 24 hours before luminescence analysis. Samples were tested in triplicate, and the mean (μ) and standard deviation (σ) were calculated. The cut-off used to determine the dilution at which the compound was no longer cytotoxic was defined based on the negative control distribution, calculated as μ and σ. Values below $\mu-2\sigma$ were considered indicative of a significant reduction in cell viability. The results showed that the developed method is sensitive, accurate, and reliable. Quantitative data on the cytotoxicity of each analysed vaccine were obtained without requiring *in vivo* testing. A cut-off value of 6.24×10^6 relative luminescence units (RLU) was established based on the negative control data distribution, demonstrating that vaccine composition, the tested antigen, and the respective strain significantly affect cell viability. For example, papillomavirus-based vaccines exhibited cytotoxicity at all tested dilutions due to their preparation process. Conversely, *Streptococcus suis*-based vaccines displayed strain-dependent toxicity. Specifically, out of the 31 vaccines tested, they were cytotoxic at ND and 1:2 concentrations in 100% of cases. Differences became evident at the 1:4 dilution, where 55% of the vaccines were no longer cytotoxic; this decreased to 36% at the 1:8 dilution, while the remaining 9% were distributed across higher dilutions (1:16, 1:32, 1:64). In conclusion, the development of this method will enable us to maintain a safety standard for the release of autogenous vaccines and, at the same time, promote the gradual replacement of animal models.

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SEROPREVALENCE OF *BARTONELLA HENSELAE* IN CATS FROM NORTHERN ITALY: EVALUATION OF RISK FACTORS AND CLINICAL ASSOCIATIONS

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Bartonellosis is a vector-borne zoonotic disease with worldwide distribution that can infect humans and a large number of mammals, including companion animals (cats and dogs) [1]. *Bartonella henselae* (family Bartonellaceae, genus *Bartonella*) is a Gram-negative bacterium primarily transmitted by the cat flea *Ctenocephalides felis* and is the causative agent of cat scratch disease (CSD) in humans [2]. Despite the endemic circulation of the vector in Italy, data relating to the seroprevalence of *B. henselae* in northern Italy are limited [3]. This study aimed to investigate the seroprevalence of *B. henselae* in cats from Emilia Romagna region and to evaluate possible associations between serological status, signalment, lifestyle and clinical findings. A total of 68 feline serum samples were retrospectively analyzed (2023-2024). Samples were collected from cats admitted to the Veterinary hospital of the University of Bologna for reasons other than those related to this study and included 58 traumatized stray cats from the local territory and 10 owned cats with clinical signs including fever, lymphadenomegaly and cardiac alterations. Anti-*B. henselae* IgG antibodies were detected by indirect immunofluorescence antibody test (IFAT), setting a cut-off value of 1:40 for positivity. For all cats, factors such as sampling season, age, sex, reproductive status, flea infestation, fever, lymphadenomegaly, and other parasitic infections were collected and statistically analyzed using the Fisher's exact test ($P<0.05$ considered significant). Overall, 16/68 cats (23.5%) were seropositive: 15/58 (25.9%) among stray cats and 1/10 (10.0%) among owned cats. Among positive subjects, antibody titers were 1:40 (7/68), 1:80 (5/68), 1:160 (2/68), and 1:320 (2/68). No statistically significant associations were found between seropositivity and the investigated variables, although higher seroprevalence was observed in cats sampled during the cold season, older than one year, and in those with flea infestation, lymphadenomegaly, or other parasitic infections. In the owned cat population, only one case of seropositivity was detected in an adult European shorthair cat with outdoor access, not receiving regular antiparasitic prophylaxis and sharing the same household with a dog. The results of this study confirm the endemic circulation of *B. henselae* in cats of the Bologna province, with a medium-low seroprevalence in the national context, and emphasize the importance of ectoparasite control within a One Health perspective. Future studies on larger and more representative samples, integrating serology and molecular tests, and assessing the circulation of *C. felis* are needed to better define risk factors related to bartonellosis and its clinical relevance.

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FROM SURVEILLANCE DATA TO EPIDEMIC FRONTS: MODELLING AFRICAN SWINE FEVER SPREAD IN ITALIAN WILD BOAR

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African swine fever (ASF) has been spreading in Italy since December 2021, mainly involving wild boar populations and causing severe sanitary and economic impacts on the pig production sector [1]. Understanding the spatial dynamics of the epidemic and supporting prioritization of control measures require integrated analytical tools, capable of accounting for heterogeneous Surveillance Systems (SS) and imperfect detection [2, 3]. Our project aims to develop a spatially explicit modelling framework to reconstruct ASF epidemic dynamics in Italy and to support scenario analysis and risk prioritization for disease control. A Bayesian dynamic patch-occupancy model has been developed to reconstruct the spatial and temporal evolution of ASF in wild boar using surveillance data collected between December 2021 and June 2025. Epidemiological information from passive surveillance, active carcass searches and hunting activities was integrated with environmental and anthropogenic covariates. All data were harmonised on a 5×5 km spatial grid with weekly temporal resolution. The model explicitly accounts for imperfect detection by separating surveillance processes from true infection dynamics and by modelling colonisation and persistence probabilities, distinguishing between local spread and long-distance jump dispersal events. The retrospective analysis allowed reconstruction of the geographic colonisation process of ASF, providing probabilistic estimates of disease presence over time and space. Preliminary results highlighted the heterogeneous performance of SS across time and space. Surveillance was most effective — and ASF detection probability highest — at the leading edge of the epidemic expansion and during the initial months following first disease detection. Conversely, the capacity of the SS to track disease dynamics was greatly limited within the core of the ASF-affected area; detection probability declined over time, approaching very low values between 12 and 24 months after first detection. The occupancy model performed well in characterising colonisation processes at the disease front, identifying environmental and infrastructural factors associated with detection and colonisation probability, but showed limited power in accounting for persistence processes within the infected area. The modelling framework has been designed to support both retrospective analysis and future projections. Ongoing activities include model validation and short-to medium-term projections of ASF spread to identify the most plausible epidemiological scenarios in areas not yet affected. The final phase of the project, scheduled for the end of 2026, will focus on integrating model outputs with the spatial distribution of pig farms and meat-processing facilities. This analysis will enable identification of production districts at higher risk of ASF introduction, estimation of potentially affected economic activities and definition of priority areas for preventive interventions. The model is conceived as an updatable tool, allowing continuous incorporation of new surveillance data and long-term use for scenario evaluation beyond the project duration.

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ENHANCING THE NUTRITIONAL AND ANTIOXIDANT PROFILE OF GOAT CHEESE THROUGH MEDICINAL PLANT SUPPLEMENTATION

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Oxidative processes occurring in milk and cheese can lead to the development of undesirable off-flavours and a reduction in the nutritional quality of dairy products. Pasture-based dairy systems often produce milk rich in polyunsaturated fatty acids (PUFA), which are beneficial from a nutritional standpoint but also more susceptible to lipid oxidation during processing and storage. Increasing the antioxidant capacity of dairy products is therefore a key strategy to improve their oxidative stability and nutritional value. Medicinal plants rich in bioactive compounds, such as polyphenols and essential oils, may represent a natural dietary approach to enhance the antioxidant potential of milk and derived products. The aim of this study was to evaluate the effect of dietary supplementation with two medicinal plants, milk thistle (Silybum marianum) and anise (Pimpinella anisum L.), on the nutritional and antioxidant profile of cheese produced from grazing dairy goats.

Twenty-four third-parity goats were randomly divided into three groups (C: control, T: thistle, A: anise) homogeneous for live weight (50 ± 2 kg), days in milk (60 ± 7.6 days), and milk yield (1980 ± 120 g/head/day). All animals grazed on a permanent pasture from 9:00 to 16:00 for two months and received 400 g/head/day of corn meal in individual pens. In addition, groups T and A received 15 g/head/day of dried milk thistle leaves and anise seed powder respectively, mixed into the concentrate before feeding. Individual milk production was recorded daily. Cheese samples aged for 25 and 50 days were obtained monthly and analyzed for chemical composition (AOAC), cholesterol content, antioxidant activity (TPC, TAC, and DPPH), and fatty acid composition (GC). Data were analyzed by ANOVA using SPSS software.

Significant differences were observed in cheese chemical composition. Both treated groups showed significantly lower cholesterol levels compared with the control group in both sampling months and at both ripening times. The fatty acid profile of cheeses revealed some differences, particularly in SFA, MUFA, and PUFA contents in samples aged for 50 days. Antioxidant activity, evaluated through DPPH and TAC assays, was significantly higher in cheeses from the treated groups during both sampling periods and at both ripening times, indicating an improved antioxidant potential. Similarly, total polyphenol content (TPC) was significantly higher in cheeses from goats receiving plant supplementation, contributing to the enhanced antioxidant capacity of the product.

Dietary supplementation with milk thistle and anise in grazing goats improved the antioxidant potential of cheese, as demonstrated by the higher TPC and increased antioxidant activity. Moreover, the lower cholesterol content observed in cheeses from treated groups suggests an improvement in their nutritional profile. Overall, the inclusion of medicinal plants in the diet of grazing goats may represent an effective natural strategy to enhance the oxidative stability and nutritional quality of dairy products.

Posters

ANALYSIS OF THE GEOGRAPHICAL DISTRIBUTION AND POPULATION DYNAMICS OF TICKS (*IXODIDAE*) IN SICILY: IMPLICATIONS FOR PUBLIC AND ANIMAL HEALTH

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Ixodidae ticks are among the most relevant vectors of infectious and parasitic diseases affecting humans and animals in the Mediterranean basin [1,2]. Due to its climatic variability and ecological heterogeneity, Sicily represents a high-suitability environment for the persistence and expansion of multiple tick species. This study was funding by Italian Ministry of Health grant number RC IZSSi 01/2023 and provides an integrated analysis of geographical distribution, population structure, and epidemiological implications of *Ixodidae* ticks in Sicily, with specific emphasis on zoonotic risk and livestock health. Field monitoring was conducted from April to October 2024 across three macro-areas representative of Sicilian ecosystems: Bosco della Ficuzza Nature Reserve, Madonie Regional Natural Park, and Zingaro Nature Reserve. Site selection was based on habitat diversity, altitudinal gradients, and the interface between wildlife, livestock, and human activities. Monthly sampling was performed using standardized flagging and dragging protocols. Each site was surveyed by three operators for 30 minutes along random transects under controlled conditions to ensure comparability. Ticks were collected, preserved in 70% ethanol, and morphologically identified by species and developmental stage. All samples were georeferenced for spatial analysis. A total of 440 ticks belonging to seven species were identified: 200 *Rhipicephalus bursa* , 153 *Ixodes ricinus* , 52 *Haemaphysalis punctata* , 18 *Hyalomma marginatum* , 14 *Dermacentor marginatus* , 2 *Rhipicephalus sanguineus* , and 1 *Hyalomma lusitanicum* .

Adults were predominant (68%), reflecting their higher detectability during host-seeking activity. Notably, nymphs (15%) represent a critical epidemiological stage, contributing to pathogen maintenance and acting as largely undetected vectors for humans [3]. Immature stages (eggs 11%, larvae 6%) showed a clustered distribution in bushy grazing areas, identifying these microhabitats as functional environmental "nurseries" supporting tick population persistence. Species distribution was strongly associated with ecological features. *Hyalomma* spp., vectors of *Theileria* spp. and *Coxiella burnetii*, were primarily linked to open and coastal environments, whereas *Dermacentor* spp. were restricted to forested habitats with wild ungulates as key hosts. These findings reveal a structured eco-epidemiological pattern driven by habitat-host interactions and environmental conditions. Beyond the epidemiological implications, the widespread presence of tick populations in grazing systems represents a significant and often underestimated economic burden for livestock production, affecting animal health, productivity, and farm sustainability. Overall, the study highlights a dynamic and spatially structured tick system with direct implications for animal and public health. The clustering of immature stages and the widespread presence of competent vectors indicate that control strategies cannot rely solely on host-targeted treatments but must incorporate integrated land and pasture management. Furthermore, climate change and extensive grazing practices are likely to amplify tick expansion and zoonotic risk. These results strongly support the implementation of a One Health framework integrating veterinary surveillance, human health monitoring, and environmental management to effectively mitigate tick-borne diseases.

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YERSINIA PSEUDOTUBERCULOSIS-ASSOCIATED DISEASE IN AN ADULT GOAT

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Yersinia pseudotuberculosis is a potential zoonotic pathogen. In Europe, it is mainly associated with sporadic, self-limiting gastroenteritis, whereas in Japan and Russia it has been linked to outbreaks of a severe systemic condition known as Far East Scarlet-Like Fever. The bacterium is widely distributed among animals, particularly wildlife, including rodents and lagomorphs; in Europe, the brown hare is considered the primary reservoir. Among domestic species, *Y. pseudotuberculosis* has been isolated from a broad range of hosts, both healthy and diseased.

An adult female goat was found in severe hypothermia (32 °C), with marked abdominal distension, and died shortly thereafter. The carcass was submitted for necropsy to the Pathology Service of the Veterinary Teaching Hospital (OVUD), Department of Veterinary Medicine, University of Perugia. The animal was kept as a companion animal together with another goat and two sheep, all reportedly healthy.

On external examination, marked abdominal distension and poor body condition (BCS 2/5) were observed. Subcutaneous tissue exhibited a gelatinous, translucent appearance consistent with edema. The parotid lymph node was enlarged and exuded creamy yellow material on cut section.

Internal fat reserves were depleted. The mesentery and omentum appeared thickened and gelatinous, as well as pericardial fat, with a characteristic “bottle-glass” appearance. The mesenteric lymph node was markedly enlarged (5 × 4 × 3 cm) and, on sectioning, displayed multiple coalescing yellow caseous areas with sharp margins, involving 80% of the section. The intestinal wall was diffusely thickened and reddened, predominantly affecting the large intestine, with occasional pinpoint hemorrhages, while the jejunum and ileum appeared unremarkable. Representative lymph nodes were collected for bacteriological analysis that resulted in the isolation of Y. pseudotuberculosis.

Histologically, lymph nodes were characterized by extensive necrosis composed of cellular debris and degenerate neutrophils, associated with colonies of small basophilic rods, surrounded by a rim of macrophages and granulation tissue. In the large intestine, the mucosa was diffusely thickened by mixed inflammatory infiltrates, with multifocal necrotic foci associated with bacterial colonies and neutrophilic inflammation.
Infection and/or disease due to *Y. pseudotuberculosis* has been reported in goats and sheep worldwide; however, the largest case series originate from Australia, New Zealand, Japan, and California. In Italy, reports of *Y.pseudotuberculosis* predominantly involve wildlife, especially hares affected by septicemia. In domestic animals, the bacterium has been isolated from ovine milk and aborted fetuses in Sardinia, with PCR detecting bacterial DNA in 26% of examined mesenteric lymph nodes, as well as from porcine tonsils and cases of bovine mastitis. Isolated cases of septicemia have also been described in a cat and a goat.

Clinical disease associated with Y. pseudotuberculosis appears to be uncommon in goats. According to the literature, the most frequently reported lesions include enterocolitis and typhlitis, followed by lymphadenitis, hepatitis, conjunctivitis, mastitis, and abortion.

The present case underlines the need of a more comprehensive consideration in adults goats presenting with chronic wasting disease, that may also consider, among other more common agents, also potentially zoonotic *Y.pseudotuberculosis*, in line with a One-Health perspective.

ASSESSMENT OF TOTAL BACTERIAL COUNT IN RAW OVINE MILK FROM WESTERN SICILY FARMS: A THREE-YEAR STUDY (2023–2025)

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Sicily holds a long-standing tradition in relation to ovine raw milk cheesemaking, and many of these so-called “typical” products are certified by the European authorities through the assignment of the “Protected Designation of Origin” (PDO, DOP in Italian) mark which defines the requisites of authenticity for products from specific geographical areas. The assessment of dairy animal welfare is crucial to contribute to a better quality of the produced milk [1]. Total bacterial count (TBC) is a microbiological parameter used to evaluate hygienic conditions on dairy farms, including milking practices and milk storage procedures, and to check the quality of milk and dairy products. High TBC levels may result from poor husbandry and hygiene practices, inadeguate maintenance and cleaning of milking machines, or inappropriate milk refrigeration. The EU (Annex III, Section IX of Regulation EC 853/2004) established a TBC limit of 1,500,000 cfu/mL for ovine raw milk intended to be heated/pasteurized, and 500,000 cfu/mL for ovine milk used to produce raw milk cheeses. This work aimed to evaluate the microbiological quality of ewe’s bulk-tank milk samples collected in 2023, 2024 and 2025, fortnightly, from 454 ovine farms (245 of which have been found throughout each year of analysis) in western Sicily, in the provinces of Agrigento, Caltanissetta, Enna and Palermo, within the self-monitoring sampling programmes for dairy farms. Bulk milk TBC was automatically measured by Bactoscan flow cytofluorimetry, an ISO/IEC 17025:2018 accredited method.

The latest official data (ISTAT 2020) showed that 3,106 ovine farms (with n=animals >10) are located in these districts, thus 14.6% was the percentage of farms we investigated. Few dairy farms (n=4, Palermo, 2023; n=1, Agrigento and n=1, Enna, 2025) had TBC (determined by a two--month rolling geometric mean with at least two samples per month) over the EU limit (>1,500,000 cfu/mL). 12.4% (2023), 8.5% (2024), and 12.3% (2025) of analyzed ovine farms had TBC values in the 501,000-1,500,000 cfu/mL range, in accordance with the criteria for processing following pasteurization. In order to better assess the hygienic conditions of farms with TBC values in the intermediate range, we further divided it into two restricted groups, 501,000-900,000 and 901,000-1,500,000 cfu/mL, respectively. More than 80% of the previous intermediate range farms had TBC levels from 501,000 to 900,000 cfu/mL, reflecting a certainly more encouraging situation. TBC levels ≤500,000 cfu/mL were found in 86.2% (2023), 91.5% (2024), and 87.2% (2025) of farms, indicating a microbiological quality suitable for raw milk cheese production. The high percentage of sheep farms in accordance with this EU limit appears considerably stable throughout the three-year period. Overall, our study indicate good hygienic quality of milk produced in the investigated dairy farms, suggesting an improvement farm management practices and milking hygiene [2,3]. These findings also support the close relationship between animal welfare and the quality of the final dairy product..

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PEDIGREE-BASED ASSESSMENT OF GENETIC DIVERSITY AND RISING INBREEDING IN THE SOPRAVISSANA SHEEP BREED

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Local livestock breeds represent irreplaceable reservoirs of genetic diversity, yet many are threatened by progressive genetic erosion. This study assessed the genetic diversity and demographic dynamics of the Italian Sopravissana sheep breed using pedigree analysis, with particular focus on inbreeding trends and the distribution of genetic contributions from founders and ancestors.

Analyses were based on the available historical pedigree provided by Asso.Na.Pa., including 42,396 individuals. Two reference populations were defined: animals with known parents (RP1) and a recent cohort born in the last 8 years (RP2=2017–2025). Gene origin parameters and individual inbreeding coefficients were estimated to quantify changes in the genetic base over time using pedigree-based algorithms implemented in R (R Core Team 2025).

Although the pedigree includes 18,003 founders, gene origin analyses revealed a marked contraction of the effective genetic base. In RP1, the effective number of founders and ancestors were f_e=554.67 and f_a=384.44, decreasing sharply in RP2 (f_e=159.65; f_a=95.54), with founder genome equivalents f_g= 53.30±8.46. The increasing f_e/f_a ratio (1.67 in RP2) indicates bottlenecks in intermediate generations and unequal use of reproducers, leading to genetic drift and loss of founder representation.

The mean inbreeding coefficient was F=0.022 (SD=0.06), with nearly 23% of individuals inbred and a maximum value of 0.45. In recent cohorts, inbreeding more than doubled (F=0.050). A significant upward trend over time was detected (β = 0.001153; R^2 = 0.67; $p < 0.001$). Higher inbreeding in males (F=0.031) than females (F=0.021) suggest an unbalanced use of breeding animals. The average individual rate of inbreeding increase (ΔF=0.0017), computed from equivalent complete generations, yielded a realized effective population size of $N_e \approx 29$[1] This value is consistent with the reduced fe and fa and supports evidence of recent genetic erosion.

Despite still retaining moderate genetic diversity, the Sopravissana breed shows clear signals of recent genetic erosion. These findings underline the urgency of targeted management strategies, including balanced use of breeders and preservation of underrepresented lineages, to ensure the long-term sustainability of this valuable genetic resource.

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THE UNI 11952:2024 STANDARD FOR ANIMAL WELFARE, TRACEABILITY, AND ENVIRONMENTAL–ETHICAL CLAIMS IN THE ITALIAN WOOL PRODUCTION CHAIN

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Currently, approximately 83 million sheep are raised in Europe, with production concentrated mainly in seven countries: the United Kingdom, Spain, France, Romania, Greece, Italy, and Germany. Within a bioeconomic framework, wool production must rely on multidisciplinary guidelines capable of addressing all aspects of fibre production, including animal management, environmental impact, biosecurity, fibre harvesting, and product traceability.

The implementation of such standards by wool producing sheep farms enables the production of wool suitable for the textile industry, aligning with the growing consumer demand for environmentally friendly and sustainable products.

The UNI 11952:2024 standard defines the requirements for the management of wool producing sheep farms in full compliance with the principles of animal welfare, as expressed through the Five Freedoms. With regard to animal welfare, the standard also incorporates the national ClassyFarm programme, which classifies farms according to their levels of risk related to animal welfare and biosecurity.

The standard specifies housing conditions and farming practices, including grazing load based on available edible biomass, housing structures and systems (density, temperature, ventilation, etc.), cleaning procedures, staff management, feeding practices, reproduction, lactation and weaning, health management, biosecurity rules, and animal transport. Furthermore, it details the requirements for wool production management, including animal preparation for shearing, shearing procedures and necessary facilities, and the classification and sorting of wool for product identification and traceability.

The recent approval of this UNI standard has contributed to bridging the gap between the requirements of the wool market, the interests of farmers, and consumer expectations. The standard enables the supply of wool that is immediately ready for use in the initial washing phase by the textile industry, without the need for additional labour; ensures animal welfare in accordance with current national legislation; and, from an environmental perspective, establishes good practices for the sustainable management of pastures and for climate change mitigation, through increased soil carbon sequestration achieved via appropriate grazing management. Moreover, the UNI standard can enhance product qualification and improve the marketability of wool garments by introducing verifiable, transparent, and certifiable criteria that allow consumers to clearly identify wool sourced from ethical and sustainable farms.

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Posters

EFFECTS OF CALCIUM AND NALOXONE SUPPLEMENTATION IN TRIS AND LEPUS EXTENDERS ON RABBIT SEMEN QUALITY

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Artificial insemination is a key reproductive biotechnology for optimising efficiency in rabbit breeding. Maintaining sperm quality during storage is essential for success. Calcium ions (Ca^{2+}) play a fundamental role in regulating sperm physiology and processes such as motility, capacitation, and the acrosome reaction. However, alterations in Ca^{2+} homeostasis can negatively impact sperm viability and functionality. Growing evidence also suggests that the endogenous opioid system may influence sperm activity. Opioid receptors (μ, δ, κ) have been identified in spermatozoa of various species, mainly localised to the acrosomal membrane, neck, and flagellum, where they appear to regulate motility and energy metabolism (Albrizio et al., 2005). These receptors can interact with calcium channels and intracellular signalling, indicating a potential functional connection between opioid signalling and calcium metabolism in sperm (Vicente-Carrillo et al., 2016). However, this interaction has not yet been studied in rabbit spermatozoa.

The aim of this study was to evaluate the effects of calcium chloride ($CaCl_2$) and naloxone (Nx), an opioid receptor antagonist, on rabbit semen quality when diluted with two extenders: TRIS (Di Iorio, 2014) and Lepus® (Medinova© Reggio Emilia, Italia). Semen samples were collected from twenty New Zealand White bucks, pooled, and divided into two aliquots, one for each extender. Each was then subdivided into four experimental treatments: a control group and groups treated with Nx (10^{-8} M), $CaCl_2$ (1.5×10^{-3} M), or Nx + $CaCl_2$. Samples were diluted 1:20, stored at +4 °C, and evaluated using computer-assisted sperm analysis (CASA) to assess motility, kinematics, trajectory indices, vitality, and morphology abnormalities.

Naloxone supplementation significantly improved total ($p < 0.001$) and progressive motility ($p < 0.001$) and several kinematic parameters ($p < 0.001$), regardless of the extender. $CaCl_2$ alone reduced sperm motility, trajectory indices, and vitality. The combined treatment (Nx + $CaCl_2$) produced the highest values for motility ($p < 0.001$) and kinetic parameters ($p < 0.001$), indicating a potential synergistic effect between opioid receptor antagonism and calcium availability. TRIS extender generally showed slightly better results than Lepus, especially in treatments containing naloxone.
These findings suggest pharmacological modulation of the endogenous opioid system is important for regulating rabbit sperm function. Low naloxone concentrations significantly enhanced sperm motility and viability, and its combination with Ca^{2+} further improved functional parameters. This approach could optimize semen preservation and improve artificial insemination efficiency in rabbit production.

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Posters

FIRST ISOLATION AND CHARACTERIZATION OF LUNG-DERIVED MESENCHYMAL STROMAL CELLS FROM THE AMAZONIAN BAT *CAROLLIA PERSPICILLATA*

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Mesenchymal stromal cells (MSCs) are multipotent cells capable of self-renewal and differentiation into multiple cell lineages, which makes them valuable tools in preservation strategies. Applying this methodology to lung cells from bat Amazonian species, such as *Carollia perspicillata*, is an important approach, given that bats are natural reservoirs of many emerging zoonotic viruses, making their respiratory system a key target for understanding host-pathogen interactions. Studying bat lung MSCs may provide insights into disease tolerance mechanisms and tissue resilience unique to chiropteran species[1;3]. This study aimed to characterize, for the first time in *C. perspicillata*, MSCs isolated from lung. Cells were cultured under standard conditions (37°C, 5% CO_2 in humidified atmosphere) in DMEM/F12 medium supplemented with 10% FBS until reaching 80-90% of confluence prior to experimental assays at Passage 3 (P3) of *in vitro* culture. Isolated cells were evaluated through tri-lineage differentiation, Colony-Forming Unit (CFU), scratch, and spheroid formation assays[2]; all assays were performed in triplicate with negative controls. Isolated cells exhibited fibroblast-like morphology and adherence to plastic, consistent with MSC characteristics. After 10 days of exposure to differentiation media, positive staining for chondrogenic and adipogenic differentiation was observed, indicating the multipotent potential of these bat-derived cells even after a relatively short induction period. On the contrary, osteogenic staining indicated only early-stage differentiation, suggesting that a longer induction period is required for this lineage. In the CFU assay, a mean of 23.0 ± 8.7 (range: 17–33) colonies was observed after 14 days of culture, suggesting a moderate clonogenic capacity. In the scratch assay, complete closure was observed at 48 hours, demonstrating active migratory potential. After 48 hours of tested conditions, the cells did not form compact spheroids, which may indicate differences in cell-cell adhesion properties or specific growth requirements compared with mesenchymal cells from other species. Overall, this study provides the first evidence of MSC-like cells derived from bat lung tissue, highlighting their potential as a novel *in vitro* model for biodiversity conservation, including cellular biobanking strategies, as well as for applications in environmental toxicology and host-pathogen interaction research. Given the well-established immunomodulatory and anti-inflammatory properties of lung-derived MSCs[1], these cells may also contribute to the regulation of pulmonary immune responses in bats, potentially supporting their unique ability to tolerate viral infections. These findings underscore the importance of expanding MSC research to underexplored wildlife species, revealing potential biological differences across taxa.

Lung tissue samples of *Carollia perspicillata* were collected under SISBIO permit no. 13248 (issued to Julio Cesar Pieczarka), in accordance with the Federal University of Pará Animal Ethics Committee guidelines (approval no. 68/2015).

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ULTRASOUND-GUIDED AMNIOCENTESIS FOR PRENATAL DIAGNOSIS IN SHEEP: AFEASIBILITY STUDY

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Ultrasound-guided amniocentesis represents a valuable approach in sheep for obtaining foetal fluids for diagnostic and research purposes. In ewes, this minimally invasive procedure has been described as a method for prenatal sampling, including the detection of infectious agents [1]. Evidence from human medicine indicates that genetic amniocentesis does not significantly affect Doppler measurements of utero-placental and feto-placental circulations, supporting the short-term safety of the procedure [2]. This preliminary study aimed to describe our experience with ultrasound-guided prenatal fluid sampling in pregnant ewes as a screening tool for molecular analyses, including Scrapie susceptibility and Robertsonian translocations. The study was approved by the Ethical Committee of the Department of Veterinary Sciences, University of Messina (approval no. 04/2026). Eight clinically healthy pregnant ewes were enrolled, and sampling was performed during the second trimester of gestation under transabdominal ultrasonographic guidance using a 22 G spinal needle. Prior to sampling, each ewe underwent ultrasonographic examination to confirm pregnancy, estimate gestational age, assess foetal viability, and identify the most suitable puncture site. Foetal heart rate and umbilical cord Doppler indices—including peak systolic velocity, end-diastolic velocity, pulsatility index, resistance index, and systolic/diastolic ratio—were recorded before and after the procedure. Prenatal fluid sampling was successful in 6 out of 8 ewes, yielding four whitish amniotic fluid samples and two yellowish allantoic fluid samples, while sampling was unsuccessful in two animals. Immediately after sampling, foetal viability was reassessed ultrasonographically by evaluating heart rate and movements. The animals were subsequently monitored for possible side effects. Statistical analysis showed no significant differences between pre- and post-procedural values. No major procedural complications were observed, and no abortions occurred in the enrolled animals. Spectrophotometric analysis (NanoDrop) confirmed the extraction of genomic DNA from both amniotic and allantoic fluids. DNA concentrations were sufficient for downstream molecular analyses, including PCR. Overall, this preliminary experience demonstrated that ultrasound-guided prenatal fluid sampling is feasible in sheep. However, specific technical training is required to optimize sample collection. These findings support the potential use of this approach as a screening tool for selected genetic conditions, infectious diseases, and transplacental infections in ovine medicine.

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Posters

MATERNAL ETHOLOGY AND FETAL HEMODYNAMICS IN GESTATING EWES SUBJECTED TO HEAT STRESS

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Climate change and increasing heatwaves pose significant challenges to livestock reproduction, as heat stress during late gestation can compromise maternal physiology and negatively impact fetal outcomes [1]. This study evaluated the effect of direct solar radiation on maternal ethology, gestational hemodynamics, and lambing parameters in fourteen pregnant Santa Inês ewes. The study was approved by the Ethics Committee for Animal Use (CEUA-FZEA USP) under protocol number 1169131124 and financial support by The São Paulo Research Foundation (FAPESP n. 2024/00982-2; 2025/04213-6) and Coordination for the Improvement of Higher Education Personnel (CAPES n. 001). The animals were divided into two groups: Control (shaded) and Experimental (unshaded, exposed to sun from day 70 to 120 of gestation). Maternal well-being was monitored via behavioral and physiological parameters, while fetal evaluation was performed using B-mode and Doppler ultrasonography [2]. The parameters assessed included heart rate and Doppler flowmetry (Pulsatility Index - PI, Resistance Index - RI, and End-Diastolic Velocity - EDV) of the umbilical artery on days 70, 80, 100, 120, and 135. Maternal ethological data confirmed significant thermal discomfort in the Experimental group, characterized by a higher frequency of tachypnea (43.75% vs. 25% in Control), open-mouth panting (3.10%), and episodes of apathy. Regarding fetal hemodynamics, although HR, PI, and RI followed physiological reduction patterns with gestational age in both groups ($p > 0.05$), a significant reduction in umbilical EDV was observed in the Experimental group at Day 120 (16.8 cm/s) compared to the Control group (35.72 cm/s; $p = 0.011$). This reduction in diastolic flow suggests increased peripheral vascular resistance and subclinical placental insufficiency induced by thermal stress. Dopplerfluxometric indices are essential for monitoring the integrity of maternal-fetal structures in sheep [3]. Despite these alterations, no significant differences were found in the induction-to-lambing interval ($p = 0.63$) or placental expulsion time ($p = 0.48$). We conclude that Triplex Doppler is a sensitive tool for detecting subclinical placental insufficiency, evidenced by reduced diastolic flow. Although solar radiation triggers maternal stress and hemodynamic changes, the fetoplacental unit demonstrates robust compensatory mechanisms that preserve neonatal vigor.

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Posters

DETECTION OF MICROPLASTICS IN EQUINE AND BOVINE FOLLICULAR FLUID AND IN EMBRYO CULTURE MEDIA

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This study aimed to investigate the presence of MPs in bovine and equine follicular fluid collected from slaughtered ovaries and in media for in vitro embryo production (IVEP). The effect of species, age, and origin were evaluated on follicular fluid samples and different media commonly used in IVEP, routinely stored at 4°C in plastic tubes (TCM, DMEM, SOF) or glass bottles (DPBS), were analyzed before and after incubation under standard conditions (38.5°C, 5% CO2 for 24 hrs) in plastic petri dishes. All samples were treated with KOH, for removing organic matter, and filtered using MF-MILLIPORE™ filters (pore size: 0.025 µm; non-sterile MCE membranes). For equine follicular fluid, Anodisc™ filters (diameter: 47 mm, pore size: 0.02 µm) were used. Before filtering, the filter was pre-wetted with 5 ml of Milli-Q water to promote adhesion of the filter to the flask and ensure proper homogenization of the sample. Filters were then stained with Nile Red [1] and analyzed with a fluorescence microscopy at 400x magnification. Ten fields per cm^2 were scanned. The maximum length (µm) and area ($µm^2$) of the fluorescent particles were measured using ImageJ (version 1.54p). Given the presence of particles also in some blanks, the number of particles found on each filter was corrected by subtracting the number present in the blank from that in the sample (C-B), allowing the removal of any environmental or MilliQ water contamination. Based on the dilutions and the examined areas, the number of particles/cm^2 (part/cm^2: (C-B)×100/2.38) and the number of particles/ml (part/cm^2/17.4) were calculated. Data (% or mean±SD) were analyzed using SPSS, and significance was considered at $p<0.05$. Microscopic observation revealed particles in all analyzed samples, except for DPBS, with heterogeneous morphology and sizes >100nm, which can be classified as MPs. No differences between filamentous and polygonal forms were observed in bovine follicular fluid (43.30% vs 57.7%) and IVEP media (51.40% vs 48.60%; binomial test, $p>0.05$). In equine follicular fluid, polygonal particles were significantly more abundant than filamentous ones (81.80% vs 18.20%; binomial test, $p<0.05$). No differences were observed in MPs concentration between bovine (2047.00±1406.28) and equine (2548.34±1444.67) follicular fluid (Mann-Whitney U test for independent variable, $p>0.05$), nor in the percentage of positive samples (25.00% vs 45.00%; Mann–Whitney U test, $p>0.05$). In the bovine species, no differences were found in the MPs concentration between young (2193.28±1579.34) and adult animals (1462.18±0.00) or based on origin (GLM test, $p>0.05$). In the equine species, mares >15 years showed a significantly higher percentage of positive samples (85.72%) compared to those <15 years (14.28%; GLM test, $p<0.05$), while MP concentrations tended to be higher in older animals (GLM test, $p=0.063$). No significant differences were observed based on origin (GLM test, $p>0.05$). After incubation, significantly higher amounts of MPs were detected in all IVEP media samples (T-test, $p<0.05$). This study provides novel evidence of microplastic contamination in the reproductive system of domestic animals and in IVEP systems, highlighting that MPs contamination is widespread, even in controlled laboratory environments. The presence of MPs in the ovarian microenvironment raises concerns about their potential impact on oocyte quality, fertilization and embryo development. Further investigations are required to better understand their biological effects on fertility and reproductive outcomes.

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BEHAVIORAL MONITORING OF TWO MALE GREY SEALS (*HALICHOERUS GRYPUS*) UNDERGOING REVERSIBLE REPRODUCTIVE CONTROL IN A CONTROLLED ENVIRONMENT

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Zoos play a relevant role in the conservation of threatened species by supporting both in situ and ex situ conservation efforts through reproductive management programmes aimed at preserving genetic diversity. Maintaining animals under controlled conditions for conservation purposes requires constant welfare monitoring, as inadequate management may lead to maladaptive behaviors, pathological conditions and reduced reproductive success [1].

The aim of this preliminary study was to evaluate the efficacy of a subcutaneous Deslorelin acetate implant in modulating behavior in two male greyseals (Halichoerus grypus) housed at Bioparco di Roma, hypothesising that treatment could reduce mutual aggression and positively affect welfare.

The study was conducted between February and June 2025. Behavioral observations were performed in two Phases: 1) after previous implantation of Deslorelin acetate in subject 1; 2) after the same treatment in subject [2] Behavioral data were collected using scan sampling with continuous recording. A specific reproductive ethogram [2] was used to monitor interactions and behavioral patterns exhibited by the two subjects during the reproductive period. Observations were carried out in two daily sessions of 20 minutes each, for a total of 3560 minutes. Videos were analysed using Behavioral Research Interactive Software BORIS [3].

Observed behaviors were grouped into three categories: Active, Inactive and Agonistic. Duration and frequency of occurrence were exported to an Excel spreadsheet and analysed using non-parametric statistics (Shapiro-Wilk and Wilcoxon signed-rank tests), with significance set at $p<0.05$.

The results showed specific behavioral modifications associated with treatment. Active behaviors differed significantly between Phase 1 (mean = 6363.87 s; median = 2657.26 s) and Phase 2 (mean = 5430.7 s; median = 3443.79 s; $p = 0.038$). Agonistic behaviors decreased from Phase 1 (mean = 157.07 s; median = 79.62 s) to Phase 2 (mean = 41.85 s; median = 19.22 s), although the difference was not statistically significant ($p = 0.320$). Inactive behaviors showed no significant differences between Phase 1 (mean = 582.93 s; median = 484.66 s) and Phase 2 (mean = 570.76 s; median = 429.42 s; $p = 0.820$).
These findings indicate greater expression of behaviors compatible with improved welfare, as well as a redistribution of time towards activities more suitable for a controlled environment. This suggests that the implant not only reduced aggression, but also promoted a broader, more natural, and less conflict-related behavioral repertoire.

In conclusion, the study suggests that the hormonal implant had a marked impact on behavioral modulation, particularly on agonistic behaviors. Hormonal monitoring results, obtained through non-invasive biological sampling collected during the same study period, will be essential to support the initial hypothesis and confirm the welfare conditions of these animals in their ex situ habitat.

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ESSENTIAL OILS IN THE TREATMENT OF EQUINE ENDOMETRITIS: A PRELIMINARY *IN VITRO* STUDY ON ENDOMETRIAL EPITHELIAL CELLS

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Equine endometritis is one of the main causes of infertility in mares and represents a significant issue in equine reproduction, leading to reduced fertility, premature luteolysis, and embryonic loss, with economic and welfare implications [1]. Conventional treatments rely on antibiotics, but their extensive use has driven antimicrobial resistance, highlighting the need for alternative therapies [2]. Essential oils have gained interest for their antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory properties and may offer alternative treatment options [3]. This study evaluated *in vitro* the effects of thyme (*Thymus vulgaris*), oregano (*Origanum vulgare*), cinnamon (*Cinnamomum cassia*), and clove (*Syzygium aromaticum)* essential oils, devil's claw (*Harpagophytum procumbens*) extract, and two mixtures of unknown composition on equine endometrial epithelial cells (EECs). The study consisted of two experimental phases: isolation, culture, and characterization of EECs, followed by the assessment of the cytotoxicity of these compounds on the selected epithelial cell population. Cells were isolated using two different isolation methods (slicing and scraping) and two digestion protocols (Trypsin-EDTA and Type I Collagenase). The resulting population showed heterogeneous morphology (epithelial- and fibroblast-like cells) and variable growth rates depending on fetal bovine serum (FBS) concentration. Colony-forming ability and *in vitro* differentiation were not observed. Cells cultured with 10% FBS formed spheroids, demonstrating their ability for cell–cell adhesion and 3D organization, while 2.5% FBS did not. All cells were positive for CD90 and CD73, negative for CD34 and CD45. Immunocytochemistry confirmed heterogeneity, with higher pan-cytokeratin expression in 2.5% FBS cells. This population was selected for subsequent experiments. Cell viability was measured by MTT assay at 6, 24, and 48 hours of *in vitro* culture. At 6 hours, thyme (0.5%), clove (0.5% and 1%), and Mixture 1 were non-cytotoxic; however, the solvent DMSO (0.1%) negatively affected cell viability. After repeating the assay, clove at 0.5% remained non-cytotoxic, and at 1% significantly increased viability (~300%; $P<0.05$). At 24 hours, clove maintained a non-cytotoxic profile, while cinnamon (1%) induced a marked increase in cell viability. After 48 hours, a significantly positive effect of clove essential oil (0.5% and 1%) on cell viability was confirmed ($P<0.05$), with increases of approximately 20–30% for 0.5% and up to 200% for 1% compared to the control, whereas all other essential oils showed cytotoxic effects.

In conclusion, this study allowed the development of an *in vitro* model based on EECs to evaluate the effects of essential oils on uterine cells. Among the tested compounds, clove essential oil showed the most promising results, maintaining or enhancing cell viability, while most other essential oils showed cytotoxic effects under the tested conditions. These results suggest that some essential oils may represent potential candidates for alternative treatments for equine endometritis, but further studies are needed to assess metabolism, optimal doses, and safety before *in vivo* use.

Research founded by: APA-CT S.r.l

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MICROBIOLOGICAL PROFILE AND ANTIMICROBIAL RESISTANCE IN EQUINE UTERINE INFECTIONS: A FIELD STUDY

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Antimicrobial resistance (AMR) is a growing global concern in veterinary medicine, requiring a more prudent use of antimicrobial agents. In equine reproduction, uterine infections are frequently managed with empirical treatments, despite increasing evidence supporting the need for culture and antimicrobial susceptibility testing to guide therapy (1,2). The aim of this study was to evaluate the prevalence of uterine bacterial infections and antimicrobial susceptibility patterns in mares under field conditions. A total of 48 uterine swabs collected from pluriparous sport mares (jumping and trotting) in the province of Ragusa (Sicily, Italy) were analyzed. Sampling was performed during the breeding season of 2025. Sampling was performed during estrus, with the cervix open. A sterile double-guarded swab was used. After passing through the cervix, the inner swab was exposed to collect the uterine sample under aseptic conditions. The swab was then retracted into its protective sheath before removal from the uterus. Samples were delivered to the laboratory within 24 hours (Biogene Laboratory, Catania, Italy). All samples were subjected to aerobic and anaerobic bacterial culture, followed by identification and antimicrobial susceptibility testing (Vitek 2 system, CLSI/VetCAST guidelines). Descriptive statistical analysis was performed using SAS software (ver. 9.4, SAS Institute Inc., USA). Overall, 62.5% of samples showed no bacterial growth, while 37.5% were culture-positive. Mixed infections were detected in 10.5% of positive cases. Anaerobic bacteria were identified in 23% of positive samples. The most frequently isolated bacteria included Streptococcus equi subsp. zooepidemicus, Streptococcus dysgalactiae subsp. equisimilis and Streptococcus gallolyticus, followed by Staphylococcus xylosus, Staphylococcus schleiferi and Staphylococcus pseudintermedius. Gram-negative isolates included Escherichia coli, occasionally detected in mixed infections with Enterococcus gallinarum. Anaerobic isolates included Anaerococcus prevotii and facultative anaerobic streptococci. Antimicrobial susceptibility patterns were heterogeneous across isolates. Ampicillin showed an overall effectiveness of 60%, with resistance observed in 40% of isolates, particularly in Escherichia coli and Anaerococcus prevotii, while Streptococcus spp. remained largely susceptible. Resistance to tetracyclines (>50%) was mainly associated with Staphylococcus spp. and Streptococcus spp. Fluoroquinolone resistance (33%) was observed primarily in Escherichia coli and opportunistic staphylococci. Multi-drug resistance patterns (25%) were identified especially in Staphylococcus xylosus, Staphylococcus schleiferi and Anaerococcus prevotii. In contrast, Streptococcus spp. retained high susceptibility to beta-lactams, while Anaerococcus prevotii remained susceptible to metronidazole and clindamycin. These findings demonstrate that uterine infections in mares are microbiologically heterogeneous. The presence of AMR across different bacterial groups reinforces the importance of culture and antimicrobial susceptibility testing prior to treatment. A targeted approach is essential to improve clinical outcomes and support antimicrobial stewardship in equine practice.

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BEYOND REPRODUCTIVE EXAMINATION: OXIDATIVE STRESS AND IMMUNE INDICATORS ASSOCIATED WITH FERTILITY AT FIRST ARTIFICIAL INSEMINATION IN DAIRY COWS

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Reproductive inefficiency in high-producing dairy cows remains a major constraint under field conditions and cannot be fully explained by clinical findings alone. Increasing evidence indicates that systemic factors, including oxidative stress and immune activation, play a key role in fertility, particularly during the transition period, when metabolic imbalance and inflammatory processes may impair ovarian function, uterine environment and embryo survival. This study evaluated the association between fertility at first artificial insemination (AI) and systemic parameters, including antioxidant status, metabolic indicators and immune activation, in Holstein cows under field conditions. A total of 149 cows (85–150 DIM) from a commercial herd were enrolled and divided into first AI (n = 96) and cows with ≥1 previous unsuccessful AI (>1 AI; n = 53). All animals underwent rectal palpation and transrectal ultrasonography (TUS), with evaluation of uterine status and ovarian structures, including dominant follicle diameter. Blood samples were analyzed for progesterone, fructosamine, leptin, vitamin C, vitamin E, total polyphenols and leukocyte counts. Data were analyzed using ANOVA, chi-square test and Cohen's kappa. Agreement between palpation and TUS was limited (63%), with κ = 0.15, indicating poor concordance. Using leukocyte count (>11,000 WBC/µL), 44 cows were classified as inflamed and 103 as normal; diagnostic sensitivity of both methods was 52%. Non-pregnant cows showed higher neutrophil proportions, supporting the presence of subclinical inflammatory conditions potentially affecting fertility. Dominant follicle diameter was not associated with pregnancy outcome, confirming the limited predictive value of morphological assessment alone under field conditions. In contrast, antioxidant parameters were associated with fertility. Total polyphenols were higher in pregnant vs. non-pregnant cows (1 AI: 1.76 vs. 1.59; >1 AI: 1.77 vs. 1.59; $p < 0.05$). Vitamin C was higher in pregnant cows at first AI (3.79 vs. 3.59 mg/L; $p < 0.05$), while in >1 AI values were 3.84 vs. 3.77 mg/L. Vitamin E was higher in pregnant cows in >1 AI (3.12 vs. 2.77 mg/L; $p < 0.05$), with no difference in 1 AI (2.8 vs. 2.8 mg/L). Metabolic indicators were not associated with fertility (fructosamine: 222 vs. 221 µmol/L in 1 AI; 235 vs. 229 µmol/L in >1 AI; leptin: ~2.8–2.9 ng/mL; $p > 0.05$). Progesterone at AI was higher in non-pregnant cows (1 AI: 0.9 vs. 0.75 ng/mL; >1 AI: 1.4 vs. 1.1 ng/mL), suggesting incomplete luteolysis or altered endocrine dynamics may reduce conception. Overall, these findings indicate that fertility is strongly influenced by systemic oxidative and inflammatory status rather than metabolic or reproductive tract factors alone. Postpartum antioxidant supplementation and evaluation of antioxidant biomarkers may support evidence-based herd management and improve identification of at-risk cows.

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OPTIMIZATION OF SEPARATION METHODS FOR FROZEN–THAWED OVINE SEMEN IN IVF APPLICATIONS

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The use of frozen–thawed semen is a key tool in ovine assisted reproduction, particularly for in vitro fertilization (IVF), which remains the most widely applied technique as intracytoplasmic sperm injection (ICSI) is not yet fully optimized in this species [1]. However, cryopreservation introduces structural and functional damage to spermatozoa and leaves residual cryoprotectants that may exert toxic effects on oocytes and early embryos [2]. Therefore, the identification of effective sperm processing protocols able to remove these compounds while preserving sperm quality is essential to improve IVF outcomes.

This study evaluated different centrifugation protocols applied to frozen–thawed ovine semen to identify the most suitable method for IVF applications. After thawing, semen samples were diluted 1:1 with SOFM medium to facilitate the removal of cryoprotectants. Four experimental conditions were tested: (i) dilution only without centrifugation (control), (ii) centrifugation at 150 × g for 10 minutes, (iii) centrifugation at 100 × g for 10 minutes, and (iv) centrifugation at 100 × g for 15 minutes, all performed at room temperature. Sperm motility parameters were analyzed using a Computer-Assisted Sperm Analysis (CASA) system, including total motility, progressive motility, and the percentage of rapid cells.

The mean motility values recorded immediately after thawing were 38% for total motility, 10% for progressive motility, and 12% for rapid cells. The dilution-only protocol maintained the initial levels of progressive motility and rapid cells, but resulted in a decrease of total motility of approximately 16%. Centrifugation at 150 × g for 10 minutes at room temperature yielded the best results, with an average increase in total motility of 7.7%, along with a 2% increase in both progressive motility and rapid cells percentage, indicating an overall improvement in sperm kinetic performance. Conversely, centrifugation at 100 × g for 10 minutes negatively affected sperm quality, leading to a reduction of total motility by 16%, progressive motility by 7%, and rapid cells by 8%. Increasing the centrifugation time at the same force (100 × g for 15 minutes) resulted in a smaller decrease in total motility (−10.8%) and no variation in progressive motility, although the percentage of rapid cells still declined by 8%.

These findings demonstrate that centrifugation conditions markedly influence the quality of frozen–thawed ovine spermatozoa. Among the tested protocols, centrifugation at 150 × g for 10 minutes appears to represent the most effective compromise between removal of cryoprotectants and preservation, or even improvement, of sperm motility parameters. Ongoing studies are currently assessing the impact of these treatments on fertilization rates and embryo development in IVF systems to validate their practical application in ovine reproductive technologies.

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Posters

UNILATERAL CRYPTORCHIDISM IN A XY GOAT: SURGICAL APPROACH AND HISTOLOGICAL FINDINGS

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A 30-month-old mixed breed goat of 21 kg, with unknown clinical history, was submitted to the Clinic for Ruminants and Swine of the Veterinary Teaching Hospital (University of Milan) after a confiscation. During general examination, the right testicle was not appreciable in the scrotum. The buck had a general childish appearance, with no horns or signs of dehorning, and infantile penis. After a transabdominal ultrasound exam, the cryptorchid testis (CT) was identified in the abdomen, closer to the right kidney than to the inguinal ring. Blood samples were taken for blood gas and biochemical analyses before surgery, together with a sample for the karyotype exam.

After sedation and general anesthesia, the upper area of the right flank was shaved and aseptically prepared for surgery. Also, preparation for the scrotal testicle removal was performed. After incision of the skin, subcutaneous and muscular layers, peritoneum was dissected and access to the abdomen was gained. The CT was easily palpated and, once isolated with sterile gauzes, two ligatures were applied, one on the pampiniform plexus and one on the deferent duct. After removal, closure of the body wall and skin layers were performed. The scrotal testis was excised using a classical open technique. After this, testes were fixed in 10% neutral buffered formalin and sent to the Pathology Unit for histopathology examination. Goat recovered well from surgery, with the support of antibiotic treatment for mild infection of the scrotal site. Macroscopically, both testicles appeared undersized as compared to an adult and sexually mature buck, with the CT being way smaller than the contralateral.

Histologically, the CT was characterized by the complete absence of germ cells and spermatogenesis, as opposed to the normal counterpart. The only cell population present in the seminiferous tubules of the retained testis consisted of Sertoli cells adherent to the basement membrane. The “Sertoli cell only” pattern was confirmed by immunohistochemistry with vimentin, which positively marked Sertoli cells in both testicles.

The unilateral right cryptorchidism is more frequent than the left one, whilst the abdominal localisation, although relatively common, is normally described as behind the bladder neck, mildly different from what found in this case. About the surgical technique, the flank approach allowed an optimal recovery of the wound, in contrast with literature, that, albeit scarce, recommended a paramedian ventral approach [1]. In the Authors’ opinion, however, the lack of contact with the hay and floor and the less abdominal pressure on this side when compared to the ventral one, reduce the risk of dehiscence and infection. Another advantage is the possibility of examining the surgical wound without need of restraining the animal, and this is of great help especially in field conditions.

Lastly, karyotype exam revealed normal 60,XY chromosomal configuration, and this is another peculiarity as infantile penis and appearance, together with cryptorchidism, are generally found in subjects affected by polled intersex syndrome [2]. In addition, PCR analyses confirmed the presence of the Sry gene as well as Amelx/y genes.

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CAN PRE-INSEMINATION ENDOMETRIAL CYTOLOGY PREDICT FERTILITY IN DAIRY CATTLE?

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Improving reproductive efficiency in dairy cows remains a major challenge for the sustainability and profitability of modern dairy farming. Within a broader research project aimed at reducing unnecessary hormone use and identifying biomarkers of infertility, this study focused on endometrial cytology as a potential indicator of subfertility. The trial was conducted in two medium-sized dairy farms, involving 137 multiparous Holstein cows monitored from dry-off until the second artificial insemination (AI). Animals were managed using either a Double-Ovsynch synchronization protocol or a precision-based strategy relying on natural heat detection through automated monitoring systems. Based on pregnancy outcomes, cows were retrospectively classified as fertile (pregnant after first (F1) or second (F2) AI) or subfertile (SF, two consecutive negative pregnancy diagnoses). Endometrial cytology samples were collected at key timepoints during the transition period (+10, +35, +45 DIM, and at first and second AI) using a cytobrush technique. Polymorphonuclear cell (PMN) percentages were determined to evaluate uterine inflammatory status in relation to fertility outcomes and reproductive management strategy. Data are expressed as mean ± standard error. A binomial generalized mixed model (GLMM) with a logit link function was applied, and pairwise comparison were performed using the Wald test (IBM SPSS Statistics 29). Statistical significance was set at $p<0.05$. Parity (secondiparous vs. multiparous), farm, and reproductive management were not significant ($P>0.05$). Time had a strong effect ($P < 0.001$), a higher rate of PMN was observed at +10 (15.81 ± 2.42) than all other timepoints (+35: 4.05 ± 0.97; +45: 4.10 ± 1.06; AI1: 2.88 ± 0.69; AI2: 4.01 ± 1.18), as well as at +35 PMN compared to AI1 and AI[2] Fertility had also a significant effect ($P = 0.005$), with a lower PMN percentage for F1 (3.92 ± 1.30) than SF (7.38 ± 0.94), while no differences were found for F2 (5.3 ± 1.20). The fertility x time interaction was significant ($P = 0.015$). Pairwise comparisons showed no differences between fertility groups at +10 and +35 ($P > 0.05$). At +45, F1 (1.50 ± 0.63) differed from SF (6.17 ± 1.96). At AI1, F1 (0.65 ± 0.26) differed from both F2 (2.87 ± 0.94) and SF (3.99 ± 1.22). At AI2, a difference was observed between F2 (0.68 ± 0.18) and SF (5.10 ± 1.55). In conclusion, endometrial cytology proved to be a sensitive tool for detecting differences in uterine inflammatory status associated with fertility outcomes. Although the conventional threshold for subclinical endometritis is set at 5% PMN, fertility-related differences emerged even below this cutoff, indicating that mild inflammation may still be biologically relevant. Notably, subfertile cows showed PMN values below the 5% threshold at first AI, but exceeded this threshold by the second AI, suggesting a worsening or persistence of uterine inflammation over time. This temporal pattern points to the presence of underlying processes occurring between inseminations that may compromise uterine health and fertility. Altogether, these findings highlight the importance of monitoring PMN dynamics rather than relying solely on fixed thresholds, to enable earlier identification of cows at risk of subfertility and to support more targeted and sustainable reproductive management strategies.

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LIPIDOMIC PROFILE OF DAIRY COWS ACROSS KEY REPRODUCTIVE TIMEPOINTS

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Dairy cows experience important physiological changes from late gestation to lactation and ovarian cyclicity resumption, which are associated with changes in lipid metabolism. The aim of this study was to characterize lipidomic variations across defined reproductive timepoints in dairy cows.

The study was conducted on 137 pluriparous Holstein cows monitored from dry-off until the second artificial insemination (AI); cows not pregnant after first AI were subsequently re-inseminated. Blood samples were collected at -21 days before calving, 10 and 35 days in milk (DIM), at first AI and second AI. Erythrocyte membrane fatty acids were analysed by gas chromatography and lipid indices were calculated. Statistical analyses were performed using RStudio (version 2023.12.1).

Several fatty acids and lipid indices showed significant changes across timepoints. Oleic acid (18:1 9c) and monounsaturated fatty acids (MUFA) were higher at 35 DIM and first AI compared with -21, 10 DIM and second AI ($P < 0.05$). Vaccenic acid (18:1 11c) increased from -21 and 10 DIM to reach the highest values at first AI, followed by a decrease at second AI ($P < 0.05$). Docosahexaenoic acid (DHA) was higher at -21 and 10 DIM and progressively decreased at first and second AI ($P < 0.05$). Linoleic acid (18:2 9c,12c) and polyunsaturated fatty acids (PUFA) were lower at 10 and 35 DIM and higher at second AI ($P < 0.05$). Daihomo-gamma-linolenic acid (20:3 8c,11c,14c) was higher before calving and decreased during early lactation and at first AI, with a partial increase at second AI ($P < 0.05$). Arachidonic acid (20:4 5c,8c,11c,14c) showed higher values at -21 and 10 DIM, decreased at 35 DIM and reached the lowest levels at first AI, followed by a slight increase at second AI ($P < 0.05$). The ω6 fraction decreased during early lactation and increased to the highest values at second AI ($P < 0.05$). The ω6/ω3 ratio increased at first and second AI, while the PUFA balance ω3/(ω3+ω6) decreased at these timepoints ($P < 0.05$). The peroxidation index was highest at -21 and lowest at first AI ($P < 0.05$).

In conclusion, the lipidomic profile of dairy cows changes significantly across reproductive timepoints, with distinct patterns observed from late gestation through lactation and ovarian cyclicity resumption. These findings suggest dynamic modulation of membrane lipid composition and inflammatory related lipid mediators across key stages of the production cycle.

Posters

EQUINE MESENCHYMAL STEM/STROMAL CELL SECRETOME: A POTENTIAL FBS ALTERNATIVE FOR EQUINE *IN VITRO* MATURATION

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In vitro maturation (IVM) of equine cumulus–oocyte complexes (COCs) is a critical step in equine embryo production. Despite the use of various supplements, maturation rates remain limited, likely due to non-physiological culture conditions compared to the natural follicular environment. Conditioned medium (CM) from mesenchymal stromal/stem cells (MSCs) may enhance IVM, as it contains growth factors and bioactive molecules. This study aimed to assess the effect of CM derived from equine Wharton's jelly mesenchymal stromal/stem cells (eWJ-MSCs) on nuclear maturation of equine oocytes. For CM production, eWJ-MSCs were isolated from the umbilical cord of three healthy mares after physiological parturition and expanded before freezing. The CM was harvested from re-expanded thawed cells cultured for 6 h in DMEMF12 without FBS (eWJ-MSC-CM). Equine COCs were collected by aspiration from follicles of abattoir-derived ovaries. Compact COCs were selected and randomly allocated either to direct IVM or to an overnight holding at controlled room temperature (22°C) in Hepes-Synthetic Oviduct Fluid (HSOF). Following holding, oocytes were subjected to IVM for 20 h or 26 h in a basal DMEM/F12-based medium supplemented with 50 ng/ml EGF, 100 ng/ml IGF-1, 0.1 IU/ml porcine FSH-LH, and 10% FBS (direct IVM: n = 85; holding: n = 73) or CM (direct IVM: n = 101; holding: n = 93). After 20 h or 26 h of IVM, COCs were denuded, stained with 10 µg/mL bisbenzimide (Hoechst 33342), and examined under an epifluorescence microscope to assess nuclear maturation (mature oocytes: metaphase II stage (MII); immature oocytes: from germinal vesicle to metaphase I stage (IMM); degenerate oocytes: DEG). The experiment was done in 3 replicates. Data were analysed using a generalized estimating equation (GEE) with logit link and Wald pairwise tests (IBM SPSS Statistics 29), with significance set at $P<0.05$. Data are presented as mean ± standard error. Maturation rates were significantly influenced by time ($P=0.003$) and group ($P=0.006$), with a significant interaction among time, medium, and holding ($P<0.001$). Considering direct IVM, MII rates at 20 h in CM (42.1 ± 2.6%) were higher ($P = 0.01$) than in FBS (35.5 ± 7.1%), while at 26 h maturation increased in both CM (65.2 ± 4.5%, $P < 0.001$) and FBS (57.2 ± 10.5%, $P = 0.029$). Notably, after holding, MII rates at 20 h were higher in CM (55.7 ± 3.0%) compared with its direct IVM counterpart, whereas no difference was observed in FBS (42.0 ± 2.4%, $P = 0.449$), which also remained lower than CM at 20 h after holding ($P < 0.001$). Moreover, in both groups, MII rates at 26 h after holding did not significantly increase compared with those at 20 h (CM: 58.2 ± 3.2%; FBS: 56.9 ± 7.7%). The percentage of immature oocytes decreased over time ($P < 0.001$) and was lower ($P < 0.001$) in the holding groups compared with direct IVM counterparts. Conversely, holding tended to increase degeneration rates ($P = 0.081$), with a significant time effect ($P < 0.001$) and higher values in FBS than in CM ($P = 0.003$). Despite the limited sample size, CM appears to improve maturation kinetics, as indicated by higher MII rates at 20 h, and to exert a protective effect, as reflected by lower degeneration rates. In contrast, holding at 22 °C did not fully prevent meiotic resumption, as demonstrated by MII rates at 20 h, and was associated with increased oocyte degeneration. Overall, these findings suggest that CM could serve as an alternative to FBS for equine oocyte IVM. Further studies are needed to evaluate the impact of CM on oocyte developmental competence.

Posters

MINERAL ION CONCENTRATIONS CAN AFFECT HYDROXYAPATITE PRODUCTION IN CANINE MESENCHYMAL STEM CELLS: NEW PERSPECTIVE FOR BONE REGENERATION

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Mesenchymal stem cells (MSCs) are applied in regenerative medicine owing to their ability to differentiate into osteogenic lineages, a process marked by hydroxyapatite deposition. Calcium (Ca^{2+}) is a well-established driver of mineralization, while magnesium (Mg2+) affects osteogenic activity, mineral metabolism, and enzyme functionality. Mg is vital for the activation of vitamin D, maintaining the balance of Ca and P to promote healthy bones [1]. Nevertheless, the combined impact of Mg2+ and Ca2+ on hydroxyapatite deposits remains unclear [2] in canine in vitro osteogenesis. This study aims to identify the optimal ratio of Mg2+ to Ca2+ for hydroxyapatite accumulation in canine MSCs (cMSCs), exploring whether Mg2+ acts synergistically with Ca2+ to promote deposition in the presence of vitamin D3 and K2 under various osteogenic conditions. In dogs the normal plasma Ca2+ and Mg2+ concentrations range from 9.4-11.1 mg/dL and 1.5-2.1 mg/dL respectively. For this investigation, cMSCs isolated from the synovial cavity of the stifle joint were cultured in a medium supplemented with three different combinations of Ca2+ (10 mg/dL, 22 mg/dL, and 3 mg/dL) and Mg2+ (1.8 mg/dL, 4.2 mg/dL, and 0.4 mg/dL), representing normal, hyper, and hypo concentrations during the osteogenesis process. Osteogenic differentiation was assessed by osteo-image analysis of hydroxyapatite formation and gene expression of osteogenic markers, including CD90 (Thy-1), osteocalcin (BGLAP), and SP7 (osterix), reflecting MSC phenotype and both early and late differentiation stages. Our findings indicate the absence of the CD45/leukocyte common antigen marker, as verified by FACS analysis on the cells, suggesting that the cells were negative for hematopoietic origin. Additionally, cMSC exhibited bi-lineage differentiation capabilities (adipogenesis and osteogenesis), which is another essential characteristic confirming their MSC identity. The cells demonstrated increased mineral accumulation at higher concentrations combination (Ca^{2+} 22mg/dL; Mg^{2+} 4.2mg/dL). The gene expression levels of CD90 ($P = 0.038$) and SP7 ($P = 0.020$) were significantly influenced by treatments, indicating that the experimental conditions primarily impacted early osteogenic gene expression (SP7), however the MSC phenotype (CD90) gene expression also enhanced. In contrast, the expression of OCN ($P = 0.336$) remained stable across the groups, suggesting no significant effect on late-stage osteogenic differentiation. These findings indicate that Ca^{2+} significantly enhanced hydroxyapatite accumulation, whereas Mg^{2+} did not contribute synergistically and rather, exerted a dose-dependent inhibitory impact without influencing ultimate mineralization. This study implies that the interaction between Ca^{2+} and Mg^{2+} plays a crucial role in the early osteogenic commitment of MSCs [3] without markedly affecting the final mineralization process. In conclusion, the present study shows that low levels of Mg^{2+} and Ca^{2+} decrease early and late osteogenesis, but when both minerals are increased, there is a higher probability of enhanced in vitro osteogenesis.

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Posters

BIOBANKING AND ONE HEALTH: OPTIMISING THE INTERCONNECTION OF HUMAN, ANIMAL, AND ENVIRONMENTAL HEALTH RESEARCH

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In its 2025–2027 Work Programme, **BBMRI-ERIC** (Biobanking and BioMolecular Resources Research Infrastructure – European Research Infrastructure Consortium) aims to integrate the **One Health** approach across different components of its research infrastructure (RI). As a first step, a Europe-wide mapping of non-human biobanks and related networks was carried out to identify potential partners and foster transdisciplinary collaborations within the One Health framework.

Within **BBMRI.it**, the Italian national node of BBMRI-ERIC, a national scouting activity was conducted to identify relevant initiatives and infrastructures operating in the veterinary and environmental domains. Among these, the **Italian Biobank Network of Veterinary Resources (BVR)** was identified as a particularly relevant initiative. This network was established with the aim of collecting, preserving, and managing different types of biological resources generated through veterinary health, food security, and research carried out by the Istituti Zooprofilattici Sperimentali (II.ZZ.SS.). In particular, the biobank network includes materials produced within the activities Laboratories belonging to the Italian Ministry of Health and the **WOAH** (World Organisation for Animal Health) National Reference Centres for important veterinary diseases, including zoonoses. Currently, five Istituti Zooprofilattici Sperimentali (IZS) contribute to the network (https://biowarehouse.net/), providing a distributed infrastructure for veterinary biological resources. The Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER) also hosts the WOAH Collaborating Centre for Veterinary Biological Biobank.

The presentation of this initiative to BBMRI-ERIC created the opportunity to include the Italian veterinary biobank network in the recently funded Horizon Europe project **CaBRIOH (Capacity Building in Research Infrastructures towards One Health)**, coordinated by BBMRI-ERIC and involving the **University of Bologna** as a partner.

The project recognises that the success of the One Health approach largely depends on researchers and technical and scientific professionals who ensure scientific excellence and global competitiveness, respond rapidly to emerging threats, and integrate knowledge across disciplinary domains. Since collaborative action across sectors is essential to achieve greater impact, CaBRIOH aims to address the current lack of cross-sectoral training by developing a comprehensive training programme for technical staff working in research infrastructures. The project will support **upskilling and reskilling across different RI professional sectors** and will implement a dedicated **training platform ("one-stop-shop") within BBMRI-ERIC**, designed to facilitate unified access to training opportunities related to One Health and research infrastructures.

Colleagues from veterinary biobanks will be actively involved, together with experts from **VetMedUni Vienna**, in the development of a **One Health Competence Matrix**, which will provide a shared European reference framework for research infrastructures working in this field. In addition, the University of Bologna will be responsible for organising a **One Health Summer School** in 2027, supported by the project and aimed at strengthening interdisciplinary competencies among RI professionals.

The successful inclusion of the Italian veterinary biobank network in this project demonstrates how participation in European research infrastructure consortia can provide valuable opportunities to showcase national initiatives and foster cross-domain interaction, collaboration, and capacity building in the One Health ecosystem.

Posters

BIODISTRIBUTION OF SOME HEAVY METALS (LEAD, MERCURY, ARSENIC AND CADMIUM) IN THE PLUMAGE OF THE CANARY (*SERINUS CANARIA*) AND THE EFFECT OF AGE AND SEX: A BIOLOGICAL MODEL FOR ONE HEALTH

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Heavy metal pollution is a major global concern due to environmental persistence, bioaccumulation and toxicity. Chronic exposure to lead (Pb), mercury (Hg), arsenic (As) and cadmium (Cd) has been associated with neurological, renal and developmental disorders in both humans and animals. Therefore, reliable non-invasive biomonitoring tools are increasingly needed within the One Health framework. This study investigated feathers as bioindicator matrices using the canary (Serinus canaria) as an experimental sentinel species. A total of 24 clinically healthy domestic canaries from a private breeding farm were enrolled and equally allocated into four groups: adult males (n=6), adult females (n=6), juvenile males (n=6) and juvenile females (n=6). Birds were maintained under standardized husbandry conditions, with controlled environmental parameters including temperature, relative humidity and air exchange, regulated through thermostats, dehumidifiers and natural/forced ventilation systems. Feathers were collected non-invasively during routine handling from four anatomical regions (nape, tail, flight feathers and rectrices) in order to assess spatial and temporal patterns of metal deposition. Feed samples were also analyzed to discriminate dietary intake from environmental exposure. [1] Concentrations of Pb, Hg, As and Cd were determined in feather and feed samples. Statistical analysis was performed using one-way and factorial ANOVA with GraphPad Prism software. Differences among feather regions, sex, age classes and relative accumulation of each metal were evaluated. Statistical significance was set at $p<0.05$. Metal concentrations were significantly higher in feathers than in feed for all investigated elements ($p<0.05$), with highly significant differences for Pb and Hg ($p<0.001$). Feed concentrations (Pb: 0.105 mg/kg; Hg: 0.005 mg/kg) were insufficient to explain the observed burdens, supporting a predominant contribution of environmental exposure and efficient sequestration during feather keratinization. Pb was the most abundant contaminant, reaching the highest levels in tail (0.38±0.07 mg/kg) and flight feathers (0.37±0.07 mg/kg), particularly in adult females. Hg, As and Cd showed comparable distribution patterns, with greater accumulation in tail and flight feathers. Mercury concentrations increased with age, peaking at 0.027±0.007 mg/kg in adult females. Age represented the main determinant of bioaccumulation, whereas sex significantly influenced only Pb levels. The detection of metals in juvenile birds indicates early-life exposure, suggesting contamination sources within confined/domestic environments potentially shared with humans. Tail and flight feathers appear to be the most informative sampling sites, supporting standardized non-invasive monitoring protocols. Canary plumage may represent a practical and reliable biomonitoring matrix for environmental surveillance, risk assessment and preventive public health strategies within the One Health perspective. [2,3]

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Posters

DETERMINING THE STAGE OF A BITCH'S ESTRUS CYCLE USING INFRARED THERMOGRAPHY: METHODOLOGY AND SETTINGS

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Infrared thermography is a non-invasive diagnostic technique capable of detecting variations in tissue surface temperature by recording the infrared radiation emitted from the body surface. In veterinary medicine, this technology has increasingly been used to monitor physiological processes associated with changes in peripheral blood flow and metabolic activity, which are known to occur during the oestrus cycle of the bitch. In the present study, the applicability of infrared thermography for identifying the oestrus stage in bitches was evaluated by analysing thermal variations in the vulvar region. Thermographic images were acquired using a calibrated digital infrared thermal camera with a minimum resolution of 320 x 240 pixels. The emissivity parameters were appropriately set for biological tissues, and acquisitions were performed under controlled environmental conditions in order to minimize potential thermal interference. Thermographic recordings were obtained while maintaining a constant distance of approximately 0.8-1 m between the thermal camera and the perivulvar area of the animal, with the camera oriented perpendicular to the body surface to ensure measurement reproducibility. Video sequences were recorded to allow the subsequent selection of the most representative thermograms. Particular attention was devoted to the preparation of the skin surface and to the reduction of potential interfering factors, including the presence of fur, environmental variations and the animal's movements. The reproductive phase of each of the 9 bitches was determined using a multimodal approach. In particular, the classification of the oestrus cycle phase was carried out by integrating anamnestic and behavioural data with the clinical assessment of the external genitalia, including the combined interpretation of vulvar parameters, the Amantea sign, the vaginal cytological examination (Diff-Quick staining) and serum progesterone concentration (by means of Chemiluminescence). This integrated approach allowed for the accurate assignment of each subject to the specific phase of the oestrous cycle. Thermographic analysis revealed measurable temperature variations in the external genital region, reflecting physiological changes in local vascularization that occur during the different phases of the oestrus cycle. Mean temperature values calculated on thermographic vulvar regions of interest (ROI), increased from pro-oestrus (32.9 °C) to oestrus (34.7°C) and subsequently decreased during cyclical diestrus (30.4°C). These findings indicate that infrared thermography could represent a valuable complementary diagnostic approach for monitoring the reproductive status of the bitch, providing a rapid, non invasive and repeatable method for detecting the physiological changes associated with the different phases of the oestrus cycle.

NEUROMUSCULAR AGING IN DOGS: PRELIMINARY ELECTROPHYSIOLOGICAL EVIDENCE

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Aging induces progressive changes in neuromuscular function, but its specific impact on peripheral nerve integrity and skeletal muscle physiology remains insufficiently understood in veterinary medicine. This study aimed to investigate whether aging is associated with measurable functional changes in peripheral nerve conduction and muscle activity in dogs, using integrated electrophysiological approaches. Neuromuscular disorders represent a significant diagnostic challenge, requiring the integration of clinical and neurological evaluation with advanced tools such as electromyography (EMG) and nerve conduction studies (NCS) [1]. EMG enables the detection of muscle electrical abnormalities and differentiation between neurogenic and myopathic conditions, whereas NCS assess the integrity and conduction properties of peripheral nerves, distinguishing axonal from demyelinating processes [2]. Together, these techniques provide a functional assessment of the neuromuscular system, complementing structural evaluation.

Dogs represent a valuable translational model, as they spontaneously develop neuromuscular conditions with clinical and pathological features comparable to those observed in humans [3]. However, the physiological effects of aging on peripheral nerve function and muscle electrophysiological properties in dogs remain poorly defined, limiting the ability to distinguish age-related changes from early pathological alterations. To address this gap, 10 clinically healthy dogs of different breeds and sexes were stratified into three age groups: young (<2 years), adult (3–7 years), and senior (>8 years). Written informed owner consent was obtained for all dogs undergoing routine diagnostic procedures (including MRI), during which EMG was performed under the supervision of a board-certified veterinary anesthesiologist. Dogs were prospectively enrolled on an opportunistic basis according to predefined inclusion criteria and in compliance with institutional guidelines. EMG was conducted in the triceps brachii and biceps femoris muscles to assess muscle activity, while NCS evaluated radial, ulnar, and sciatic-tibial nerves. Supramaximal stimulation was used to record compound muscle action potentials (CMAPs). Preliminary findings indicate that aging is associated with a progressive decline in neuromuscular function. Senior dogs exhibited reduced CMAP amplitudes, prolonged latency, and decreased conduction velocity, suggesting age-related axonal loss and impaired myelin integrity. Increased temporal dispersion further indicates reduced synchronization of motor unit activation, reflecting alterations at both nerve and muscle levels. Together, these findings support the existence of an electrophysiological signature of peripheral nerve and muscle aging in dogs. Establishing age-specific reference values will improve diagnostic accuracy and facilitate the differentiation between physiological aging and neuromuscular disease, enhancing the translational relevance of canine models for studying neuromuscular aging.

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Posters

SALIVARY CORTISOL LEVELS IN DOGS INVOLVED AND NOT INVOLVED IN ANIMAL-ASSISTED SERVICES DURING AN APTITUDE TEST

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Currently, the evaluation of the suitability of dogs for their participation in Animal-Assisted Services (AAS) is not standardized and generally does not include the assessment of physiological parameters.

This preliminary study, part of the PNRR-funded project THE spoke 10, compared the cortisol response of dogs involved and not involved in AAS during a test aimed at assessing of their suitability for AAS.

A total of 52 dog-owner dyads participated in the Suitability Test (SuiTe) [1], including 17 dogs involved in AAS and 35 dogs not involved, balanced for age and sex [AAS: 5.8±3.0 years old; 7 females (2 intact, 5 spayed); 10 males (4 intact, 6 neutered); not-AAS: 5.9±3.1 years old; 17 females (6 intact, 11 spayed); 18 males (12 intact, 6 neutered)].

SuiTe is a 45 minutes pre-screening test aimed at assessing suitability of dogs in AAS. It consists in gradually exposing dogs to physical and social stimuli, such as food, toys, sounds, moving objects, handling and brushing performed by caregiver or strangers (both males and females), as a simulation of common and potentially stressful stimuli during AAS sessions. Salivary cortisol samples were collected before (T0) and after (T1) SuiTe and analysed using salivary cortisol ELISA kit (Salimetrics®). Data did not follow normal distribution. Mann-Whitney U test was used for between-group analysis (AAS dog vs not-AAS dogs) of cortisol levels at T0 and T1, and delta ($p<0.05$). Wilcoxon signed-rank test was used for within-group analysis (T0 vs T1 in both groups) ($p<0.05$).

Cortisol values (ng/ml) were all within physiological range (median [IQR] T0: 1.31 [1.02-1.66]; T1: 1.43 [1.10-1.98]) [2]. Cortisol values at T0 were slightly higher in AAS dogs that in not-AAS dogs (W=385, p=0.09, estimate: 0.31; 95% CI [-0.06; 0.61]; median [IQR] AAS: 1.52 [1.47-1.82]; not-AAS: 1.20 [0.92-1.53]), while no differences were observed at T1 ($p>0.05$; AAS: 1.58 [1.38-2.07]; not-AAS: 1.31 [1.06-1.88]). However, delta cortisol (T1-T0) did not differ between the two groups and, accordingly, no significant changes in cortisol were observed from T0 to T1 in each group ($p>0.05$).

Despite a slight increase at T1 was observed in both groups, the SuiTe did not induce a substantial peak in cortisol levels, and therefore it can be regarded overall as low stressful. The fact that AAS dogs arrived at the test (T0) with higher cortisol could be related to physiological arousal, i.e. excitement before the start of an activity, as found before AAS sessions. Some limitations of this preliminary study include a relatively small sample size, as well as the fact that the not-AAS group included dogs with a wider variability in terms of breed, size, previous experiences, and T0 cortisol levels.

Future analyses will include a larger sample size and additional physiological (e.g., blood pressure, heart rate, respiratory rate, temperature, salivary oxytocin) and behavioural parameters, alongside repeated testing to assess whether this pattern persists over time.

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Posters

AN OOCYTE CELL EXTRACTION SYSTEM TO STUDY EARLY POST-FERTILIZATION EVENTS OF SPERM STRUCTURES

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The sperm cell is highly specialized, with structures including the perinuclear theca (PT) surrounding the nucleus, outer dense fibers, the fibrous sheath around the flagellar axoneme, and a midpiece containing 50–80 mitochondria in a helical arrangement. While essential for fertilization, the post-fertilization fate of these structures is not fully understood. Soluble sperm factors trigger oocyte activation, meiotic completion, and the cortical reaction, whereas paternal mitochondria are eliminated according to the Mitochondrial Eve Paradigm, ensuring strict maternal inheritance of mtDNA [1]. This selective removal is critical, as mixed mtDNA can lead to severe disorders and is often incompatible with embryonic development [2]. To study these events, we adopted a previously established porcine cell-free system that mimics the early post-fertilization environment [3]. Sperm cells were washed, immobilized, and permeabilized, while oocytes from slaughterhouse ovaries were matured, denuded, and snap-frozen. The homogenized oocyte lysate, supplemented with a creatine kinase ATP system, was applied to sperm slides, allowing simultaneous tracking of numerous sperm–cytoplasm interactions. Sperm mitochondria were labelled with Mitotracker, and perinuclear and nuclear remodelling were monitored by immunolabeling and DNA staining. This system reproduces critical events, including PT solubilization marked by CRISP2 dispersal, a prerequisite for sperm nuclear decondensation and male pronucleus formation. The study focuses on mitophagy, where paternal mitochondria are degraded via the Ubiquitin-Proteasome System (UPS) and oocyte autophagy. SQSTM1 (p62) acts as an early adaptor, recognizing ubiquitin tags on paternal mitochondria exposed in the ooplasm. Validation of the system was achieved by monitoring CRISP2 dispersal, and we show that SQSTM1 selectively associates with sperm mitochondria, directing them to autophagosomes. Integrating these findings with real-time metabolic analyses will allow us to define the timing of paternal mitochondrial loss and its impact on the embryo's energy transition.

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INFLUENCE OF ATTACHMENT STYLE AND BEHAVIORAL TEST ON SALIVARY CORTISOL IN DOGS

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Animal Assisted Interventions have increased in number in the last decade. Still, little is known about the characteristics that dogs should have to be suitable for this kind of activity and even less on the strength and type of bond with their handler.

The aim of the study (approval of the organism in charge of animal welfare n. 69/2025), which is part of the PNRR funded project THE spoke 10, was to analyze possible associations between dogs' attachment style toward the caregiver, as assessed through the Ainsworth Strange Situation Test (ASST), and their cortisol response during two different behavioural tests: the Suitability Test (SuiTe) and the ASST.

The SuiTe is a pre-screening assessment designed to evaluate the suitability of dogs for Animal-Assisted Interventions (AAIs) by exposing them to social and physical stimuli; according to the evaluation performed by Russo et al. (2025) dogs were assessed as suitable (S) or unsuitable (U) for AAIs. The ASST was used to assess dog attachment styles; based on Riggio et al. (2021) dogs were classified as securely or insecurely attached to their caregiver.

Twenty dogs, not previously involved in AAIs, (11 females and 9 males; mean age 4.65 ± 2.47; size: large=11, medium=5, small=4) were evaluated both in Suite and ASST. Saliva was collected with Salivette® swabs before (T0) and after (T1) each test for cortisol quantification.

The qualitative assessment revealed 15 dogs as securely attached and 5 as insecurely attached to their caregiver. As for the Suite, 14 dogs were classified as unsuitable and 6 as suitable.

No significant difference was found for delta cortisol comparing securely and insecurely attached dogs in both SuiTe and ASST (Mann-Whitney test W=45 p=0.39 estimate:0.26, 95% CI [-0.351 0.764]). No association emerged between attachment style and suitability of the dogs (Fisher's exact test p=1 95% CI [0.008 7.421] OR=0.5).

Although after SuiTe there was a slight increase in cortisol and after ASST a slight decrease, delta cortisol did not significantly differ between the two tests (Wilcoxon signed-rank test V=51, p=0.079, estimate: -0.407, 95% CI [-0.864 0.0454]; median Suite: 0.029 vs median ASST: -0.35).

These results should be interpreted cautiously due to the small sample size; however, they suggest that attachment style alone may not be a predictor of physiological activation or of dogs' suitability for AAIs.

Interestingly, a non-significant trend toward different cortisol concentrations between the two tests was observed, with a decrease in cortisol levels during the ASST and a slight increase during the SuiTe. This pattern may reflect differences in the structure of the two procedures. In the ASST, cortisol levels may peak during the separation phase and subsequently the number and intensity of stimuli progressively decrease until the reunion with the caregiver, leading to an overall reduction between pre- and post-test measures. In contrast, the SuiTe exposes dogs to progressively more challenging stimuli, which may sustain or increase arousal levels throughout the test, resulting in relatively higher cortisol concentrations at the end of the procedure. This trend may also be influenced by sample composition, with a higher proportion of "secure" dogs (n=15) and "unsuitable" dogs (n=14), potentially leading to lower activation during the ASST and higher activation during the SuiTe.

The results suggest a potentially more stressful nature of the SuiTe test compared to ASST; further research on larger samples is needed to clarify or confirm these preliminary observations.

ANY INFORMATION FROM HAIR ABOUT CALVES AROUND WEANING?

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Non-esterified fatty acid (NEFA; also called Free fatty acids, FFA) concentrations are a valuable indicator of ketosis in adult cows [1] but in calves they could be used to evaluate metabolic effects of suckling and feeding strategies [2].

This preliminary study aimed to detect NEFA in hair of calves in three crucial phases of animal life, namely pre-weaning, weaning, and post-weaning phases.

18 Italian Simmental female calves were enrolled in the experiment. After birth, all the calves were immediately separated from their dams and received 4 L of colostrum, thereafter they were individually housed for 25 days and fed with pasteurized milk twice a day. From birth to the beginning of weaning (60 days of age), calves were fed 8 L of milk per day; subsequently, milk intake was reduced to 4 L/d for one week up to 2 L/d until weaning. Weaning process began when the animal ingested at least 450 g/d of pelleted complementary feed and ended with an ingestion of at least 1.5 kg per day meeting the National Research Council (NRC) requirements.

Hair samples were collected by shaving with the razor close to the skin at birth (60 days before weaning started; T1), 10 days (T2) and 20 days (T3) after the beginning of the weaning. Samples collected at T2 and T3 were re-growth hair.

After washing and extraction, hair samples were evaluated for NEFA with the Wako NEFA-HR(2) test (FUJIFILM Wako Chemicals Europe GmbH, Germany), an enzymatic colorimetric method validated by our research group for bovine hair.

NEFA (µg/mg) was log-transformed and analysed using a linear mixed-effects model, the sampling time was included as a fixed effect, while the animal was included as random intercept.

Data are means ± standard error.

Significant differences emerged between the three sampling times. In particular, NEFA concentrations before weaning (T1: 2.33±0.20 µg/mg) described an increment at weaning (T2: 3.98±0.33 µg/mg; $p<0.01$) and eventually a slight decrease was highlighted at T3 (3.04±0.20 µg/mg; $p<0.05$).

This study allowed to observe that even if the plasmatic half-life of NEFA is very short (2–4 min; [3]), the analyte was able to bind the keratin hair structure and provide insight into animal metabolic status. Also, the nutritional requirements must be met by the adopted weaning strategy and the present study pointed out the importance of recording data about individual dry and liquid matter intake to manage the gradual weaning process and the new diet in the most appropriate way from a metabolic point of view. As a matter of fact, calves play a crucial role in the future of any herd, making them an essential component of each farm.

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TOURISM CARRIAGE HORSES UNDER REAL WORKING CONDITIONS IN SICILY: A LOOK AT THEIR STRESS AND INFLAMMATORY RESPONSE

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The use of horses in tourism carriage activities raises questions regarding their walfare, particularly under chalenging environmental conditions as hot climate [1-3]. Heat stress is known to significantly affect physiological responses and welfare in horses [2].The purpose of the present study aimed to evaluate some markers of stress and inflammatory response in carriage horses working under real conditions in Sicily (Italy) during the seasons of late spring and summer. Twenty-two Standardbred horses were monitored during routine working days in May, June and July. Blood samples were collected before work (07:00) and after work (17:00). Serum cortisol, total proteins and protein fractions were analysed. Environmental conditions were recorded, and the temperature-humidity index (THI) was calculated to assess heat stress. Protocol of animal husbandry and experimentation were reviewed and approved in accordance with the standards recommended by the Guide for the Care and Use of Laboratory Animals and Directive 2010/ 63/EU for animal experiments. No significant effect of work was detected on cortisol levels or protein fractions $P>0.05$), suggesting an adequate physiological adaptation to the workload. Conversely, a significant effect of month was observed, eith higher values of cortisol, total proteins, and α1- and α2-globulins recorded in July compared to May and June ($P<0.05$). The THI values recorded during the months of investigations showed mild stress in June and high stress in July. Overall, the results suggest that tourism carriage horses are physiologically adapted to their routine work. However, environmental conditions, particularly heat stress, significantly influence stress and inflammatory responses. Therefore, management strategies such as avoiding work during peak heat hours and ensuring adequate hydratrion are recommended to improve animal walfare.

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EFFECTS OF HEAT STRESS ON DAIRY CATTLE REPRODUCTIVE PERFORMANCE: PRELIMINARY RESULTS FROM A CASE STUDY

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Heat stress, assessed by the bioclimatic index THI (temperature-humidity index), is defined as the critical physiological condition triggered by any combination of environmental variables, such as temperature, relative humidity, wind speed, and solar radiation, which causes the animal to exceed the thermoneutrality zone [1][2]. In dairy cattle, this state occurs when the accumulation of metabolic and environmental heat exceeds the body's capacity for dissipation, no longer being able to be compensated by the natural mechanisms of thermoregulation (conduction, convection, radiation and evaporation); this induces an alteration of homeostasis and an increase in body temperature that forces the cow to profound behavioral, physiological and neuroendocrine changes to compensate [3]. The aim of this study is to evaluate the impact of heat stress in reproductive performance and milk production in Holstein cows farmed in a large-size herd (600 lactating animals on average) located in Anzola dell'Emilia (BO). The study examined the period between June 2024 and February 2025 when the data were collected from 480 lactating Holstein cows. Climatic parameters were measured through CMP environmental control PLC (Mod. QU0075, Gussago, BS, Italy) and THI were calculated for each day: THI = (0.8 x Air Temperature) + [Relative Umidity (Air Temperature-14.4)] + 46.4. For each cow, days in milk and daily milk production were available along with farm's daily milk production and number of mastitis events retrieved from the Afimilk management software (Afimilk, Israel). Reproductive data (Estrus detection, number of services, positive pregnancy diagnosis, pregnancy rate, gestation length, and number of calvings) were collected during the study period. For the statistical analysis all data were averaged by month. Two different periods of the year were identified: No heat-stress period (Monthly THI < 68) and heat-stress period (Monthly THI > 68). The differences between the two periods were analyzed by non-parametric Mann-Whitney test. To correlate THI with reproductive parameters and milk production data, nonparametric Spearman's rank correlation test was performed; significance was set at $P<0.05$. The results showed a statistically significant difference between the two periods for estrus detection ($P=0.024$), number of services ($P=0.024$), positive pregnancy diagnosis ($P=0.038$), the total number of calving ($P=0.032$), and the total days in milking ($P=0.033$). Milk production was mildly higher during heat stress, but not statistically significant ($P=0.352$). All the reproductive parameters were lower during heat-stress period. However, the number of calvings occurring in the herd is also related and influenced to other factors too, like management choices other than health status. Our results highlighted a negative correlation between THI and estrus detection ($P=0.017$), number of services ($P=0.017$), and positive pregnancy diagnosis ($P=0.011$). Supporting the literature, the data revealed that reproduction (cycling parameters) is mainly involved and negatively influenced by heat stress. However, the incidence of mastitis did not show statistically significant difference between the two periods. Further analyses are necessary to evaluate how heat stress affects complex parameters regulated by multiple factors, like fertility and health status, including udder health.

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DIETARY SUPPLEMENTATION WITH EXTRUDED LINSEED AND PADINA PAVONICA EXTRACT PRESERVES RABBIT SPERM MOTILITY UNDER *IN VITRO* LIPOPOLYSACCHARIDE CHALLENGE

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IInflammatory responses triggered by endotoxins severely impair sperm function and male fertility [1]. This study evaluated the effects of dietary n-3 polyunsaturated fatty acids (n-3 PUFAs), derived from extruded linseed alone or combined with the macroalga Padina pavonica, on rabbit sperm motility resilience under an in vitro lipopolysaccharide (LPS) challenge. Following ethical approval (Authorization no. 325/2021-PR of 19 May 2021), twelve rabbit bucks were randomly assigned for 60 days to three dietary treatments: control (CNT), 5% extruded linseed (L), and 5% extruded linseed plus 0.2% P. pavonica extract (LPP). Semen was collected using an artificial vagina, and ejaculates were exposed in vitro to LPS (0, 400, and 600 µg/mL). Sperm motility was evaluated by computer-assisted sperm analysis (CASA) immediately after exposure and after 1, 2, and 4 h. Analyses focused on key motility parameters: percentage of static spermatozoa, progressive motility, curvilinear velocity (VCL), and linearity (LIN).

Results demonstrated that dietary n-3 PUFA supplementation positively modulated sperm motility, particularly under LPS challenge. LPS induced a marked dose- and time-dependent impairment of motility in CNT samples. After 4 h at 600 µg/mL, progressive motility declined to 0% and static sperm reached 100% ($p < 0.001$), with VCL approaching 0 µm/s and a significant reduction in LIN ($p < 0.01$). In contrast, dietary supplementation significantly mitigated these effects. Across LPS concentrations and incubation times, sperm from L and LPP groups maintained lower percentages of static spermatozoa (≈20%) and higher progressive motility (>30–40%) compared with CNT ($p < 0.05$). Moreover, supplemented groups preserved higher VCL (≈200 µm/s) and LIN values under LPS challenge, indicating sustained kinematic efficiency. The LPP group showed slightly superior performance compared to L, suggesting an additional contribution of algal bioactive compounds in sustaining sperm motility. Even in the absence of LPS, both supplemented groups exhibited improved baseline motility, particularly higher progressive motility and linearity, supporting the role of n-3 PUFAs in optimizing sperm function [2].

Overall, dietary n-3 PUFA supplementation attenuated LPS-induced sperm dysfunction by preserving key motility parameters. The inclusion of P. pavonica further enhanced these effects, indicating a synergistic interaction between plant-derived PUFAs and marine bioactive compounds. This nutritional strategy may represents a promising approach to improve sperm motility and mitigate LPS-induced impairment of sperm function.

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PRELIMINARY ANALYSIS OF THE BIOLOGICAL ACTIVITY OF MICROENCAPSULATED *VACCINIUM FLORIBUNDUM* KUNTH IN AN *EX VIVO* PORCINE WHOLE BLOOD MODEL

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Natural bioactive compounds are increasingly investigated for nutraceutical and therapeutic applications in human and veterinary medicine. *Vaccinium floribundum* Kunth (mortiño), an endemic Andean "superfruit", is rich in polyphenols and flavonoids with documented antioxidant and anti-inflammatory properties [1]. Previous *in vitro* studies on microencapsulated *Vaccinium floribundum* (VFM) demonstrated its safety and bioactivity in primary porcine Aortic Endothelial Cells (pAEC), where 1 mg/ml VFM significantly modulated the inflammatory response in LPS-challenged cells [2].

This study aimed to evaluate the potential anti-inflammatory and anti-apoptotic effects of VFM using a more complex *ex vivo* pig whole blood model in accordance with the 3R principles.

Whole blood samples were collected in lithium heparin from three pigs at a local slaughterhouse and diluted 1:1 with RPMI 1640 medium. Baseline cell viability (T_0) was assessed via flow cytometry. Samples were divided into four groups: control (CTR), VFM (1 mg/ml), LPS (10 µg/ml), and co - treatment (LPS+VFM). Samples were incubated at 38°C under slow and continuous agitation and withdrawn at different time points (1, 5, and 24 h) for subsequent analysis.

Interleukin-8 (IL-8) gene expression (pro-inflammatory marker) was quantified via RT- qPCR at 1 and 5 h post-treatment. Apoptosis was assessed 24 h post-treatment by flow cytometry using Annexin V/Propidium Iodide (PI) staining.

All three subjects showed an up-regulation of IL-8 following LPS stimulation, confirming the responsiveness of the ex vivo pro-inflammatory model. Notably, VFM treatment appeared to modulate the inflammatory response in all samples, a down-regulation of IL-8 gene expression was observed precisely at the time points of LPS activation for each subject. However, the intensity and timing of the response varied between individuals. One subject responded at 1h, while the others responded at 5 h.

Flow cytometric analysis at 24 hours revealed a trend toward a reduced apoptotic rate in VFM-treated samples, particularly under LPS-induced inflammatory stress, suggesting a potential cytoprotective role.

This heterogeneous kinetic pattern is consistent with the transient nature of LPS-induced signalling, characterized by a rapid peak followed by a timely resolution [3], and likely reflects the intrinsic biological variability of slaughterhouse-derived animals (e.g. age, sex, pre-analytical conditions). While these findings suggest potential anti-inflammatory and anti-apoptotic effects of VFM, the high variability likely masked statistically significant trends in pooled data. This *ex vivo* model represents a biologically complex system in accordance with the 3R principles, but further studies with refined experimental designs and larger sample sizes are necessary to confirm VFM's bioactivity and define its precise mechanism of action.

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DIETARY OMEGA 3 MODULATE LIPID MOBILIZATION AND METABOLIC STATUS IN RABBIT DOES DURING THE FIRST REPRODUCTIVE CYCLE

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The welfare and productivity of breeding rabbits depend on their ability to cope with the substantial metabolic demands of the first reproductive cycle [1]. This study evaluated the effects of dietary n-3 polyunsaturated fatty acids (n-3 PUFA), provided by extruded linseed alone or combined with the marine macroalga Padina pavonica, on metabolic status and lipid mobilization in nulliparous rabbit does. Following ethical approval (OPBA_18_2021), thirty nulliparous New Zealand White females were monitored throughout their first reproductive cycle and randomly assigned (n = 10/-group) to three isocaloric and isonitrogenous diets: control (CNT), 5% extruded linseed (L; rich in α-linolenic acid), and 5% extruded linseed plus 0.2% P. pavonica extract (LPP; rich in EPA, DHA, and other bioactive compounds). To assess metabolic adaptation, blood samples were collected at five physiological stages: baseline (before a 60-day adaptation period), artificial insemination (AI), parturition, mid-lactation (day 18), and weaning (day 35). Non-esterified fatty acids (NEFA), glucose, insulin, and leptin were analyzed using RIA and enzymatic colorimetric assays. Data were analyzed using generalized linear mixed models ($p < 0.05$). Reproductive stage significantly influenced all metabolic parameters. Glucose concentrations were affected exclusively by time ($p < 0.001$), predictably decreasing from AI to parturition to support fetal demands before stabilizing during lactation and decreasing again at weaning, reflecting shifting energetic demands. NEFA concentrations increased from AI to parturition and remained elevated throughout lactation ($p < 0.001$), indicating lipid mobilization to support energy demands. However, LPP animals showed significantly lower NEFA levels at AI compared to CNT ($p < 0.01$), suggesting reduced early reliance on body reserves. Leptin varied across physiological stages ($p < 0.05$), decreasing at parturition and peaking at mid-lactation, consistent with metabolic adaptations supporting hyperphagia and lactogenesis [2]. Notably, LPP does exhibited lower leptin concentrations than CNT at parturition ($p < 0.01$), suggesting improved peripheral sensitivity and attenuated adipose inflammation. Insulin concentrations were influenced by both diet and physiological stage ($p < 0.05$), decreasing postpartum due to the catabolic demands of lactation. The L group maintained relatively higher insulin levels than CNT from parturition to weaning ($p < 0.05$), suggesting improved metabolic regulation. Conversely, LPP animals showed a transient increase at mid-lactation, coinciding with maximal lactose synthesis, followed by lower concentrations at weaning. This supports the hypothesis that algal supplementation improves insulin sensitivity, aligning with its known antidiabetic effects [3]. Overall, the combination linseed and Padina pavonica enhanced endocrine regulation and reduced lipid mobilization, offering a promising strategy to improve metabolic resilience in breeding rabbit does.

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FIRST REPORT OF *ANGIOSTRONGYLUS VASORUM* IN A WOLF FROM THE EMILIA-ROMAGNA REGION, ITALY

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Angiostrongylus vasorum is a metastrongylid nematode of dogs and other canids that reside in the pulmonary arteries and right heart of infected hosts. The life cycle is indirect, infection is through ingestion of intermediate (gastropod mollusks) or paratenic (frogs) hosts. Clinical manifestations of *A. vasorum* infection have been described mainly in dogs and include respiratory signs and bleeding disorders. The red fox is considered the natural reservoir of this parasite and in Italy, the infection is reported with increasing frequency in dogs [1]. *A. vasorum* in wolves has been described in the regions of Lazio, Abruzzo and Tuscany, with prevalences ranging from 2-66% [2]. The present case report is the first, to the authors' knowledge, of *A. vasorum* infection in a wolf from the Emilia-Romagna region.

In December 2025, a wolf was found dead in the Correggio municipality area of Emilia Romagna. The animal was submitted to the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna for postmortem examination to investigate the cause of death. The wolf was an an adult female with no external signs of trauma. At necropsy, lungs showed several extensive areas of reddish-brown coloration with increased consistency in the diaphragmatic lobes and foci of pleural fibrosis with adhesions to the parietal pleura. Examination of the heart revealed ten nematodes of approximately 2 cm. in length in the right ventricle. The worms and lungs were sent to the Parasitology laboratory of the Dipartimento di Scienze Medico-Veterinarie dell'Università di Parma for morphological identification of the parasites and histopathological evaluation of the lungs. The nematodes were slender and pinkish with a "barber pole" appearance (from the red intestine intertwined with the white reproductive tract), typical of female *A. vasorum.*

Histopathology of the lung showed parasitic granulomatous foci characterized by peripheral fibrosis and inflammatory infiltrates of lymphocytes, plasma cells, macrophages, granulocytes and a few multinucleated giant cells. Numerous larvae and adult nematodes were observed.

The anatomo-pathological and histological features from the present report are similar with those usually shown by dogs harboring *A. vasorum* [3]. Although death of the infected wolf could not be ascribed to angiostrongylosis, no other cause of death was ascertained, and the parasite is known to be highly pathogenic in dogs. *A. vasorum* is endemic in canine populations in many regions of central and southern Italy as well as in Sardinia [1]. There is no data on the prevalence of *A. vasorum* in dogs or wild canids in Emilia-Romagna. Further studies are needed to elucidate the role of wolves in the epidemiology of infection in this area. Indeed, recent estimates put the wolf population density in the region at10 wolves/100km[2] Moreover, angiostrongilosis should be considered as a wildlife health issue in regions where wolves are a conservation issue.

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Posters

FIRST EVIDENCE OF THE CESTODE *OOCHORISTICA* SP. IN A CAPTIVE YUCATÁN SPINY-TAILED IGUANA (*CACHRYX DEFENSOR*) IN ITALY

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The global trade in exotic pets has led to an increased presence of reptiles in private households, raising concerns for animal and public health due to the potential introduction of non-native parasites. Despite growing scientific interest in captive reptiles, knowledge of their endoparasites remains limited, and infections are often subclinical, presenting with nonspecific symptoms such as weight loss, lethargy, or diarrhea. Reptiles can serve as definitive, intermediate, or paratenic hosts for several parasites. Among these, cestodes of the genus *Oochoristica* (Cyclophyllidea, Anoplocephalidae) are a taxonomically complex group primarily reported in tropical and subtropical areas, with few records in Europe [1].

Here, we report the first identification of *Oochoristica* sp. in a captive-bred Yucatán spiny-tailed iguana (*Cachryx defensor*) in Italy. The iguana, purchased at a reptile exhibition in Austria and housed in northern Italy, had never undergone parasitological screening prior to its arrival in Italy.

During routine fecal examination, a live proglottid was observed, and cestode eggs were detected using the Mini-FLOTAC® technique with zinc sulphate flotation [2]. Morphological evaluation suggested a cestode of the genus *Oochoristica*, which was confirmed through molecular analyses targeting partial 18S rRNA, 12S rRNA, and NADH dehydrogenase subunit 1 genes. Phylogenetic analysis of the 18S rRNA sequence positioned the isolate in a sister relationship with *O. hemidactyli*, indicating potential genetic divergence and highlighting the need for further taxonomic investigation. The iguana was successfully treated with praziquantel, and follow-up fecal examinations were negative for cestode eggs and proglottids.

This case highlights the risks associated with the international reptile trade, including the introduction of parasites into new geographic areas and gaps in preventive health management [3]. Outdoor access to an unfenced garden and feeding live insects, which may act as intermediate hosts in the life cycle of *Oochoristica* sp., likely contributed to the animal's infection.

These findings underscore the importance of quarantine protocols, routine parasitological screening, and the use of sensitive diagnostic techniques, with appropriate flotation solutions, to detect infections that may be overlooked by conventional methods [3]. Moreover, the integration of morphological and molecular analyses was essential for accurate parasite identification and phylogenetic placement, thereby contributing to the broadening of available genetic data for *Oochoristica* sp.

In conclusion, this report represents the first documentation of *Oochoristica* sp. in *Cachryx defensor* in Italy, expanding both the known host range and geographic distribution of the cestode. It emphasizes the critical need for enhanced awareness among veterinarians, exotic pet owners, and regulatory authorities regarding parasitic diseases in captive reptiles to safeguard animal welfare and limit parasite circulation.

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CRYPTOSPORIDIUM SPP., *GIARDIA DUODENALIS*, AND *ENTEROCYTOZOON BIENEUSI* SURVEILLANCE IN SURFACE WATER SAMPLES: DEVELOPMENT OF A COST-EFFECTIVE METHOD FOR DNA RECOVERY

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Waterborne diseases caused by protozoa and microsporidia are regarded as a public health concern and are responsible for many outbreaks worldwide. *Cryptosporidium* spp., *Giardia duodenalis* and *Enterocytozoon bieneusi* are relevant waterborne pathogens due to their environmental persistence and zoonotic potential, particularly in livestock-intensive areas, and have been implicated in waterborne outbreaks [1]. Current methodologies for parasites detection in water are largely based on standardized protocols such as International Standard ISO 15553, which combine large-volume filtration, immunomagnetic separation (IMS) and immunofluorescence microscopy [2]. While these approaches are considered reference methods, they are labor-intensive and costly, and are limited to the detection of specific protozoa, thereby excluding other relevant pathogens such as microsporidia. Furthermore, their performance can be affected by factors such as clogging, which depends on water turbidity and composition [3]. Therefore, simplified and cost-effective approaches that allow the detection of diverse parasites in water matrices are needed.

The aim of this study was to develop a cost-effective method for the detection of multiple parasites (*Cryptosporidium* spp., *Giardia duodenalis* and *Enterocytozoon bieneusi*) in small volumes of surface water.

Ten litres of surface water were collected monthly over a two-month period from four different sites. Samples were transported refrigerated and processed within 24–48 hours. An initial centrifugation step was performed (10 min at 3730×g) to separate the sediments from the supernatant. The sediments recovered from 10L were then concentrated through two further centrifugation steps (10 min at 3730×g; 5 min at 7460×g) to remove the remaining supernatant and increase the particle density. The supernatant was filtered within 24–48 h using vacuum filtration with cellulose membranes (pore sizes 11 µm and 3 µm) to recover different parasite elements. Filtration blanks with distilled water were included to monitor potential contamination. DNA was extracted from both the sediments and the filters using matrix-specific kits, and nested PCR assays were performed to detect each target parasite.
No positivity was observed in any of the filter membrane samples across all sites. In contrast, all sediment samples tested positive for at least one of the investigated parasites, suggesting that this method effectively concentrates target parasite elements in the pellet.

These findings support the development of a cost-effective approach to detect multiple waterborne microparasites. The protocol reduces the need for large sample volumes, thereby improving the efficiency of environmental monitoring. While this method only indicates the presence of the target parasites rather than their abundance, it can serve as a practical screening tool to identify potentially contaminated areas and prioritize sites for further quantitative assessment, aiding in the evaluation of waterborne zoonotic risks.

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Posters

EPIDEMIOLOGICAL UPDATES ON PARASITIC AND FUNGAL INFECTIONS IN DOMESTIC FERRETS (*MUSTELA PUTORIUS FURO*)

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The domestic ferret (*Mustela putorius furo*) is a small carnivorous mammal of the Mustelidae family. It is popular in Italy as a pet and for hunting. Certain management and feeding practices can lead to parasitic infections in ferrets, potentially making them reservoirs for parasites that affect other animals or humans.

This study aims to assess the prevalence and risk factors of parasitic and fungal infections in pet ferrets in Italy, and evaluate the level of owner awareness of these pathogens and the effectiveness of current preventive strategies.

Biological samples (faeces, tape impressions, ear swabs and hair) were collected from 172 domestic ferrets in various contexts (private individuals, breeders, pet shops, and volunteer associations) across Italy over one year, from November 2023 to November 2024. The owner completed a questionnaire to collect data on management, diet, preventive strategies, and animal health status.

Copromicroscopic examinations were performed using the MINI-FLOTAC dual technique with flotation solutions FS2 (sodium chloride, NaCl; s.g.=1.200) and FS7 (zinc sulphate, ZnSO4; s.g.=1.350). Coccidian oocysts were sporulated in 2.5% potassium dichromate for species identification. Diarrheic samples were also analysed for the detection of coproantigens of *Cryptosporidium* spp. and *Giardia duodenalis* (RIDA® QUICK *Cryptosporidium/Giardia* Combi). Tape impressions and ear swabs were microscopically observed to detect ectoparasites. Fungal cultures with Sabouraud Dextrose Agar at 25°C for 15 days were performed.

Of the 172 ferrets examined, 89 were female (51.7%) and 83 were male (48.3%). The mean age was 2 years and 7 months (± 737.1 days), with an age range of 24 days to 9 years. The animals belonged to private owners (157), voluntary associations (7), a pet shop (4), an organic rodent control company (4), and a breeding farm (4).

Regarding the geographical origin, 157 subjects came from Italy, and 15 subjects came from other countries: Czech Republic (1), Slovak Republic (1), Russia (4), Switzerland (5), Hungary (2), Spain (1), and Romania (1).

Overall, 147 fecal samples were collected, comprising 139 individual samples and eight pools from individuals living together. Five of them were positive for intestinal protozoa (3.40%). 5 OPG were counted in two samples, with values of 30, 40, and up to 56560 in the other three samples. *Eimeria ictidea* was identified in three samples (2.04%) and *Cystoisospora* spp. in two samples (1.36%). *Sarcocystis* spp. was also detected in one of these samples (0.68%).

Out of the 15 diarrheic samples, four were positive for *Giardia duodenalis* (26.67%), one for *Cryptosporidium* spp. (6.67%) and two (13.33%) for both protozoa.

One animal tested positive for *Otodectes cynotis* (0.59%).

170 hair samples were submitted for fungal culture. Six animals presented macroscopic skin lesions (3.49%), of which one ferret with an erythematous, pruritic and alopecic lesion on the 4th and 5th fingers of the right hand associated with onychogryphosis resulted positive for *Trichophyton mentagrophytes* (0.60%).

Even if these data demonstrate a limited prevalence of parasites and fungi in ferrets in Italy, probably attributable to appropriate management practices and regular use of drugs, zoonotic agents were also found. However, critical factors, such as feeding them raw meat and their cohabitation with other animals and different species, underline that their role in the epidemiology of parasitic diseases and the potential impact on animal and public health should be further investigated.

WILDLIFE DERMATOPHYTOSES IN CENTRAL ITALY (UMBRIA AND MARCHE REGIONS): A FIFTEEN-YEAR INVESTIGATION (2010–2024)

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Urbanization is reshaping ecosystems, transforming cities into complex habitats where wildlife increasingly coexists with humans. This growing interaction elevates the importance of monitoring wild animals as reservoirs of zoonotic pathogens and as indicators of environmental health within a One Health framework [1]. In this context, dermatophytes are of particular significance, as their epidemiology is influenced by changing patterns of human–animal interaction, including contact with wildlife and contaminated environments [2]. However, data on dermatophyte infections in wildlife remain limited. This study aimed to investigate the occurrence and diversity of dermatophytes in wild fauna from Central Italy and to characterize the isolates using molecular methods. Between 2010 and 2024, a total of 704 wild animals (111 wild ruminants, 257 grey squirrels, 116 hedgehogs, 96 foxes, 40 wolves, 33 badgers, 30 porcupines, 10 martens, 7 red squirrels, 3 European wild cats and 1 Eurasian beaver) from the Umbria and Marche regions were examined through a combination of passive (e.g. animals admitted to wildlife rescue centres, veterinary hospitals, and diagnostic laboratories) and active surveillance (e.g. LIFE program targeting squirrel populations) [3]. After clinical assessment of the integumentary system, samples consisting of fur or spines were collected using non-invasive brushing techniques in live animals or during necropsy in deceased individuals. Laboratory analyses involved both cultural and molecular approaches; in particular, an end-point PCR assay using universal fungal primers ITS1 and ITS4 was performed to amplify the ITS1–5.8S–ITS2 region flanked by conserved regions of the 18S and 28S rRNA genes. Additional molecular typing was performed to differentiate strains within the *Trichophyton mentagrophytes* complex. Dermatophytes were predominantly detected in grey squirrels, which accounted for the majority of positive cases (n = 60, 8.52%), followed by foxes (n = 8, 1.14%) and red squirrels (n = 6, 0.85%), while only sporadic cases were observed in badgers, hedgehogs, and porcupines (each n = 1, 0.14%). Overall, seven dermatophyte species were identified through a combination of conventional and molecular techniques. *Paraphyton mirabile* was the most frequently isolated species (n = 42, 5.96%), followed by the *Trichophyton mentagrophytes* complex (n = 17, 2.41%) and *Microsporum canis* (n = 5, 0.71%), whereas species of the genus *Arthroderma* were detected at lower frequencies. A case of co-infection involving *T. mentagrophytes* and *M. canis* was recorded in a red squirrel. None of the 111 wild ruminants tested positive for dermatophytes. Molecular characterization revealed both *T. mentagrophytes* var. *mentagrophytes* genotype III* and var. *interdigitale* genotype II*. Interestingly, only 5 animals exhibited clinical skin lesions (e.g. crusted dermatitis and/or non-exudative alopecic lesions) suggesting that most infected wildlife act as asymptomatic carriers. However, the absence of reported human infections among personnel handling these animals emphasizes the effectiveness of basic protective measures in reducing zoonotic risk. The study highlights the ecological diversity of dermatophytes in wildlife and reports the presence of both common and less frequently described species, including *P. mirabile*, whose ecological role and zoonotic potential require further investigation. The findings also reinforce the importance of integrating molecular diagnostics with traditional methods to improve accuracy in species identification. Despite limitations such as geographical restriction and sampling bias, these findings contribute to understanding dermatophyte ecology and emphasize the need for continued surveillance and interdisciplinary collaboration to mitigate zoonotic risks.

[1] Goulet et al. An operational framework for wildlife health in the one health approach. One Health, 19:100922, 2024

[2] Segal and Elad, Human and zoonotic dermatophytoses: epidemiological aspects. Front. Microbiol., 12:3532, 2021

[3] Cruciani et al. Health Status of the Eastern Grey Squirrel (*Sciurus carolinensis*) Population in Umbria: Results of the LIFE Project 'U-SAVEREDS.' Animals, 12: 2741, 2022

Posters

IVET-PRO: PREDICTIVE TEACHING IN VETERINARY SCIENCES WITH THE HELP OF A LEARNING ANALYTICS DASHBOARD SUPPORTING ADAPTIVE AND INTERDISCIPLINARY TEACHING STRATEGIES

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Veterinary education is increasingly required to prepare professionals able to manage complex challenges related to zoonoses, food safety and public health within the One Health framework. In this context, teaching innovation should not only introduce new educational methods but also support lecturers in selecting the most effective strategies according to the characteristics of each student cohort (1-3). This project aims to develop and test a predictive teaching approach based on learning analytics, centred on the creation of a dashboard designed to assist lecturers in adapting teaching strategies to the learning profiles of their classes.

The project involves three closely related veterinary disciplines: inspection of foods of animal origin, veterinary infectious diseases, and veterinary parasitology and parasitic diseases. Learning data will be collected from multiple sources, including online quizzes, formative assessments, laboratory activity checklists and short questionnaires on learning preferences and self-perceived difficulties. These indicators will be integrated into a learning analytics dashboard capable of identifying patterns of engagement, performance and learning behaviour within the class group.

Based on these patterns, the dashboard will support the categorisation of student cohorts according to their prevailing learning dynamics, helping lecturers select the most appropriate innovative and participatory teaching strategies. Depending on the identified profile, teaching activities may include flipped classroom sessions on key concepts, micro-learning modules, cooperative learning activities, or project-based interdisciplinary tasks.

The approach will be implemented in pilot courses where students will address integrated case studies reflecting real veterinary challenges, such as the management of zoonotic outbreaks requiring the combined interpretation of parasitological identification, clinical implications and food inspection procedures. Educational outcomes will be evaluated through student performance indicators, engagement metrics, questionnaires and focus groups, and compared with cohorts not involved in the project.

The expected impact includes improved alignment between teaching strategies and students' learning needs, increased engagement and participation and the strengthening of interdisciplinary reasoning in veterinary problem solving. In addition, the project will promote the development of a community of practice among lecturers and produce a transferable toolkit for predictive and adaptive teaching in veterinary education.

The present work is ongoing, and the poster will aim to disseminate the project idea that was awarded university funding for the development and support of innovative teaching. The poster will present the project and the timeline of its different phases, which will be completed by March 2027.

[1] Paulsen L., Lindsay E. Learning analytics dashboards are increasingly becoming about learning and not just analytics: a systematic review. Education and Information Technologies, 29:14279–14308, 2024.

[2] Ramaswami G., Susnjak T., Mathrani A., Umer R. Use of predictive analytics within learning analytics dashboards: a review of case studies. Technology, Knowledge and Learning, 28:959–980, 2023.

[3] Cabral L., Pinto R., Gonçalves G. AI-powered learning analytics dashboards: a systematic review of applications, techniques, and research gaps. Discover Education, 4:525, 2025.

BACTERIAL TICK-BORNE PATHOGENS AND TICK BACTERIAL MICROBIOTA: COMPARISON BETWEEN TARGETED PCR AND 16S rRNA METABARCODING IN WILDLIFE-ASSOCIATED TICKS FROM NORTHWESTERN ITALY

Alice Giusti[1], Gabriele Spatola[1], Chiara Nogarol[2], Accorsi Annalisa[2], Serena Robetto[2], Barbara Moroni[2], Aitor Garcia Vozmediano[2], Emily Sel[1], Valeria Listorti[2], Santina Di Bella[3], Simona Zoppi[2], Riccardo Orusa[2], Elisabetta Razzuoli[2], Andrea Armani[1], Annalisa Guercio[3], Maria Lucia Mandola[2], Lisa Guardone[1|2]

[1]Dip. di Scienze Veterinarie, Università di Pisa; [2]IZS del Piemonte, Liguria e Valle d'Aosta; [3]CRN per Anaplasma, Babesia, Rickettsia e Theileria (C.R.A.Ba.R.T.), IZS della Sicilia, Palermo

Ticks are major vectors of bacterial pathogens of veterinary and human health importance. Routine surveillance still relies mainly on targeted PCR assays, while broader approaches such as 16S metabarcoding remain insufficiently evaluated in operational settings. This study compared targeted PCR assays and 16S rRNA metabarcoding for the detection of bacterial tick-borne pathogens and investigated whether pathogen infection status was associated with shifts in tick bacterial microbiota. Ninety tick pools collected from wildlife in northwestern Italy between 2020 and 2023 were analysed in an ongoing research project (GR-2021-12374932). Most pools consisted of *Ixodes ricinus* from roe deer, fallow deer, wild boar and chamois. DNA extracts were tested by targeted PCR for *Anaplasma* spp., *Borrelia burgdorferi* sensu lato, *Coxiella burnetii* and *Rickettsia* spp., and the same samples underwent V3-V4 16S rRNA metabarcoding. Agreement between methods was assessed by Gwet's AC1 and McNemar's test; microbiota differences were evaluated through alpha and beta diversity analyses and differential abundance testing. By targeted PCR, 54/90 pools were positive for at least one pathogen, with *Rickettsia* spp. the most frequent finding (49/54), followed by *Anaplasma phagocytophilum* (6/54) and *B. burgdorferi* s.l. (1/54); *Coxiella burnetii* was not detected. Metabarcoding showed a bacterial community dominated by Burkholderia-Caballeronia-Paraburkholderia and Rickettsia, with additional detection of environmental, commensal and tick-associated taxa. Concordance between methods was strongly pathogen-dependent. Substantial agreement was observed only for *Rickettsia* spp. (AC1=0.732), whereas agreement was moderate for *Anaplasma* spp. (AC1=0.449) and absent for *Borrelia* spp. and *Coxiella* spp., with marked asymmetry in discordant results. These differences likely reflect the combined effect of low prevalence, distinct molecular targets, and the limited taxonomic resolution of the analysed 16S region, particularly for Coxiella-like endosymbionts. Infection status was associated with microbiota structure: coinfected pools showed significantly higher richness and Shannon diversity than uninfected pools, and beta diversity differed significantly among uninfected, single-infected and coinfected groups. Multivariable analyses indicated that *Rickettsia* spp. was the pathogen most consistently associated with microbiota variation, while differential abundance analysis showed enrichment of *Rickettsia* spp. in coinfected pools and depletion of several environmental genera in infected ticks. Overall, our results suggest that targeted PCR and metabarcoding should be considered complementary rather than interchangeable tools. Their integration may improve surveillance by combining robust detection of known pathogens with broader characterization of the tick pathobiome.

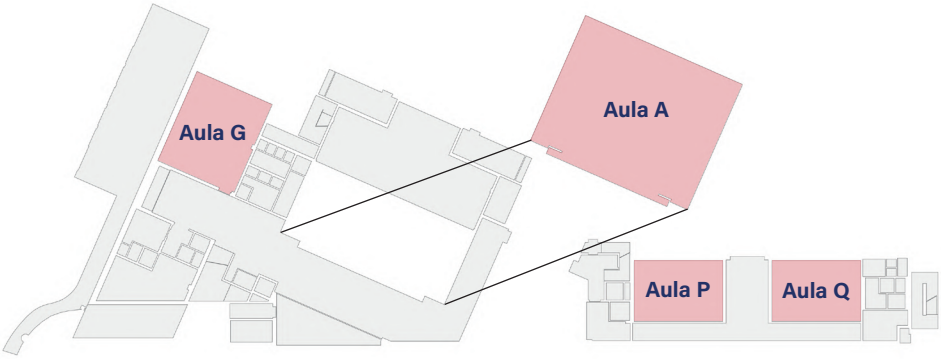
[1] Boulanger et al. Front Cell Infect Microbiol, 9:248, 2019;

[2] Eisen and Paddock. Annu Rev Entomol, 66:495-514, 2021;

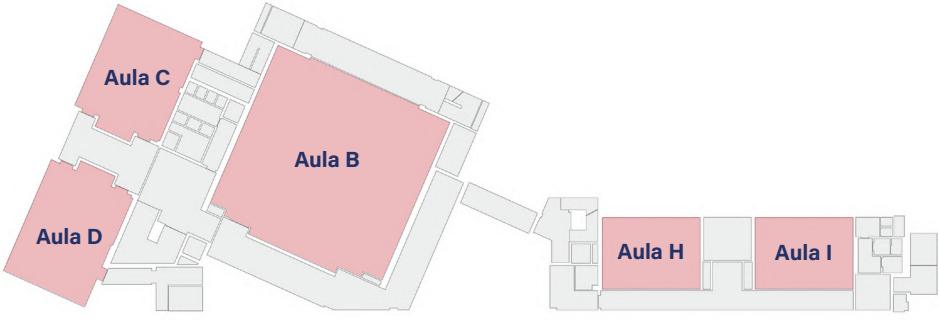
[3] Guardone et al. Ticks Tick Borne Dis, 15:102411, 2024.

Alma Mater Studiorum

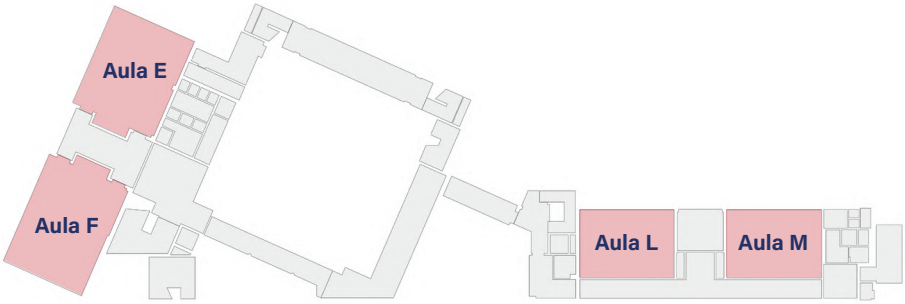
Piano Terra



Primo Piano



Secondo Piano



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