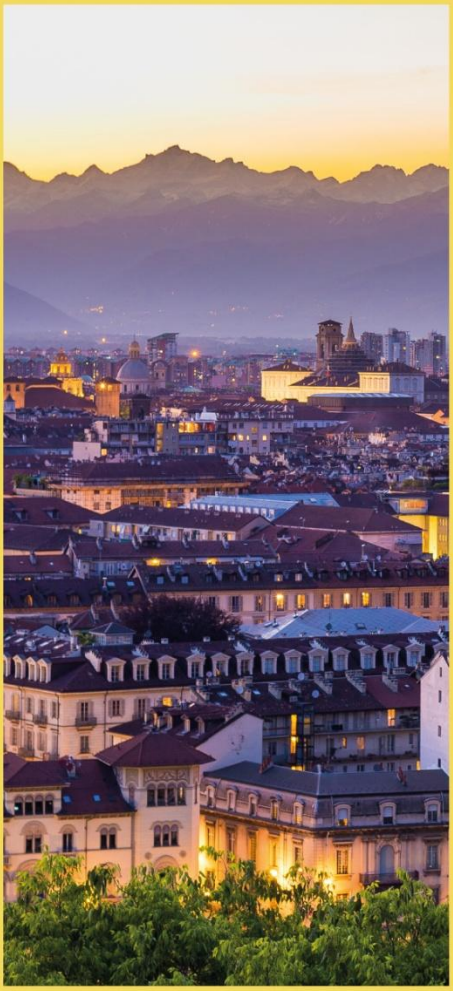
TURIN 2025
ANNUAL ESVP-ECVP CONGRESS

27-30 AUGUST 2025
TURIN-ITALY

PROCEEDINGSBOOK



European Society of Veterinary Pathology

European College of Veterinary Pathologists

TABLE OF CONTENTS

SPEAKER ABSTRACTS

RESIDENT DAY

TUMOR CLASSIFICATION AND GRADING SYSTEMS

Valentina Zappulli [1], Laura Peña [2]

[1] University of Padua, Legnaro, Italy
[2] Complutense University of Madrid, Madrid, Spain

Classification systems in canine/feline oncology have mainly been adapted from human classifications. The internationally applied human systems have been published by the International Agency for Research on Cancer of the World Health Organization (WHO), and are recently available also in a very convenient digital format (https://tumourclassification.iarc.who.int/welcome/). They are regularly updated and renowned and constitute an indispensable resource for pathologists, with detailed histological descriptions and images. Adapted WHO histological classifications of tumors of domestic animals have been published by the Armed Forces Institute of Pathology (AFIP, Washington DC, USA) and have been world widely used for decades, together with additional published books on domestic animal tumors. More recently, the 4th edition of the WHO Fascicles, retitled as the Surgical Pathology of Tumors of Domestic Animals have been renewed for some tissue categories and proposed by the Davis-Thompson Foundation (Gurnee, IL, USA) (https://davisthompsonfoundation.org/product-category/surgical-pathology-of-tumors-of-domestic-animals/). A few other classification systems have been proposed in the literature for some animal tumor types but a few comparative studies have been performed. Classification of tumors is based on differential morphological features which categorize heterogeneous tumors derived from a specific tissue/cell type. A standardized diagnostic classification is fundamental for prognostic and predictive studies and strongly influences the clinical decisions. However, the application of classification systems suffers subjective interpretation and biases of pathologists, so that consensus studies and guidelines should be proposed to reduce observer variability. Additionally, intrinsic features of tumorigenesis, such as a continuum in the tumor progression with no defined morphological threshold, can make tricky the categorization of benign versus malignant tumors, to improve which, precise follow up data are fundamental. Lastly, each tumor is unique, also morphologically, and can be very variable and undifferentiated as well, making classification difficult and subjective, and ancillary diagnostic techniques indispensable. The needed standardization is therefore the result of a multidisciplinary effort among clinicians, oncologists, pathologists and biologists. In this lecture some classifications will be briefly presented, indicating upsides and downsides, showing in a practical way the intrinsic difficulties, exemplifying particularly mammary cases.

In addition to histopathological classifications, recent years have seen the implementation of histological grading systems for tumours in domestic animals, offering prognostic information alongside the traditional histopathological diagnosis. These systems allow for the identification, within the same histological subtype, of those tumours that will behave more aggressively than their histotype counterparts, thereby enabling more appropriate therapy for each oncology patient. In human oncology, these systems are standardised and well-validated, guiding therapeutic decisions. In veterinary oncology, grading systems are still evolving, with some already established (e.g., mast cell tumours, soft tissue sarcomas, mammary carcinomas) and others under development or validation. Grading systems should use standardised criteria that are easy for pathologists to apply, rely solely on histopathological features, be validated through properly conducted prognostic studies, and be accepted for use by the veterinary pathology community. Common histopathological grading criteria

include: cell differentiation, mitotic count, nuclear pleomorphism, tumour necrosis, and architectural patterns (e.g., gland or tubule formation in adenocarcinomas). Histological grading, often combined with emerging molecular markers, is key to advancing personalised cancer care in veterinary medicine. For example, in canine mast cell tumours, the grading system is frequently used alongside immunohistochemical markers such as Ki-67, c-KIT expression patterns, and mitotic index to refine the prognosis and guide therapeutic decisions. To unify and standardise available grading systems, two international organisations are working from different perspectives: the Oncology-Pathology Working Group (OPWG), sponsored by the VCS and the ACVP, and more recently, the Veterinary Cancer Guidelines and Protocols. In this presentation, the available grading systems for tumours in domestic animals will be reviewed from a practical and interactive approach, with examples in mammary cases.

E-mail: valentina.zappulli@unipd.it

SETTING THE STAGE: WRITING EFFECTIVE TITLES, ABSTRACTS, AND KEYWORDS FOR SCIENTIFIC PUBLICATIONS

Joshua Webster

Titles and abstracts provide readers with their first impression of a paper. When doing literature searches, readers will evaluate the title and abstract to determine if the paper is worth reading or if they should move on to another paper. The title should be a factual representation of the paper, highlighting the species, disease, and nature of the work or findings. It should be specific and accurate, without over- or underselling the paper. The abstract should summarize the key components of the paper, introducing the goal of the study, the methods and study design, the key results, and the significance of the findings. By providing a descriptive title that represents the study, and by accurately summarizing the study in the abstract, readers can identify relevant papers and further engage with your work. Keywords complement the title and abstract because they can be used in searches to identify papers. Specific keywords including the species, disease, disease process or lesions, and technique can help readers identify relevant papers. The use of keywords in the title and abstract can further help increase the likelihood of readers finding a paper during a literature search. Given the tremendous numbers of papers published each month, it is important that the title and abstract are accurate representations of the paper and to stand out to relevant readers during literature searches.

E-mail: webste28@gmail.com

THE PEER REVIEW PROCESS: THE AUTHOR'S, EDITOR'S, AND REVIEWER'S PERSPECTIVES

Joshua Webster

Peer review is an essential part of scientific publications. The peer review process serves as an expert evaluation of manuscripts before they are disseminated to the broader scientific community. The goal of this process is to ensure a standard of quality and validity of the scientific literature. The peer review process should be aimed at improving manuscripts before publication and identifying critical flaws in studies that could significantly impact their interpretation or conclusions. For authors, the peer review process provides new insights into their work that can help improve a manuscript before it is published. As authors, we are often so engaged in our study and our interpretations that we can overlook key components of a study. External evaluations by peers can identify the weak spots in a paper, which can range from issues with experimental design to a simple lack of clarity, that are critical to address before publication. As a reviewer, the peer review process helps to build expertise in scientific writing, builds a reputation as a subject matter expert, and allows one to have impact on their field of study. The goal of a reviewer should not be to be overly critical of a paper, but to identify fatal flaws and ways that paper can be improved prior to publication. Peer reviewers are serving as subject matter experts and should focus on the scientific content of the paper. For example, they should consider the following questions: Is the purpose of the study clearly stated? Does the study design and methods properly address the goals of the study? Are the Materials and Methods detailed in such a way they could be independently repeated? Are the Results presented clearly and appropriately with consideration to the methods, study design, and data? Is the interpretation of the Results correct without over-interpretation? Does the Discussion provide insights into the interpretation and significance of the Results, and is this supported by the data in the paper? While clarity of the presentation is important, grammar should not be the focus of the peer review. The roles of editors are to identify appropriate experts to serve as reviewers and to use the input of the reviewers to make recommendations for the paper. The editor should mediate the reviewers' recommendations, identifying key points that are essential, beneficial, or potentially out of scope for the paper. The editor has the challenging role of arbitrating the reviewers and authors, but they are the final decision maker. Professionalism is critical for all aspects of the peer review process. All parties should understand that the goal is to improve the manuscript and to ensure the validity of the scientific literature. A high quality paper can have a longstanding impact, so it is important to ensure the quality and validity of each paper.

E-mail: webste28@gmail.com

DIAGNOSTIC APPROACH TO NEW INFECTIOUS CHALLENGES

Mariano Domingo [1], Alessio Bortolami [2]

[1] Universitat Autònoma de Barcelona, Bellaterra, Spain
[2] Istituto Zooprofilattico Sperimentale delle Venezie, Venezie, Italy

Infectious diseases are a common problem in domestic and wild animals and represents a diagnostic challenge for clinicians, pathologists, and microbiologists. On the face of an outbreak of a suspected infectious disease, multiple diagnostic strategies are applied with the aim of revealing infectious agents and other factors which could be implicated. Age of affected animals and clinical course may offer clues for understanding the disease process. Necropsy allows recognition of macroscopic and microscopic patterns of lesions, which may have a strong association to a specific pathogen or group of pathogens, and a causative role can be reinforced detecting them inside lesions by immunohistochemistry (IHC). Sampling of organs and tissues at necropsy for performing additional laboratory techniques, as well as additional diagnostic workout in live animals (serology, antigen, or genome detection) help to establish etiological diagnosis, orienting to measures to control and prevent disease. In some organ systems, coinfections are a frequent finding, and the role of detected organism need to be balanced. A good example of this complex situation is respiratory disease in farm animals like swine or cattle. For example, in swine, viruses like PRRSV and PCV2 act synergistically and often interact with bacteria to increase pathogenicity. Lesional pattern, IHC and laboratory molecular diagnosis are crucial to establish a diagnosis.

In recent years, the capabilities of veterinary diagnostic laboratories have greatly expanded, offering unprecedented sensitivity and specificity in the detection of infectious agents. This progress spans a wide range of platforms, from molecular assays to immunodiagnostics and microbial culture. However, these tools must be carefully selected and interpreted in light of pathological findings, particularly when lesions suggest specific agents or complex coinfections.

Targeted approaches, such as real-time PCR or immunological methods based on monoclonal antibodies, are highly effective when the pathogen is well-defined and stable. However, high genetic and antigenic variability, especially in RNA viruses, can lead to diagnostic escape, affecting both nucleic acid- and antibody-based tests. Similarly, for bacterial infections, where multiple organisms may be present in the same lesion or organ system, pathogen quantification and localization become crucial for establishing causality. In such cases, immunohistochemistry (IHC) remains an invaluable technique, offering spatial resolution by confirming the presence of a specific pathogen within the lesions, even when culture or molecular results are equivocal. In contrast, untargeted methods such as metagenomic sequencing provide a powerful alternative, enabling detection of both known and novel agents without prior assumptions. These approaches are particularly useful when pathology suggests infectious etiology, but routine tests are inconclusive. However, they remain expensive, technically demanding, and can suffer from reduced sensitivity in the presence of mixed infections, especially when bacterial and viral loads vary significantly.

The integration of pathology-driven hypotheses with laboratory testing, whether targeted or untargeted, remains essential. Collaborative interpretation between microbiologists and pathologists

is crucial for optimizing diagnostic yield, especially in the context of emerging infections and increasingly complex disease ecologies.

E-mail: mariano.domingo@uab.cat

CAREER COUNSELING IN VETERINARY PATHOLOGY: HOW CAN WE HELP YOU?

Silvia Benali [1], Christopher Palgrave[2] , Massimo Castagnaro[3], Anna Oevermann[4] , Laetitia Dorso[5], Cecilia Gola [6], Filippo Torrigiiani [7], Silvia Guionaud [8], Giovanni Pellegrini [9]

[1] MyLav Laboratory, Milano, Italy
[2] IDEXX, South Africa
[3] Department of Comparative Biomedicine, University of Padua
[4] Division of Neurological Sciences, Bern, Switzerland
[5] Oniris, Ecole Nationale Vétérinaire, Agroalimentaire et de l'Alimentation, Nantes, France
[6] Merck, Colleretto Giacosa, Italy
[7] Merck, Ivrea, Italy
[8] Astra Zeneca, Cambridge, UK
[9] EVOTEC, Verona, Italy

Meet and interview successful experts at different career statuses and from diverse professional fields, including industry, diagnostic labs, academia and others. We invite you to listen to their personal experiences and recommendations, and to ask them all your questions regarding your own personal career goals and strategies.

THURSDAY 28TH AUGUST 2025

AUTOMATING TASKS THROUGH AI-BASED IMAGE UNDERSTANDING IN PATHOLOGY – WHY? WHY NOT? AND WHAT TO CONSIDER

Marc Aubreville [1]

[1] Flensburg University of Applied Sciences, Flensburg, Germany

Artificial intelligence (AI) has rapidly emerged as transformative tool in the field of pathology, offering the promise of more efficient workflows, enhanced diagnostic accuracy, and novel insights into disease processes. In both human and veterinary pathology, image understanding through AI-based approaches is increasingly being explored for tasks such as tissue segmentation [1], mitotic figure detection [2], or tumor grading [3]. Despite promising results, often matching or surpassing expert performance, widespread clinical adoption remains limited. This keynote will examine a central question: which image-based tasks in pathology are appropriate candidates for automation through AI, and what challenges must be addressed before such tools become clinically viable?

One common misconception is that the most frequently performed tasks should be prioritized for automation. However, routine tasks like tumor classification are typically handled quickly and accurately by trained pathologists. In contrast, the most impactful opportunities for machine learning lie in tasks that are time-consuming, prone to significant inter-observer variability, or fundamentally difficult for humans to perform reliably. For instance, the quantification of histological features across large tissue areas, such as the assessment of mitotic activity or microvascular density, can be highly labor-intensive and are additionally subject to substantial variability between observers. At the same time, these tasks are well suited for assessment through an algorithm. Moreover, AI opens possibilities for identifying complex patterns that may not be perceptible to human observers at all, such as predicting treatment outcomes based on subtle morphological cues, which might otherwise require molecular or genomic data.

One obstacle on the way to clinically applicable AI solutions is robustness. Digital pathology images show a high diversity beyond what is caused by different use cases. Numerous sources of variation, such as differences in tissue preparation, staining protocols, or scanning equipment, introduce significant visual differences into the resulting digital slides. These variations can substantially alter the appearance of the image and disrupt the assumptions that machine learning models make about the underlying data distribution. As a result, models trained on data from one laboratory or scanner often fail to generalize well to data from another—a phenomenon known as domain shift. Despite considerable research efforts and recent progress in mitigating these effects [2], domain shift remains a fundamental obstacle to the robust and widespread deployment of AI in pathology, and, so far, can only be effectively mitigated by acquiring more diverse training data collections. It is essential to evaluate the robustness on these highly heterogeneous data corpora in order to show generalization and hence to build trust.

This crisis is largely driven by a lack of transparency throughout the development pipeline, including insufficient reporting on model architectures, training data, preprocessing steps, and evaluation procedures. While the machine learning community has taken important steps to address these issues, such as encouraging the open release of code, pretrained models, and standardized benchmarks, these practices are still not consistently applied in the domain of pathology.

A key reason for this gap can be seen in the increasing availability of easy-to-use software tools for training AI models, which often encourage interactive, ad hoc experimentation, where models are tuned iteratively and evaluated subjectively and informally. This process may lack rigorous documentation and objective benchmarks. As a result, it becomes difficult to reproduce the results or understand the underlying sources of performance. Moreover, without structured evaluation protocols and external validation, such workflows are susceptible to a range of cognitive and methodological biases, including confirmation bias [4] (favoring results that align with prior expectations) or selection bias (reporting only successful cases). To promote awareness and help mitigate common methodological shortcomings, a group led by the editors of Veterinary Pathology recently introduced minimum reporting guidelines for AI-related manuscripts submitted to the journal, which will also be discussed in the keynote.

References:
Wilm, F., et al. *Pan-tumor CAnine cuTaneous Cancer Histology (CATCH) dataset*. Sci Data **9**, 588 (2022).

Aubreville, M., et al. *Domain generalization across tumor types, laboratories, and species—insights from the 2022 edition of the mitosis domain generalization challenge*. Medical Image Analysis 94 (2024): 103155.

Ganz, J, et al. *Automatic and explainable grading of meningiomas from histopathology images*. MICCAI Workshop on Computational Pathology. PMLR, 2021.

Rosbach, E., et al. *When Two Wrongs Don't Make a Right"-Examining Confirmation Bias and the Role of Time Pressure During Human-AI Collaboration in Computational Pathology.* Proceedings of CHI 2025.

E-mail: marc.aubreville@hs-flensburg.de

BIOMARKERS AND THE BATTLE OF THE BLACKBOX AND BIAS

Inti Zlobec [1]

[1] University of Bern, Institute of Tissue Medicine and Pathology, Bern, Switzerland

Pathology is going digital! On the one hand, digitization of glass slides and automated lab workflows are meant to improve efficiency and open the gateway to AI applications. On the other, pathologists are faced with interpreting images for diagnosis in a completely different way (on a monitor rather than microscope) yet still assuring the quality of their diagnoses with no change in turn-around-times. Is AI meant to help here? Generative AI and large language models (LLMs) have shown promising capabilities in delivering precise pathology diagnoses and suggesting effective treatment plans when paired with histological images and textual input. Foundational models have attracted attention for their ability to recognize complex tissue patterns and to be fine-tuned for enhanced accuracy in specific applications. With AI making headlines across the field, we would imagine that AI in pathology is ready for prime time. Is it? In today's talk, we explore the answer to this question, taking a bird's eye-view on where the community stands with the transition to digital pathology. We will then take a critical approach to the current market for AI applications and associated challenges. We will ask ourselves, which medical professionals do tissue-based AI algorithms benefit (pathologists or oncologists?), and how will pathologists and oncologists deal with the rise in increasingly complex biomarker algorithms? Indeed, pathologists seem ready to use AI tools based on hand-crafted features, leading to verifiable AI outputs, but what about those tools using end-to-end deep learning for predicting molecular aberrations, therapy response or prognosis? The computational companion diagnostic tests are starting to gain momentum, but how will pathologists trust tests whose results they cannot verify- should they? Finally, we will look at how bias in AI algorithms can lead to inequity in patient care and although computational pathology is still a relatively new and evolving field, emerging evidence suggests that it is not exempt from biases that may occur at any level of the AI development lifecycle. The technologies are at our fingertips! And still up-and coming are spatial transcriptomics and multiplex immunofluorescence, among others. To conclude, we look at what we can expect in the near to mid future.

E-mail: Inti.zlobec@unibe.ch

INTEGRATIVE MODELING OF TRANSCRIPTOMICS AND PATHOLOGY TOWARDS DATA-DRIVEN PRECISION ONCOLOGY

Federica Eduati[1]

[1] Eindhoven University of Technology, Eindhoven, The Netherlands

Histopathology images contain rich information about the spatial organization of cells and their microenvironment, yet much of this information remains underutilized in routine diagnostics. In this talk, I will present an AI-based framework that extracts spatial maps of tissue composition—such as immune infiltration, stromal content, or tumor subtypes—directly from standard H&E-stained slides. While originally developed for human oncology, the approach may hold relevance for veterinary pathology applications.

The models are trained using transcriptomic data to learn associations between tissue morphology and underlying biological processes. However, once trained, they can be applied to new cases using only histology images—without the need for molecular profiling. This enables the generation of interpretable spatial maps that align with established histological features while providing additional biological insight, supporting both diagnostic and research applications.

Finally, I will briefly discuss how such AI-derived spatial representations could inform dynamic spatial models of tissue behavior—offering a path toward simulating disease progression or treatment response in a mechanistically grounded way. These tools could help bridge molecular and morphological data, enhancing precision oncology.

References:
Lapuente-Santana, Ó., Kant, J. & Eduati, F. Integrating histopathology and transcriptomics for spatial tumor microenvironment profiling in a melanoma case study. *npj Precis. Onc.* **8**, 254 (2024). https://doi.org/10.1038/s41698-024-00749-w

E-mail: f.eduati@tue.nl

ARTIFICIAL INTELLIGENCE IN SCIENTIFIC PUBLICATIONS: A VETERINARY PATHOLOGY PERSPECTIVE

Joshua Webster

While artificial intelligence has been incorporated into many work processes and facets of life for many years, the last several years have seen rapid progress in artificial intelligence technologies and in their impact on how we work. As pathologists, artificial intelligence can be used for quantitative image analysis, analysis of 'omics data (eg, transcriptomics, proteomics, metabolomics), literature searches and summaries, and scientific writing. The use and impact of these applications is continually evolving, but it is important to consider how these approaches can be used safely, effectively, and ethically. Artificial intelligence software and approaches are one set of many tools that a pathologist has to conduct their work. Similar to the other tools available to pathologists, it is essential that the pathologist understands how to appropriately use the tools, their strengths and limitations, and takes full responsibility for the use of the tools. While artificial intelligence tools can be used to analyze data as part of the scientific method, they need to be used appropriately, and the associated methods need to be fully disclosed and described in a paper, just as one would do for any other method. This is essential to allow another investigator to repeat the study and is consistent with long-held standards for scientific reporting. While artificial intelligence tools can help with grammar and language in a paper, and should be disclosed if they are used, they cannot serve as authors, as they cannot confirm or refute conflicts of interest, attest to the originality and accuracy of the data, or hold the copyright for the work. Furthermore, the interpretation, context, and implications of a study relies on the expertise of the authors, who are responsible for the content of a paper. While artificial intelligence tools can aid in the process, it is up to the authors to drive and own the data and interpretation of the data in a paper. Furthermore, while artificial intelligence tools like large language models can be used for literature searches, these tools should serve as starting points rather than finishing lines, as there are risks of bias and inaccuracies (eg, hallucinations) that come with these tools. Cross-referencing and original thought are integral components of literature reviews, and again, it is the author's responsibility to ensure the accuracy, originality, and validity of their work.

E-mail: webste28@gmail.com

SIMULATION OF INFECTION IN NON-ANIMAL MODELS – DATA INTEGRATION AND VALIDITY IN 3R RESEARCH

Andreas Hocke [1]

[1] Charité - Universitätsmedizin Berlin, Berlin, Germany

The 3R principle ("Replacement, Reduction, Refinement") demands the avoidance, substitution or improvement of animal experiments whenever possible. In line with this objective, human-based non-animal models (NAM) are gaining increasing importance in biomedical research. Particularly in the study of human diseases, NAM have become essential, as traditional animal models often fail to accurately reflect human pathophysiology, leaving a significant translational gap in the preclinical pipeline. This is especially evident in infectious diseases including zoonoses, where host-specific factors critically affect disease progression and are only partially transferable across species.

Similarly, for animal-specific infections, suitable species-specific models are often lacking; established animal models in pigs, rodents, or ferrets show similar translational limitations. While species-specific *in vitro* and *ex vivo* models offer an alternative, due to limited cellular complexity, the absence of, e.g., systemic immune components and lack of vascularization, they generally cannot fully replace cross-species animal models on a 1:1 basis.

Today, key NAM include organotypic tissue cultures and stem cell-derived organoids, which provide critical insights into physiology and pathophysiology. However, their scientific value fundamentally depends on meeting quality criteria such as validity, robustness, and reproducibility. Central to this is the question of construct validity: does the model truly measure what it claims to measure?

Achieving high validity, robustness, and reproducibility requires deeply characterized, species-adapted models. In our research, we develop human lung organoids derived from adult stem cells, extensively characterized at the molecular, cellular, and morphological levels against native lung tissue. Our goal is to establish and validate these models for the targeted study of severe respiratory infections - such as those caused by Influenza A viruses, coronaviruses, or *Streptococcus pneumoniae* - thus enabling applications such as pre-pandemic risk assessment for emerging pathogens.

In addition to classical morphological, molecular and protein biochemistry assays, high-resolution technologies such as single-cell RNA sequencing, multiplex imaging, or spatial transcriptomics are employed both to characterize the models and to analyze the disease processes they simulate. However, the true added value emerges through the integration of these multimodal datasets, ideally linking molecular information at the RNA and protein levels to the cellular-morphological and spatial-temporal context.

Based on these integrated multimodal data, virtual representations of real organoid models - so-called digital twins - could be generated in the future. A digital twin is a dynamic, data-driven model that accurately maps and predicts the biological state and response of an organoid to external influences such as infection. These digital twins open new avenues for simulating disease progression, testing interventions virtually, making personalized predictions, and optimizing experimental design

strategies. Thus, they offer a promising perspective for further reducing animal experiments and improving the translational value of biomedical research.

Drawing on our own experimental data, the presentation outlines approaches and challenges in data generation and integration for the validation of NAM using the example of severe human lung infections. Furthermore, based on our findings from a systematic review on COVID-19 research, it discusses the current obstacles regarding data quality, validation strategies, and generalizability that must be overcome to fully exploit the potential of human-based NAM - offering a forward-looking perspective on future developments such as AI-assisted data analyses and the establishment of digital twins for complex infectious disease processes.

References:
https://pubmed.ncbi.nlm.nih.gov/36538516/

https://pubmed.ncbi.nlm.nih.gov/35876564/

https://pubmed.ncbi.nlm.nih.gov/39231629/

https://pubmed.ncbi.nlm.nih.gov/35728978/

https://pubmed.ncbi.nlm.nih.gov/36008580/

https://pubmed.ncbi.nlm.nih.gov/39320092/

E-mail: andreas.hocke@charite.de

AVIAN INFLUENZA BEYOND BIRDS: CROSS-SPECIES TRANSMISSION AND IMPLICATIONS

Alessio Bortolami [1]

[1] Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy

Avian influenza viruses, especially highly pathogenic strains such as H5N1, have historically caused severe outbreaks in avian populations characterized by rapid dissemination, high mortality rates, and substantial ecological repercussions. Recent occurrences of cross-species transmission into diverse mammalian hosts, ranging from marine mammals and carnivores to domestic cats and livestock, primarily associated with H5NX viruses belonging to clade 2.3.4.4b, are redefining our understanding of HPAI. These emerging patterns pose critical questions about viral evolution, host adaptation mechanisms, and the unpredictable clinical presentations of avian influenza viruses in novel hosts.

Traditionally, mammals infected by avian influenza viruses were considered incidental or "dead-end" hosts, incapable of efficient viral amplification or sustained transmission. However, this perspective is now outdated. Since 2020, more than 50 mammalian species have been documented with confirmed infections of Highly Pathogenic Avian Influenza (HPAI) virus H5N1 (clade 2.3.4.4b), with some cases demonstrating clear mammal-to-mammal transmission (Peacock et al., 2025). A notable example includes outbreaks among farmed minks in Spain where genetic analyses from these outbreaks identified viral mutations of public health significance (Aguero et al., 2023). Similarly, significant mortality events in seals along South American coasts exhibited classic pathological features of H5N1 infection, including necrotizing encephalitis and extensive hepatic necrosis (Fiorito et al., 2025). Collectively, these findings indicate that HPAI viruses of clade 2.3.4.4b are not merely incidental in mammals but are actively undergoing genetic adaptations enabling exploitation of novel physiological niches, directly impacting surveillance strategies and diagnostic methodologies.

Ecological interactions, particularly scavenging behavior, facilitate the increasing incidence of spillover events. Mammals, including foxes and bears, ingest substantial viral loads through consumption of infected bird carcasses, potentially leading to gastrointestinal and respiratory infections. Notably, evidence of reverse spillback from infected mammals to avian populations adds complexity to transmission dynamics. Seals shedding HPAI viruses into marine environments could subsequently reinfect coastal seabird colonies, perpetuating viral circulation within ecosystems. For pathologists, recognizing this bidirectional transmission is crucial for expanding diagnostic criteria beyond avian reservoirs, considering mammals as potential reservoirs or vectors of infection.

Clinically, HPAI manifestations in mammals differ significantly from the classical avian presentation of acute respiratory or neurological syndromes. Mammals frequently display heterogeneous, sometimes severe pathologies. Domestic cats infected with clade 2.3.4.4b H5N1 have exhibited fatal neurological disorders, including ataxia, seizures, and pulmonary edema. Recently documented outbreaks in cattle revealed previously unrecognized presentations such as viral mastitis and reduced milk production, associated with direct viral replication within mammary gland tissues (Baker et al., 2025). This unprecedented clinical variability complicates timely diagnosis, particularly when dealing with unknown presentations that might delay prompt recognition of the disease, with catastrophic consequences. Additionally, asymptomatic infections, present significant diagnostic challenges, raising concerns about undetected viral circulation. Pathologists must therefore increasingly rely on

advanced molecular diagnostic approaches, including reverse transcription-polymerase chain reaction (RT-PCR) assays and whole-genome sequencing, to accurately confirm HPAI infections in atypical, non-avian hosts.

The zoonotic potential of these evolving viruses remains a critical concern. Genetic mutations facilitating enhanced mammalian replication, notably PB2-E627K, have already been observed in human H5N1 infections associated with dairy cattle, underscoring adaptive evolution towards efficient mammalian infection. For pathology professionals, this underscores the importance of closely monitoring tissue tropism and viral shedding patterns in mammalian species to refine zoonotic risk assessments. Concurrently, mass mortality events in wildlife populations, illustrate the broader ecological threat posed by HPAI viruses, highlighting their potential to disrupt biodiversity, ecosystem stability, and complex trophic interactions.

In response to these dynamic challenges, pathologists play a central role. Effective detection and diagnosis of HPAI in novel mammalian hosts require heightened awareness of atypical clinical and pathological presentations. Integrated collaboration between veterinary and human health sectors in a One Health perspective is also essential for comprehensive tracking of novel hosts and viral mutations facilitating cross-species transmission.

References:
Peacock, T. P., et al. *The global H5N1 influenza panzootic in mammals.* Nature 637.8045 (2025): 304-313.

Agüero, M, et al. *Highly pathogenic avian influenza A (H5N1) virus infection in farmed minks, Spain, October 2022*. Eurosurveillance 28.3 (2023): 2300001.

Fiorito, C., et al. *Pathology of Influenza A (H5N1) infection in pinnipeds reveals novel tissue tropism and vertical transmission.* bioRxiv (2025): 2025-02.

Baker, A. L., et al. *Dairy cows inoculated with highly pathogenic avian influenza virus H5N1.* Nature 637.8047 (2025): 913-920.

E-mail: Abortolami@izsvenezie.it

FROM DIAGNOSIS TO PROTECTION: ENHANCING CETACEANS CONSERVATION STRATEGIES THROUGH VETERINARY PATHOLOGY

Sandro Mazzariol [1]

[1] University of Padova, Padova, Italy

Pathology is a vital component in the conservation of marine mammals, providing critical insights into health conditions, causes of mortality, and environmental threats. By studying the effects of human activity on marine mammals, pathology enables researchers and policymakers to understand the pressures faced by these species and to implement appropriate conservation measures.

Stranding events offer important opportunities to assess the health of marine mammal populations. Through necropsies—post-mortem examinations—pathologists can determine if deaths are linked to human activities These findings are essential for designing targeted mitigation policies. Systematic post-mortem investigations and long-term data collection enable researchers to track mortality trends, detect new threats, and assess cumulative impacts on marine mammal health. These informations support conservation strategies and allows authorities to measure the success of policies over time.

The European Union's **Marine Strategy Framework Directive (MSFD)** relies on pathological data to assess indicators such as biodiversity, contaminants, and marine litter. Similarly, the **EU Habitats Directive** uses pathology to evaluate the conservation status of marine species and determine whether they meet the criteria for Favorable Conservation Status. These directives consider data derived from necropsies to measure pollution levels, the impact of marine debris, and signs of declining health.

The **ACCOBAMS Agreement**—covering cetaceans in the Mediterranean and Black Seas—emphasizes the importance of harmonized post-mortem protocols among member states. It promotes national stranding networks and shared necropsy methods, facilitating regional comparisons of mortality data and threats. The adoption of a standardized protocol (Resolution 6.22, updated in 2019) encourages a multi-tiered approach involving professionals at different skill levels, enhancing the collection and use of forensic data across borders. On a global scale, the **International Whaling Commission (IWC)** and the **International Union for Conservation of Nature (IUCN)** also use pathology data to assess anthropogenic threats and inform species status on the Red List of Threatened Species.

Forensic pathology has revealed several leading causes of human-related mortality in marine mammals. Bycatch and entanglement in fishing gear are among the most significant. Detailed necropsies reveal evidence of drowning, deep wounds, and trauma, allowing the identification of gear types and fishing practices responsible for fatalities. These findings have influenced the redesign of gear and promoted the use of safer techniques to reduce accidental captures. Ship strikes are another major concern, particularly for large whales. Necropsy can reveal signs such as blunt force trauma, fractures, and propeller injuries. This has prompted regulations like ship speed restrictions and rerouting of vessels in whale migration corridors. Underwater noise—especially from sonar and seismic surveys—can cause acoustic trauma in cetaceans. In some species, this may lead to embolic

syndromes involving gas and fat bubbles in the bloodstream. Necropsies showing such lesions have triggered reviews of noise-producing activities in sensitive marine habitats.

Despite the value of necropsies, investigations face challenges such as rapid decomposition of carcasses, inaccessible stranding sites, and a lack of trained personnel. Recent advances like **CT scans and MRI** now allow for "virtual necropsies," helping document internal injuries and preserve digital records for future analysis. These tools, along with improved training and international collaboration, are expanding the capacity for thorough and standardized investigations.

In conclusion, pathology is a fundamental pillar of marine mammal conservation. It offers direct evidence of human impact and environmental stress, guiding legal protections, conservation strategies, and global policies. As ocean ecosystems face increasing threats, the role of pathology in identifying problems, supporting legislation, and developing mitigation solutions becomes ever more critical. Continued investment in this field will be essential to ensure the survival and health of marine mammal populations in the years to come.

References:
ACCOBAMS. (2016). Resolution 6.22: Guidelines for a Coordinated Cetacean Stranding Response. Adopted at the Sixth Meeting of the Parties.

ACCOBAMS. (2019). Joint ACCOBAMS-ASCOBANS "Best Practice" on Cetacean Post-Mortem Investigations and Tissue Sampling. Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and Contiguous Atlantic Area.

European Commission. (1992). Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora (Habitats Directive).

European Commission. (2008). Directive 2008/56/EC of the European Parliament and of the Council establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive).

International Union for Conservation of Nature (IUCN). Red List of Threatened Species. https://www.iucnredlist.org

International Whaling Commission (IWC). Scientific Committee Reports. https://iwc.int.

E-mail: sandro.mazzariol@unipd.it

PRION DISEASES IN FARMED AND WILD ANIMALS: CURRENT SITUATION IN EUROPE

Sylvie Benestad [1]

[1] Norwegian Veterinary Institute, Ås, Norway

Prion diseases, or transmissible spongiform encephalopathies (TSEs), are a group of progressive neurodegenerative disorders that affect both animals and humans. They are caused by the misfolding in the brain of a normal protein called prion protein, into “prions”, which accumulate and cause neurological symptoms leading to the death of the host.

Prion strains can differ in several characteristics, including their: 1) Biological properties: Different strains may exhibit variations in their ability to induce disease, the severity of symptoms, and the incubation period (generally very long) before symptoms appear; 2) Molecular characteristics: Strains can have distinct conformations or structures of the prion protein (PrP), which can influence how they replicate and how they interact with the host's cellular machinery; 3) Host Range: Some prion strains may be more effective at infecting certain species than others, which can affect the epidemiology of prion diseases.

The most well-known prion disease, Bovine Spongiform Encephalopathy (BSE), emerged in the United Kingdom in the 1980s and subsequently led to a widespread crisis affecting cattle populations across Europe. BSE gained significant attention in the 1990s due to the tragic occurrence of human cases of variant Creutzfeldt-Jakob disease (vCJD) after consuming beef products contaminated with BSE prions.

The BSE crisis led to significant regulatory changes in livestock management and feed practices across the globe. Countries implemented strict controls on animal feed, surveillance programs, and testing of cattle for BSE. These measures effectively reduced the incidence of BSE, and the disease has since been largely controlled in many regions. However, sporadic cases continue to be reported in older animals, and the risk of re-emergence of the disease remains a concern. Scientists have emphasized the importance of ongoing surveillance and monitoring to prevent any resurgence of BSE and protect public health.

In addition to BSE, other prion diseases such as scrapie in sheep and goats and Chronic Wasting Disease (CWD) in cervids, pose significant challenges due to their high horizontal transmissibility. Scrapie has been endemic in some European sheep populations for decades, with efficient research aimed at understanding its transmission dynamics and genetic resistance in livestock. The European Union has established control measures, including the culling of affected animals and the implementation of breeding programs to enhance resistance against scrapie.

Chronic wasting disease (CWD), which affects wildlife animals, emerged in the 1960s in the USA. It has since spread to 36 American States and 5 Canadian provinces, where the situation is now totally out of control.

In 2016, the first case of CWD was discovered in a reindeer in Norway, marking the emergence of a significant concern regarding the disease in Europe. Currently, CWD has been detected in reindeer,

moose, and red deer within Norway, as well as in moose in Finland and Sweden. Substantial efforts have been undertaken to characterize the various prion strains associated with this disease, especially in Europe. Notably, while the CWD strains in North America appear to exhibit a high degree of uniformity, the strains identified in Europe are multiple and are all distinct from their North American counterparts.

International research is ongoing to better understand the potential transmissibility of CWD to humans (zoonotic potential) and other farmed animal species. This includes studies on the susceptibility of various animal models and the detection of environmental contamination. Current research efforts in Europe are focused on understanding the mechanisms of prion transmission, the environmental stability of prions, and the development of diagnostic tools. Advances in molecular biology and genetics are paving the way for improved surveillance and control strategies, which are crucial for managing these diseases in both farmed and wild populations.

In conclusion, while significant progress has been made in managing prion diseases in farmed animals in Europe, ongoing vigilance is required to address the challenges posed by both domestic livestock and wildlife. The interconnectedness of animal health, human health, and environmental factors underscores the need for a One Health approach in tackling prion diseases.

E-mail: sylvie.benestad@vetinst.no

A BRIDGING PERSPECTIVE BETWEEN CLINICAL NEUROLOGY AND NEUROPATHOLOGY IN CATTLE

Antonio D'Angelo [1],

[1] Department of Veterinary Sciences - University of Turin, Grugliasco, Italy

The aim of this lecture is to present the field experience of the author of common neurological diseases of cattle. Focus will be on clinical presentation, neurological findings, correlation to neuroanatomical localization and main pathological findings.

As soon as a neuroanatomical localization is reached, it is important to establish a list of differential diagnosis. In this context, it can be helpful use the acronym VITAMIN D (Vascular; Inflammatory; Traumatic; Anomaly; Metabolic/Toxic; Idiopathic; Neoplastic; Degenerative), in order to put the patient's history and clinical signs (neuroanatomical localization) in one or more the over mentioned categories, in descending order of importance.

VASCULAR: Vascular problems are a rather rare event in bovines. Aortic thrombosis, among these, should be considered in the differential diagnosis for calves with acute flaccid paralysis, loss of spinal reflexes and deep pain sensation in the pelvic limbs. Isolation of E. coli from several affected tissues and long bone (septicemia) suggests that it may be the etiological cause for thrombus formation.

INFLAMMATORY: Suppurative Meningitis-Meningoencephalitis, Otitis Media-Media Interna, Listeriosis and Thromboembolic Meningoencephalitis are the most frequent bacterial inflammatory diseases. Other causes include Brain abscess/Basilar Empyema, Vertebral Suppurative Osteomyelitis-Discospondylitis and Septic Polymyositis. Viral (Pseudorabies, Rabies, Bornavirus, Bovine herpesvirus encephalomyelitis, Bovine paramixovirus encephalomyelitis, European Sporadic bovine encephalomyelitis, Malignant catarrhal fever) as well as parasitic (Sarcocystosis, Toxoplasmosis, Neosporosis) inflammatory diseases are seldom found.

TRAUMATIC: the most common traumatic diseases are Head Trauma, Spinal cord and Vertebral Trauma, Sacral and Sacrococcygeal Trauma, Avulsion of the Cauda Equina, Injury to peripheral nerves, Postpartum paralysis, Dehorning brain injury, Lightning strike and electrocution.

ANOMALY: there are many congenital defects of the nervous system. Many of them are lethal. Causes may include genetic and environmental factors. Frequent conditions are Cerebellar Hypoplasia, Hydrocephalus and Hydranencephaly. Occasionally, Congenital tremor, Cerebellar Abiotrophy, Congenital pendular nystagmus, Arthrogryposis, Meningoencephalocele, Bicephalus.

METABOLIC/NUTRITIONAL/TOXIC: they include Hypocalcaemia, Hypomagnesaemia, Hepatoencephalopathy, Ketosis, Hypokalemic syndrome, Uremic encephalopathy, Polioencephalomalacia or cerebrocortical necrosis, Hypovitaminosis A, White muscle disease, Botulism, Tetanus, Lead poisoning, Sodium salt poisoning, Water intoxication, Toxic plants.

IDIOPATHIC: Nervous form of coccidiosis

NEOPLASTIC: nervous system neoplasia is seldom reported in cattle. Lymphoma is relatively the most frequent.

DEGENERATIVE: Bse-Base represent the most common degenerative diseases even if their importance is decreased over time.

References:
Mayhew, J. Mackay, R. Large Animal Neurology, 3rd edition. Wiley & Sons, 2022

De Lahunta, A., Glass, E., Kent M. De Lahunta's veterinary neuroanatomy and clinical neurology 5th ed., Elsevier, January 2021

E-mail: antonio.dangelo@unito.it

THE INVISIBLE PALETTE: AI-POWERED VIRTUAL STAINING IN HISTOPATHOLOGY

Laura Setyo [1]

[1] AstraZeneca, United Kingdom

This lecture provides an accessible yet in-depth overview of virtual staining, with a special emphasis on virtual staining methods and their implications for diagnostic and research pathology. It is designed for delegates with a range of technical backgrounds—including those new to artificial intelligence (AI)—and aims to demystify the core technologies, practical applications, and evaluation strategies in this rapidly evolving field.

Introduction to Virtual Staining
Virtual staining refers to the digital recreation and analysis of tissue sections using advanced computational algorithms. It offers a practical, scalable solutions that enhances diagnostic workflows, conserves samples and reagents, and enables data-driven insights from both contemporary and archival tissue collections. Examples discussed in this talk include virtual staining (synthetic PAS staining) and virtual immunofluorescence (synthetic macrophage marker visualisation).

Methods in Virtual Staining: Paired and Unpaired Approaches
A core focus of this lecture is on the two main strategies for developing virtual staining models: the paired and unpaired sample approaches.

Paired Sample Method:
Here, matched images are used for model training. We will discuss image registration as a part of this pipeline, and the importance of aligning tissue sections (serial or same) pixel by pixel. Both traditional mathematical algorithms and deep learning-based models (such as VoxelMorph) are discussed as solutions to this computationally intensive but vital task. This high-fidelity mapping allows for high accuracy, but is limited by the need for carefully paired datasets.

Unpaired Sample Method:
Recognizing that perfect image pairs are often unavailable in large or historical datasets, we will then touch on the unpaired method, through the use of CycleGAN. This approach learns stain translation from collections of images without 1:1 alignment. In the featured case study, a CycleGAN architecture allowed the synthesis of virtual PAS stains from H&E images. The unpaired method is scalable and flexible, though it may sacrifice some pixel-level precision.

Challenges
We address the unique challenges of virtual staining, including limited or variable training data, inconsistencies caused by different staining protocols, repetitive tissue patterns, and imperfections from tissue processing. The lecture emphasizes that robust training data and thoughtful algorithm design are crucial to attain reliable, useful results, as even the most sophisticated model will struggle without quality inputs.

Evaluating Model Performance
We will briefly touch on evaluation of virtual staining models using quantitative metrics and qualitative pathologist review. The validation process also includes assessing the model's impact on pathologist decision-making and its generalizability to new samples, stains, and scanners.

Take-Home Messages
Overall, virtual staining enables label-free, rapid, and resource-efficient tissue analysis; it maximizes the diagnostic utility of both new and existing slides and preserves precious clinical material. As AI and imaging continue to advance, virtual staining holds the promise to automate routine pathology tasks, enhance throughput, and enable novel types of quantitative tissue analysis.

References:
Denholm, J., Hamidinekoo, A., Burlutskiy, N., Setyo, L., Zhang, I., Yousefi, F., Mortimer, J., Palés Huix, J., Bagnall, C., Lewis, A., Hulme, H., Unwin, R., Barry, S., Goodwin, R., Gallagher, F., Söderberg, M., Qaiser, T. (2025) *Virtual histological staining as a tool for extending renal segmentation across stains.* Modern Pathology (in press).

E-mail: laura.setyo@astrazeneca.com

BEYOND THE HYPE: GENERATIVE AI IN VETERINARY PATHOLOGY DOCUMENTATION

Gregor von Dulong[1]

[1] ReportAssistant, Berlin, German

Introduction

While much attention has focused on AI's diagnostic capabilities in image recognition, the more immediate and practical applications lie in text- and voice-based generative AI tools that are transforming how veterinary pathologists document findings, generate reports, and optimize their daily workflows[1][2].

The administrative burden of documentation consumes substantial portions of pathologists' time that could otherwise be dedicated to diagnostic interpretation and patient care [5][6]. Here, the integration of generative artificial intelligence (AI) represents a paradigm shift.

This article examines how generative AI technologies—specifically large language model (LLM) copilots, AI scribes, and voice-based reporting systems—can address these challenges while acknowledging the current limitations and ethical considerations that must guide their implementation.

Current Challenges in Veterinary Pathology Documentation

Veterinary pathology documentation presents several distinctive challenges that make it particularly suitable for AI-assisted solutions. Unlike in human medicine, veterinary pathologists routinely work across multiple species with varying anatomical structures and pathologies, requiring documentation systems that can accommodate diverse taxonomic classifications and reference intervals[3]. Further, documentation frequently must be created in hands-free environments such as postmortem examinations.

The workload burden in veterinary pathology contributes significantly to professional burnout, with studies indicating that administrative tasks, including documentation, represent a major source of stress among veterinary professionals[6]. This administrative burden not only affects individual pathologists but also impacts the overall efficiency of diagnostic services and can contribute to delays in patient care.

AI Solutions Automate Reporting

Large language models trained on medical text have demonstrated remarkable capabilities in generating clinical documentation[2][13]. Further, voice recognition technology has demonstrated significant potential in veterinary medicine, with specialized systems like ReportAssistant achieving 98-99% accuracy in veterinary terminology recognition.

Combining the two trends of increasingly powerful LLMs and improvements in voice recognition technologies, these AI scribes represent an evolution of workflow enhancement. They move beyond simple transcription to intelligent structuring of clinical reports[11][12].

In addition to acting as a scribe, these systems excel in automatically generating standardized sections of pathology reports, such as gross descriptions, microscopic findings summaries, and diagnostic conclusions. They can maintain consistency in terminology, ensure appropriate inclusion of relevant negative findings, and adapt writing style to institutional preferences while maintaining clinical accuracy[14]. Research has shown that LLM-generated medical summaries can achieve accuracy levels comparable to those produced by medical residents, suggesting significant potential for routine documentation tasks[15].

Different from legacy transcription software, these systems can capture entire consultation dialogues, identify relevant clinical information, and generate structured reports that follow established pathology reporting formats.

Benefits and Workflow Optimization

Studies in human medicine have shown that AI scribes can reduce documentation time by up to 70%, freeing clinicians to focus on patient care rather than administrative tasks[12]. This time reduction allows pathologists to handle higher caseloads or dedicate more time to complex diagnostic challenges.

Further, standardization of report formats and terminology represents another significant benefit [16][14]. AI systems can ensure consistent use of diagnostic terminology, complete inclusion of required report elements, and adherence to institutional or professional guidelines. This standardization not only improves report quality. In the long term, it facilitates data mining for research and other quality improvement initiatives.

Current Limitations and Challenges

Despite promising capabilities, current LLM systems exhibit limitations that must be acknowledged. Studies have identified several failure modes, including hallucination of clinical information, and inflexible reasoning patterns that may miss important clinical nuances[17][18][19]. These limitations require significant engineering efforts from companies like ReportAssistant, as well as the training of custom models to satisfy the high demand for accuracy in veterinary medicine.

Integration challenges with existing veterinary practice management systems and laboratory information systems represent practical barriers to implementation[16]. The veterinary field often utilizes specialized software systems that may not readily interface with AI documentation tools, requiring custom integration solutions or workflow modifications.

Future Directions and Regulatory Considerations

The successful integration of generative AI into veterinary pathology documentation requires a measured approach that acknowledges both opportunities and limitations. Initial implementations should focus on routine documentation tasks where AI assistance can provide clear benefits while maintaining some human oversight[18][24].

Training and education programs for veterinary pathologists should provide practitioners with an understanding of how to use these models and address both the capabilities and limitations of AI systems[23]. Professional development should emphasize the role of AI as a tool to augment rather than replace human expertise.

Further, the implementation of AI systems in veterinary medicine raises important regulatory and ethical considerations. While veterinary AI systems may face less stringent regulatory oversight than human medical devices, they still require careful evaluation to ensure patient safety and diagnostic accuracy[21][22].

Data privacy and security concerns are particularly relevant. As a result, leading companies implement appropriate safeguards and anonymization procedures to maintain compliance with relevant privacy regulations such as GDPR.

Conclusion

Generative AI technologies offer significant potential to enhance veterinary pathology documentation through improved efficiency, standardization, and accessibility. Voice-based reporting systems and LLM copilots can address challenges faced by veterinary pathologists. A successful implementation requires appropriate planning, attention to system limitations, and oversight protocols.

The path forward involves gradual integration of AI tools into existing workflows, with emphasis on augmenting rather than replacing human expertise. Already today, AI-based documentation systems such as ReportAssistant can cut specialist veterinarian documentation times by up to 90% for some cases. As these tools become widely integrated with industry software, adoption will surge, leading to gains in overall efficiency of veterinary services.

References

1. Rouse M., Nelson H. 5 use cases for generative AI in healthcare documentation, Newton,USA: TechTarget, Informa Tech; 2025, April 28; used throughout.

2. Biswas A., Talukdar W. Intelligent Clinical Documentation: Harnessing Generative AI for Patient-Centric Clinical Note Generation, Ithaca, USA: arXiv, Cornell University; 2024, 2405.18346; 994–1008.

3. Sacchini F., Freeman K.P. Quality documentation challenges for veterinary clinical pathology laboratories, Thousand Oaks, USA: SAGE Publications; 2008, 20(3); 302–306.

4. Sharma R. AI in healthcare documentation: The future of medical records, San Francisco, USA: Markovate Inc.; 2024, Nov 18; used throughout.

5. Parsons B.L. Workplace burnout in veterinary staff: A student's perspective and analysis, Corvallis, USA: Oregon State University, Honors College; 2024, May 22; used throughout.

6. Sharma R. How AI medical scribes are transforming healthcare workflows: Beyond voice to EHR, San Francisco, USA: Markovate Inc.; 2025, Jun 17; used throughout.

7. Heilmeyer F., Böhringer D., Reinhard T., Arens S., Lyssenko L., Haverkamp C. Viability of open large language models for clinical documentation in German health care: real-world model evaluation study, Toronto, Canada: JMIR Publications; 2024, 12:e59617; 1–

8. Perkins S.W., Muste J.C., Alam T., Singh R.P. Improving clinical documentation with artificial intelligence: a systematic review, Chicago, USA: AHIMA Press; 2024, 21(2); used throughout.

9. Lee C., Britto S., Diwan K. Evaluating the impact of artificial intelligence (AI) on clinical documentation efficiency and accuracy across clinical settings: a scoping review, Cureus. 2024;16(11):e73994. doi: 10.7759/cureus.73994. Used throughout.

10. Bracken A., Reilly C., Feeley A., Sheehan E., Merghani K., Feeley I. Artificial Intelligence (AI) – powered documentation systems in healthcare: a systematic review, New York, USA: Springer; 2025, 49(1); used throughout.

11. Kim J., Podlasek A., Shidara K., Liu F., Alaa A., Bernardo D. Limitations of large language models in clinical problem-solving arising from inflexible reasoning, Ithaca, USA: Cornell University Library; 2025, arXiv:2502.04381; used throughout.

12. Chiang A., Ghassemi M., Rajpurkar P. Evaluating the clinical utility of large language models, London, UK: Nature Publishing Group; 2024, 30; 1206–1215.

13. Reese J.T., Danis D., Caufield J.H., Groza T., Casiraghi E., Valentini G., Mungall C.J., Robinson P.N. On the limitations of large language models in clinical diagnosis, medRxiv [preprint]. 2024 Feb 26;

14. Hager P., Jungmann F., Bhagat K., Hubrecht I., Knauer M., Vielhauer J., Holland R., Braren R., Makowski M., Kaisis G., Rueckert D. Evaluating and mitigating limitations of large language models in clinical decision making, London, UK: Nature Publishing Group; 2024, Nature Medicine

15. Prinz, T.(2024, December 10). Approval of AI-based medical devices in Europe: What regulatory requirements exist in MDR & Co. for AI-based medical devices in Europe? VDE Health(2023, November 15). The ethics of AI in healthcare. HITRUST. Retrieved from Boudi, A. L., Boudi, M., Chan, C., & Boudi, F. B. (2024, November 26). Ethical challenges of artificial intelligence in medicine. Cureus, 16(11)

16. Busch, F., Hoffmann, L., Rueger, C., van Dijk, E. H. C., Kader, R., Ortiz-Prado, E., Makowski, M. R., Saba, L., Hadamitzky, M., Kather, J. N., Truhn, D., Cuocolo, R., Adams, L. C., & Bressem, K. K.(2025). Current applications and challenges in large language models for patient care: A systematic review. Communications Medicine, 5, Article 26

WISDOM: WINE-INFUSED SLIDE DISCUSSION ON NEUROPATHOLOGY MYSTERIES. TRAIN YOUR BRAIN

Anna Oevermann [1]

[1] Division of Neurological Sciences, Bern, Switzerland

Join us for the Vespertine WISDOM Neuropathology Session, which spices up this year's ECVP/ESVP meeting in Turin! This dynamic evening event complements the traditional mystery slide session with a fresh twist: three intriguing and challenging neuropathology cases presented by fellow pathologists, followed by interactive discussion with an international panel of veterinary neuropathology experts.

The goal? To conquer the diagnostic "pink desert" and stimulate collaborative learning in a relaxed setting—refreshments included. Let's solve some brain mysteries—together!

E-mail: anna.oevermann@unibe.ch

FRIDAY 29TH AUGUST 2025

FROM CODE TO CARE: APPLYING GENOMIC INSIGHTS IN VETERINARY ONCOLOGY

William Hendricks [1, 2, 3]

[1] Translational Genomics Research Institute, Phoenix, United States
[2] Actual Labs, Phoenix, United States
[3] MI:RNA Diagnostics, Edinburgh, United Kingdom

The overwhelming clinical need and opportunity for innovation in oncology needs little introduction. Most of us have experienced cancer ourselves, in our family members, in our pets, and in our clients, and we have faced the daunting task of making clinical decisions with limited data and imprecise tools. Yet, we are experiencing a paradigm-shift, begun in human cancer medicine, built on understanding cancer as a genetic disease, that is revolutionizing patient care. New diagnostics and interventions have reduced the human cancer death rate by over 33% since 1991. Canine cancer genomics is also entering a pivotal era, where expansive datasets, advanced tools, and translational insights are converging. This presentation outlines the current landscape of canine cancer genomics, bridging discovery and application, and identifies new opportunities to harness these insights.

Cancer arises when genetic mutations drive excessive cell growth and tumor formation. As mutations accumulate, tumors become malignant, then invade and metastasize. Next-generation sequencing studies have now mapped the complex mutational landscapes of hundreds of thousands of human and thousands of canine tumors. Each patient's cancer has a unique spectrum of mutations, even between same tumor types. These mutations are often diagnostic and prognostic biomarkers or actionable drug targets. This genomic understanding has become foundational to human oncology through the field of molecular pathology. Thousands of genomic tests guide cancer screening, diagnosis, and treatment, supported by resources like OncoKB that link mutations to clinical utility. In veterinary oncology, similar momentum is building. Resources such as the Integrated Canine Data Commons and the Canine Cancer Genome Atlas are making annotated genomic data and harmonized tools more accessible.

Although many individual mutations have consistent diagnostic, prognostic, or therapeutic value, every patient's cancer bears a unique signature that warrants profiling of their unique signature. In clinical veterinary genomics, this specific process depends on application, i.e. screening, tumor profiling, or monitoring, but regardless is typically accomplished by extracting DNA from affected tissue (and sometimes unaffected tissue as well), typically from an FFPE block, aspirate, or even blood sample, which is then analyzed on genetic sequencers with varying degrees of breadth and depth. Bioinformatic analysis and biomarker knowledgebases are used to identify mutations and return predictions (for screening and monitoring) and/or annotated findings (for tumor profiling and monitoring) to veterinarians, often within weeks, informing diagnosis, prognosis, and treatment planning. Screening results may guide additional clinical evaluation. Tumor profiling may facilitate diagnosis and prognostication and may even guide selection of targeted therapies, although, unlike in human oncology, these therapies are often used off-label and with limited data.

A growing suite of such tests is available through academic centers, commercial startups and large diagnostics laboratories. Studies show promising clinical utility. For example, in one tumor profiling series, genomic analysis for tumor profiling clarified diagnosis in 54% of challenging cases and offered

therapeutic or prognostic guidance in 69% of the remainder. Nearly 90% of cases yield clinically actionable insights. Furthermore, in real-world studies, more than 20% of dogs treated with genomics-guided therapies remained progression-free for up to two years.

Despite this promise, challenges remain. Genomic tools are still costly, logistically complex, and hard to integrate into everyday veterinary workflows. More critically, diagnostics have advanced faster than therapeutics. We can now better detect and understand canine cancers, but our ability to intervene remains limited. Innovation is continuing to emerge, particularly in multi-omic research integrating DNA, RNA, protein, and epigenomic data, and in non-invasive sampling techniques using blood, saliva, and urine. These studies are refining cancer subtypes, identifying new targets, and expanding our testing toolkit. However, development of companion therapeutics remains an unmet need. We must build pipelines for biomarker-driven drug development alongside these diagnostics.

Progress is further constrained by limited infrastructure and funding in animal health. Many academic discoveries stall due to lack of support for rigorous, prospective validation while commercial products may outpace evidence, eroding clinician trust, or overlook logistical considerations in daily clinical practice. We need collaborative platforms spanning academia, startups, and industry that move fast, validate thoroughly, and gather user (veterinarian) needs up front. Pharmaceutical companies, large and small, must also adopt the genomic mindset. And veterinary pathologists, central to diagnosis, must be equipped and empowered to bring molecular insights into routine care.

Ultimately, the future of veterinary oncology lies in integration - of omics layers, clinical tools, academic discovery, commercial translation, and, most importantly, patient care. We stand at a generational inflection point. The tools are emerging. Now we must ensure they lead to impact on care not just insight from code.

E-mail: whendricks@tgen.org

LEVERAGING CELLULAR IMMUNITY TO INDUCE DURABLE RESPONSES IN CANINE CANCER PATIENTS

Nicola Mason [1]

[1] University of Pennsylvania, Philadelphia, United States

The potential for harnessing the power of the immune system to specifically eliminate cancer cells and provide durable clinical remissions was realized in 2017 when the first genetically engineered, autologous cellular therapy was approved by the FDA for the treatment of relapsed/refractory B cell acute lymphoblastic leukemia (B-ALL). This approach involved isolating a patient's own T cells, activating, and transducing them with a lentiviral vector that delivered a chimeric antigen receptor (CAR) into the T cells. This re-directed the antigenic specificity of the T cell towards target cell-surface expressed antigens such as CD19, leading to their elimination through CAR-T cell cytotoxicity. Unprecedented response rates and durable remissions in patients with B-non-Hodgkin's lymphoma (B-NHL), chronic lymphocytic leukemia (CLL) and multiple myeloma followed. However, not all patients with hematological malignancies are "cured" and current success using CAR-T cells to treat solid tumors that exhibit a myriad of immunosuppressive barriers, has been limited.

Significant advances have been made in understanding immune suppression within the tumor microenvironment (TME) as well as the phenotype and functional attributes of autologous T cell products that correlate with clinical success.[1] This understanding has led to innovative next-generation CAR design and manufacturing strategies that aim to improve clinical responses to engineered immune cells. Immune competent pet dogs with hematological and solid tumors are being increasingly utilized to evaluate the safety and effectiveness of these next-generation strategies. Several groups have now established robust protocols for generating canine CAR-T cells. The antigen targeting moiety of the CAR consists of a single chain variable fragment of an antibody enabling MHC-independent antigen recognition. Canine specific or cross-reactive CARs have been described against canine CD20,[2] HER2,[3] IL-13Ralpha2[4] and B7H3,[5] providing opportunities for therapeutic effect in canine B-NHL, HER2^{+} carcinomas, high grade gliomas and sarcomas respectively. However, while canine CART-19 and CART-20 cells show antigen-specific targeting and effective killing in vitro, their success in B-NHL has been limited, despite reports of cytokine release following administration[6] and elimination of target antigen-positive disease.[2] This failure is in part associated with a reduced ability of the CAR-T cells to expand and persist in vivo, to penetrate solid masses and to function within an immune suppressive microenvironment. These barriers are being addressed through different approaches that include optimizing manufacturing protocols for CAR-T cells, arming CAR-T cells with pro-inflammatory cytokines that are constitutively secreted to aid proliferation, persistence and promote a favorable microenvironment, or endowing them with switch receptors that turn inhibitory signals within the TME into activating signals.[7] In addition, to address additional challenges of patient T cell dysfunction, product cost and availability, off-the-shelf, adoptive cell transfer platforms are being developed that that do not require editing for successful allo-transfer. These include invariant NKT cell[8] and NK cell[9] platforms that are being actively pursued in the veterinary space.

In this lecture we will discuss current experiences with canine CAR-T implementation in the canine oncology clinic. We will discuss factors that influence the success of this approach in the dog,

including target antigen selection, T cell fitness and features of the tumor microenvironment that may serve as correlative biomarkers of response.

References:

Fraietta, J.A*., et al.* Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia. *Nat Med* **24**, 563-571 (2018).

Panjwani, M.K*., et al.* Establishing a model system for evaluating CAR T cell therapy using dogs with spontaneous diffuse large B cell lymphoma. *Oncoimmunology* **9**, 1676615 (2020).

Mata, M*., et al.* Toward immunotherapy with redirected T cells in a large animal model: ex vivo activation, expansion, and genetic modification of canine T cells. *J Immunother* **37**, 407-415 (2014).

Yin, Y*., et al.* Checkpoint Blockade Reverses Anergy in IL-13Ralpha2 Humanized scFv-Based CAR T Cells to Treat Murine and Canine Gliomas. *Mol Ther Oncolytics* **11**, 20-38 (2018).

Cao, J.W*., et al.* Targeting osteosarcoma with canine B7-H3 CAR T cells and impact of CXCR2 Co-expression on functional activity. *Cancer Immunol Immunother* **73**, 77 (2024).

Atherton, M.J., *et al.* Case Report: Clinical and Serological Hallmarks of Cytokine Release Syndrome in a Canine B Cell Lymphoma Patient Treated With Autologous CAR-T Cells. *Front Vet Sci* **9**, 824982 (2022).

Labanieh, L. & Mackall, C.L. CAR immune cells: design principles, resistance and the next generation. *Nature* **614**, 635-648 (2023).

Rotolo, A*., et al.* Unedited allogeneic iNKT cells show extended persistence in MHC-mismatched canine recipients. *Cell Rep Med* **4**, 101241 (2023).

Razmara, A.M*., et al.* Preclinical evaluation and first-in-dog clinical trials of PBMC-expanded natural killer cells for adoptive immunotherapy in dogs with cancer. *J Immunother Cancer* **12**(2024).

E-mail: nmason@vet.upenn.edu

NEW PARADIGMS FOR COLORECTAL CANCER TREATMENT AND MONITORING

Alberto Bardelli

FROM HUMAN TO NON HUMAN: THE NEW CHALLENGES OF PATHOLOGY AND FORENSIC SCIENCES

Cristina Cattaneo [1]

[1] Labanof, Università degli Studi di Milano, Italy

Human forensic medicine has spent decades developing rigorously validated methods for trauma interpretation, fracture, wound ageing and courtroom presentation. Veterinary forensics, by contrast, still faces uneven standards and scarce peer-reviewed data. Current literature therefore calls for systematic “technology transfer” from human to animal investigations

In this presentation the experience gained from human clinical and post-mortem practice—including high-resolution CT/MRI use, immunihistochemistry, criminalistics and quantitative blunt- and sharp-force trauma libraries, fracture-chronology algorithms and strict chain-of-custody protocols—will be illustrated with suggestions for the adaptation to the veterinary arena. Multidisciplinary case reports, from scene of crime, through autopsy, to the labs (toxicology, genetics, balistics etc.), will further demonstrate how joint work between veterinary pathologists and forensic anthropologists and pathologists clarifies mechanism, timing and intent in complex abuse scenarios, leading to more robust legal outcomes. In fact by weaving validated human forensic know-how into animal investigations we can move beyond basic welfare assessment toward full legal recognition of animals as sentient victims, while enriching both medical and veterinary science through shared research and teaching agendas.

Keywords: veterinary forensics; forensic pathology, forensic anthropology, criminalistics, trauma analysis; post-mortem imaging; immunihostochemistry, animal welfare; cross-disciplinary collaboration.

References:
Parry N.M.A., Stoll A. “The rise of veterinary forensics.” *Forensic Science International* 306 (2020): 110069.

Kulnides N., Lorsirigool A. “The role of veterinarians in forensic science: a review.” *World’s Veterinary Journal* 13 (3) (2023): 452-458

Yamada K. *et al.* “Role of autopsy imaging in veterinary forensic medicine: experiences in 39 cases.” *J. Vet. Med. Sci.* 85 (3) (2023): 301-307

Smith-Blackmore M., Bethard J.D. “A multidisciplinary investigation of chronic animal abuse: collaboration between veterinary forensics and forensic anthropology.” *J. Forensic Sci.* 66 (1) (2021): 389-392.

Sainsbury A. *et al.* “Two case studies in veterinary forensic imaging and a brief literature review.” *Forensic Imaging* 2 (2020): 100031.

E-mail: cristina.cattaneo@unimi.it

FORENSIC HISTOPATHOLOGY – ANYTHING SPECIAL ABOUT IT?

Anja Kipar [1]

[1] University of Zurich, Zurich, Switzerland

Histological examinations are daily routine of a veterinary pathologist involved in diagnostic work. As part of the work-up of post mortem cases, the tool is often used selectively, eg. to confirm gross findings, to clarify gross differential diagnoses, or to screen for any pathological changes. They are also a component of the work-up of forensic cases that diagnostic veterinary pathologists undertake. Under the latter circumstances they might serve different or additional purposes and may, depending on the circumstances of the case and the questions that need to be addressed, require very specific approaches. Some of the latter will be discussed in talks of this workshop.

The present introductory lecture will, based on examples, present a range of case scenarios in which a (routine) histological examination provided essential results, helped to clarify specific questions, and/or has likely been the essential factor for a decision whether the case was taken further towards prosecution or not. Ultimately, it tries to highlight the histological examination as an essential component of the unbiased approach that we take as forensic pathology experts contributing to a case.

E-mail: anja.kipar@uzh.ch

SEROUS FAT ATROPHY

Henrik Elvang Jensen [1]

[1] University of Copenhagen, Copenhagen, Denmark

The presentation will provide a comprehensive overview of serous fat atrophy, also referred to as gelatinous transformation, when identified in bone marrow. It will cover key terminology and provide characterization of gross lesions and histopathological manifestations. Studies involving necropsies/autopsies will be presented, with respect to tissues that are preferably examined for the presence of serous fat atrophy. Also, the progression of serous fat atrophy will be elucidated. In the presentation various histological stains being applied for stating the diagnosis of serous fat atrophy will be shown.

E-mail: elvang@sund.ku.dk

HISTOPATHOLOGICAL FINDINGS IN BYCATCH DOLPHINS

Antonio Fernandez [1]

[1] University Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain

Bycatch, defined as the accidental entanglement of non-target species in fishing gear and it widely recognized as being the leading manmade cause of small cetacean mortality both globally (Reeves *et al.,* 2013). There is a growing public, scientific and policy recognition that assessing the impacts of human activities on marine animal welfare is as important as more traditional assessments based on health, causes of mortality and population dynamics. This is particularly relevant for bycatch, the unintended capture of non-target species including whales, dolphins, and porpoises in fishing gear.

Diagnosing bycatch, particularly in cases involving peracute underwater entrapment (PUE) syndrome, remains challenging. Diagnosis relies on an increasingly recognized set of gross and microscopic diagnostic criteria, though none are pathognomonic. The most frequent histopathological findings included mild acute degenerative changes in skeletal muscle, fragments of striated muscle within the alveolar spaces, alveolar oedema and emphysema in the lungs, hemorrhages and acute degenerative changes in the myocardium, the presence of intracytoplasmic hyaline globules in the liver (IEGs), and intravascular (gas embolism) and intratissular clear spaces. These lesions were associated with the empty round spaces observed histologically, which were interpreted as gas bubble emboli, indicative of agonal gas embolism. Furthermore, immunohistochemical analysis was conducted in heart samples using an antibody against heat shock protein 70 (HSP 70). This analysis revealed localized immunostaining, particularly in the blood vessels. Such findings are consistent with previous studies reporting the expression of HSPs in response to acute ischemia in cardiac tissue. The presence of HSP immunostaining in this case may indicate a stress response due to ischemic injury, correlating with the vascular pathology observed during the analysis.

All these findings provide valuable insights into the pathophysiology of PUE syndrome and its effects on vital organs. Immunohistochemically and using proteomic analyses confirmed an association between the IEGs and acute phase proteins, suggesting a relationship between acute stress (i.e., bycatch), disease, and cellular protective mechanisms.

References:

Reeves R, McClellan K and Werner T (2013) Marine mammal bycatch in gillnet and other Entangling Net Fisheries, 1990 to 2011. *Endangered Species Research* 20(1) 71–97 https://doi.org/10.3354/esr00481

Wessels, M.E. (Ed.) 2024. Use of pathology to better inform the welfare impact assessment of bycatch and entanglements. Report from the European Cetacean Society Conference Workshop, Catania, Sicily, Italy. 8th April 2024. *European Cetacean Society Special Publication* Series 63.

Fernández A, Sierra E, et al. Cetacean Intracytoplasmic Eosinophilic Globules: A Cytomorphological, Histological, Histochemical, Immunohistochemical, and Proteomic Characterization. *Animals (Basel).* 2023. Jun 27;13(13):2130. doi: 10.3390/ani13132130. PMID: 37443929; PMCID: PMC10340075.

E-mail: antonio.fernandez@ulpgc.es

HISTOPATHOLOGY OF MECHANICAL TRAUMA

Lorenzo Ressel [1]

[1] University of Liverpool, Neston, United Kingdom

This lecture presents a synthesis of the approach to the interpretation of mechanical trauma in veterinary forensic pathology, with emphasis on the morphological characteristics of lesions caused by blunt and sharp force trauma. The histological appearance of these injuries is critical for identifying the type and mechanism of trauma, particularly in forensic contexts involving suspected abuse.

Blunt force Trauma
Abrasions are resulting from tangential friction or compressive forces and are characterized histologically by loss of the superficial epidermis, often exposing the dermis. The epidermal margins may appear irregular or necrotic. The dermis beneath the abrasion commonly contains disrupted collagen bundles, capillary congestion, and extravasated erythrocytes, sometimes with embedded particulate material (e.g., soil). Contusions are identified by intradermal or subcutaneous haemorrhage, with erythrocytes diffusely distributed among intact collagen fibres. There may be disruption of small vessels, but the overlying epidermis remains intact. Contusions may vary in depth and extent depending on the force applied and the resilience of the tissue. The margins are usually ill-defined, and haemorrhage may dissect along fascial planes or muscle bundles. Lacerations, which result from tearing rather than cutting, exhibit irregular wound edges and micro-bridging structures such as strands of intact tissue such as nerves, vessels, or connective fibres spanning the wound gap. Histologically, the surrounding tissue shows signs of mechanical distortion, including crushed muscle fibres, hemorrhage, and interstitial edema. The dermal collagen may appear frayed or fragmented, and muscle tissue may show fibre separation.

Sharp Force Trauma
Sharp force injuries, in contrast, display clean, sharply demarcated edges. There is an absence of tissue bridging across the defect, and the margins appear smooth under low magnification. Incisions extend through skin and subcutaneous tissues in a linear or curved pattern depending on the instrument used. Damage to adjacent tissues is minimal, with little evidence of crushing or distortion. Vascular disruption is evident at the site of incision, but surrounding structures remain largely unaffected. With chop wounds there is an overlap between sharp and blunt features. Differentiating between blunt and sharp force trauma histologically hinges on recognizing these specific architectural patterns—namely, the presence or absence of bridging, the regularity of wound edges, the pattern and distribution of hemorrhage, and the extent of adjacent tissue distortion.

Wound age estimation
While morphological criteria for lesion classification are well established, the precise estimation of wound age remains a significant challenge in veterinary forensic histopathology. Variability between species, individual immune responses, and the influence of environmental factors complicate the interpretation of temporal markers, underscoring the need for further research and validation of adjunctive diagnostic tools.

References:
Ressel L., Hetzel U., Ricci E. *Blunt Force Trauma in Veterinary Forensic Pathology*: Veterinary Pathology; 2016, 53(5); 941–961.

Hilbe M., Sydler T., Hugentobler L., Heller O., Stadler T., Kuratli J. *Forensic wound age estimation in dog tissue correlated with newly formed collagen fibres: a retrospective study*: Journal of Comparative Pathology; 2024, 215; 81–90.

Dettmeyer R.B., *Forensic histopathology: fundamentals and perspectives*, Berlin, Germany: Springer; 2011, 5; 215–221

E-mail: ressel@liverpool.ac.uk

HISTOPATHOLOGY OF THERMAL INJURY

Udo Hetzel [1]

[1] Institute for Veterinary Pathology (IVPZ), Zürich, Switzerland

Thermal injuries in animals are challenging aspects in veterinary forensic pathologic cases due to the high variability of species and possible given circumstances of occurring injuries. Often aetiological circumstances are not known or only reported in fragments, and depending on incomplete observations of third parties. Thermal injuries can occur due to application of fire, hot steam or hot fluids, by radiation heat, microwave radiation and inhaled hot and possibly toxic gases. Localized and generalized heat lesions, i.e. burns, scalds, and inhalation trauma are a histological challenge to the veterinary pathologist. The severity of lesions is related to intensity, depth, and the tissue area involved and are related to temperature, time span of application, heat conduction, and thermal capacity of the tissue and often the differentiation between intravital and post mortem findings are partially impossible. A summary of the literature with examples from veterinary and human forensic medicine will attempt to provide some clarification on this topic of veterinary forensic pathology.

References:

Hanglin Ye, Suvranu De Thermal injury of skin and subcutaneous tissues: A review of experimental approaches and numerical models. Science direct 2016; Volume 43, 5: 909-932.

Pielesz A, Gawłowski A, Biniaś D, Bobiński R, Kawecki M, Sama E, Waksmańska W. A Histologic Perspective on Electrical and Thermal Burn-Injured Human Skin. Adv Skin Wound Care. 2019 May;32(5).

Schulze C, Peters M, Baumgärtner W, Wohlsein P. Electrical Injuries in Animals: Causes, Pathogenesis, and Morphological Findings. Vet Pathol. 2016; 53(5):1018-29.

Wohlsein P, Peters M, Schulze C, Baumgärtner W. Thermal Injuries in Veterinary Forensic Pathology. Vet Pathol. 2016; 53(5):1001-17.

E-mail: udo.hetzel@uzh.ch

HOW RELIABLE IS YOUR DIAGNOSIS? TYPES, CAUSES AND CONTROL OF INSECURITIES IN DIAGNOSTIC AND FORENSIC PATHOLOGY

Achim Gruber [1]

[1] Free University of Berlin, Germany

The accuracy and justification of every clinical or judicial decision are based on a correct diagnosis or expert opinion, respectively. However, many types of personal assessments and test results may contain uncertainties that may lead to incorrect clinical decisions. The same applies to the reliability of expert opinions for legal disputes. Uncertain or even erroneous assessments can have various causes, only some of which are known to the examining or commissioning person.

This presentation provides an overview of three different types of susceptibility to errors using the example of pathological biopsy and cytology examinations, which can be transferred to other veterinary or legal disciplines. A solid understanding of the possible sources of errors results in a spectrum of preventive measures that should be implemented in professional environments.
In principle, three different types of uncertainties can be distinguished, which have different consequences for their recognition and communication:

Type 1 Insecurities
Uncertainties that are inherent in the method and are generally known to professionals. In principle, they are foreseeable and should therefore be taken into account when selecting the method used. Such uncertainties can be influenced to a limited extent both during sampling and when carrying out the test procedure.

Type 2 Insecurities
Case-typical or case-specific uncertainties resulting from insufficient diagnostically relevant information from the sample or from the information on which the expert opinion is based. The examiner recognizes this uncertainty and formulates a vague opinion. Non liquet statements are used in the examination report or an intuitive, subjective and rough assessment of a probability based on specific terms. This expresses the competence and uniqueness of the investigator. The consequence of such uncertainties may be a recommendation for further investigations or the procurement of information in court proceedings.

Type 3 Insecurities
Uncertainties due to bias and noise that are usually not apparent to either the person commissioning the study or the person conducting the study. These can distort the results of the study systematically and directionally (bias) or randomly and undirectedly (noise). Both often remain unrecognized, but can be reduced by procedural hygiene measures.

Risks associated with diagnostic uncertainties can be reduced by, among other measures,
- knowledge of their types and causes,
- high quality training, experience, and continuing education,
- structured procedural hygiene,
- an actively self-questioning working style and
- open communication with the client

Importantly, adequate communication and discussion of case-specific, limited probabilities in pathology reports and expert opinions significantly contribute to avoiding incorrect decisions. This can also fundamentally affect the accountability of the investigator or expert. Proper communication of insecurities enables the decision maker, if necessary, to initiate further diagnostic tests or gather further evidence in the context of all other available data in order to reduce the risk of error as far as possible. This complex topic likely deserves more attention in the training of pathologists and forensic experts.

References:
Gruber AD, Wehrend A, Steidl T, Kramer K, Merbach S, Baumgärtner W, Buyle T, Weisser NF, Beineke A. *How reliable is your diagnosis?* Tierarztl Prax Ausg G. 2024 Oct;52(5):281-288. doi: 10.1055/a-2395-6662

E-mail: achim.gruber@fu-berlin.de

DIAGNOSTIC UNCERTAINTY IN VETERINARY PATHOLOGY REPORTS: TOWARDS A STANDARDISED USE AND INTERPRETATION OF MODIFYING PHRASES

Giuseppe Giglia [1]

[1] University of Perugia, Perugia, Italy

Identifying, describing, and interpreting macroscopic and microscopic features of pathology specimens are foundational to detecting lesions and establishing diagnoses in veterinary pathology. However, there are cases where specimens do not exhibit sufficient criteria for a definitive diagnosis. These limitations may arise from various factors, including, but not limited to, inadequate tissue sampling, the presence of artifacts (Lindley et al., 2014), or pathologist-related variables such as professional experience, familiarity, and expertise with the species, tissue, or organ under examination. In such cases, veterinary pathologists often rely on modifying phrases (MPs) to express uncertainty and communicate their level of diagnostic confidence (Galloway and Taiyeb, 2011; Bracamonte et al., 2016; Gibson et al., 2022; Amin, 2023). While commonly used, MPs can introduce ambiguity, as their interpretation may vary due to cultural and linguistic differences, potentially complicating the understanding of diagnostic pathology reports.

In human pathology, these concerns have guided investigations into the perception of MPs diagnostic confidence and their associated levels of certainty (Attanoos et al., 1996; Galloway and Taiyeb, 2011; Lindley et al., 2014; Bracamonte et al., 2016), ultimately leading to the proposal of standardized terminology to improve clarity in communication (Amin et al., 2021; Amin, 2023). In veterinary medicine, however, research on this topic remains limited. Only a few studies have examined how MPs are perceived by veterinary clinical (Christopher and Hotz, 2004; Rishniw and Freeman, 2023) and anatomic (Giglia et al., 2025) pathologists, as well as by other veterinary professionals (Giglia et al., 2025). Despite their frequent use in veterinary pathology diagnostic practice, MPs are typically employed at the discretion of the individual pathologist, without standardized guidance. This lack of consistency may hinder effective communication between pathologists and other veterinary professionals, potentially leading to misinterpretation of pathology reports, an issue potentially exacerbated in today's digital era, where reports are readily shared across geographic and professional boundaries. This workshop starts with a discussion on the MPs-related literature and findings of a recent online survey investigating how veterinary anatomic pathologists and other veterinary professionals perceive the diagnostic confidence associated with selected MPs (Giglia et al., 2025). Additionally, it incorporates discussion of real diagnostic cases to highlight the practical use and implications of MPs use. Participants will explore the challenges in articulating diagnostic uncertainty, share perspectives, and discuss practical approaches for improving the clarity of MPs usage in daily diagnostic work. Recommendations from the workshop include the preferential use of clearly defined and standardized terms (e.g., diagnostic of, compatible with, consistent with) in pathology reports to facilitate better communication. Notably, when a definitive diagnosis is established, the use of MPs is discouraged. This workshop invites participants to contribute to the ongoing effort to standardize the definition, interpretation, and use of MPs in veterinary pathology to enhance communication between pathologists and other veterinary professionals, ultimately supporting informed clinical decision-making and improved patient care.

References:
Amin A. *Communication of Certainty in Surgical Pathology Reports*. Arch Pathol Lab Med. 2023;147(4):384–385.

Amin A, DeLellis RA, Fava JL. *Modifying phrases in surgical pathology reports: introduction of Standardized Scheme of Reporting Certainty in Pathology Reports (SSRC-Path).* Virchows Arch. 2021;479(5):1021–1029.

Attanoos RL, Bull AD, Douglas-Jones AG, Fligelstone LJ, Semararo D. *Phraseology in pathology reports. A comparative study of interpretation among pathologists and surgeons.* Journal of Clinical Pathology. 1996;49(1):79–81.

Bracamonte E, Gibson BA, Klein R, Krupinski EA, Weinstein RS. *Communicating Uncertainty in Surgical Pathology Reports.* Academic Pathology. 2016;3:2374289516659079.

Christopher MM, Hotz CS. *Cytologic diagnosis: expression of probability by clinical pathologists.* Veterinary Clinical Pathol. 2004;33(2):84–95.

Galloway M, Taiyeb T. *The Interpretation of Phrases Used to Describe Uncertainty in Pathology Reports.* Pathology Research International. 2011;2011:1–6.

Gibson BA, McKinnon E, Bentley RC, et al. *Communicating Certainty in Pathology Reports.* Arch Pathol Lab Med. 2022;146(7):886–893.

Giglia G, Porcellato I, Lo Giudice A, Brachelente C, Lepri E, Leonardi L, Mechelli L, Sforna M, Meuten D, Mandara MT. *Modifying phrases in veterinary pathology diagnostic reports: The veterinary professionals' perception on the diagnostic confidence.* Veterinary Pathology. 2025:03009858251334346.

Lindley SW, Gillies EM, Hassell LA. *Communicating diagnostic uncertainty in surgical pathology reports: Disparities between sender and receiver.* Pathology - Research and Practice. 2014;210(10):628–633.

Rishniw M, Freeman KP. *Preferences of clinical pathologists for probability-modifying terms describing cytologic sample evaluations: A survey study.* Vet Clin Pathol. 2024;53 Suppl 1:60–64.

E-mail: giglia.giuseppe.93@gmail.com

WHAT DO CLINICIANS AND PATHOLOGISTS NEED FROM EACH-OTHER

Pompei Bolfa [1], Laura Marconato [2]

[1] Department of Biomedical Sciences, Ross University School of Veterinary Medicine, Saint Kitts And Nevis
[2] Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy

In oncology, a precise and confident diagnosis is not merely a formality: it's the foundation on which all treatment decisions rest. Every stage of cancer treatment, from the initial diagnosis to the final therapeutic steps, relies on the information provided by pathology reports.

The accuracy and clarity of these reports determine the pathway for intervention, influencing whether patients receive the correct treatment, at the right time, and with the best chance for success. As such, the pathologist's role is pivotal, making their ability to deliver reliable diagnoses not just a professional responsibility, but a critical component of patient care.

For oncologists, the pathologist's report guides the entire course of care, from surgical planning to the need of adjuvant chemotherapy. When a pathology report presents a well-substantiated diagnosis, it allows clinicians to act decisively. In contrast, ambiguous findings can stall the clinical process, introduce diagnostic uncertainty, and increase the risk of inappropriate or delayed therapy.

Sampling
Sampling is a critical first step in the diagnostic process, and the goal should be to submit a sample that is representative of the tumor and provides sufficient tissue for histologic evaluation. The oncologist, by ensuring proper sampling techniques, enables the pathologist to provide a detailed, accurate report.

This report, in turn, becomes the backbone for all subsequent treatment decisions.

Beyond histotype
A pathology report should include the histopathologic description and diagnosis.
It is recommended to also include the mitotic count, lymphovascular invasion (If present), surgical margin evaluation (if applicable), and lymph node status (if the lymph node has been submitted alongside the primary tumor).

For margin evaluation, it is essential that the margins are inked in order to provide accurate measurements (in mm). The responsibility for inking the margins typically falls to the submitter, but this can vary depending on institutional protocols. The role of surgical margins in predicting cancer recurrence is still a subject of study, and more research is needed to understand their true impact. For some tumors, factors such as histologic grade and the biologic behavior may be more important than the margins themselves in predicting prognosis.

Differential diagnoses
One of the major challenges in pathology is navigating the landscape of histologic mimics, in other words tumors that closely resemble one another but require very different treatment. It's essential

that these complexities are addressed within the report, in the comment section. A well-considered list of differential diagnoses, when appropriate, does not undermine the diagnosis; rather, it reflects thoughtful analysis. When there is any degree of uncertainty, suggesting specific immunohistochemical stains or ancillary testing helps oncologists plan the next steps with clarity.

Level of confidence
The pathology report is, in many ways, the bridge between two specialties. From the oncologist's perspective, an ideal report includes not just a diagnosis but the level of confidence in that diagnosis. It is particularly helpful when the pathologist includes a concise comment, especially when findings are unexpected or when there is a known discrepancy with clinical suspicion. The pathologists should clearly communicate the level of diagnostic certainty (or uncertainty) in their reports. Transparency regarding uncertainty allows for further investigation or a second opinion, reducing the risk of clinical missteps.

What should be avoided
There are certain elements that oncologists would prefer not to see in a pathology report, as they may fall outside the pathologist's scope or create confusion in clinical decision-making.

One such example is the inclusion of specific treatment recommendations or prognostic statements. Pathologists provide a microscopic diagnosis and report parameters that are prognostic, while clinicians assign the prognosis based on the overall clinical context. Indeed, prognostication is nuanced and depends not only on the histologic diagnosis but also on multiple patient-specific variables, including tumor location, clinical stage, biologic behavior, and response to therapy. Oncologists are trained to integrate all of these factors when counseling owners and tailoring an individualized treatment plan.

Second opinion
Another important message is that a request for a second opinion is not a critique of the original pathologist's work. In oncology, where decisions are high-stakes and emotions often run high, a second opinion can offer reassurance for both the clinician and the owner. It should be seen as an opportunity for collaboration rather than a challenge to the pathologist's expertise.

Ultimately, the shared goal of both pathologists and oncologists is to provide the best possible care to patients. By fostering open communication, respecting each other's roles, and striving for clarity and precision, we can ensure that every report becomes a reliable stepping stone toward informed decision-making.

E-mail: laura.marconato@unibo.it

DECODING CANINE CANCER: HARNESSING GENETIC INSIGHTS AS TOOLS FOR PROGNOSIS AND THERAPEUTIC ADVANCEMENTS IN ONCOLOGY CARE

Lucas Rodrigues [1]

[1] One Health Company, Palo Alto, United States

This presentation will offer a comprehensive overview of the evolving landscape of canine oncology, centering on the molecular targets that have revolutionized treatment strategies. We will explore the critical signaling pathways and genetic aberrations, such as KIT, PIK3CA, RAS-family, BRCA1/2, PDGFR, EGFR, ERBB2 and others, that drive tumor in dogs. By understanding these molecular mechanisms, we gain valuable insight into how targeted therapies have emerged as precise tools for intervention.

We will illustrate how small molecule inhibitors, designed to interact with these defined targets can potentially transform clinical outcomes in veterinary oncology. Real-world applications and clinical protocols will be discussed, demonstrating how molecular targeting translates into practice and improves patient care.

The presentation will also delve into the future of personalized medicine, highlighting ongoing research into novel targets and biomarkers. At the heart of this shift is the essential role of **genetic testing**. Genetic profiling not only identifies actionable mutations but also guides treatment selection, enhances therapeutic precision, and reduces adverse effects.

This session underscores the powerful intersection of molecular biology, clinical innovation, and translational research. Attendees will gain insight into the current and emerging molecular targets shaping the future of canine cancer care and learn how to apply this knowledge to elevate diagnostic and therapeutic decisions for companion animals.

References:
Rodrigues L, Wu K, Harvey G, Post G, Miller A, Hull A, Lambert L, Lopes C, Zou J. Real-world evidence couples genomic biomarkers with therapeutic outcomes for canine hemangiosarcoma. Sci Rep. 2025 May 14;15(1):16442.

Min, HY., Lee, HY. Molecular targeted therapy for anticancer treatment. *Exp Mol Med* 54, 1670–1694 (2022).

Wu P, Nielsen TE, Clausen MH. FDA-approved small-molecule kinase inhibitors. Trends Pharmacol Sci. 2015 Jul;36(7):422-39.

Chon E, Novel genomic prognostic biomarkers for dogs with cancer. J Vet Intern Med. 2023 Nov-Dec;37(6):2410-2421.

Hanazono K, et al., Epidermal growth factor receptor expression in canine transitional cell carcinoma. J Vet Med Sci. 2015 Jan;77(1):1-6.

Tsuboi M, et al., Assessment of HER2 Expression in Canine Urothelial Carcinoma of the Urinary Bladder. Vet Pathol. 2019 May;56(3):369-376

Rodrigues L,et al., Shared hotspot mutations in oncogenes position dogs as an unparalleled comparative model for precision therapeutics. Sci Rep. 2023 Jul 6;13(1):10935.

Maeda S, Lapatinib as first-line treatment for muscle-invasive urothelial carcinoma in dogs. Sci Rep. 2022 Jan 13;12(1):4.

Lorch G, et al., Identification of Recurrent Activating *HER2* Mutations in Primary Canine Pulmonary Adenocarcinoma. Clin Cancer Res. 2019 Oct 1;25(19):5866-5877.

Morini M, et al., Mutational Analysis of *c-KIT* and *PDGFRA*in Canine Gastrointestinal Stromal Tumors (GISTs). Vet Sci. 2022 Jul 21;9(7):376.

Gregory-Bryson E, et al., Canine and human gastrointestinal stromal tumors display similar mutations in c-KIT exon 11. BMC Cancer. 2010 Oct 15;10:559.

Frost D, Lasota J, Miettinen M. Gastrointestinal stromal tumors and leiomyomas in the dog: a histopathologic, immunohistochemical, and molecular genetic study of 50 cases. Vet Pathol. 2003 Jan;40(1):42-54.

Wang G, et al., Molecular subtypes in canine hemangiosarcoma reveal similarities with human angiosarcoma. PLoS One. 2020 Mar 25;15(3):e0229728.

Estabrooks T, et al., Identification of genomic alterations with clinical impact in canine splenic hemangiosarcoma. Vet Comp Oncol. 2023 Sep 21

Hédan B, et al., PTPN11 mutations in canine and human disseminated histiocytic sarcoma. Int J Cancer. 2020 Sep 15;147(6):1657-1665.

Takada M, Hix JML, Corner S, Schall PZ, Kiupel M, Yuzbasiyan-Gurkan V. Targeting MEK in a Translational Model of Histiocytic Sarcoma. Mol Cancer Ther. 2018 Nov;17(11):2439-2450.

Palma SD, et al., Review on Canine Oral Melanoma: An Undervalued Authentic Genetic Model of Human Oral Melanoma? Vet Pathol. 2021 Sep;58(5):881-889.

Hardwick L. A Comparative View on Molecular Alterations and Potential Therapeutic Strategies for Canine Oral Melanoma. Vet Sci. 2021 Nov 22;8(11):286.

E-mail: lucas@fidocure.com

COMPREHENSIVE GENOMIC PROFILING FOR CANINE CANCERS: VETOMICS' APPROACH TO PRECISION MEDICINE

Guannan Wang [1]

[1] VetOmics, United States

Current status of genomics-based precision medicine in veterinary medicine
In human oncology, precision medicine has transformed cancer care, replacing one-size-fits-all approaches with personalized strategies that account for individual genomic and molecular differences. Tumor genomic profiling is now a routine part of cancer care for human patients, delivering profound benefits in diagnosis, prognostication, and personalized treatment planning.

In veterinary oncology, however, precision medicine is still in its infancy. Efforts to enable genomic diagnostics and targeted therapies for dogs face significant limitations and challenges:

- Incomplete understanding of canine cancer genomes and biology
- Limited primary canine clinical and drug data
- Access and cost barriers to drugs

VetOmics's vision and approaches
VetOmics was founded with a clear mission: to tackle the challenges in veterinary genomic diagnostics and precision medicine, bridging critical gaps to transform cancer care for pets and deliver innovative precision medicine solutions for better outcomes.

- A comprehensive approach to understand canine cancer genomes
- Hypothesis-driven clinical study leveraging real-world patients' response and outcome data
- Advancing veterinary drug development and repurposing

Flagship test: Canine CGP
Canine CGP (Comprehensive Genomic Profiling) is a whole exome sequencing test that analyzes all major mutation types across 20,257 genes, covering the entire coding exome of the canine genome. It is the world's largest, most advanced tumor genomic diagnostic test for dogs with cancer, providing unparalleled insights into tumor genomics to support personalized cancer management and effective treatment.

Applicable across all canine cancer types, Canine CGP aids in diagnosis, prognostication and personalized treatment planning, including both single-agent and combination therapy strategies across chemotherapy, targeted therapy, immunotherapy and radiotherapy.

In clinical practice, Canine CGP delivers comprehensive genomic insights for each dog, maximizing the likelihood to identify putative pathogenic mutations and clinically actionable biomarkers, driving novel and effective treatment options for canine cancer patients.

In research settings, Canine CGP provides unprecedented insights into the genomic landscapes of common and rare canine cancers, paving the way for groundbreaking discoveries and translational breakthroughs.

Conclusion
VetOmics stands out as the first company to integrate comprehensive genomic profiling and real-world clinical data to revolutionize veterinary oncology with an expert team. By addressing critical gaps in understanding cancer genomics in pets, clinical data generation, and drug access, we aim to enable the full potential of precision medicine to companion animals, providing better outcomes for patients and their families.

References:
Clinical, pathologic and molecular findings in 2 Rottweiler littermates with appendicular osteosarcoma. Res Sq. Preprint. 2024 Apr 11

Genomic analysis across 53 canine cancer types reveals novel mutations and high clinical actionability potential. Veterinary and Comparative Oncology, 2023 Dec 5. *Corresponding author
Novel Genomic Prognostic Biomarkers for Canine Cancer Patients. Journal of Veterinary Internal Medicine, 2023 Oct 6. *Corresponding author

Standing in the canine precision medicine knowledge gap: Improving annotation of canine cancer genomic biomarkers through systematic comparative analysis of human cancer mutations in COSMIC. Veterinary and Comparative Oncology, 2023 May 8. *Corresponding author.

Genomic tumor analysis provides clinical guidance for the management of diagnostically challenging cancers in dogs. Journal of American Veterinary Medical Associations, 2023 Mar 1.

Canine Oncopanel: A capture-based, NGS platform for evaluating the mutational landscape and detecting putative driver mutations in canine cancers. Veterinary and Comparative Oncology, 2021 Jul 19. *Corresponding author.

Molecular subtypes in canine hemangiosarcoma reveal similarities with human angiosarcoma. Plos One, 2020; 15(3): e0229728. *Corresponding author

Actionable mutations in canine hemangiosarcoma. Plos One, 2017, Nov 30; 12(11): e0188667

E-mail: DrWangVetOmics@outlook.com

RELIABLE SOURCES FOR EXAM PREPARATION AND TIME MANAGEMENT STRATEGIES

Edouard Reyes Gomez [1]

[1] Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France

The annual certifying ECVP Examination is divided in 5 sections that are, in chronological order: HISTOPATHOLOGY, GROSS PATHOLOGY, GENERAL PATHOLOGY, VETERINARY PATHOLOGY and COMPREHENSIVE PATHOLOGY.

For a good preparation to the ECVP examination, residents and future candidates have to be familiar with these different sections, especially regarding their format, scope and expectations, in order to find reliable resources. The format of questions, for all sections, is presented on the ECVP website along with some examples. Candidates also have to be well prepared to time management as time can be a critical factor especially for HISTOPATHOLOGY and COMPREHENSIVE.

GENERAL PATHOLOGY and **VETERINARY PATHOLOGY** are demanding in terms of personal work and memorization, but resources and sources for preparation (textbooks and journals) are present in a detailed and annually updated reading list that is provided on the ECVP website. This list will cover the majority of questions. In addition to these resources, making and exchanging questions among residents is a good way to improve preparation. Regular journal clubs can also help in identifying relevant papers. For the majority of candidates, time management is not a problem in these sections.

For **GROSS PATHOLOGY**, there are now innumerable resources to prepare this section. Pictures from textbooks are, of course, a good start. Several online and free photo banks made by renowned institutions can also provide good quality pictures with a wild range of organs and species. Image challenges in the *Veterinary Pathology* journal also represents a good resource. Here again, making and exchanging questions/pictures among residents is useful and stimulating for preparation. For the majority of candidates, time management is not a problem in this section.

Regarding **HISTOPATHOLOGY**, the Wednesday Slide Conference (WSC)-Veterinary Systemic Pathology Online slide collection of the Joint Pathology Center (formerly AFIP) is probably the best known free online resource. In addition to the classic WSC case description, since 2009, there are free graded descriptions for each case that represent an excellent complement for examination preparation. Residents should however be aware that not all WSC cases represent “exam cases” (e.g., if slides have multiple organs, too few lesions, etiologic diagnosis impossible without context etc.). Other resources and slide collections are available, including on or from the ECVP website. At their institution, residents might also have access to slide collections and mock exam material. Time management is critical for this section. In order to be well prepared, training in the same conditions as for the real examination is mandatory (quite room, typing description on a computer, slide set to be share among two residents etc.). Adaptation of the time limit for description (e.g., 25 min/slide for 1st year residents, 20 min for 2nd year residents, 12 min for 3rd year residents) can be a progressive way to help residents to manage their time. Using a time schedule during the examination is also very useful.

For **COMPREHENSIVE PATHOLOGY**, examples of questions are present on the ECVP website as well as a dedicated presentation given at the Madrid Congress in 2024. Time management is extremely

important in this section. A proposed schedule is given to candidates at the beginning of this section with suggested dedicated time (e.g., 20 min for abstract, 40 min for Tox Path etc.). It is also critical that residents and candidates be familiar with the type and format of questions (describe, summarize, interpret etc.) in order to give appropriate, precise and concise answers. Otherwise, candidates may lose too much time writing excessive and unnecessary text.

E-mail: edouard.reyes-gomez@vet-alfort.fr

SATURDAY 30TH AUGUST 2025

FROM CLINIC TO LAB: OPTIMIZING THE DERMATOLOGIST–PATHOLOGIST COLLABORATION

Chiara Brachelente [1], Stefano Borio [2]

[1] Department of Veterinary Medicine, Perugia, Italy
[2] Clinica Veterinaria Nervianese, Nerviano, Italy

Histopathology is a powerful diagnostic tool in veterinary dermatology—but only when used correctly. Accurate diagnoses rely not only on the pathologist's interpretive skills but also on effective communication and collaboration with the clinician. This interactive seminar highlights common errors in dermatologic biopsy sampling and submission, and offers practical strategies to improve clinician-pathologist interaction.

When and Why to Biopsy?
Skin biopsy should be based on clinical reasoning. Appropriate contexts include atypical or persistent lesions, suspected neoplastic or paraneoplastic syndromes, failure of empirical therapy, or inconclusive cytology. Biopsy is discouraged when lesions are chronic with extensive secondary changes, during corticosteroid therapy, or when relevant clinical data are missing.

Common Sampling and Communication Errors
Several technical pitfalls can compromise biopsy value:

- Sampling from non-representative lesions (e.g., crusts or secondary changes)
- Using too small punch sizes (e.g., 4 mm for deep or extensive lesions)
- Poor sample orientation or fragmentation
- Surgical scrubbing (except for nodules), which may remove key superficial lesions like pustules or crusts, including acantholytic cells or etiological agents
- Inadequate fixation or improper packaging

The tools and technique should be chosen according to the lesion's type and location (punch biopsies vs scalpel biopsies). While a common belief is that biopsies should be taken at the border between normal and lesional skin, in many cases the most diagnostic yield is achieved when the sample is taken from the center of the lesion.

A critical component often underestimated is the submission form. Pathologists work without access to the patient and rely entirely on the information provided. Clinicians must include a concise clinical history, anatomical distribution, biopsy site description, prior treatments, response to therapy, and, if possible, images.

Submitting a large enough tissue sample does not ensure diagnosis. What really makes the difference is the clinical context provided by the dermatologist. The pathologist is not a clinician and should not reconstruct the full diagnostic framework or differential list based on minimal or vague input.

A well-prepared submission should include a summary of the clinical problem and a list of differential diagnoses formulated by the clinician through appropriate workup. By the time a biopsy is taken, the clinician should have already performed a thorough diagnostic workup and excluded a range of differentials—so that the biopsy is not the starting point, but rather a targeted tool. At biopsy

submission, the clinician should be able to pose a focused set of diagnostic questions to the pathologist, based on a narrowed list of well-considered hypotheses.

The Pathologist's Approach: Pattern Recognition and Clinical Integration
Since the late 1970s, pattern analysis has provided a structured method for diagnosing inflammatory skin diseases. Rather than classifying by etiology, this approach starts with low-power examination to identify architectural patterns (e.g., perivascular, pustular, nodular), followed by higher magnification to assess inflammatory cells and changes in the epidermis, dermis, and adnexa. These patterns do not equate to individual diseases but represent groups with shared pathogenetic mechanisms. Thus, interpretation must always be contextualized with clinical data.

The dermatopathologist's role is not only to provide a detailed morphological description and formulate a morphologic diagnosis—as required by professional standards—but also, like the clinician, to interpret findings in a broader diagnostic framework. This means translating microscopic patterns into a list of differential diagnoses to support the clinician's clinical reasoning.

In some cases, histopathologic findings strongly support a specific diagnosis; in others, they are more generic and must be interpreted by the clinician. The pathologist may also recommend further tests—immunohistochemistry, special stains, or molecular analyses—to support diagnosis.

Conclusion
Biopsies do not guarantee diagnosis. Their success depends on appropriate lesion selection, sound sampling technique, and, most critically, effective bidirectional communication. This seminar highlights the contributions and responsibilities of both parties and outlines common mistakes that reduce diagnostic value.

These concepts will be further emphasized through selected clinical case presentations. Case studies will illustrate the value of collaboration, showing how accurate sampling and well-structured clinical information can significantly influence interpretation and outcome. The interactive format offers participants the opportunity to apply these principles to practical scenarios and reinforce the importance of a coordinated clinicopathologic approach.

References:
Welle M.M., Linder K.E. *Integumentary system*. In: Zachary JF, ed. *Pathologic Basis of Veterinary Disease*. 7th ed. 2022.

Mauldin E.A., Peters-Kennedy J. *Integumentary System.* In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals. Vol. 1. 6th 2016.

Jasmin P. *Clinical Handbook on Canine Dermatology*. 3rd ed. 2011.

Gross TL, Ihrke PJ, Walder EJ, Affolter VK. *Skin Diseases of the Dog and Cat: Clinical and Histopathologic Diagnosis*. 2nd ed. Blackwell Publishing, 2005.

E-mail: chiara.brachelente@unipg.it

FROM ONE HEALTH TO CIRCULAR HEALTH TO FACE CONTEMPORARY CHALLENGES

Ilaria Capua [1]

[1] Johns Hopkins University SAIS Europe, Bologna, Italy

The *One Health* concept, although it has gained renewed prominence in recent years, has its origins more than six decades ago. Originally introduced to emphasise the interdependence of human, animal, and environmental health, One Health has provided a valuable conceptual and operational framework for addressing zoonotic diseases, antimicrobial resistance, and ecological degradation. The COVID-19 pandemic underscored its importance, spotlighting the interface between species and environments in disease emergence and the necessity of coordinated cross-sectoral approaches.

Despite its continued relevance, One Health—formulated in a markedly different scientific and ecological context—no longer fully captures the scope and complexity of contemporary health threats. These include climate change, disruptions in global food systems, demographic shifts affecting animal and human populations, the acceleration of novel pathogen emergence, public mistrust in science, and the evolving role of information technologies. While One Health has emphasised the role of interspecies transmission and ecosystem health, its capacity to address systemic drivers of pathology—particularly in the veterinary and comparative domains—has become increasingly limited.

The Circular Health approach builds on One Health, providing a wider and more connected way to tackle the complicated health and disease issues in related biological systems. It draws philosophical inspiration from the Hippocratic tradition of the four classical elements—earth, air, water, and fire—which were historically considered fundamental to both human physiology and environmental balance. In the context of contemporary comparative pathology, these elements serve as proxies for the environmental forces that influence disease expression, transmission, and susceptibility across species.

Circular Health asserts that these elements are not merely symbolic but represent concrete environmental determinants of health outcomes. For instance, air quality has well-documented effects on both human and animal respiratory pathology; particulate matter and atmospheric pollutants contribute to a range of inflammatory and degenerative conditions in species from rodents to livestock. Soil degradation impacts not only food safety and nutrition but also exposure to mycotoxins, helminths, and soil-borne pathogens, which affect both domestic and wild animal populations. Water scarcity and contamination increase the burden of enteric and parasitic diseases, particularly in intensively managed animal systems and regions with compromised infrastructure. Fire—reframed as thermal and energy-related stress—affects immune function, metabolic balance, and vector distribution in a wide range of species.

Comparative pathology, with its trans-species diagnostic lens, is uniquely positioned to observe and interpret how these environmental and social determinants manifest differently across taxa. The Circular Health framework invites veterinary pathologists to consider not only lesions and aetiologies but also upstream ecological, climatic, and socioeconomic factors that shape disease landscapes.

Moreover, Circular Health is distinctly forward-looking. It embraces data science and artificial intelligence as essential tools for veterinary epidemiology, syndromic surveillance, and diagnostic precision. The integration of genomic, environmental, and clinical datasets enables more refined comparative analyses and predictive modelling, enhancing both animal health monitoring and the early detection of zoonotic spillovers.

Importantly, Circular Health aligns with the global agenda outlined by the United Nations Sustainable Development Goals (SDGs) and foresees them as implementation tools. While One Health has largely concentrated on zoonoses and surveillance, Circular Health broadens this scope to promote human and animal health and welfare, sustainable agriculture, preservation of biodiversity, and climate resilience through the objectives and targets of the SDGs. SDGs related to food security (SDG 2), clean water (SDG 6), climate action (SDG 13), and ecosystem protection (SDG 14 and 15) are directly relevant to veterinary science and comparative pathology.

In conclusion, while One Health continues to offer a vital framework for managing interspecies health risks, it must evolve to remain effective in the face of an increasingly complex knowledge base and contemporary threats. Circular Health represents a next-generation paradigm—one that integrates ancient perspectives with contemporary veterinary science and emerging technologies. For the fields of veterinary medicine and comparative pathology, it offers a powerful lens through which to investigate disease, inform intervention, and advance a sustainable vision of health that includes all species and their shared environments.

References:
Capua I.**,** *Circular Health: Empowering the One Health Revolution***,** Milano Italy, Bocconi University Press 2021

Mantegazza, L., De Pascali, A.M., Munoz, O., Manes, C., Scagliarini, A., Capua, I. *Circular Health: Exploiting the SDG roadmap to fight AMR*. Lausanne, Switzerland: Frontiers Media S.A.; 2023. 13:1185673.

Gullino ML, Pasquali M, Pugliese M, Capua I. *Positioning plant health within the evolving human-animal-environmental health paradigms. One Health*. 2024 Nov 7;19:100931. doi:10.1016/j.onehlt.2024.100931.

E-mail: icapua@jhu.edu

POSTER FLASH ABSTRACTS

119 | INFLUENZA A VIRUS RECEPTORS IN THE BOVINE AND PORCINE MAMMARY GLANDS

C. Kristensen [1] K. Pedesen [1], D. Selnihhin [1], P. Petric [1], R. Trebbien [2], R. Webby [3], L. Larsen [1], P. Ryt-Hansen [1], H. Jensen [1]

[1] Department of Veterinary and Animal Sciences, University of Copenhagen, Frederiksberg, Denmark
[2] Department of Virus and Microbiological Special Diagnostics, Statens Serum Inst, Copenhagen, Denmark
[3] Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, United States

Background
This study investigates the *in situ* expression of α2,3/α2,6-linked sialic acids (SA) to galactose (Gal) in the mammary glands of lactating cows and sows. Influenza A viruses (IAVs) isolated from birds prefer SAs linked to Gal in an α2,3 linkage (SA-α2,3-Gal, avian receptor) as their host receptor, whereas IAVs isolated from humans and pigs prefer SA-α2,6-Gal (human receptor). Additionally, IAVs isolated from chickens prefer SA-α2,3-Gal-β1,4 (chicken receptor) while those from ducks prefer SA-α2,3-Gal-β1,3 (duck receptor).

Materials & Methods
Mammary glands from lactating cows (*n* = 9) and sows (*n = 5*) were investigated by lectin histochemistry. The chicken receptor was detected by Maackia Amurensis Lectin I (MAA-I), the duck receptor by MAA-II, and the human receptor by Sambucus Nigra Lectin (SNA).

Results
In both cows and sows, the human and duck receptors were highly expressed on the surface of active lactocytes. The human receptor was detected on the surface of the mammary ducts of all cows, whereas one cow also expressed the chicken receptor. All sows expressed the human and duck receptors on the surface of the mammary ducts.

Conclusion
The presence of IAV receptors on the surface of active lactocytes of both species suggests that some IAVs, as in cows, can also attach to the epithelial cells of the sow udder. Ongoing viral attachment studies are needed to confirm this. Furthermore, knowledge of the presence and influence of IAV immunity in sow milk must be elucidated to understand the potential of the sow udder as a replication site.

261 | CARDIAC PERIPHERAL NERVE SHEATH TUMOURS IN CATTLE: A SLAUGHTERHOUSE-BASED STUDY (2017–2025)

M. Hilbe [1], G. Rosato [1], F. Seehusen [1]

[1] Institute of Veterinary Pathology, Zurich, Switzerland

Background
Since 2017, as part of the 'Organ Alterations at Slaughter' project, pathological changes identified during meat inspection, including 60 bovine heart samples or whole hearts were investigated. Peripheral Nerve Sheath Tumours (PNSTs) are among the more frequently reported neoplasms in cattle, particularly affecting the brachial plexus and intercostal nerves as well as the heart. According to literature, Hereford and Holstein breeds appear overrepresented. Based on histological and immunohistochemical findings, PNSTs in cattle can be classified as schwannomas, neurofibromas, or hybrid neurofibroma-schwannomas.

Materials & Methods
Of the 60 heart samples, 9 exhibited whitish nodules on the epicardium or myocardium, around the great vessels, and/or on the endocardium near the atrioventricular valves. These samples were processed for histological examination using HE staining and were further analysed by immunohistochemistry using antibodies against GFAP, vimentin and S100.

Results
All 9 cases were diagnosed as PNSTs. In three cases, age and breed data were unavailable. The remaining six animals were between 9 and 19 years old and included one Red Holstein, one Brown Swiss, two Aberdeen Angus, one Angus crossbreed, and one mixed breed. All animals were female.

Conclusion
Cardiac PNSTs in cattle appear to be relatively frequent. Two tumours originated from the endocardium near the atrioventricular valves, potentially impairing intracardiac blood flow. Within this small cohort, the Angus breed was overrepresented. All tumours were S100 and vimentin positive and expressed at variable extent GFAP. Two of the PNSTs were malignant.

365 | CORRELATION OF RECTAL BIOPSY FINDINGS WITH MUCOSAL CHANGES ELSEWHERE IN THE EQUINE INTESTINE

H. Pekkarinen [1, 2], P. Syrjä [2]

[1] Finnish Food Authority, Helsinki, Finland
[2] University of Helsinki, Helsinki, Finland

Background
Rectal biopsy is the most used diagnostic tool for diagnosis of equine inflammatory bowel disease. However, there are few studies on how rectal inflammation correlates with inflammation in other parts of the equine intestine and about the normal leukocyte numbers in equine rectal mucosa.

Materials & Methods
Histological samples from jejunum, ventral colon, small colon, and the routine rectal biopsy site were obtained from 20 horses, with or without intestinal signs, during routine autopsy. Samples were stained with HE and categorised according to inflammation type (eosinophilic, neutrophilic, lymphoplasmacytic), site (proprial, submucosal), and severity (mild, moderate, severe), using one scoring system for all examined areas. Ventral colon was substituted with dorsal colon in one case with severe autolysis. Statistical association between sites and severity were analysed using Fisher's exact test (significance level $p<0.05$).

Results
14 rectal samples showed inflammation, five of them moderate, nine mild. In contrast, 19 large colon samples showed inflammation, 11 of them severe. Increased rectal submucosal eosinophils ($p=0.0281$) and moderate/severe inflammation of the small colon ($p=0.0378$) correlated with severe large colon inflammation. However, moderate or severe inflammation in either rectal mucosa ($p=1.0000$) or small intestine ($p=1.0000$) did not correlate with large colon inflammation of any severity.

Conclusion
This small pilot study indicates that submucosal eosinophils are noteworthy in rectal biopsies and that mucosal changes in the small colon, accessible for biopsy per rectum, may better represent changes in the equine large colon. Mild or absent proctitis does not exclude severe large intestine inflammation.

397 | AI WITHOUT TRAINING: GPT-4O IN CANINE TISSUE IMAGE CLASSIFICATION

L. Ressel [1]

[1] University of Liverpool, Liverpool, United Kingdom

Background
Recent developments in generative AI, such as GPT-4o's image recognition capabilities, introduced the possibility of using general-purpose models for histological image analysis without specialized training. This study investigates GPT-4o's ability to identify tissue types and classify them as normal or pathological in canine histological samples.

Materials & Methods
108 images of normal, inflammatory, and neoplastic canine tissues were obtained from histology slides or textbooks, using four acquisition methods: mobile phone-mounted microscope, professional microscope camera, digital slide scanner, and textbook photography. Slides were stained with two HE protocols and captured at various magnifications. GPT-4o was prompted with the same questions for each image, and its responses were compared to diagnoses by a board-certified pathologist.

Results
GPT-4o identified all images as histological sections (100%) and correctly classified tissue types in 75% of cases. It distinguished normal from pathological samples with 84% accuracy and identified lesion type (inflammatory vs neoplastic) with 77% accuracy. Accuracy varied by tissue type, with lower performance for endocrine, reproductive, and some vascular tissues. No significant differences were observed across stain types, magnifications, or acquisition methods.

Conclusion
GPT-4o demonstrated strong generalization in classifying canine histology images without domain-specific training. Compared to CNNs, which require large, curated datasets, multimodal LLMs like GPT-4o leverage broad pretraining to interpret diverse inputs. These findings suggest a potential broader role for such models in veterinary histopathology classification.

403 | FILAMENTOUS BACTERIA CAUSING PERITONITIS IN DOGS AND CATS

A. Demeter [1], A. Rebollada-Merino [1]

[1] Cornell University, Ithaca, United States

Background
Nocardia spp. and *Actinomyces spp*. are Gram-positive filamentous bacteria that commonly cause indolent localized cutaneous or subcutaneous infections in dogs and cats; systemic infections are rarely reported.

Materials & Methods
Cases of peritonitis caused by filamentous bacteria in dogs (n=5) and cats (n=5) submitted to the Animal Health Diagnostic Center (Cornell University) were reviewed retrospectively. Disseminated infection was excluded based on clinical signs and imaging for biopsy cases (n=6) and post-mortem examination for necropsy cases (n=4).

Results
Clinical history included recent abdominal surgery (enterotomy, n=1; nephrectomy, n=1), chronic disease (copper-associated hepatitis, n=1), or both chronic disease and recent surgery (hyperadrenocorticism and enterotomy; n=1) in most dogs (4/6; 67%). Two cats (40%) had undergone a recent surgery (enterostomy, n=1; spay, n=1). Necropsy revealed a diffusely thickened, pale-tan, and firm mesentery. Histological examination showed severe, diffuse, chronic, pyogranulomatous peritonitis and omentitis containing large intralesional colonies of filamentous bacteria. Bacteria were gram-positive (10/10), argyrophilic (6/6), and strongly (1/6), weakly (2/6), or negative (3/6) on acid-fast stains. One cat had concurrent non-B, non-T cell leukemia. *Nocardia nova* was isolated from the mesentery of a necropsied cat.

Conclusion
Peritonitis caused by filamentous bacteria is a rare presentation in dogs and cats that may clinically and grossly mimic primary peritoneal neoplasms (mesothelioma), dissemination of other neoplasia (carcinomatosis, sarcomatosis), and steatitis (vitamin E deficiency). Surgery, causing disruption of the gut barrier, and preexisting co-morbidities leading to immunosuppression were predisposing factors in most of our cases. As microbiological examinations were not performed in all cases, accurate identification of bacteria may be hindered.

410 | DIFFERENT COMPARTMENTS, DIFFERENT ROLES? IMMUNOPHENOTYPING EPITELIAL AND LAMINA PROPRIA COMPARTMENTS IN FELINE INTESTINAL LYMPHOMA.

S. Vieira de Carvalho [1, 2], R. Noiva [1, 2], C. Marques [3, 4]

[1] CIISA, Interdisciplinary Centre of Research in Animal Health, Lisbon, Portugal
[2] Associated Laboratory AL4AnimalS, Lisbon, Portugal
[3] INSA, Instituto Nacional de Saúde Dr. Ricardo Jorge, Porto, Portugal
[4] CECA, Center for Study in Animal Science, Porto, Portugal

Background
Alimentary lymphoma is the most common feline lymphoid tumor. Epitheliotropism can be found in both high-grade (HGITL) and low-grade intestinal T-cell lymphomas (LGITL) and an apical-to-basal gradient has been described, suggesting chronic endoluminal stimulation.
We aim to investigate differential expression of Bcl-2, DNA mismatch repair components and PCNA between epithelial lymphocytes (EL) and lamina propria lymphocytes (LPL) in feline intestinal lymphomas.

Materials & Methods
Formalin-fixed paraffin-embedded tissues from 11 laparoscopic and 10 endoscopic biopsies of T-cell intestinal lymphoma (8 HGITL, 13 LGITL), diagnosed at the Pathology Laboratory of the Faculty of Veterinary Medicine, University of Lisbon, between 2017 and 2023, were phenotyped by immunohistochemistry for expression of CD3, CD20 or Pax5, Bcl-2, MLH1, MSH6, MSH2 and PCNA.

Results
10 lymphomas were transmural. EL were predominantly CD3+ (20/21), with only one case showing identical CD3+ and CD20/Pax5+ expression. EL moderate/strong expression of markers was frequent for Bcl-2 (16/21) and MSH2+ (13/21), occasional for MLH1 (10/21), and uncommon for MSH6 (5/21). EL PCNA expression was weak in 10/21 cases and negative in all others.
LPL had stronger expression of MLH1, MSH6, MSH2 and PCNA than EL in over 65% of cases, while LPL Bcl-2 expression was equal to or weaker than EL in over 70% of cases.
No significant differences were evident between HGITL and LGITL, or transmural and non-transmural lymphomas.

Conclusion
Our findings suggest that EL have a more predominant anti-apoptotic immunophenotype while LPL have a stronger proliferative immunophenotype, possibly suggesting different roles for each compartment in feline intestinal lymphoma.

ORAL ABSTRACTS

MARINE MAMMALS

212 | PATHOLOGICAL FINDINGS IN FREE-RANGING DIDELPHIS SPP. FROM URBAN AREAS IN BRAZIL: A ONE HEALTH PERSPECTIVE

E Ferreira Machado [1, 2], M. Lima Borges [2], J. Garcia [2, 3], M. Albuquerque Landi [2], J. Carvalho [2], J. Fernandes Takahashi [2], I. Jesus [2], B. Bernardes da Silva [4], C. Castagna [4], C. Lima [4], T. Zwarg [5], T. Sanches [5], M. Frediani [5], M. Siconelli [6, 7], R. Friciello Teixeira [8, 9, 10], A. Costa [8], R. Ressio [2], C. Borges [2], J. Catão-Dias [1], J. Guerra [1, 2], N. Azevedo Fernandes [2]

[1] Laboratory of Wildlife Comparative Pathology (LAPCOM), São Paulo, Brazil
[2] Pathology Centre, Adolfo Lutz Institute, São Paulo, Brazil
[3] School of Medicine, University of São Paulo (FMUSP), São Paulo, Brazil
[4] Surveillance Unit for Zoonoses, Campinas, Brazil
[5] Wildlife Management and Conservation Centre, São Paulo, Brazil
[6] Zoonosis Surveillance Unit , Ribeirão Preto, Brazil
[7] Laboratory of Molecular Virology, Ribeirão Preto, Brazil
[8] Municipal Zoological Park, Sorocaba, Brazil
[9] Universidade de Sorocaba, Sorocaba, Brazil
[10] Universidade Paulista (UNESP/Botucatu), Botucatu, Brazil

Background
Marsupials of the genus *Didelphis* serve as important reservoirs of zoonotic agents and as bioindicators of environmental health. This study aimed to characterise pathological processes in free-ranging Brazilian opossums from São Paulo State, Brazil, within a One Health perspective.

Materials & Methods
A total of 400 necropsied and biopsied marsupials were evaluated retrospectively and prospectively between 2004 and 2024. Histopathology was the primary diagnostic tool, supplemented by immunohistochemistry (IHC), polymerase chain reaction (PCR), microbiological cultures, and electron microscopy in selected cases.

Results
The majority of cases involved *Didelphis albiventris* (48.8%) and *Didelphis aurita* (48.0%), predominantly adults from urban areas. Infectious agents were detected in 40.6% (162/400) of cases: bacteria (26.2%) in most cases causing acute sepsis, metazoans (15.5%) causing enteritis and bronchopneumonia, protozoans (4.5%) encysted in different tissues, fungi (1.2%) causing dermatitis, and viruses (1.0%) causing encephalitis and dermatitis. Zoonotic pathogens identified included *Toxoplasma gondii* and rabies virus. Neoplastic lesions were observed in 23 animals, including hepatocellular carcinoma, haemangiosarcoma, lymphoma, and squamous cell carcinoma. *Streptococcus didelphis* was detected in six cases by PCR, highlighting bacterial sepsis as a relevant cause of mortality. Most cases originated from the cities of São Paulo and Campinas.

Conclusion
This study highlights the broad spectrum of pathological processes affecting *Didelphis* spp. in urbanised regions of Brazil and reinforces their role in zoonotic pathogen surveillance. Histopathological examination proved to be a valuable tool for broad pathological investigations, supporting One Health surveillance strategies.

263 | FIRST REPORT OF ENCEPHALOMYOCARDITIS VIRUS INFECTION AND DISEASE IN A COMMON HIPPOPOTAMUS (HIPPOPOTAMUS AMPHIBIUS)

L. Sanz-Matamala [1, 2], L. Martino [2], M. Domingo [2], V. Almagro [1], A. Palomo [1, 2], B. Oliveras [1], A. Luppi [3]

[1] Parc zoològic de Barcelona (Barcelona zoo), Barcelona, Spain
[2] Autonomous University of Barcelona, Cerdanyola del Vallès, Spain
[3] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Parma, Italy

Background
Viral encephalomyocarditis (EMC) is caused by a Cardiovirus of the Picornaviridae family, named encephalomyocarditis virus (EMCV). Rodents are considered the natural hosts and reservoirs of this virus throughout the world. The main route of transmission is the consumption of food or water contaminated with their urine or feces. EMCV infection has been described in a wide range of mammals, including suids, primates, elephants and pygmy hippopotamuses (Choeropsis liberiensis). This study presents the first case of EMCV infection and associated disease in a captive common hippopotamus (Hippopotamus amphibius) at the Barcelona Zoo.

Materials & Methods
A 33-year-old female common hippopotamus presented with acute-onset anorexia followed by recumbency and sudden death. A complete necropsy was performed, and formalin-fixed tissue samples were histologically studied. For the detection of EMCV, reverse transcription polymerase chain reaction (RT-PCR) was conducted on frozen heart samples.

Results
Macroscopically, no significant lesions were observed that could explain the sudden death. Histologically, the most relevant finding was a severe, subacute, multifocal to diffuse, non-suppurative myocarditis, consistent with a viral infection. No lesions were observed in the skeletal muscle or brain. RT-PCR analysis of heart tissue was positive for EMCV.

Conclusion
While this was an isolated case, this finding highlights the potential susceptibility of previously unreported species to EMCV and underscores the importance of pest control and biosecurity measures in zoos for infectious disease control.

394 | A COMPREHENSIVE REVIEW OF CAUSES OF DEATH OF MARINE MAMMALS KEPT UNDER HUMAN CARE IN ITALY, 1999-2024

G. Baviera [1], C. Centelleghe [1], S. Mazzariol [1]

[1] Department of Comparative Biomedicine and Food Science, University of Padua, Padua, Italy

Background
This study examines necropsies of marine mammals housed in Italian marine parks, conducted under the EU Animal Health Law (AHL) framework. The main goal is to better understand under human care (UHC) marine mammals' health status by analysing pathological results, disease prevalence, physiological abnormalities, trauma, and causes of death identified in the necropsy reports.

Materials & Methods
The review included 33 marine mammals (23 *Tursiops truncatus*, one *Grampus griseus*, one *Halichoerus grypus*, two *Phoca vitulina*, five *Otaria flavescens*, one *Zalophus californianus*) examined by the trained veterinarians of the Dept. of Comparative Biomedicine and Food Science (University of Padua) since 1999. Necropsies, data and sample collection followed standard protocols. Pathogen-positive tissues were flagged for zoonotic potential and relevance under the EU-AHL.

Results
Adult deaths (n=21) were mainly attributed to infectious diseases (n=13 bacterial; n=1 fungal). Other causes identified were dilated cardiomyopathy (n=1), congenital urogenital anomaly (n=1), high-grade poorly differentiated squamous cell carcinoma with pseudosarcomatous features (n=1), parturition-related trauma (n=1), blunt spinal trauma (n=1), acute necrotising pancreatitis (n=1) and one undetermined case.
Among young and neonates (n=12), the main causes of death included bacterial infections (n=5), blunt trauma (n=2; one cranial, one abdominal), meconium aspiration syndrome (MAS) (n=2), meconium plug syndrome (MPS) (n=1), gastric ulceration with secondary debilitation (n=1) and one undetermined case.

Conclusion
The findings highlight two main aspects: adult deaths were mainly related to infectious diseases; neonatal deaths were linked to perinatal vulnerability. Three isolated pathogens emerged as major concerns for public health and animal husbandry: Methicillin-Resistant *Staphylococcus Aureus* (MRSA), *Mycobacterium avium*, and Dolphin morbillivirus.

LIVESTOCK NEUROPATHOLOGY

406 | HIPPOCAMPAL SCLEROSIS: A HALLMARK OF EMERGING RETROVIRAL ENCEPHALITIS IN CATTLE?

M. Polidori [1, 2], T. Seuberlich [1], A. Oevermann [1]

[1] Division of Neurological Sciences, DCR-VPH, Vetsuisse, University of Bern, Bern, Switzerland
[2] Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland

Background
Non-suppurative encephalitis is a common cause of neurological disease in cattle, frequently caused by ovine herpesvirus 2 (OvHV-2), bovine astroviruses (BoAstV-CH13 and CH15), or bovine β-retrovirus (BoRV-CH15) in Europe. While hippocampal sclerosis, characterized by chronic neuronal loss and astrogliosis, has been observed in affected cattle, its prevalence and significance have not been systematically evaluated.

Materials & Methods
This study retrospectively examines H&E-stained hippocampal sections from 55 cattle diagnosed with non-suppurative encephalitis. In 27 cases, infections by BoRV-CH15 (*n* = 9), BoAstV CH13 or CH15 (*n* = 10), or OvHV-2 (*n* = 8) were confirmed using high-throughput sequencing, PCR, or ISH during routine surveillance. The remaining cases had an unknown or unconfirmed etiology.

Results
Lesions of varying severity were observed in the hippocampus of 33 animals. Histological findings included lymphoplasmacytic and histiocytic perivascular cuffs (mild, *n* = 10; moderate, *n* = 17; or severe, *n* = 6), often associated with gliosis in CA1–CA4 regions. Hippocampal sclerosis with severe neuronal loss and astrocytic hypertrophy and hyperplasia was observed in five BoRV-CH15-positive cases and four unconfirmed cases, while mild (*n* = 3) or no hippocampal sclerosis was observed in animals infected with other pathogens.

Conclusion
Hippocampal inflammation is a common finding in cattle with non-suppurative encephalitis, with hippocampal sclerosis primarily linked to retroviral infections. While the sample size is limited, our findings suggest a potential association between hippocampal sclerosis and BoRV-CH15. Future studies will further clarify the clinical significance of hippocampal involvement, particularly in relation to seizure activity and diagnostic implications.

AI AND DEEP-LEARNING APPROACHES

98 | ARTIFICIAL INTELLIGENCE FOR FELINE INTESTINAL PATHOLOGY: SMALL CELL LYMPHOMA VS LYMPHOPLASMACYTIC ENTERITIS

S. Defourny [1], S. Sabattini [2], M. Wright [3], A. Rigillo [4], H. Crosby-Durrani [5], E. Ricci [5], G. Rocchigiani [5], E. Faroni [2], L. Marconato [2], M. Pietra [2], A. Petrini [1], L. Ressel [5]

[1] Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy
[2] Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Bologna, Italy
[3] University of Liverpool, Neston, United Kingdom
[4] I-vet Diagnostics, Cellatica, Italy
[5] University of Liverpool, Liverpool, United Kingdom

Background
Low-grade intestinal T-cell lymphoma (LGITL) and lymphoplasmacytic enteritis (LPE) are common causes of feline chronic enteropathy. While histopathology remains the diagnostic gold standard, distinguishing between LGITL and LPE is challenging due to overlapping morphological features and high interobserver variability. Convolutional Neural Networks (CNNs) may improve consistency and accuracy in histopathological assessments.

Materials & Methods
A total of 161 formalin-fixed, paraffin-embedded endoscopic intestinal biopsies from cats with LGITL or LPE were retrospectively selected, digitized, and blindly re-evaluated by two board-certified veterinary pathologists using HE and IHC. Discordant cases were excluded. An InceptionV3 CNN, trained with transfer learning, was applied to 8,026 manually-selected image tiles (1024×1024 pixels, RGB), obtained from HE-stained slides. Training included tile balancing, image augmentation, and a 5-fold cross-validation strategy. A test set of 23 cases was classified by the CNN, which generated a final diagnosis based on the majority vote of correctly classified tiles per case. The same cases were independently reviewed by three board-certified pathologists, and performance was compared.

Results
A total of 142 cases (104 LGITL, 38 LPE) were included. The CNN achieved an average tile-level accuracy of 85.3% in cross-validation. At the case level, it correctly diagnosed 92% of test cases in 1 minute 20 seconds, compared to average 82% accuracy in 9 minutes for the pathologists. Notably, the case misclassified by the CNN was also misdiagnosed by two of the three pathologists.

Conclusion
CNN-based analysis demonstrates strong potential as a diagnostic support tool for distinguishing feline LGITL from LPE. Further model optimization and expanded datasets may enhance its performance.

105 | "LYMPHOM-AI": A PRELIMINARY APPLICATION OF DEEP LEARNING FOR LYMPH NODES AND CANINE LYMPHOMA CLASSIFICATION

C. Romanello [1], A. Levov [2], P. Roccabianca [1], L. Ressel [2]

[1] Department of Veterinary Medicine and Animal Science, University of Milan, Lodi, Italy
[2] University of Liverpool, Liverpool, United Kingdom

Background
Canine lymphoma is among the most common neoplasms in dogs, classification based on morphologic, phenotypic and histological grade attribution is essential for adequate staging, prognosis and treatment. Deep learning (DL) has proven high diagnostic accuracy for several tumour types including lymphoma in Human Medicine. Its application in Veterinary Medicine offers a promising diagnostic tool for lymphomas.

Materials & Methods
A total of 28 reactive lymph nodes and 186 nodal lymphoma HE stained slides were retrieved from the Archive of the University of Liverpool, diagnosed by board-certified pathologists with immunohistochemical phenotyping (B, T), and scanned. Representative areas were annotated on 105 cases (83 lymphoma, 22 reactive) and used to train a binary convolutional neural network (CNN) with a semantic segmentation approach (8xSubsampling DSnet), while 109 cases (103 lymphoma, 6 reactive) were used as test. Pixel distribution analysis on generated segmentation masks was performed using Orbit Image Analysis software, to establish the most likely (>50% of the area) diagnosis.

Results
The network achieved 85,8% accuracy and 85,4% mean F1. Performance in diagnosing lymphoma was higher in large cell lymphomas compared to other subtypes and in areas with high mitotic activity. The WHO lymphoma subtypes present in the training set seems to influence performance on the test set.

Conclusion
This preliminary study highlights the potential of DL as a promising support tool in diagnosing lymphomas. Further studies are required to assess its reproducibility and explore effectiveness of more complex networks focusing on multi-class networks with different WHO lymphoma types and specific reactive lymph node areas.

435 | ARTIFICIAL INTELLIGENCE-ASSISTED KI67 CELL COUNTING TO AID CANINE CUTANEOUS MAST CELL TUMOUR PROGNOSTICATION

D. Walker [1], A. Herrmann [1], K. Erles [1], S. Haugland [1]

[1] Veterinary Pathology Group, Bristol, United Kingdom

Background
Cutaneous mast cell tumours (MCTs) are among the most common malignant skin neoplasms in dogs. Accurate prognostication of tumour behaviour is crucial as part of tailored treatment strategies. Though grading systems are well-established and routinely used, some MCTs behave in a more aggressive manner than predicted. The Ki67 index, a marker of growth fraction, is commonly used as an additional prognostic test with higher expression being statistically correlated with poorer outcomes. However, manual Ki67 cell counting is time-consuming, suffering from interobserver and intraobserver variability. This study aimed to develop an artificial intelligence (AI)-assisted, automated system for cell counting, to improve accuracy, reduce pathologist workload and reduce interobserver variability.

Materials & Methods
QuPath, an open-source image analysis platform, was used to analyse Ki67-stained MCT samples. Using machine learning, the system was trained on representative images from 244 MCTs , with identification of positive and negative staining mast cells. The automated results were compared with manual counts on a dataset of 77 images, provided to 3 board-certified veterinary pathologists to determine accuracy and reproducibility, through interobserver variability.

Results
When fully automated, the AI-assisted system demonstrated good interobserver agreement with pathologists (mean kappa 0.71), comparable to pathologists amongst themselves (mean kappa 0.65), with significantly reduced analysis time.

Conclusion
The automation of Ki67 cell counting for canine cutaneous mast cell tumours is a promising tool for improving prognostic assessments and supporting clinical decision-making. This approach offers a reliable and consistent method to enhance diagnostic workflows, through significant time savings and reduced interobserver variability.

460 | DEEP LEARNING-BASED CLASSIFICATION OF C-KIT IMMUNOHISTOCHEMICAL PATTERNS IN CANINE CUTANEOUS MAST CELL TUMOURS

M. Monteiro [1], J. Almeida [2], N. Papanikolaou [2], T. Carvalho [1], P. Faísca [3, 4, 5]

[1] Histopathology Platform, Champalimaud Foundation, Lisbon, Portugal
[2] Computational Clinical Imaging Group, Champalimaud Foundation, Lisbon, Portugal
[3] FMV/Universidade Lusófona- Centro Universitário de Lisboa, Lisbon, Portugal
[4] DNAtech Veterinary Laboratory, Lisbon, Portugal
[5] Gulbenkian Institute For Molecular Medicine, Oeiras, Portugal

Background
C-kit immunohistochemistry expression in canine cutaneous mast cell tumours (cMCTs) correlates with prognosis and histological grade. This project aimed to train a deep learning model (DLM) to classify c-kit staining patterns in cMCTs.

Materials & Methods
32 c-kit stained cMCT slides were scanned. Four tumour regions of interest (ROIs) per slide were randomly selected, covering 13.88% tumour cells. StarDist was used for segmentation, yielding 7307 cell tiles annotated by three observers as Pattern I, II, III, undetermined, or discard (unstained, artefacts). One observer's annotations (14% I, 13% II, 17% III, 11% undetermined, 45% discard) were used for DLM training and validation. Inter-rater variability, model performance, and correlation between slide-level predominant model-predicted patterns and diagnostic pathologist-assigned patterns were evaluated.

Results
Observer pattern agreement was good (κ=0.70), with full consensus in 71% and partial in 98% of cells. The model reached 66% accuracy and 0.86 one-vs-one area under-the-curve (AUC). Class sensitivities were: discard 81%, Pattern I 73%, II 68%, III 57%, and undetermined 11%. Low sensitivity for undetermined patterns reflects inherent ambiguity from staining or image quality. Misclassification with other cytoplasmatic patterns and background staining in Pattern I contribute to lower Pattern III sensitivity. At the slide level, model-pathologist agreement was moderate (κ=0.50), with 66% accuracy and sensitivities of 50%(I), 88%(II), and 75%(III).

Conclusion
Further work and external validation are warranted to improve model performance and demonstrate generalizability. This proof-of-concept DLM shows promising correlation with pathologist assessments and could support pattern classification and quantification in cMCTs, potentially contributing to prognostic evaluations.

LIVESTOCK /INFLUENZA

117 | TRANSMISSION AND PATHOGENICITY IN FERRETS AFTER EXPERIMENTAL INFECTION WITH HPAI H5N1 CLADE 2.3.4.4B VIRUSES

L. Bordes [1], N. Gerhards [1], M. Roose [1], S. Venema [1], M. Engelsma [1], W. van der Poel [1], E. Germeraad [1], N. Beerens [1], S. Vreman [1]

[1] Wageningen Bioveterinary Research, Wageningen University and Research, Lelystad, Netherlands

Background
In 2021, there was a marked increase in mortality of wild mammals attributed to infection with highly pathogenic avian influenza (HPAI) H5N1 clade 2.3.4.4b viruses in Europe. Neurological signs and high viral antigen levels in the brain of infected mammals indicate that the virus causes severe disease, but serological analysis suggest infections may be more widespread and asymptomatic. The clinical manifestation and transmissibility of HPAI H5N1 viruses among mammals represent critical risk factors for potential zoonotic transmission to humans.

Materials & Methods
We examined the pathogenicity, viral tissue tropism, and associated pathology of three HPAI H5N1 viruses in ferrets. Ferrets were experimentally inoculated with an H5N1 poultry virus (genotype C) and two H5N1 fox viruses (genotype BA), one of which carries the zoonotic PB2-E627K mutation.

Results
The fox isolates, but not the poultry isolate, caused high morbidity, viral shedding and mortality in ferrets. Necrosis of liver, bile duct epithelial cells and pancreas as well as mild encephalomalacia in cerebrum, olfactory bulb and trigeminal ganglion were observed for most ferrets inoculated with the mammalian isolates. Transmission to co-housed ferrets was investigated in a group setting for the virus carrying the PB2-E627K mutation and caused neurological signs accompanied by more pronounced changes in the CNS in recipient ferrets.

Conclusion
This study showed that HPAI H5N1 clade 2.3.4.4b viruses can infect ferrets with varying pathogenicity, and that mammal-to-mammal transmission can occur. This highlights the need for enhanced surveillance in mammals for early detection of HPAI infection and vigilance of potential zoonotic threats.

282 | NATURAL INFECTIONS OF HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS H5N1 IN WILD BIRDS IN THE UK: HISTOPATHOLOGY, VIRAL DISTRIBUTION AND NEUROTROPISM

B. Martí Garcia [1], F. Lean [1], A. Núñez [2], N. Majó Masferrer [3, 4]

[1] The Royal Veterinary College, Hatfield, United Kingdom
[2] Animal and Plant Health Agency (APHA-Weybridge), Addlestone, United Kingdom
[3] Centre de Recerca en Sanitat Animal (CReSA), Bellaterra, Spain
[4] Facultat de Veterinària, Universitat Autònoma de Barcelona (UAB), Bellaterra, Spain

Background
Since the emergence of highly pathogenic avian influenza (HPAI) H5N1 clade 2.3.4.4 Goose/Guandong lineage in Europe in 2014, an unprecedented outbreak occurred during the 2021-2022 epidemiological year, causing mass mortalities in wild birds, including birds of prey, but also increasingly affecting Charadriiformes, imposing substantial ecological and infection pressure at the wild bird-poultry and also avian-mammalian interface.

Materials & Methods
In this study we have thoroughly assessed the histopathological lesions and antigen distribution by IHC from 115 PCR positive wild birds that died naturally from highly pathogenic avian influenza (HPAI), including Charadriiformes, birds of prey, gamebirds, waterfowl and captive wild birds.

Results
The commonest histological lesion was pancreatic necrosis followed by splenic necrosis, encephalitis or neuronal necrosis, myocardial necrosis or myocarditis, necrosis of the respiratory tract and, lastly, hepatic necrosis. Ninety-six birds were positive by IHC in multiple organs and most of the viral antigen was detected in the brain followed by the respiratory tract, heart, pancreas and kidney. In the brain, neurons, neuropil and endothelium were the most commonly labelled structures and they showed the highest predicted probabilities to detect viral antigen by IHC.

Conclusion
In conclusion, the mortality in different wild birds can be associated with multisystemic viral dissemination and resultant tissue damage and endothelial tropism is a key feature in the pathogenesis of the natural infection. The most likely neuroinvasive mechanism is the vascular route, with rapid parenchymal tropism and spread, although further studies are warranted.

418 | NATURAL PORCINE AND EXPERIMENTAL MURINE INFLUENZA A VIRUS INFECTION – A COMPARATIVE MORPHOLOGICAL APPROACH

R. Bachmann [1], F. Seehusen [1], S. De Neck [1], G. Rosato [1], D. Mega [2], J. Stewart [2], A. Kipar [1]

[1] Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
[2] Institute of Infection, Veterinary and Ecological Sciences, Liverpool, United Kingdom

Background
In most cases of natural porcine influenza A virus (IAV) infection, the development of lesions in the lower respiratory tract cannot be clearly classified. This study therefore employs an experimental murine model to investigate whether the progression of morphological lesions in pigs can be more precisely characterised.

Materials & Methods
Porcine lungs were tested positive by PCR, mice were inoculated intranasally with IAV X31. The pulmonary inflammatory response was specified by immunohistochemistry using antibodies against Iba1 (macrophages), CD3 and CD20/CD45R (T and B lymphocytes), and myeloperoxidase (MPO)/Ly6-G (neutrophils), followed by morphometric analysis.

Results
In naturally infected pigs with undetermined infection onset, the earliest lesion observed was necrosis of the bronchiolar epithelium — findings similar to murine lungs at 2–3 days post-infection (dpi). In advanced stages, pigs exhibited predominantly acute inflammation (5-22% MPO-positive area per total lung area) with only mild interstitial lymphocytic infiltration (1-4% CD3-positive area), resembling lesions observed in mice at 4–5 dpi. During the regenerative phase, pigs showed marked lymphohistiocytic infiltration around vessels and bronchioles (5-18% CD3- and 12-34% Iba1-positive area), along with bronchiolar epithelial hyperplasia. By 7 dpi, mice demonstrated epithelial hyperplasia in some bronchioles, whereas others appeared necrotic. The inflammatory infiltrate was dominated by lymphocytes, primarily located perivascularly and to a lesser extent peribronchially (4-15% CD3-positive area).

Conclusion
The progression of infection, in terms of epithelial damage and inflammatory cell infiltration, is comparable between mice and pigs. A subsequent transcriptomic analysis will determine whether infection-associated gene expression profiles are conserved across both species.

464 | COMPARATIVE AVIAN INFLUENZA IMMUNO-PATHOLOGY BETWEEN CHICKENS, PEKIN DUCKS AND EURASIAN WIGEONS INFECTED WITH DUTCH H5 HPAI STRAINS OF CLADE 2.3.4.4

S. Vreman [1], E. Germeraad [1], M. Roose [1], S. Venema-Kemper [1], F. Harders [1], M. Hulst [1], N. Beerens [1]

[1] Wageningen Bioveterinary Research, Wageningen University and Research, Lelystad, Netherlands

Background
Highly pathogenic avian influenza (HPAI) H5 viruses are a constant threat for the poultry sector. In this study, domestic chickens, domestic Pekin ducks (Pd) and Eurasian wigeons (Ew- model for wild birds), were infected with three HPAI H5 viruses to investigate species difference in innate immunity and pathology.

Materials & Methods
Chickens, Pd and Ew (n=12 per group) were inoculated with H5N8-2014 (clade 2.3.4.4c), H5N8-2016 and H5N6-2017 (both clade 2.3.4.4b) virus. At 12, 24 and 72 hours post inoculation (hpi), birds (n=4) of each group were sacrificed. Histopathology of lung, brain and ileum, viral protein and RNA expression (e.g. IL-6, IFN-γ and MDA-5) were evaluated. Additionally, transcriptional profiles of lung and brain were analysed 24hpi. Results were compared with non-infected control birds (n=4).

Results
Only Ew and Pd survived all three HPAI H5 infections till 72 hpi. In lungs and brains of chickens and Pd, especially after H5N6-2017 infection, high RNA levels of IL-6, IFN-γ and MDA-5 were detected in combination with differentially expressed genes (DEGs) related to e.g. virus recognition and antiviral pathways. In contrast, DEGs in lungs and brains of Ew were mostly upregulated after H5N8-2014 infection. Histopathologic changes and in-situ virus expression in lung and brain supported these species and virus differences.

Conclusion
Chickens show a higher mortality compared to Ew and Pd, which can be related to the cytokine storm shortly after HPAI H5 infection. Domestic chickens and Pd display a different virus sensitivity compared to “wildlife” Ew as represented by the difference in lung and brain responses after H5N8-2014 infection.

MISCELLANEOUS

160 | FIRST CLINICOPATHOLOGICAL CHARACTERIZATION OF AN EXTREME BODY WRINKLE PHENOTYPE IN MERINO SHEEP AND ITS ASSOCIATION WITH ORF VIRUS INFECTION

A. Gómez [1], P. Quílez [1], M. Ruiz de Arcaute [1], S. Sanz [1], I. Moncayola [2], R. Reina [2], E. Pérez [1], S. Villanueva-Saz [1], L. Monteagudo [1], P. Windsor [3], D. Lacasta [1], L. Luján [1]

[1] Universidad de Zaragoza, Zaragoza, Spain
[2] 3Instituto de Agrobiotecnología (CSIC-Gobierno de Navarra), Mutilva, Spain
[3] University of Sydney , Camden, Australia

Background
Merino sheep exhibit a high follicular density, enhancing wool yield but predisposing them to inherited cutaneous disorders such as breech wrinkle, characterized by a high number of conspicuous skin folds (wrinkles) in the area around the tail and hind legs (breech). Body wrinkle is a severe phenotype of breech wrinkle, affecting the entire body. Due to the significance of body wrinkle, gross lesions and genetic basis have been investigated in Merino sheep. However, to the best of the authors' knowledge, pathological progression of lesions and histopathological characteristics remain poorly studied.

Materials & Methods
Four body wrinkle affected neonatal lambs underwent periodic clinical examinations, hematological analyses, and skin biopsies over a period of 1 to 2 months previous to natural death and postmortem studies. A retrospective pedigree analysis was also carried out in animals from the original farm to assess the inheritance pattern of this cutaneous disorder.

Results
Affected lambs displayed generalized alopecia with excessive skin folds. Hematological analysis showed neutrophilia, lymphopenia, and eosinopenia. Histopathological examination revealed follicular dysplasia, follicular keratinization and follicular keratosis and degeneration of follicular epithelium, particularly in the outer root sheath. Pedigree analysis suggested an autosomal inheritance pattern. All affected lambs developed severe Orf lesions on the muzzle, leading to marked reduction in feed intake, progressive debilitation, and ultimately death at 1-2 months-old.

Conclusion
This study provides the first clinicopathological characterization of body wrinkle in Merino lambs and its association with Orf virus (ORFV) infection, suggesting that this syndrome increases susceptibility to severe ORFV infection.

177 | MICROVASCULAR NETWORK ALTERATIONS IN FELINE HYPERTROPHIC CARDIOMYOPATHY: INSIGHTS FROM QUANTITATIVE IMAGE ANALYSIS AND 3D VESSEL MODELLING

F. Prisco [1], A. Luchian [2], M. Baron Toaldo [3], S. Fonfara [4], L. Ressel [2], A. Kipar [1, 2]

[1] Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
[2] Institute of Infection, Veterinary and Ecological Scien, University of Liverpool, Liverpool, United Kingdom
[3] Clinic for Small Animal Medicine, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
[4] Ontario Veterinary College, University of Guelph, Guelph, Canada

Background
Feline hypertrophic cardiomyopathy (HCM) is the most common cardiac disease in domestic cats. The condition is associated with reduced microvascular density, with evidence of architectural alterations of the microvasculature. To characterise the latter, we applied two-dimensional (2D) and three-dimensional (3D) image analysis.

Materials & Methods
Forty serial formalin-fixed paraffin embedded sections of left ventricular free wall myocardium from each four HCM and control cats were immunostained for CD31, then digitalised. Blood vessel segmentation, section alignment, and volume reconstruction were performed using the HeteroGenius Medical Image Manager. Vascular morphometry was assessed in 2D via Groovy scripting in QuPath 0.5.1, while Python scripting in 3D Slicer 5.8.1 and VesselExpress 1.1.0 served for 3D reconstruction, modelling, and quantitative analysis.

Results
In HCM, we observed a trend toward reduced overall vessel density (not statistically significant) despite comparable total vessel area in both groups. Mean vessel area and diameter were significant higher, and vessels were more widely spaced. These changes were mainly attributed to lower numbers of small-calibre vessels (capillaries, <7 µm diameter). VesselExpress confirmed increased mean vessel diameter and volume and found a trend towards a higher number of branching points and branches per branching point in HCM where, vessels also exhibited reduced straightness and increased branching angles; here, the differences were minimal but statistically significant.

Conclusion
The combined 2D and 3D analyses confirms substantial reorganisation of the microvasculature in HCM. In light of capillary rarefaction, the increased size of remaining vessels may reflect an adaptive attempt at maintaining myocardial perfusion in HCM.

401 | IN VITRO STUDIES ON THE INTERACTION OF RESPIRATORY SYNCYTIAL VIRUS-INFECTED BOVINE WELL-DIFFERENTIATED AIRWAY EPITHELIAL CELLS WITH FRESHLY ISOLATED NEUTROPHILS

N. Gerhards [1], M. Vrieling [1], L. Bordes [1], S. Nguyen-van Oort [1], R. Dresken [1], R. De Jong [1], R. De Swart [1]

[1] Wageningen Bioveterinary Research, Wageningen University and Research, Lelystad, Netherlands

Background
Bovine respiratory syncytial virus (BRSV) is an important component of the bovine respiratory disease complex, and causes bronchointerstitial pneumonia associated with infiltration of neutrophils into the airway epithelium. *In vitro* models to study the pathogenesis of BRSV are scarce and often lack an immunological component.

Materials & Methods
We developed an *in vitro* BRSV infection model in bovine bronchial well-differentiated airway epithelial cells (WD-AECs) cultured at air-liquid interface, using a recombinant BRSV strain that expresses enhanced green fluorescent protein (rBRSV-EGFP). WD-AECs consist of a differentiated pseudostratified epithelium containing basal, mucus-forming and ciliated cells that form tight junctions. Freshly isolated bovine neutrophils were stained with cell tracker deep red to study interactions with EGFP-expressing BRSV-infected epithelial cells. Co-culture was performed for a period of 1-4 hours, after which the co-localization was assessed by confocal laser scanning microscopy.

Results
BRSV replicated efficiently on bovine WD-AECs without acquiring cell-culture adaptations, enabling the generation of a rEGFP-BRSV strain. BRSV predominantly infected ciliated epithelial cells, which occasionally formed syncytia. During co-culture performed five days after rEGFP-BRSV infection, neutrophils migrated from the basolateral side through the pores of the transwell filter into the respiratory epithelium. In BRSV-infected cultures, the neutrophils were found to cluster near EGFP-positive cells.

Conclusion
We have developed bovine bronchial WD-AECs that can be used as *in vitro* infection models to study viral replication, tropism and tissue changes. By introducing immune cells to bovine WD-AECs infected with rEGFP-BRSV, an additional layer of complexity was added to investigate inflammatory pathogenesis *in vitro*.

FORENSIC PATHOLOGY

240 | PATHOLOGICAL ALTERATIONS AND GLYCOPHORIN A AND C EVALUATION AS INNOVATIVE TOOLS FOR INVESTIGATING WOUND VITALITY IN VETERINARY FORENSIC PATHOLOGY

G. Piegari [1], L. Longobardi [1], V. Vestuto [2], M. Miranda [2], G. Salanti [1], D. De Biase [2], O. Paciello [1]

[1] University of Naples "Federico II", Naples, Italy
[2] University of Salerno, Salerno, Italy

Background
Glycophorin is a highly sensitive forensic marker for assessing wound vitality in human forensic pathology. Nevertheless, studies focusing on glycophorin evaluation are still lacking in veterinary forensic medicine. This study aims to 1) examine the pathological changes and glycophorin expression levels in both ante- and post-mortem traumatic injuries in dogs and 2) assess the post-mortem morphological changes of the wound and the glycophorin degradation rate over time.

Materials & Methods
10 dog cadavers were enrolled for the study and divided into three groups. Group A included four dog cadavers that died from polytrauma, Groups B and C included three cadavers each that died from causes unrelated to trauma. In groups B and C, a single post-mortem incision was made 30 minutes (group B) or three days (group C) after death. Ante- and post-mortem wounds were evaluated over time, collecting samples at 1-, 24-, 48-, 72- and 96h for histopathology. Furthermore, swabs from the wound were collected for ELISA and immunoblot analysis using the anti-glycophorin A and C antibodies.

Results
Group A cases showed a moderate to severe haemorrhage, whereas a mild or absent erythrocyte extravasation was observed in other groups. Glycophorin expression levels were higher in Group A than in Groups B and C ($p<0.01$). A time-dependent Glycophorin degradation was also observed in all assessed cases.

Conclusion
Our results suggest that haemorrhages and elevated glycophorin expression are consistent with vital wounds; thus, their evaluation may be a valid tool for investigating wound vitality in veterinary forensic pathology.

293 | VIRTUAL REALITY AND 3D PRINTING TECHNOLOGY AS INNOVATIVE APPROACHES IN VETERINARY FORENSIC PATHOLOGY EDUCATION: A MULTICENTRIC EVALUATION STUDY

F. Parisi [1], N. Lattero [2], D. De Biase [3], N. Fonti [1], L. Meomartino [2], M. Millanta [1], O. Paciello [2], G. Piegari [2]

[1] University of Pisa, Pisa, Italy
[2] University of Naples "Federico II", Napoli, Italy
[3] University of Salerno, Fasciano, Salerno, Italy

Background
Animal-based practical classes have traditionally been central role in veterinary education. In recent years, increasing efforts have focused on developing alternative teaching methods, although limited research has explored students' perceptions of these innovations. The present study aimed to develop a protocol for the 3d reconstruction of gunshot wounds and to evaluate its educational contribution using a multicentric approach.

Materials & Methods
Two pig cadavers were experimentally shot using a semi-automatic pistol. Photogrammetry and CT scans were used to obtain a virtual wound reconstruction (VR)and a 3D-printed bone model. These models were presented to 137 students: third-year students and trainees in Veterinary Medicine programs at the Universities of Pisa (UNIPI) and Naples Federico II (UNINA), as well as students in the Animal Pharmaceuticals and Nutraceuticals program at the University of Salerno (UNISA). Participants completed a questionnaire to gather general impressions and specific feedback on the models. Data were analysed using nonparametric tests.

Results
Students reported strong confidence in identifying injuries using VR and 3d models. However, the 3d-printed model received higher scores than the VR simulation for accuracy and clarity of anatomical reference points. Among the participants, trainees gave more favourable evaluations of the models' anatomical reference points than students. Trainees and students from UNISA were also more likely to view the models as valid alternatives to cadavers, compared to students from veterinary faculties.

Conclusion
Our results suggest that models are perceived as effective tools for practical training, although evaluations appear to be influenced by students' academic backgrounds.

325 | ASSESSMENT OF NUCLEI DENSITY IN A PORCINE WOUND MODEL BY APPLICATION OF INSTANSEG

K. Barington [1], H. Jensen [1]

[1] University of Copenhagen, Copenhagen, Denmark

Background
With advancements in slide scanners and softwares for evaluating tissue slides, digital pathology may offer a tool for an objective age assessment of wounds. This study aimed to evaluate cell density in granulation tissue (GT) over time through the application of a novel, deep learning-based algorithm for nuclei segmentation, which potentially may be used for estimating wound age in veterinary forensic pathology.

Materials & Methods
In total, 47 pigs were anesthetized, and four full-thickness wounds were established on the back by surgical excision. Wounds were left to heal by second intention until pigs were euthanized on day 5, 10, 15, 20, 25, 30, or 35. HE-stained sections of GT from 188 wounds were scanned using the Axioscan 7 with a 20x/0.8 objective (Zeiss, Germany). In QuPath (version 0.6.0-rc3), each wound was divided into two approximately equal-sized areas by creating annotations that covered the superficial half and the deep half of the GT, respectively. Nuclei were detected using the InstanSeg extension, and nuclei per mm^2 were calculated. Nested one-way ANOVA was used to detect differences between the age groups.

Results
A total of 179 wounds were included, and nine wounds were excluded due to sampling errors, artefacts, or poor scanning quality. Nuclei density decreased with increasing wound age ($P<0.05$), which was most pronounced in the superficial half of the GT.

Conclusion
The density of nuclei in GT, based on automated detection, showed time dependent changes and can potentially be used as a tool for obtaining objective age assessments of wounds.

CANCER IN DOGS AND CATS

106 | NOT ALL LYMPHOMAS ARE BORN THE SAME. DIFFERENT PERSISTENCE STRATEGIES IN FELINE LYMPHOMA

S. Vieira de Carvalho [1, 2], C. Marques [3, 4], R. Noiva [1, 2]

[1] CIISA, Interdisciplinary Centre of Research in Animal Health, Lisbon, Portugal
[2] Associated Laboratory AL4AnimalS, Lisbon, Portugal
[3] INSA, Instituto Nacional de Saúde Dr. Ricardo Jorge, Porto, Portugal
[4] CECA, Center for Study in Animal Science, Porto, Portugal

Background
Sustaining proliferative signaling, evading growth suppressors, resisting cell death and enabling replicative immortality are four classical hallmarks of cancer. Recently, the unlocking of phenotypic cellular plasticity has also been included as a distinct hallmark.
While small T-cell alimentary lymphoma is the most common form of feline lymphoid tumor, large B-cell lymphoma is the most common nasal cavity tumor in cats.
We aim to investigate differences in expression of Bcl-2, DNA mismatch repair components and proliferating cell nuclear antigen (PCNA) between alimentary and nasal feline lymphomas.

Materials & Methods
Formalin-fixed paraffin-embedded biopsy tissues from 15 cases of B-cell nasal lymphoma and 22 cases of alimentary lymphoma (21 T-cell and 1 B-cell), diagnosed at the Pathology Laboratory of the Faculty of Veterinary Medicine, University of Lisbon between 2017 and 2023, were immunophenotyped by immunohistochemistry regarding expression of Bcl-2, MLH1, MSH6, MSH2 and PCNA.

Results
The nasal lymphomas were large cell, frequently moderately to strongly MLH1+, MSH6+, MSH2+ and PCNA+, and only rarely BCL-2+ (2/15). Conversely, alimentary lymphomas were mostly small T-cell, Bcl-2+ (20/21), frequently moderately to strongly MLH1+, MSH6+ and MSH2+, and only occasionally PCNA+ (7/22). The only B-cell alimentary lymphoma was large cell, and its immunophenotype closely resembled the nasal lymphomas.

Conclusion
Our findings suggest that cell persistence strategies vary greatly between different lymphoma phenotypes which may help explain differences in prognosis and response to treatment.

314 | DECODE-STS: DECIPHERING THE LANDSCAPE OF CANINE SOFT-TISSUE SARCOMA USING SPATIALLY DEFINED PROTEOMICS AND TRANSCRIPTOMICS

D Fuchs [1], E. Markkanen [1], H. Ech-Cherif [1], S. Aghaabbasi [1], A. Pöschel [1], E. Beebe [1], L. Laura [2], W. Wolski [2], F. Guscetti [1], M. Nolff [1]

[1] University of Zürich, Zürich, Switzerland
[2] ETH Zürich/University of Zurich, Zürich, Switzerland

Background
Soft-tissue sarcomas (STS) are a heterogeneous group of frequent canine tumors displaying locally invasive growth and variable metastatic potential. Progress in STS treatment is impeded by very sparse molecular data, limiting the identification of reliable diagnostic and prognostic markers and the distinction of STS from adjacent normal tissue (NT) for targeted interventions. This study employed spatially resolved molecular profiling of STS and NT to identify tumor-specific targets and diagnostic features.

Materials & Methods
Combining laser-capture microdissection, RNAsequencing and liquid-chromatography tandem mass spectrometry, we profiled a retrospective cohort of 106 STS (30 fibrosarcoma (FSA), 31 myxofibrosarcoma (MFS), 19 perivascular wall (PWT), 26 peripheral nerve sheath tumors (PNST)) and 148 patient-matched adjacent NT samples (skeletal muscle, adipose and connective tissue). PWT/PNST were differentiated by IHC (GFAP, NGFR, SOX10).

Results
Analysis of 254 tissue samples revealed both significantly upregulated and tumor-exclusive features, representing potential diagnostic and therapeutic targets. We identified distinct transcriptomic clusters that align with histopathological classification in numerous cases. Interestingly, additional distinct, yet undefined molecular subtypes emerged across all histopathological entities independent of anatomical location, suggesting biological heterogeneity not captured by the current classification. However, limited clinical follow-up data constrained interpretation of their prognostic relevance.

Conclusion
We provide the first comprehensive proteomic and transcriptomic overview of the frequent canine STS subtypes FSA, PWT, PNST and MFS and their adjacent NT, providing a molecular framework for biomarker discovery and therapeutic target development. Further validation in clinically annotated cohorts is necessary to clarify the clinical implications of the novel molecular subtypes.

335 | THE OVERLOOKED VARIABLES: SLIDE THICKNESS AND 3D TUMOUR HETEROGENEITY ON MITOTIC COUNTS OF CANINE MAST CELL TUMOURS

P. Faísca [1, 2,-3], L. Silva [4], I. Carvalho [3], J. Santos [2, 3], J. Catarino [2, 3], D. Ramilo [2], P. Rodrigues [1], A. Gama [4, 5]

[1] Gulbenkian Institute For Molecular Medicine, Oeiras, Portugal
[2] FMV/Universidade Lusófona- Centro Universitário de Lisboa, Lisboa, Portugal
[3] DNAtech Veterinary Laboratory, Lisboa, Portugal
[4] Departamento de Ciências Veterinárias, CECAV, UTAD, Vila Real, Portugal
[5] CECAV & AL4Animals , Vila Real, Portugal

Background
Mitotic count (MC) is one of the most widely used methods to predict tumour behaviour. Given the high inter- and intra-observer variability, several strategies have been developed to improve and standardize this procedure, including the definition of the evaluation area, the use of immunohistochemistry (IHC), and the application of computer-assisted image analysis. However, in previous studies, neither 3D tumour heterogeneity nor the impact of slide thickness, that is often either not mentioned or varies between 2 to 5µm, has been assessed. In this study, we aim to evaluate both the 3D tumour variability and the effect of slide thickness on MC of Canine Mast Cell Tumours (cMCTs).

Materials & Methods
Six cMCTs were uniformly and serially sectioned into 5 to 8 equally spaced slabs. From each slab, two consecutive sections (3µm and 5µm thick), collected in random order, were obtained. Phosphohistone H3 (PHH3) IHC was performed, and after manually reviewed, mitotic figures were quantified using QuPath. MCs were calculated for the entire tumour, for software-defined hotspots, and within 2.37 mm² regions of interest (MC-ROIs). Hotspot locations were also evaluated qualitatively.

Results
Within each tumour, a statistically significant variation in MC was observed between slabs, both in the whole-slide image (MC/area), in hotspots, and in MC-ROIs. Slides sectioned at 5µm consistently showed higher MCs compared to 3µm. Hotspot locations differed between slabs and were sometimes influenced by section thickness within the same slab.

Conclusion
In cMCTS, MCs show considerable 3D intra-tumoral heterogeneity. Slide thickness has a substantial impact on MC quantification and should be standardized.

354 | SPLICE ME UP, SCOTTY: EXTRA DOMAIN-B FIBRONECTIN IN CANINE TUMOURS PUTS A SPOTLIGHT ON MELANOMA

S. Dell'Aere [1], D. Stefanello [1], E. Gariboldi [1], A. Ubiali [1], R. Ferrari [1], L. Auletta [1], M. Bocci [2], A. Galbiati [2], S. Cazzamalli [2], E. Gilardoni [2], A. Verdi [1], D. Neri [3], P. Roccabianca [1]

[1] Department of Veterinary Medicine and Animal Sciences, University of Milan, Lodi, Italy
[2] Philochem AG, R&D Department, Otelfingen, Switzerland
[3] Philogen S.p.A., Siena, Italy

Background
Extra Domain-B fibronectin (EDB+FN) is a splice variant of fibronectin involved in embryogenesis, angiogenesis, wound healing, and tissue remodelling. Normal adult tissues lack EDB+FN, while it is overexpressed by cancer-associated fibroblasts (CAFs) and vessels (CAVs) in many aggressive human tumours including carcinomas, lymphomas, and melanomas. Considering its selective expression, EDB+FN represents a target for tumour-directed therapies, particularly through antibody-drug conjugates (ADCs). EDB+FN studies in canine oncology are lacking. This study aims to investigate EDB+FN expression in different canine tumours and to assess its potential as a therapeutic target.

Materials & Methods
EDB+FN expression was assessed by immunohistochemistry on retrospectively collected canine tumours using an L19-based recombinant antibody, cross-reactive with canine EDB+FN. Expression was semi-quantitatively scored in neoplastic cells, CAFs, CAVs, and tumour stroma.

Results
Sixty-six cases from seven tumour types were included. EDB+FN expression was most intense and consistent in neoplastic cells, CAFs, and CAVs of melanomas (6/6 cases). Of ten apocrine gland anal sac adenocarcinomas (AGASACs) EDB+FN was expressed in neoplastic cells (7/10) and CAVs (8/10). Of ten soft tissue sarcomas EDB+FN was expressed in neoplastic cells (5/10) and CAVs (6/10). Expression was mostly cytoplasmic. Other tumour types (lymphomas, osteosarcomas, hemangiosarcomas, mast cell tumours; ten each) were EDB+FN low to negative. Stroma was negative.

Conclusion
The comparative analysis of EDB+FN expression across different canine tumour types revealed variable expression with only melanomas consistently exhibiting high positivity in all cases. Our results highlighted melanoma and AGASAC as candidate tumours for future studies on the efficacy of EDB-targeted ADCs.

387 | CHARACTERISATION OF TLSS MATURATION STAGE IN CANINE MAMMARY CARCINOMAS

G. Giambrone [1], R. Puleio [2], C. Vullo [1], S. Monti [1], G. Marino [1], A. Sfacteria [1]

[1] University of Messina, Messina, Italy
[2] Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Palermo, Italy

Background
Tertiary lymphoid structures (TLSs) are ectopic lymphoid aggregates developing in various diseases, including neoplasms. TLSs exhibit three maturation stages. The most immature stage features unorganised tumour-infiltrating lymphocytes (TILs). Primary follicle-like TLSs (PF-TLSs) are characterised by the presence of follicular dendritic cells but lack germinal centres (GCs), unlike the most mature secondary follicle-like TLSs (SF-TLSs). This study aims to characterise TLSs at different maturation stages in canine mammary carcinomas (CMCs).

Materials & Methods
Stromal and peritumoral TLSs were assessed in the most representative sections for each sample of CMCs (n=154). Different immune cells were identified using CD3, CD20, CD21, Macrophage Marker (MAC/387), CD204, and Mast Cell Tryptase antibodies. The positivity for CD21 and GC presence were used to differentiate TLS stages. The statistical analysis was conducted using a chi-square test to evaluate the correlation between TLSs and tumour grade and histotype.

Results
TILs (n cases=136) were composed of T and B lymphocytes. CD21+ cells were in inflammatory cell groups often with a follicular-like disposition. PF-TLSs (n cases=66) were composed of T and B lymphocytes, plasma cells, dendritic cells, macrophages, and mast cells. Only 4 cases presented SF-TLSs with GCs of CD20+ B cells surrounded by CD3+ T lymphocytes intermingled with some CD21+ cells. The PF- and SF-TLS presence was positively correlated with tumour grade ($p<.000001$).

Conclusion
TLSs present different maturation stages in CMCs. The PF-TLS heterogeneous composition may relate to the macrophage and mast cell roles in recruiting and regulating their maturation. The TLS maturation correlates with grade in CMCs.

COMPANION ANIMALS W/O CANCER

296 | LOSS OF EPITHELIAL MITOCHONDRIA AND OPENING OF TIGHT JUNCTIONS, A KEY POINT IN CANINE INFLAMMATORY BOWEL DISEASE

L. Biagini [1], G. Rossi [1], S. Preziuso [1], R. Ranieri [2], D. De Bellis [1], S. Sara [3], A. Agnese [1], L. Galosi [1], A. Gavazza [1], G. Pengo [4]

[1] School of Biosciences and Veterinary Medicine, University of Camerino, Matelica, Italy
[2] Department of Veterinary Sciences, University of Pisa, Pisa, Italy
[3] Christian Doppler Laboratory for Innovative Gut Health Concepts of Livestock, I, Vienna, Austria
[4] Clinic "St. Antonio", Cremona, Italy

Background
Mitochondria, the primary energy producers of the cell, have a bacterial origin. Mitochondrial DNA (mtDNA) is recognized as a pro-inflammatory damage-associated molecular pattern, with implications in various inflammatory conditions, particularly in the gut. We hypothesized that mtDNA is released during the active phase of canine IBD, acting as a pro-inflammatory factor.

Materials & Methods
From 2020 to 2022, plasma was collected in an interval of 2 hours, from 10 dogs diagnosed with IBD (based on clinical, endoscopic, and histopathological findings) and 10 healthy controls matched by breed, age, sex, size, and diet. Circulating mtDNA levels were measured using qPCR (targeting COX1/GAPDH). Intestinal biopsies from IBD dogs were compared with archived healthy mucosal samples for histological scoring, junctional protein expression. Additionally, we examined mitochondrial damage using Transmission Electron Microscopy (TEM) and expression of TLR9, the target of mtDNA, in human intestinal mucosa affected by IBD.

Results
mtDNA levels were significantly elevated in IBD dogs ($p < 0.0001$), correlating with disease severity markers, including CCECAI, CRP, albumin, and leukocyte counts. IBD dogs also showed increased TLR9+ inflammatory cells ($p < 0.05$), reduced expression of tight junction proteins occludin ($p = 0.002$) and zonulin ($p = 0.01$), and increased claudin-2 ($p = 0.002$). TEM revealed a significant reduction in both number and diameter of mitochondria ($p = 0.002$).

Conclusion
This study demonstrates for the first time in dogs that mtDNA is released during active IBD and may act as a mechanistic biomarker. Mitochondrial restoration may offer a novel therapeutic strategy for canine IBD.

339 | SCORING AND DISTRIBUTION OF CARTILAGE DEGENERATION AND OSTEOPHYTOSIS IN CERVICAL FACET JOINT ARTHROSIS IN WARMBLOOD HORSES WITH AND WITHOUT WOBBLER

W. Bergmann [1], T. Spoormakers [1], S. Veraa [1], W. Back [2], K. Vanderperren [3], C. Delessalle [3], I. Wijnberg [1], S. Journée [3], H. Journée [1, 4, 5], H. Vernooij [1], A. Gröne [1], G. Grinwis [1]

[1] Utrecht University, Utrecht, Netherlands
[2] Royal Friesian Horse Association, Drachten, Netherlands
[3] Ghent University, Ghent, Belgium
[4] University of Groningen, Groningen, Netherlands
[5] Amsterdam University Medical Center, Amsterdam, Netherlands

Background
Cervical vertebral stenotic myelopathy (CVM) can be caused by articular process joint (APJ) arthrosis. For objective evaluation a validated gross scoring scheme for cartilage degeneration was developed. Cartilage degeneration and osteophytes were associated with age, spinal region, sex and presence of neurological signs due to CVM.

Materials & Methods
The APJs of C2-Th2 of 35 warmblood horses (1-21 years; 20 males, 15 females; 5 neurologically sound, 16 with CVM, 14 of unknown neurological status), were photographed post-mortem, and a gross cartilage degeneration grading scheme was developed. Of 26 horses, cervical osteophytes were scored post-mortem using computed tomography.

Results
Intra (kappa 0.66, 0.69, 0.77) - and inter (kappa 0.53, 0.61) -observer reliability scores for cartilage degeneration were moderate to substantial. Association between cartilage degeneration and osteophytes was poor (r_s 0.09-0.61). Cervical cartilage degeneration was significantly higher than thoracic (cranial neck: estimate -0.53, 95% confidence interval (95%CI) -0.86- -0.19 ; caudal neck: -0.38, 95%CI -0.71- -0.04). Cranial cervical cartilage degeneration and osteophytes were more severe than caudal cervical (cartilage: -0.15, 95%CI -0.29- -0.01; osteophytes: -0.31, 95% CI -0.49 - -0.14), and not correlated with neurological signs or sex. Cartilage degeneration was correlated with age (1-4 versus 5-12 [0.35, 95%CI 0.05-0.64] and 1-4 versus 13-21 years [0.63, 95%CI 0.30-0.96]), osteophytes were not.

Conclusion
Contrary to current assumptions, cartilage degeneration and osteophytes were more pronounced cranially cervical then caudally cervical and not associated with neurological signs or sex. Osteophytes showed poor correlation with cartilage degeneration, highlighting limitations of morphological markers in diagnosing APJ arthrosis.

378 | ATHEROSCLEROSIS AND FAMILIAL HYPERCHOLESTEROLEMIA IN KORAT CATS

V. Karkamo [1], N. Airas [2], M. Hytönen [2], J. Lindén [2], K. Hagner [1], M. Mäyränpää [2], P. Kovanen [3], A. Knuuttila [4], H. Kairento [2], S. Nguyen [4], K. Öörni [2], M. Jauhiainen [5], J. Metso [5], T. Grönthal [2], L. Kareinen [1], S. Hundi [2], M. Kaukonen [2], L. Lyons [6], H. Anderson [7], H. Lohi [2], A. Sukura [2], I. Kareinen [2]

[1] Finnish Food Authority, Helsinki, Finland
[2] University of Helsinki, Helsinki, Finland
[3] Wihuri Research Institute, Helsinki, Finland
[4] Orion Corporation, Espoo, Finland
[5] Minerva Foundation Institute for Medical Research, Helsinki, Finland
[6] University of Missouri, Columbia, United States
[7] Mars Petcare Science & Diagnostics, Helsinki, Finland

Background
Atherosclerosis, a chronic condition marked by lipid accumulation and inflammation in arterial walls, is a leading cause of morbidity and mortality in humans. While primary atherosclerosis is rare in most domestic animals and has not been previously documented in felines, this study presents the first comprehensive clinical, metabolic, and genetic characterization of naturally occurring atherosclerosis in the Korat breed of domestic cats (*Felis catus*).

Materials & Methods
The study utilized tissue (n=2), selected (via availability) blood (n=76), and DNA (n=309) samples from Korat and available un-matched control cats to investigate cardiovascular disease, lipid metabolism and genetic cause. Human samples for comparision, histological techniques, whole genome sequencing, and lipid assays depending on feline sample volumes available were applied, with ethical approval and statistical analysis.

Results
We identified atherosclerosis in two affected autopsied Korat cats with vascular pathology comparable to human atherosclerosis. Genetic analysis revealed a recessive loss-of-function variant in the low-density lipoprotein receptor (LDLR) gene (n_{WT}=239; n_{HETZ}=62; n_{HOMZ}=8) and all studied homozygous individuals exhibiting severe hypercholesterolemia with widespread arterial disease, mirroring human familial hypercholesterolemia. Lipid profiling and autopsy showed no significant abnormalities or vascular lesions in heterozygous cats.

Conclusion
This study presents a novel, naturally occurring double-knockout model of atherosclerosis in a companion animal species. We propose that the evolutionary absence of the proprotein convertase subtilisin/kexin type 9 (PCSK9) gene in *Carnivora* may preserve LDLR function in these carriers, mitigating disease progression. This study has already contributed to improved feline health through the development of genetic testing.

WILD LIFE INCL. MARINE MAMMALS

134 | A NOVEL AMYLOIDOSIS IN TIGERS: IDENTIFICATION OF ODORANT-BINDING PROTEIN-DERIVED AMYLOID

N. Kobayashi [1] O. Fasina [2], Y. Itoh [1], S. Kadekaru [3], L. Linden [4], Y. Yumi [5], T. Murakami [1]

[1] Tokyo University of Agriculture and Technology , Tokyo, Japan
[2] Iowa State University, Ames, United States
[3] Okayama University of Science, Ehime, Japan
[4] University of Tennessee, Knoxville, United States
[5] The Animal Disease Research and Support Association, Tokyo, Japan

Background
Amyloidosis refers to a group of disorders characterised by extracellular deposition of misfolded proteins known as amyloid. To date, 21 amyloid precursor proteins have been identified in animals. In this study, we encountered cases of previously uncharacterised systemic amyloidosis in captive tigers and investigated its pathogenesis using proteomic analysis.

Materials & Methods
Seven tigers from zoos were examined postmortem. Histological analysis (H&E and Congo red staining), proteomic analysis using LC-MS/MS, and immunohistochemistry (IHC) were performed. For IHC, an anti-odorant-binding protein (OBP) rabbit polyclonal antibody was newly developed against the N-terminal sequence (Leu10-Pro27) of feline OBP. Structural prediction (AlphaFold3 and PlaToLoCo) was performed for OBP.

Results
Histopathological examination revealed amyloid deposits in the heart (myocardial interstitium, tunica media and adventitia of the small arteries, and lamina spongiosa of the atrioventricular valves) in five tigers, and three tigers had amyloid deposits expanding the synovial intima and subjacent stroma, elevating and occasionally disrupting the synovial membrane. LC-MS/MS of two tigers detected OBP, particularly its N-terminal peptides, as the main component of amyloid deposits. IHC demonstrated that amyloid deposits are positive for OBP in all tigers. Structural prediction showed that OBP has a low complexity region (LCR) in the N-terminus and a β-barrel motif in the central to C-terminus region.

Conclusion
This study identifies OBP as a novel amyloid precursor responsible for systemic amyloidosis in tigers. The presence of an N-terminal LCR suggests a potential role in amyloid formation, consistent with mechanisms seen in neurodegenerative diseases involving protein misfolding.

137 | POST-MORTEM EXAMINATIONS AND DIAGNOSES OF REINDEER IN FINLAND FROM YEARS 2020 – 2025

L. Itkonen [2], M. Huttunen [1], T. Korkea-Aho [2], H. Nordgren [1], A. Oksanen [2], A. Sukura [1], M. Nylund [2]

[1] University of Helsinki, Helsinki, Finland
[2] Animal Health Diagnostic Unit, Finnish Food Authority, Oulu, Finland

Background
Post-mortem examinations of reindeer are scarce in Finland, as the transport distances are long. Oulu is the northernmost city in Finland where the Finnish Food Authority (FFA) performs post-mortem examinations of reindeer. Since 2023 additional field necropsies have been conducted in PORAUS research project that aims to develop a remote digital necropsy (RDN) method for reindeer.

Materials & Methods
Post-mortem examinations of reindeer at the FFA Oulu include a necropsy and samples for histology, parasitology, bacteriology, virology, and chronic wasting disease (CWD) sampling. In PORAUS project, local veterinarians in the reindeer herding area were trained to perform a field necropsy, fill in a form of findings, and collect above-mentioned samples to be examined at the FFA Oulu. Photos and online video connection were utilized in the project.

Results
A total of 59 whole reindeer carcasses were examined at the FFA Oulu in years 2020-2025. The number of whole carcasses declined during these years almost linearly. Since the PORAUS project started, there have been 42 additional field necropsies by April 2025. Out of all reindeer carcasses examined at the FFA Oulu and within the PORAUS project between January 2020 and April 2025 (n=101), the most common reasons of death have been cachexia, peritonitis and ruminal acidosis.

Conclusion
Still ongoing PORAUS project of field necropsies has likely increased the total number of reindeer post-mortem examinations and disease results recorded by the FFA. Implementing RDN for reindeer in Finland will hopefully ensure data of reindeer diseases also in the future.

218 | HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERISATION OF INFLAMMATORY LESIONS IN FLYING FOXES NATURALLY INFECTED WITH AUSTRALIAN BAT LYSSAVIRUS (ABLV).

Y. Lin Tan [1], M. Gimeno [1, 2], P. Pinczowski [2], S. Gestier [2], E. Hall [1], M. Krockenberger [1]

[1] The University of Sydney, Sydney, Australia
[2] Elizabeth Macarthur Agricultural Institute, Department of Primary Industries, Menangle, Australia

Background
Flying foxes are recognised reservoirs of multiple zoonotic viruses, including Australian Bat Lyssavirus (ABLV), a neurotropic virus capable of causing fatal disease in humans and animals. Despite its low prevalence, ABLV poses a public health risk due to increasing interactions between flying foxes and both humans and domestic animals. This study aimed to characterise the neuroinflammatory response to natural ABLV infection using histopathology and IHC.

Materials & Methods
Formalin-fixed brain tissues from 25 ABLV qPCR-positive and 5 ABLV-negative flying foxes were examined. Lesion severity was scored on HE sections. IHC using CD3, CD79b, and IBA-1 identified T-lymphocytes, B-lymphocytes, and macrophages, respectively. Digital image analysis using QuPath v0.5.1 quantified immunolabelled cells in meninges, perivascular areas and neuropil.

Results
In ABLV-positive flying foxes, meningitis (96%), gliosis (88%), and perivascular cuffing (80%) were the most frequent lesions. Macrophages were the most abundant inflammatory cell (average 48% of cells), with a 3-fold increase compared to negative controls. T-lymphocytes and B-lymphocytes exhibited a 10-fold increase in ABLV-positive animals and were significantly more numerous in the meninges ($p<0.05$). No significant variation in macrophage distribution was observed between anatomical regions. Increased lymphocyte counts correlated with greater lesion severity (CD3 $p=0.005$; CD79b $p=0.043$), and increased T-lymphocytes were significantly associated with reduced viral load ($p=0.045$).

Conclusion
ABLV-positive flying foxes develop variable meningoencephalitis with mixed mononuclear inflammation. While macrophages predominate numerically, T-lymphocytes, particularly within the meninges, are closely associated with both inflammation severity and viral clearance. These findings enhance our understanding of ABLV-associated neuropathology in flying foxes.

445 | HISTOPATHOLOGY AND IMMUNOLABELLING OF VIRAL ANTIGEN IN TISSUES OF HOODED CROWS NATURALLY INFECTED WITH WEST NILE VIRUS.

A. Kycko [1], J. Niczyporuk [1]

[1] National Veterinary Research Institute, Puławy, Poland

Background
West Nile Virus (WNV) is an emerging mosquito-borne zoonotic virus in central Europe. From 2022 to 2024, several summer outbreaks of WNV associated with deaths of corvids in central Poland were reported. The present study aimed to assess the histopathological changes and virus distribution in the internal organs of the fallen birds confirmed to be WNV-positive by RT-PCR.

Materials & Methods
Formalin-fixed tissues of brain, lung, liver, kidney and heart collected from 20 hooded crows were subjected to histopathology using HE staining and immunohistochemistry using an antibody against WNV antigen to determine the localisation of the virus in the tissue sections.

Results
Histopathological examination revealed multifocal nonsuppurative encephalitis of varying severity, mild to severe, characterised by gliosis and lymphohistiocytic infiltrations around blood vessels in all the brains. Hyperaemia and mild interstitial lymphohistiocytic infiltrations were occasionally visible in in the heart, kidney, lungs and liver in which necrotic foci were additionally observed in 5 cases. WNV immunolabeling was detected in single macrophages present in the interstitial infiltrates, in single neurons in the brain, as well as in single cardiomyocytes.

Conclusion
To our knowledge, this is the first report describing histopathological changes associated with WNV infection in hooded crows in Poland. Cases of infection of wild birds with the WNV-related virus Usutu were reported in Poland during the same period, but not in the crows studied. The results of our study confirm the need to monitor the circulation of WNV in the wild crow population in Central Europe.

452 | ANTHRACOSILICOSIS IN ASIAN HOUBARA BUSTARD AND ITS INFLUENCE IN RESPIRATORY DISEASE

A. Perez de Vargas [1], M. Al Daheri [1], M. Carrasco [1], M. Silvestre [1]

[1] Reneco International Wildlife Consultants LTD, Abu Dhabi, United Arab Emirates

Background
In birds, dust particles can accumulate in the lungs often without causing disease. While usually incidental, clinically significant pneumoconiosis has been reported in some species. We have observed anthracosilicosis in captive houbara bustards.

Materials & Methods
Samples of 95 captive Asian houbara (*Chlamydotis macqueenii*) were collected during necropsy. Birds were outdoors, exposed to their steppic habitat, which includes desert sand. They were classified into two categories: presence or absence of respiratory clinical signs. Samples were collected for microbiology, PCR, and histopathology. Lung samples were microscopically evaluated and graded for the degree of anthracosilicosis and fibrosis present. Generalised linear models were developed to determine the influence and relations between different parameters.

Results
The analysis revealed 54.7% of anthracosilicosis; significant effect of age on severity, and data suggesting that older individuals are more likely to develop severe forms. A significant association between fibrosis severity and clinical status was seen. Clinically sick individuals were more likely to have more severe fibrosis than clinically healthy individuals. GLMs models showed no significant effect of pneumoconiosis grade on the probability of having the following bacteria: *E.coli*, *Pseudomonas* and *Klebsiella*.

Conclusion
Even though respiratory tract diseases can occur alongside anthracosilicosis, there is no evidence in the samples analysed that link an increase susceptibility to clinical respiratory tract infections when is present. The results suggest that the progression in fibrosis severity is steep highlighting the need of early diagnosis. The results of this study improve the knowledge of the possible interactions between this lung pathology and different microorganisms.

POSTER ABSTRACTS

EXOTIC, WILDLIFE & ZOO ANIMALS

53 | IS YOLK EMBOLISM REALLY CAUSED BY YOLK? - A PROTEOMIC ANALYSIS IN A MILK SNAKE (LAMPROPELTIS TRIANGULUM)

M. Ikeda [1], H. Kondo [1], T. Murakami [2], N. Kobayashi [2], Y. Itoh [2], H. Shibuya [1]

[1] Nihon University, Kanagawa, Japan
[2] Tokyo University of Agriculture and Technology, Tokyo, Japan

Background
Yolk embolism, an uncommon condition in certain reptiles and birds, is hypothesized to result from the entry of yolk-derived material into the bloodstream, although this has not been demonstrated to date. Diagnosis currently depends exclusively on HE staining, and molecular assays for confirmation are lacking. This study aimed to characterize the proteomic composition of yolk emboli to provide insight into their origin.

Materials & Methods
A captive female milk snake, estimated to be over nine years old, was found dead in water. Necropsy and histopathological examination were performed. Embolic material within blood vessels was collected by laser microdissection and analysed by LC-MS/MS. Due to the absence of a species-specific protein database, MS/MS data were collated to a Colubridae peptide database from the NCBI.

Results
Grossly, white, flexible material surrounding the ovary was observed. Histologically, amorphous to globular, hyaline material was observed within blood vessels of various organs, including the cerebral ventricles. The material was also deposited in the renal interstitium and in vessel walls of the ovary. LC-MS/MS identified ovotransferrin, α_2-macroglobulin, and vitellogenin as constituent proteins present in the emboli.

Conclusion
Proteomic analysis confirmed the presence of egg-associated proteins in the embolic material. Vitellogenin, a yolk protein, as well as ovotransferrin and α_2-macroglobulin, which were associated with egg white, were detected. The detection of these proteins suggests that yolk embolism may involve not only yolk-derived components, but also egg white proteins. These findings indicate that the pathogenesis of yolk embolism is more complex than previously considered.

61 | LUNGWORM INFECTION IN DUTCH WILD-LIVING SEALS: EVALUATING QUANTITATIVE MEASUREMENTS

N. Eijk [1] C. Roos [1], J. Brand [1], A. Gröne [1], L. IJsseldijk [1]

[1] Utrecht University, Utrecht, Netherlands

Background
Seals in Dutch waters frequently have lungworms, specifically *Parafilaroides gymnurus* (PG) and *Otostrongylus circumlitus* (OC). Lungworm infections can induce severe respiratory disease, jeopardizing health and survival of affected seals. We evaluated quantitative lungworm measurements to determine prevalence and severity of lungworm infestations in stranded seals.

Materials & Methods
Postmortem examinations were conducted on 22 harbour seals (*Phoca vitulina*) and two grey seals (*Halichoerus grypus*) found dead on Dutch beaches (n=17) or euthanased (n=7), between October 2021 and July 2023. Macroscopic lungworm infestation was assessed semi-quantitatively as none, mild, moderate, or severe depending on the visible lungworms within tissue. From a subset of harbour seals (n=20), one lung was weighed and flushed with digestive solution as per modified Inderbitzen method (MIM) to determine lungworm type and quantity. Lung samples were collected from all specimens for histology.

Results
Macroscopic examination revealed lungworm infestations in 13/24 seals, with five mild, four moderate, and four severe infestations. MIM identified lungworms in 16/20 seals, thirteen showing PG and fifteen showing OC infestations. Histological examination detected lungworms in 17/24 seals, all PG, three combined with OC. PG was observed in cranial (15/24), middle (16/24), and caudal (14/24) lung lobes. Consolidated measurements identified lungworms in 17/24 seals. Pearson's correlation analysis suggested histology as the most sensitive method for identifying PG (correlation coefficient 1.00; $p<0.01$), whereas OC is better detected by MIM (correlation coefficient 0.87; $p<0.01$).

Conclusion
We found lungworms in 71% of examined seals. Future examinations are needed to better understand the implications of lungworm infections.

165 | FIRST DESCRIPTION OF PENTASTOMIASIS IN EUROPEAN FRESHWATER TURTLES

M. Crispo [1, 2], E. Lienard [2, 3, 4], E. Monge [1, 2, 4], N. Gaide [1, 2], L. Bortot [1, 2], Y. Abou Monsef [2], A. Poujade [1, 2], G. Le Loc'h [1, 2]

[1] IHAP, Toulouse , France
[2] ENVT, Université de Toulouse, Toulouse , France
[3] UMR INTHERES, Toulouse , France
[4] INRAE, Toulouse , France

Background
Pentastomes are worm-like, crustacean parasites with an indirect life cycle and a zoonotic potential, residing in the respiratory tract of a variety of species, including reptiles.

Materials & Methods
Between November 2023 and February 2025, a total of 10 freshwater turtles, including 6 European pond turtles (*Emys orbicularis*), 3 Mediterranean pond turtles (*Mauremys leprosa*) and 1 razor-backed musk turtle (*Sternotherus carinatus*) originating from a sanctuary located in the South West of France, were submitted to the National Veterinary School of Toulouse for diagnostic investigation, due to the onset of increased mortality. The majority of turtles (8/10) died at the sanctuary and were submitted frozen. Two live animals exhibited marked respiratory distress and were euthanized soon after hospitalization.

Results
Necropsy showed pulmonary congestion, hemorrhages, and consolidation, with 2-6 cm long yellow-white worms found in the respiratory tract of all animals. Histopathological analysis revealed bronchointerstitial pneumonia and the presence of adult pentastomes within the bronchial lumen. PCR targeting the 18S rRNA gene of pentastomids (Pent629F and Pent1011R) followed by Sanger sequencing, strongly supported the involvement of *Levisunguis subaequalis*. Histology on mosquitofish (*Gambusia* spp.) sharing the same pond, highlighted pentastome nymphs in two specimens, further confirmed by molecular analysis.

Conclusion
This study represents the first documented case of pentastomiasis in European freshwater turtles. A spill-over event from exotic turtle species was highly suspected, given the parasite's detection in soft-shell turtles in the USA and the cohabitation of a variety of turtle species at the sanctuary, facilitated by a competent intermediate host like mosquitofish.

220 | CETACEANS FROM THE MEDITERRANEAN MAY HAVE SPECIFIC PHOTOBACTERIUM DAMSELAE SUBSP. DAMSELAE STRAINS

L. Martino [1, 2, 3], M. Domingo [1, 2, 3], M. Abarca [2, 3, 4], L. Pérez [3, 5], À. Cobos [2, 6, 7], A. Barca [8, 9, 10], A. Vences [8, 9, 10], C. Osorio [8, 9, 10]

[1] Servei de Diagnòstic de Patologia Veterinària, Facultat de Veterinària, UAB, Bellaterra, Spain
[2] Departament de Sanitat i Anatomia Animals, Facultat de Veterinària, UAB, Bellaterra, Spain
[3] Universitat Autònoma de Barcelona (UAB), Bellaterra, Spain
[4] Veterinary Mycology Group, Facultat de Veterinària, UAB, Bellaterra, Spain
[5] Facultat de Veterinària, UAB, Bellaterra, Spain
[6] IRTA, Animal Health, Centre de Recerca en Sanitat Animal (CReSA), Bellaterra, Spain
[7] Unitat mixta d'investigació IRTA- UAB en Sanitat Animal, CReSA, Bellaterra, Spain
[8] Departamento de Microbioloxía e Parasitoloxía, Santiago de Compostela, Spain
[9] Aquatic One Health Research Center (iARCUS), Santiago de Compostela, Spain
[10] Universidade de Santiago de Compostela, Santiago de Compostela, Spain

Background
Photobacterium damselae subsp. *damselae* (*Pdd*) causes mortality in aquaculture and is an opportunistic pathogen in cetaceans. We describe a fatal septicemia in a free-ranging Mediterranean striped dolphin (*Stenella coeruleoalba*) caused by a *Pdd* strain lacking all known hemolysins. Additionally, we characterize *Pdd* strains in other Mediterranean cetaceans.

Materials & Methods
Complete necropsy of the juvenile male dolphin was performed. Formalin-fixed tissue samples were microscopically evaluated. Bacteriology and morbillivirus detection was studied in different tissue samples using culture and RT-PCR. Transmission electron microscopy (TEM) was performed on liver sections. Genetic and phenotypic analyses of *Pdd* in additional isolates of striped dolphins (n=22) and Risso's dolphins (*Grampus griseus*) (n=2) were investigated. Further molecular phylogenetic analysis based on partial *toxR* gene sequences was performed.

Results
The animal was in good conservation status. Macroscopically, loss of body condition, jaundice, generalized lymphadenomegaly and splenomegaly were observed. Microscopically, severe multifocal necrosuppurative hepatitis, splenitis, lymphadenitis, and meningitis were found, associated with 2-3 um thick Gram-negative rods. Ultrastructurally, a bacterial polysaccharide capsule was identified. A *Pdd* strain with mild hemolytic activity but lacking all known hemolysins was isolated. Similar results were obtained of Pdd strains isolated from other studied cetaceans from the Mediterranean. Samples were negative for cetacean morbillivirus on RT-PCR.

Conclusion
Cetacean *Pdd* strains from the Mediterranean include very unusual *Pdd* genotypes and phenotypes compared to typical fish and human isolates. Further investigations are needed to understand the pathogenic role, species and geographic distribution of these strains, and to characterize the so-far unidentified hemolysins.

232 | MORPHOLOGIC DESCRIPTION OF MICROSPORIDIANS SEVERE NECROTIZING MYOSITIS AND XENOMA FORMATION IN SEABREAM (DIPLODUS SARGUS) FROM NAPLES'S GULF

D. De Biase [1], V. Russo [2], E. Vaccaro [2], G. Piegari [2], S. Papparella [2], A. Anastasio [2], O. Paciello [2]

[1] Department of Pharmacy, University of Salerno, Fisciano (SA), Italy
[2] Department of Veterinary Medicine and Animal Production, University of Naples, Naples, Italy

Background
Microsporidia are obligate intracellular parasites belonging to the phylum Microspora, further classified according to their ability to form xenomas. The emerging presence of this parasite in fish meat may be problematic to the seafood industries due to the loss of their commercial value. This study aimed to describe the morphological features of myositis in seabream (*Diplodus sargus*) from Naples Gulf, possibly caused by a xenoma-inducing Microsporidia.

Materials & Methods
Thirty freshly caught seabreams from Naples Gulf were collected from marketplaces and divided into groups (A and B) based on the presence or absence of gross pathologic findings. Sixty epaxial muscles were snap-frozen and stained with H&E for the pathological assessment and periodic acid Schiff (PAS) and Giemsa for parasite identification.

Results
muscles from group A showed mild discolouration with multifocal haemorrhages. All muscle biopsies from group A showed several stages of xenomas from the earliest cyst-like forms to the most advanced granulomatous parasitic lesions. A chronic, moderate to severe, multifocal, polyphasic histiocytic and lymphoplasmacytic myositis and necrosis were observed. Microorganisms were strongly positive for PAS and Giemsa stain. In group B, no pathologic alterations were observed.

Conclusion
Microsporidian infections are a significant problem in fish health, responsible for diseases and economic losses. Macroscopic and microscopic examination of muscle can significantly help diagnose and prevent this parasitic disease. There is still a considerable lack of information regarding the immune response, biology, pathogenesis, and control of fish microsporidia. Further molecular analyses are needed to support the morphological and etiologic diagnosis genetically.

245 | POSTMORTEM FINDINGS IN TWO PORPOISES (PHOCOENA SPINIPINNIS) STRANDED ALONG THE COAST OF CHILE

C. Flores Olivares [1], V. Neira [3], N. Ariyama [2, 3], J. Ruíz [1], S. Marambio [4], M. Zamorano [4], R. Reyes [4], P. Oyarzún Ruiz [5], M. Pérez [6], M. Saldías [6]

[1] Laboratorio de Investigación de Ciencias Veterinarias, Universidad del Alba, La Serena, Chile
[2] Programa de Doctorado en Ciencias Silvoagropecuarias y Veterinarias, Universidad, Santiago, Chile
[3] Departamento de Medicina Preventiva Animal, Facultad de Ciencias Veterinarias y, Santiago, Chile
[4] SERNAPESCA, La Serena, Chile
[5] Departamento de Microbiología, Facultad de Ciencias Biológicas, Universidad de Concepción, Chile
[6] Universidad del Alba, La Serena, Chile

Background
*Phocoena spinipinnis*is a small, robust cetacean measuring between 1.5 and 1.8 meters in length, with an average weight ranging from 50-70 kilograms, and is native to South America. During the second half of 2024, the Chilean National Fisheries and Aquaculture Service (SERNAPESCA) found two stranded individuals of this species along the coast of Coquimbo, Chile. Necropsies were performed, and samples were collected for various analyses.

Materials & Methods
The animals were frozen in accordance with SERNAPESCA's internal protocols, and necropsies were performed at the Universidad del Alba in La Serena under its authorization, in collaboration with the Universidad de Chile. Samples were collected for influenza virus PCR, formalin-fixed tissues for histopathology, and parasites preserved in alcohol for identification.

Results
Relevant findings are as follows: **Animal 1:** Mandibular fracture associated with a focus of severe hemorrhage and edema. Evidence of low numbers of pulmonary and intracardiac parasites. **Animal 2:** Severe pulmonary parasitism with a marked presence of nematodes of the genus *Pseudalius*, extending from the distal third of the trachea to the terminal portions of the apical lung lobes, as well as gastrointestinal parasites. This animal was pregnant, carrying a fetus in the final third of gestation. Both individuals presented in excellent body condition.

Conclusion
Although pulmonary parasitoses have been reported in marine mammals, the authors are not aware of previous reports describing such severity as observed in Individual 2. It is essential to perform necropsies on fresh individuals, as the freezing and thawing process causes significant damage to both histopathological and virological analyses.

249 | FALLOW ME – OLFACTION NOT INFECTION: TEMPORARILY PROMINENT VERRUCOUS PREPUCE ALTERATIONS IN FALLOW DEER (DAMA DAMA) DURING RUT

G. Wibbelt [1], A. Joehnk [2], T. Wahler [3]

[1] Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany
[2] Neuwittenbek, Germany
[3] Rathmannsdorf, Germany

Background
Reports on infectious fibro-/papillomatosis in cervid species like roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*) seem to increase in Austria, Hungary or Slovenia. Alerted, hunters in Northern Germany noted prominent verrucous prepuce alterations in Fallow deer (*Dama dama*) and collected samples for further investigation.

Materials & Methods
In October and November 2024 six genital tracts of male fallow deer and one entire male, all either road kills or shot, were collected for investigation. Tissue samples of the preputial sheath of all seven individuals were fixed in 4% formalin solution and processed routinely for histological examination.

Results
Upon opening, the preputial sheath of all seven individuals had differing degrees of the same tissue alteration in the frontal part of their prepuce revealing a transition zone of yellow stained fine filamentous proliferations of the cutaneous membrane. These increased in size and height towards the preputial orifice while changing to a dark brown colour. Restricted in space this filamentous hyperplasia caused an invertion of the inner layer through the preputial orifice to the outer aspect, resulting in a radially symmetrical broad based verrucous alteration of the preputial opening. Histologically, high numbers of bacterial colonies colonized the cutaneous hyperplastic filaments.

Conclusion
In contrast to the - mostly multifocal - (fibro-)papillomatous lesions in roe and red deer caused by transmissible host-specific papilloma viruses, prepuce alterations in fallow deer are species-specific physiologic alterations during rutting season – seemingly a widely unknown fact. Presumably, the bacterial colonization adds to olfactory cues to attract female deer.

251 | MORPHOLOGY AND HISTOLOGY OF THE SKIN OF SYMPTERYGIA BREVICAUDATA TO UNDERSTAND PATHOLOGICAL PROCESSES

C. Flores Olivares [1], V. Hevia-Hormazábal [3, 4, 5], C. Covarrubias [2], C. Gatica [4, 6], J. Córdova [4], C. Miranda [4], E. Acuña [4, 5]

[1] Laboratorio de Investigación de Ciencias Veterinarias, Universidad del Alba, La Serena, Chile
[2] Universidad del Alba, La Serena, Chile
[3] Programa de Doctorado en Biología y Ecología Aplicada (BEA), Facultad de Cienci, Coquimbo, Chile
[4] Departamento de Biología Marina, Facultad de Ciencias del Mar, Coquimbo, Chile
[5] Center for Ecology and Sustainable Management of Oceanic Islands (ESMOI), Coquimbo, Chile
[6] Programa de Magíster en Ciencias del Mar Mención Recursos Costeros, UCN, Coquimbo, Chile

Background
Sympterygia brevicaudata is distributed from Ecuador to central Chile. It is a species for which no population estimates are currently available; however, it is suspected that its numbers are declining due to bycatch being classified as Near Threatened according to the IUCN.

Materials & Methods
Three juvenile individuals were analyzed; they were euthanized following the AVMA guidelines for fish. Samples were preserved in buffered formalin, and subsequent analyses included hematoxylin and eosin (H&E) staining and histochemical techniques. Seven specific areas were selected for examination: dorsal tip of the snout, anterior end of the braincase and upper edge of the eye region, dorsal side of the pectoral fin, spines, ventral tip of the snout, gill slits, and ventral area of the pectoral-fin.

Results
It was demonstrated that the skin of the juveniles of this ray is composed of a non-keratinized stratified epithelium, just like that of other chondrichthyans, with three distinct layers (superficial, intermediate, and basal) and a variable thickness ranging from 4 to 8 cell layers. Clearly defined cell types were identified, including mucus-producing cells, protein-storing cells, germinative cells, chromatophores, and epithelial cells. These cells showed different staining affinities with histochemical techniques (P.A.S. and Alcian Blue pH 2.5). Additionally, a moderate presence of eosinophilic granular cells was observed in the tail spines.

Conclusion
This histological study of the skin of Sympterygia brevicaudata provides a fundamental basis for understanding the biology and pathology of this species, which may be crucial for addressing its population decline and developing effective conservation strategies.

257 | CORNEAL LESIONS IN RAINBOW TROUT (ONCHORHYNCHUS MYKISS) EXPOSED TO HIGH NH3 ENVIRONMENTAL LEVELS

G. Bianchi [1], G. Mirra [1], A. Giancamillo [1], A. Zurlo [1], M. Giancamillo [1], G. Chiarello [2], A. Costa [1], E. Buoio [1], G. Radaelli [3], D. Bertotto [3], L. Aidos [1], C. Giudice [1]

[1] University of Milan, Lodi, Italy
[2] University of Milan, Milan, Italy
[3] University of Padua, Legnaro, Italy

Background
Ocular tissues, mainly the cornea, are considered bioindicators for chemical threat, due to their direct exposure to the external enviroment. In this work, we aimed to describe corneal pathological changes in rainbow trout (*O. mykiss*) exposed to high levels of ammonia (NH_3) in a traditional recirculating aquaculture system (RAS) compared to the novel Titanium dioxide-photoelectrocatalysis (PEC) system.

Materials & Methods
Eye globes from rainbow trout raised in PEC and in traditional RAS system were collected and fixed in formalin. Corneal changes were analysed in enucleated eyes by evaluating 1) structural changes through a) optical coherence tomography (OCT) and b) standard histopathology; 2) tear-film mucous production with special staining (AB-PAS and HID-AB); 3) corneal ulcers and reactive fibrosis via cytokeratin IHC and Picrosirius red (F3BA) staining; 4) oxidative damage via 8-hydroxy-2-deoxyguanosine (8-OHDG) IHC; 5) cellular proliferation via Ki-67 IHC. Retinal damage was also investigated for neuronal and glial changes via IF for neurofilament and Glial Fibrillary Acidic Protein, respectively.

Results
Higher ammonia levels registered in traditional RAS (compared to PEC) resulted in more severe corneal ulceration, focal thinning, fibrosis, and neovascularization. Oxydative stress was present in both systems, while no difference regarding mucous production was found. No retinal damage was observed.

Conclusion
High levels of water NH_3 cause significant corneal lesions, more severe in traditional RAS. Corneal lesions assessment, through OCT and traditional histopathology, was a useful tool to compare different aquacultural systems. The potential role of ocular damage as a biomarker of chronic toxicity requires further investigation.

259 | FIRST REPORT OF MORBILLIVIRAL ENCEPHALITIS IN AN ADULT STRIPED DOLPHIN (STENELLA COERULEOALBA) FROM THE BAY OF BISCAY, FRANCE

S. Labrut [1], B. Thuillier [1], T. Khomasuridze [1], M. Prot [2], E. Simon-Lorière [2], S. Wund [3]

[1] LABOCEA, Ploufragan, France
[2] Institut Pasteur, Université Paris Cité, CNRS UMR2000, Evolutionary Genomics of , PARIS, France
[3] Observatoire Pelagis, Unité d'Appui et de Recherche (UAR) 3462 Centre National d, La Rochelle, France

Background
To improve understanding of marine mammal health in France, Pelagis, the observatory for the conservation of marine mammals from La Rochelle University and the French National Center for Scientific Research, has developed and implemented standardized protocols as part of the French National Stranding Network, including those applied in collaboration with departmental laboratories such as LABOCEA (Brittany). Among these, morbillivirus screening is prioritized due to its well-documented role in mass mortality events affecting cetacean populations in the Mediterranean, North, and Baltic Seas.

Materials & Methods
A subadult male striped dolphin (*Stenella coeruleoalba*) stranded on the southern coast of Brittany was subjected to a comprehensive necropsy. Samples from major organs and visible lesions were collected for bacteriological, virological, and histopathological analyses using refrigerated, frozen, and formalin-fixed specimens, respectively.

Results
The dolphin, in intermediate nutritional condition and harboring only light parasitic infestation, exhibited moderate diffuse meningeal congestion. No pathogenic bacteria were isolated. Histological examination revealed moderate lymphoplasmacytic meningoencephalitis, multifocal gliosis, and neuronal necrosis—features suggestive of viral etiology. Immunohistochemical staining demonstrated morbillivirus antigen in both neurons and glial cells throughout multiple neuroanatomical regions. Subsequent metatranscriptomic sequencing successfully generated a complete dolphin morbillivirus (DMV) genome from brain tissue.

Conclusion
This case constitutes the first documented instance of morbilliviral encephalitis in a striped dolphin stranded on the Brittany coastline, expanding the known geographic range of DMV infections and highlighting the importance of systematic health monitoring in marine mammals.

294 | NEOPLASIAS IN FREE-RANGING WOLVES IN SWEDEN: FOUR CASES FROM 77 YEARS OF WILDLIFE DISEASE SURVEILLANCE

O. Ågren [1], E. Höök [1], G. Averhed [1], A. Bowman [2], H. Uhlhorn [1]

[1] Swedish Veterinary Agency, SVA, Uppsala, Sweden
[2] Royal Veterinary College, Hatfield, United Kingdom

Background
Neoplasia in wolves (*Canis lupus*) is seldom reported and case reports are often from zoo collections. The Swedish wolf population was practically extinct due to hunting by 1966, when the species was protected. A few Finnish-Russian founder wolves immigrated and there was a litter in 1983. Since then, the population has grown to almost 400 individuals and is now managed by culling. Presently, major health concerns are inbreeding, cryptorchism and sarcoptic mange.

Materials & Methods
We used the database at the Swedish Veterinary Agency, with 77 years of pathology cases from the Swedish wildlife disease surveillance, to review diagnoses of neoplasia in free-ranging wolves.

Results
Of 875 submitted carcasses of free-ranging wolves, only four cases with a neoplasia diagnosis were found in the period 2009-2024; a 9 year old male with intestinal adenocarcinoma, a cryptorchid 4 year old male with a Sertoli cell tumour in one of the abdominal testes, a ten year old emaciated male with an humeral osteosarcoma, and a not yet aged emaciated male with worn teeth, sarcoptic mange, and an extensive thoracic lymphoma.

Conclusion
Neoplasia is a rare finding in necropsied free-ranging wolves and, as expected, is mainly found in old individuals. Abdominal cryptorchism is known to predispose for testicular neoplasia. Of 666 aged dead Swedish wolves, only 2% were eight years or older. A good monitoring of the health of the Swedish wolf population is made possible by the intensive conservation and management plan which includes necropsies of all found dead or euthanised wolves.

313 | NATURAL SARS-COV-2 INFECTIONS IN FARMED MINKS RESULTS IN SEVERE LUNG PATHOLOGY AND HIGH VIRAL LOADS IN MULTIPLE ORGANS

S. Vreman [2], G. Giglia [1], R.J. Molenaar [3], R. Hakze [2], E. Delemarre [4], S. Nierkens [4], K. Wiese [2], G. Agliani [1], W. Van der Poel [2], F. Van Kuppeveld [1], B. Bosch [1], E. De Bruin [1], A. Gröne [1], N. De Bruijn [3], J. Van den Brand [1]

[1] Utrecht University, Utrecht, Netherlands
[2] WBVR, Wageningen, Netherlands
[3] Gezondheidsdienst voor Dieren, Deventer, Netherlands
[4] UMCU, Utrecht, Netherlands

Background
In 2020, the first disease outbreak of SARS-CoV-2 in farmed minks was reported in the Netherlands, followed by outbreaks in other countries. Knowledge on the dynamics of viral spread and disease progression after natural infection of farmed minks is limited.

Materials & Methods
This study examined naturally infected farmed minks using pathology, immunohistochemistry, virology, serology, proteomics, and screening for Aleutian Disease Virus (ADV) co-infection. A total of 45 minks were divided into 4 groups based on clinical health status and time of sampling: found dead before culling (n=15), clinically healthy during culling (n=10), clinical signs during culling (n=10) and found dead during culling (n=10).

Results
Histopathology revealed interstitial pneumonia as the most prominent finding in all minks , with more severe lesions seen in both groups of found dead minks. Associated SARS-CoV-2 antigen was detected in the nose, trachea and lungs, and in extra-respiratory tissues (intestine, spleen and lymph nodes) in all groups. Highest viral RNA loads were found in nasal and throat swabs, nose and lung tissues while in found dead minks lower levels were seen in spleen, liver, and intestine. SARS-CoV-2 specific antibodies were present in all groups, whereas there was no significant difference in immune related proteomics between the groups. Based on qPCR, over 57 % of minks (26/45) were co-infected with ADV.

Conclusion
In conclusion, minks naturally infected with SARS-CoV-2 show severe lung pathology with high viral loads in several organs and severe lung lesions are also observed in animals without clinical signs.

318 | EVALUATION OF PFOA AND PFOS EFFECTS ON A TURSIOPS TRUNCATUS FIBROBLAST CELL LINE

A. Molinari [1], S. Iori [1], V. Moccia [1], V. Zappulli [1], S. Mazzariol [1], M. Giantin [1], M. Pauletto [1], C. Centelleghe [1]

[1] University of Padua, Legnaro, Italy

Background
Due to their widespread presence in the environment, 3,3,3-trifluoro-2-trifluoromethyl propionic acid (PFOA, CAS: #564-10-3) and heptadecafluorooctanesulfonic acid (PFOS, CAS: #1763-23-1) pose a serious threat to marine wildlife. As apex predators, cetaceans are particularly vulnerable to accumulating these pollutants. Therefore, developing reliable *in vitro* assays is crucial for accurately evaluating the impact of these chemicals on these species.

Materials & Methods
After determination of the growth curve of patented immortalized *Tursiops truncatus* skin fibroblasts, 50 000 cells/well were seeded on a 96 well cell culture plate and, after 24 hours, exposed to PFOA and PFOS concentrations ranging from 0.1-2000 uM and 0.1-750 uM, respectively. Cytotoxicity was assessed at 24, 48 and 72 hours after treatment. Necrosis, apoptosis and oxidative stress were examined after 48 hours of exposure, evaluating annexin V, caspase 3/7, reactive oxygen species and glutathione levels.

Results
The IC50 values for PFOA at 24, 48, and 72 hours of exposure were determined, resulting in 934.0, 837.5 and 695.2 µM, respectively. As the concentration of PFOA increased, a rise in the annexin V and caspase 3/7 levels, and a drop in the glutathione amount were observed, reflecting enhanced necrosis, late apoptosis and oxidative stress phenomena.

Conclusion
*In vitro*models of marine cetaceans offer a promising approach to study the environmental distribution and cellular effects of PFAS compounds, leading to a better understanding of their pathological and toxicological impacts on these vulnerable species, with broader implications for marine ecosystem health.

330 | THE EFFECT OF COMMONLY USED PESTICIDES ON GROWTH AND VITALITY OF A PERMANENT BOA CONSTRICTOR KIDNEY DERIVED CELL CULTURE

A. Kipar [1], L. Kipar [1], U. Hetzel [1], F. Baggio [1]

[1] University of Zurich, Vetsuisse Faculty, Zurich, Switzerland

Background
In Costa Rica, carbofuran, glyphosate, paraquat and mancozeb are commonly used pesticides on banana and pineapple plantations. By contaminating environment or prey, they could be harmful to native snakes, such as the *Boa constrictor*, who inhabitat these plantations. Using an *in vitro* approach, we investigated the effect of these compounds on growth and viability of *Boa constrictor* cells.

Materials & Methods
The I/1 Ki permanent *B. constrictor* kidney derived cell culture was incubated with carbofuran, glyphosate, paraquat (at 800/400/200 µM) or mancozeb (at 80/40/20 µM) and maintained for 12 days. On day 0 (D0) and D3, the cells were given medium with compound, followed by medium only on D6 and D9. Cells were counted and morphologically assessed (in culture and in formalin-fixed, paraffin embedded pellets) on days 3, 6 and 12.

Results
With mancozeb, cultures could be maintained up to D6, at seemingly stable, though significantly decreased numbers. However, practically all cells appeared necrotic on D3. Paraquat exposure was associated with a significant decrease in cell numbers at any time point, accompanied by overt cell necrosis. With carbofuran, a significant reduction in cell number and morphological evidence of cell necrosis was observed at the high dose. Glyphosate did not seem to affect cell growth and viability.

Conclusion
Mancozeb appears highly toxic for I/1 Ki cells, while paraquat and carbofuran affect cell viability to a variable extent. Glyphosate did not have an overt effect. Next, on-site studies on wild indigenous snakes are required to gather information on the potential pesticide uptake.

351 | HISTOLOGIC CHARACTERIZATION OF THE MULTIPLE CONGENITAL OCULAR ANOMALIES' SYNDROME IN COMTOIS HORSES

L. Mathy [1], N. Milhau [1], J. Lafitte [1], F. Weigel [1], G. Bellini [1], F. Vernay [1], I. Raymond-Letron [2], E. Segard-Weisse [1], M. Abitbol [1], J. Cadoré [1], S. Belluco [1]

[1] VetAgro Sup, Marcy l'étoile, France
[2] ENVT, Toulouse, France

Background
Multiple Congenital Ocular Anomalies (MCOA) syndrome, affecting over 90% of French Comtois horses, is associated with a missense mutation (PMEL:c.1849C>T) in the PMEL gene. This mutation, which also causes the silver coat colour, is possibly linked to vision impairment, as reported by owners. To date, MCOA in Comtois horses has been fully described only through ophthalmic and ultrasonographic examinations. This study aimed to provide a histological characterization of lesions in the ciliary body and iris.

Materials & Methods
Forty-six formalin-fixed enucleated eyes were collected from 25 Comtois horses. Each eye was trimmed to include nasal and temporal regions of the anterior segment. From each paraffin blocks, a 3µm HE-stained section was analysed histologically blindly with respect to the genetic results. DNA was extracted from additional sections, and part of *PMEL* exon 11 was amplified and sequenced for genotyping.

Results
Seven horses were homozygous mutant, 15 were heterozygous, and 3 were homozygous wildtype (control group) for the *PMEL*:c.1849C>T mutation. No ocular lesions were observed in wild-type animals. All mutant horses displayed cystic lesions in the ciliary body and iris, more pronounced in the temporal region, and lined by a hyperplastic, non-pigmented neuroepithelium. Lesions were more prevalent and severe in the ciliary processes than the posterior iris. No substantial differences in lesion type or severity were observed between heterozygous and homozygous mutants.

Conclusion
The *PMEL*:c.1849C>T is associated with neuroepithelium-derived cystic lesions in the anterior uvea. Histological analysis reliably distinguishes affected from unaffected horses but does not differentiate carrier status.

381 | BROAD-SPECTRUM ANTI-SAA ANTIBODIES FOR ANIMAL AA AMYLOIDOSIS DIAGNOSIS

T. Murakami [1], N. Kobayashi [1], M. Ikeda [2], H. Kondo [2]

[1] Tokyo University of Agriculture and Technology, Fuchu, Japan
[2] Nihon University, Fujisawa, Japan

Background
AA amyloidosis occurs in a wide range of mammals and birds. Immunohistochemistry (IHC) for Serum Amyloid A (SAA) is essential for definitive diagnosis, but commercially available anti-human SAA antibodies may yield false negatives or nonspecific signals depending on the species. This study aimed to develop anti-SAA antibodies applicable to diverse animal species.

Materials & Methods
The Asp32–Gly50 region of vertebrate SAA is reported as highly conserved, except for polymorphisms at position 45 (Q, K, or R). We synthesised three peptides (DKYFHARGNYDAAXRGPGG; X = Q, K, or R) and immunised rabbits to obtain anti-Q45, -K45, and -R45 sera. Purified antibodies were qualitatively tested by IHC on AA amyloid deposits of 14 species: dog, cat, cow, pig, mouse, guinea pig, rabbit, duck, flamingo, chicken, quail, cormorant, red-crowned crane, and chimpanzee; all cases were confirmed as AA amyloidosis by mass spectrometry. A commercial anti-human SAA polyclonal antibody (Cloud-Clone) was used for comparison.

Results
The commercial antibody showed variable reactivity across species and tissues, with false negatives in the flamingo. Species with SAA-Q45 (e.g., dog, cat, pig) showed strong reactivity with Q45 antibody; those with SAA-K45 (e.g., flamingo, chimpanzee) with K45 antibody; and those with SAA-R45 (e.g., duck, chicken) with R45 antibody. Cross-reactivity with non-corresponding antibodies was weak or absent.

Conclusion
We successfully developed anti-SAA antibodies that specifically and effectively label AA amyloid in various species. Reactivity patterns highlight the importance of residue 45 in the SAA epitope and support the use of sequence-matched antibodies for accurate diagnosis in veterinary pathology.

402 | DISEASE PREVALENCE IN CONFISCATED EXOTIC AND INDIGINOUS BIRDS AND REPTILES HOUSED IN A CITES-AUTHORIZED RESCUE CENTER IN SPAIN

M. Jimenez Martinez [1], A. Martin [1], A. Rabanal Soto [1], V. Hidalgo Martinez [1], A. Rodriguez Luis [2], M. Aranda Vazquez [3], J. De Andres Gamazo [1]

[1] Complutense University of Madrid, Madrid, Spain
[2] Fundación Fieb, Toledo, Spain
[3] TRAGSATEC, Madrid, Spain

Background
Illegal wildlife trafficking is a threat to global biodiversity with significant impact on ecology and public health. The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) regulates the trade of endangered species and authorizes rescue centers such as the *Fundación para la Investigación en Etología y Biodiversidad* (FIEB) in Spain, to ensure the care, rehabilitation and reintroduction of victims of illegal trade. This study provides a postmortem analysis of diseases in animals at FIEB, between 2021 and 2024, with the purpose of improving management and welfare.

Materials & Methods
A total of 17 birds (12 species) and 12 reptiles (9 species) were analyzed. Complete necropsy and histopathological analyses were carried out between FIEB and the Complutense University Veterinary Teaching Hospital.

Results
The most relevant pathologies associated with death in birds were infectious (52%), followed by metabolic/nutritional disease (17%). In reptiles 50% of deaths were associated with metabolic/nutritional disease and 41% with infections. Birds more commonly showed enteritis (n=9), followed by hepatitis (n=7), and renal gout (n=5). Hepatocellular atrophy (n=9), biliary stasis (n=6), and renal gout (n=5) stood out in reptiles. Bacterial infections occured in birds and reptiles; avian polyomavirus, herpesvirus and bornavirus were suspected (n=4 birds). *Leukocytozoon* sp. was detected in one bird.

Conclusion
Considering the diversity of the pathologies identified and the variety of species, this study highlighted the need for more individualized treatment strategies and management whenever feasible instead of population based strategies used more commonly in other types of zoological institutions.

407 | SERRATOSPICULUM SPP. INFECTION IN A JUVENILE SAKER FALCON: A CASE REPORT FROM SERBIA

I. Vucicevic [1], B. Djurdjevic [2], K. Erdélyi [3], D. Bogunovic [1], M. Pajic [2], S. Aleksic-Kovacevic [1]

[1] Faculty of Veterinary Medicine, Belgrade, Serbia
[2] Scientific Veterinary Institute "Novi Sad", Novi sad, Serbia
[3] Veterinary Medical Research Institute, Budapest, Hungary

Background
Serratospiculiasis is a parasitic disease caused by filarial nematodes of the genus *Serratospiculum*. Although commonly reported in falcons, there are no published data on its occurrence in saker falcons (*Falco cherrug*) in Serbia, where this species is endangered.

Materials & Methods
A juvenile saker falcon, originally ringed in Hungary and equipped with a tracking transmitter, was found dead in the Vojvodina region of Serbia after the device showed no movement. The carcass was submitted to the Scientific Veterinary Institute „Novi Sad“ for necropsy and cause-of-death analysis.

Results
Necropsy revealed signs of prolonged starvation and anorexia. Approximately 40 yellowish-white nematodes were observed, both freely within the coelomic cavity and attached to thickened thoracic and abdominal air sac membranes. Parasitological examination identified them as *Serratospiculum* spp. Histopathology of the lungs revealed verminous pneumonia accompanied by mild to moderate focal hemorrhages. Various developmental stages of the parasite were also observed in the heart and gizzard. Bacteriological, mycological and virological tests were negative. The liver and kidneys were enlarged and pale, indicating possible systemic compromise. Vacuolar degeneration was observed in the liver, while no microscopic changes were detected in the kidneys.

Conclusion
Although the pathogenicity of *Serratospiculum* spp. in raptors remains is debated, our findings suggest that a heavy parasitic burden likely contributed to the bird’s weakened condition and subsequent death. To our knowledge, this represents the first documented case of serratospiculiasis in a saker falcon in Serbia, emphasizing the importance of parasitological surveillance in conservation efforts for this endangered species.

416 | A CASE SERIES OF CONCURRENT INCLUSION BODY DISEASE (IBD) AND LYMPHOMA IN BOAS

S. Turchetto [1], C. Vries [1], M. Casanova [1], A. Cerezo Echevarría [1]

[1] Department of Pathology, LABOKLIN GmbH & Co.KG, Bad Kissingen, Germany

Background
Inclusion Body Disease (IBD), caused by reptarenaviruses, is a multisystemic viral disease associated with high morbidity and mortality in boid snakes. Histologically, it is characterized by intracytoplasmic inclusion bodies. On the other hand, lymphomas are among the most common tumors in snakes. This study presents a case series of boas with concurrent IBD and lymphoma.

Materials & Methods
Historical data including age, gender and affected organs of *Boa constrictors* and *Boa imperators* with histological diagnosis of IBD and lymphoma were analyzed from samples submitted to LABOKLIN GmbH & Co. KG between 2008 and 2025.

Results
Nine boas met the inclusion criteria. Four were male, four female, and one of unknown sex. One boa was 3 years old; eight were over 6 years, with age unknown for two. Six cases consisted of full or partial necropsies, while three were single biopsies from liver, pancreas and an unidentified tissue. Organs most frequently affected by IBD included liver (n=6), trachea (n=4), lung (n=4), esophagus (n=4), stomach (n=4), pancreas (n=4) and kidney (n=4). Organs most frequently affected by lymphoma were liver (n=6) pancreas (n=3) and skin (n= 3). Concurrent IBD and lymphoma were most often observed in the liver (n=6), intestine (n=2), pancreas (n=3), trachea (n=2), kidney (n=2), eye (n=2) and various other organs.

Conclusion
This study describes co-occurrence of IBD and lymphoma in boas. Further studies are needed to highlight a possible direct association between reptarenaviruses infection and neoplasia in this species.

422 | PULMONARY PNEUMOCYSTOSIS AND GASTRIC CANDIDIASIS IN A CAPTIVE KINKAJOU (POTOS FLAVUS): PATHOLOGIC FINDINGS AND MOLECULAR CHARACTERIZATION

P. Diaz Santana [1], J. Deniz Marrero [1], F. Salguero [2], S. Fingerhood [3], B. Marti Garcia [4], E. Calderon [5], A. Suarez Bonnet [4]

[1] University of Surrey, Guildford, United Kingdom
[2] UK Health Security Agency , Salysbury, United Kingdom
[3] University of Nottingham, Nottingham, United Kingdom
[4] Royal Veterinary College, London, United Kingdom
[5] Institute of Biomedicine of Seville (IBiS), Seville, Spain

Background
An adult, intact male captive kinkajou (*Potos flavus*) with emaciation and hind limb overgrooming was submitted for postmortem examination.

Materials & Methods
Hematological and biochemical analyses were performed ante-mortem by IDEXX Reference Laboratories (UK) using automated platforms. A complete postmortem examination was conducted following standard protocols. Tissues were either fixed in 10% neutral buffered formalin or frozen. Histopathological evaluation of selected organs included H&E, PAS, and GMS stains. DNA was extracted from paraffin-embedded lung tissue, and mitochondrial large and small subunit rRNA genes of Pneumocystis were amplified by PCR, sequenced, and analyzed phylogenetically using MEGA 11. PCR testing for canine distemper virus was conducted on pulmonary tissue. In situ hybridization (RNAScope) targeting SARS-CoV-2 RNA was performed on lung sections. Sequences were submitted to GenBank, and alignments were conducted using MUSCLE with model selection based on the Bayesian information criterion.

Results
Histopathology revealed lymphoplasmacytic interstitial pneumonia with alveolar fungal structures consistent with *Pneumocystis* spp. PCR and in situ hybridization of lung tissue were negative for canine distemper virus and SARS-CoV-2, respectively. PCR amplified *Pneumocystis* mtLSU (510 bp) and mtSSU (565 bp) rRNA genes, suggesting a potentially novel species. Gastric candidiasis was also identified based on morphological evaluation.

Conclusion
This case highlights the susceptibility of kinkajous to opportunistic fungal infections in captive environments. Further investigation is needed to understand their role in the ecology of fungal pathogens, especially at the human–wildlife interface. Enhanced surveillance and interdisciplinary collaboration are essential to assess zoonotic potential and guide conservation and public health strategies.

423 | PATHOLOGICAL FINDINGS IN FOUR MAGELLANIC PENGUINS (SPHENISCUS MAGELLANICUS) DURING SURVEILLANCE IN CHILE, 2024

C. Flores Olivares [1], V. Neira [4], G. Cerda [2], N. Ariyama [3, 4], J. Ruíz [1], S. Marambio [2], M. Zamorano [2], R. Reyes [2], P. Oyarzún Ruiz [5], M. Seguel [6]

[1] Laboratorio de Investigación de Ciencias Veterinarias, Universidad del Alba, La Serena, Chile
[2] SERNAPESCA, La Serena, Chile
[3] Programa de Doctorado en Ciencias Silvoagropecuarias y Veterinarias, Universidad, Santiago, Chile
[4] Departamento de Medicina Preventiva Animal, Facultad de Ciencias Veterinarias y , Santiago, Chile
[5] Departamento de Microbiología, Facultad de Ciencias Biológicas, Universidad de C, Concepción, Chile
[6] School of Veterinary Medicine, St George´s University, Grenada

Background
Spheniscus magellanicus is a migratory seabird distributed along the coasts of southern South America. Despite being classified as a species of “Least Concern,” its population is declining due to threats such as marine pollution, parasitism, and infectious diseases. This study reports pathological findings from four Magellanic penguins stranded in Coquimbo, Chile, between June and August 2024, during an active surveillance program for highly pathogenic avian influenza (HPAI) led by SERNAPESCA.

Materials & Methods
Three animals were frozen prior to necropsy, while one was examined fresh following death with neurological signs. Tissue samples were collected in formalin for histopathological and immunohistochemical (IHC) analysis. A mouse monoclonal antibody targeting the nucleoprotein of Influenza A virus, was used for IHC. Parasites were preserved in 70% ethanol for subsequent examination.

Results
All penguins were emaciated and infected with gastrointestinal nematodes, which were identified as *Contracaecum pelagicum*. Notably, one individual had a large foreign body in the intestine consisting of plastic debris (bags and fishing-nets), causing complete cloacal obstruction. One penguin exhibited severe, diffuse, multisystemic lymphoplasmacytic inflammation, primarily affecting the kidneys, duodenum, and lungs. These organs showed positive intracytoplasmic immunolabeling for Influenza A virus (IAV). Marked inflammation was observed in renal and mesenteric plexuses. In one case, there was extensive, diffuse, intracytoplasmic labeling for Influenza A virus antigens in the gastrointestinal tract, lungs, and kidneys.

Conclusion
This case is the first to describe microscopic lesions and IAV immunopositivity across multiple organ systems in Magellanic penguins. These findings highlight the complex interactions between anthropogenic pollution, parasitism, and viral infections affecting wildlife.

425 | PATHOLOGY OF STRANDED BLUE SHARKS (PRIONACE GLAUCA) IMPALED BY SWORDFISH (XIPHIAS GLADIUS)

A. Oliver-Guimera [1], A Campos-Sosa [2], J. Crespo-Picazo [2], M. Muñoz-Baquero [1, 2], P. García-Salinas [3, 4], J. Penadés-Suay [3, 4], D. García-Párraga [2], J. Ortega [1]

[1] Universidad Cardenal Herrera-CEU, CEU Universities, Alfara del Patriarca, Spain
[2] Fundación Oceanogràfic de la Comunitat Valenciana, Valencia, Spain
[3] Fundación Azul Marino de la Comunidad Valenciana, Oropesa, Spain
[4] Associació LAMNA per al estudi dels elasmobranquis, Valencia, Spain

Background
Swordfish (*Xiphias gladius*) are known for their highly agonistic behavior that might involve attacking other aquatic animals, including blue sharks (*Prionace glauca*) or inanimate objects. The rostrum of swordfish might break as a result of these interactions and it can be used to identify attacks by this species.

Materials & Methods
Ten blue sharks found stranded along the Spanish Mediterranean coast after being impaled by swordfish rostra were examined using Computed Axial Tomography (CT) and necropsied for complete gross and histological evaluation.

Results
Multiple rostral fragments belonging to juvenile swordfish were found with CT or during necropsy. The most affected organ was the encephalon, showing meningeal and brain hemorrhages, encephalomalacia, and acute and chronic encephalitis, followed by the eyes, which exhibited hemorrhage and acute panophthalmitis. Interestingly, in some cases where multiple rostral fragments were observed, some were surrounded by fibrous connective tissue, indicating non-fatal previous interactions. Other commonly observed lesions, not directly associated with the traumatic lesions, included hepatic emaciation, necrotic hemorrhagic ulcers in the spiral valve, and glomerulonephritis.

Conclusion
Swordfish interactions with blue sharks may be underestimated, as the wounds caused by the rostrum can be very small and easily go unnoticed. Therefore, a highly detailed external examination is necessary, along with the complete opening of the head during necropsy, as most fragments tend to lodge there. The use of diagnostic methods, such as CT scans, helps effectively assess the presence of rostral fragments.

LIVESTOCK

33 | ADDITIONAL CONFIRMATION OF TRANSPLACENTAL INFECTIONS OF OVGHV2 IN SHEEP

S. Headley [1], J. Jesus Lima [1], S. Zuchieri [1], G. Yoshitani [1], F. Silva [1], B. Machado [1], J. Fritzen [1], J. Lisbôa [1], A. Alfieri [1]

[1] Universidade Estadual de Londrina, Londrina, Brazil

Background
Macaviruses associated with malignant catarrhal fever (MCF) are referred to as malignant catarrhal fever virus (MCFV). Of these *Macaviruses*, Alcephine gammaherpesvirus 1 and 2 are known for their reproductive effects. However, there are few documented demonstrations of reproductive infections associated with ovine gammaherpesvirus 2 (OvGHV2). Accordingly, this report provides additional evidence of transplacental infection by OvGHV2 in sheep.

Materials & Methods
A two-year-old Santa Ines ewe that died due to the complications of pregnancy toxemia was submitted to routine post-mortem evaluations that revealed two fetuses (# 1 and 2). Selected tissue fragments of all animals were processed for histopathology, for the immunohistochemical (IHC) detection of MCFV antigens, and then used PCR assays designed to amplify OvGHV2 DNA.

Results
Pathological findings in the ewe included brain and pulmonary edema, fatty liver, and lymphoplasmacytic portal hepatitis. Pathological evaluations of the fetuses revealed widespread congestion of most organs. IHC detected widespread dissemination of MCFV antigens within epithelial cells of the kidney, lungs, small intestine, and liver of the ewe and Fetus #1, but not in Fetus # 2. PCR amplified OvGHV2 DNA from the lungs, kidneys, and liver of the ewe, as well as from the liver, lung, kidney, thymus, and small intestine of Fetus # 1, but only from the liver of Fetus # 2.

Conclusion
Both fetuses suffered transplacental infections, suggesting the possible reproductive effects of this *Macavirus*. These findings add to the relatively few documented demonstrations of vertical infections by OvGHV2 in sheep.

50 | THE MUCOSAL SHIELD: A HISTOPATHOLOGICAL PERSPECTIVE ON INTESTINAL MUCUS ALTERATIONS AND GOBLET CELL DYNAMICS IN PIGLET DIARRHOEA

C. Brandt Becker [1], M. Hansen [1], S. Saxmose Nielsen [1], M. Rydal [1], J. Nielsen [1], M. Kjelin Morsing [1], C. Bøttcher Jørgensen [1], M. Fredholm [1], H. Elvang Jensen [1]

[1] University of Copenhagen, Frederiksberg, Denmark

Background
Diarrhoea in pigs is a frequent clinical manifestation and a major reason for antibiotic use. The intestinal barrier constitutes the first line of defence against invading microbes and includes a glycoprotein-rich mucus layer that protects the epithelium. Changes in mucus composition and goblet cell populations have been described for various infections, however their role in early-life and post-weaning diarrhoea in pigs remains uncertain.

Materials & Methods
A case-control study (diarrheic versus non-diarrheic) was conducted on 30 piglets from one Danish farm. Animals were divided into five age groups (4, 14, 25, 49, and 67 days). Tissue samples from jejunum and colon were fixed in Carnoy's solution and immunohistochemically stained for MUC2 and MUC5AC (mucins). Slides were digitized and analysed in QuPath. Ten crypts per segment were evaluated for goblet cell counts and mucin-positive area using machine learning-based techniques.

Results
Goblet cell numbers displayed an age-related increase in both segments, independent of diarrhoea-status. In neonates, diarrhoea was associated with higher goblet numbers and MUC2+ areas. At 49 days, colonic goblet hyperplasia with reduced MUC2 positivity indicated mucus depletion and immature cell recruitment. At 67 days, diarrheic piglets showed lower goblet cell counts in both segments, with unchanged MUC2+ fractions. *De-novo* MUC5AC expression was observed in colon of two piglets from the oldest group.

Conclusion
Goblet cell dynamics and mucus composition in piglets are affected by age and diarrhoea-status, with distinct age-related alterations. These findings suggest that disruption of the mucosal barrier plays a role in the pathogenesis of piglet diarrhoea.

91 | DIHYDROPTEROATE SYNTHASE AND DIHYDROFOLATE REDUCTASE GENETIC PROFILING IN PNEUMOCYSTIS SUIS

B. Blasi [1], A. Breil [1], L. Ma [2], H. Weissenböck [1], C. Weissenbacher-Lang [1]

[1] Institute of Pathology, University of Veterinary Medicine Vienna, Vienna, Austria
[2] Critical Care Medicine Department, National Institutes of Health, Bethesda, United States

Background
Pneumocystis encompasses a genus of fungal species that possibly infect lungs of all mammals, including humans. The first-line treatment involves sulfonamides and trimethoprim, respectively targeting the DHPS and DHFR genes of the folic acid pathway, respectively. Mutation in those genes have been linked to prior exposure to these drugs and possibly to treatment failure. In pigs, these drugs are used to treat various bacterial and protozoan infections, resulting in an indirect exposure of P.suis to them.

Materials & Methods
DNA was isolated from lungs of *Pneumocystis*-infected pigs from Austrian farms (n= 57) and wild boars (n=7) and used for PCRs targeting *P. suis* DHPS and DHFR genes, followed by DNA sequencing. The sequences were aligned with those from *Pneumocystis jirovecii,* the human infecting species, to compare possible mutations with those already characterized.

Results
In DHFR gene no nonsynonymous mutations were detected. In DHPS gene, most of domesticated pig samples (74%) showed the same nonsynonymous mutation at codon 57 (P57S) present in *P.jirovecii*, one of the most common mutations described in a potential association to sulfonamides resistance. This mutation was absent in all analyzed wild boar samples.

Conclusion
This study is the first to explore potential drug resistance-associated mutations in *P. suis*. The widespread presence of P57S mutation in farmed pigs, contrasted with its absence in wild boars, strongly suggests selective pressure exerted by sulfonamides in domesticated swine. Further studies are needed to establish a causal relationship between the P57S mutation, sulfonamide exposure, and drug resistance.

143 | HISTOPATHOLOGICAL LESIONS AND DETECTION OF PORCINE CIRCOVIRUSES IN PRDC-AFFECTED PIGS USING NANOPORE SEQUENCING

G. Burrai [1], M. Polinas [1], C. Cacciotto [1], S. Sanna [1], C. Cubeddu [1], E. Emanuela [1], D. Dei Giudici [2], P. Pazzola [1], E. Antuofermo [1], A. Alberti [1]

[1] Department of Veterinary Medicine, University of Sassari, Sassari, Italy
[2] Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy

Background
Porcine Respiratory Disease Complex (PRDC) is a major health issue in swine production, characterized by multifactorial aetiology and significant economic impact. Among the wide array of pathogens involved in porcine respiratory disease, porcine circoviruses (PCVs) have gained increasing attention due to their frequent detection in both healthy and diseased animals, their immunomodulatory properties, and their potential role in exacerbating respiratory pathology. While PCV2 has long been established as a major pathogen in PRDC, PCV1, historically considered non-pathogenic, and PCV3—a recently discovered virus— are increasingly implicated in PRDC.

Materials & Methods
Lung tissue samples from 10 adult pigs, 10 post-weaning pigs, and 10 piglets were collected and examined macroscopically and histologically. Total DNA was extracted from the selected tissues. Samples were initially tested with a nested PCR for *Circoviridae*, followed by genome nanopore sequencing using the MinION™ Mk1C (Oxford Nanopore Technologies) to characterize viral profiles in the lung tissue.

Results
The most commonly observed lesion in the examined categories was a diffuse chronic histiocytic and lymphoplasmacytic interstitial pneumonia, which was significantly more severe in piglets compared to post-weaning pigs (*Kruskal-Wallis test*, H = 8.89, p = 0.005). No significant differences were observed in adult pigs. Nanopore sequencing revealed the presence of PCV3 and cyclovirus in piglets, PCV1 and PCV2 in post-weaning pigs, and PCV3 in adult pigs.

Conclusion
These results emphasize the importance of implementing comprehensive PRDC management strategies that address both established pathogens, such as PCV2, and emerging viruses like PCV3.

158 | THE ROLE OF CLOSTRIDIUM PERFRINGENS BETA-TOXIN IN THE PATHOGENESIS OF PORCINE NECROTIC ENTERITIS.

B. Tarek [1], F. Farhoosh [1], J. Burggisser [1], I. Iacovache [2], B. Zuber [2], H. Posthaus [1]

[1] Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Bern, Switzerland
[2] Institute of Anatomy, Medical Faculty, University of Bern, Bern, Switzerland

Background
Clostridium perfringens type C causes fatal necrotic enteritis in newborn animals and sporadically in humans. The disease is characterized by rapidly progressing hemorrhage and necrosis of the small intestine. Beta-toxin (CPB), a hemolysin-like β-pore-forming toxin, is the main virulence factor of pathogenic type C strains. CPB targets endothelial cells in the small intestinal wall, leading to vascular damage early during disease progression.

Materials & Methods
We used genome-wide CRISPR-Cas9 loss-of-function screens, CRISPR-Cas9 single gene knockout, ectopic overexpression studies and cryo-electron microscopy (EM) to investigate the molecular and structural basis of CPB target cell specificity.

Results
We unambiguously identified Platelet Endothelial Cell Adhesion Molecule-1 (CD31) as the cell surface receptor for CPB in endothelial and monocytic cells. We determined the membrane proximal Ig6 domain of CD31 as the toxin binding domain. The oligomeric pore structure of CPB was resolved as symmetrical eightfold protomers, consisting of N-terminal β-barrel protrusion, a cap, a rim, and a stem domain. The rim domain contains unique loops that define the receptor specificity of CPB. A range of recombinant CPB mutants substantiate this finding and uncover important residues in this interaction.

Conclusion
Our results explain the remarkable receptor and cell type specificity of CPB. This correlates with lesions in naturally affected pigs and humans.

201 | BRIDGING PATHOLOGY AND MATERIALS SCIENCES: MACROPHAGE-MEDIATED CRYSTALLISATION OF ALUMINIUM OXYHYDROXIDE ADJUVANT IN VACCINE GRANULOMAS

E. Pérez [1], V. Sebastián [2, 3, 4], A. Ibarra [2, 4], M. Navarro [4], Á. Gómez [1, 5], M. Pérez [5, 6], L. Luján [1]

[1] Department of Animal Pathology, University of Zaragoza, Zaragoza, Spain
[2] Institute of Nanoscience and Materials of Aragon, CSIC-University of Zaragoza, Zaragoza, Spain
[3] Biomedical Research Networking Centre-Bioengineering, Biomaterials and Nanomedic, Madrid, Spain
[4] Advanced Microscopy Laboratory, University of Zaragoza, Zaragoza, Spain
[5] AgriFood Institute of Aragon (IA2), University of Zaragoza, Zaragoza, Spain
[6] Department of Anatomy, Embryology and Animal Genetic, University of Zaragoza, Zaragoza, Spain

Background
Aluminium oxyhydroxide (AlOOH), an adjuvant in human and veterinary vaccines, induces persistent granulomas containing acicular microstructures rich in aluminium (Al), referred to as crystalloid bodies (CB). Its origin and nature remain poorly understood. In a recent ovine vaccination trial, the absence of CB in the AlOOH stock solution and vaccine, together with their consistent presence in granulomas four months post-injection, suggested in vivo formation mediated by macrophages.

Materials & Methods
Histological analysis (by HE) and Al-specific staining (by lumogallion and modified Al haematoxylin) were performed on samples from six sheep vaccinated with AlOOH and bluetongue virus (BTV-4). In four granulomas, structural and compositional characterisation of CB and AlOOH particles was conducted using TEM, SEM, STEM-HAADF, SAED, and EDS. Crystalline structure was further examined via Fast Fourier Transform of high-resolution TEM images.

Results
CB appeared as hexagonal, rod-shaped microparticles. EDS confirmed a composition predominantly of Al and oxygen (n=30 CBs). Crystallographic analysis revealed a structure inconsistent with the original AlOOH, but matching gamma-alumina (γ-Al_2O_3), a high-value industrial material used as catalyst and adsorbent.

Conclusion
This study presents the first evidence of intracellular metal crystallisation in mammalian cells, likely driven by the aggregation of adjuvant-derived nanoparticles. Results suggest that macrophages can function as biological reactors, transforming AlOOH into γ-Al_2O_3 under physiological conditions without the high temperatures (400–500 °C) currently required. From a multidisciplinary perspective, this unexpected biomineralisation offers novel avenues on the biogenic formation of γ-Al_2O_3 under more energy-efficient and sustainable strategies.

215 | DANDY-WALKER MALFORMATION IN AN ANGUS CALF

A. Demeter [1]

[1] Cornell University, Ithaca, United States

Background
A 2-day-old, 30 Kg, male Angus calf was submitted for a necropsy following euthanasia. The calf was delivered by a healthy first-time calving heifer. Clinical examination before euthanasia revealed that the calf was laterally recumbent, neurologically inappropriate, with positional nystagmus and no palpebral reflex.

Materials & Methods
Post-mortem examination under biosecurity laboratory 2+ conditions was pursued following standard technique. Tissue sections were collected for histopathology examination and ancillary testing for: aerobic and anaerobic bacteria, *Leptospira sp*., *Campylobacter fetus*, *Brucella sp*., bovine viral diarrhea, bovine herpesvirus 1, *Neospora sp.*

Results
The calf was in good body condition with moderate post-mortem autolysis. No abnormalities were noted in the thoracic, abdominal cavities, or musculoskeletal system. The cerebellum was subjectively smaller, overall approximately 1/2 of the expected size, with a distinct separation of the two cerebellar hemispheres, allowing visualization into the fourth ventricle and underlying brainstem. The fourth ventricle was severely dilated and covered by a thin, transparent membrane. The cerebellar vermis was absent (agenesis), and the edges of the separated cerebellar hemispheres were slightly undulated. The corpus callosum and hippocampus were not grossly noted. The cerebellar hemispheres had retained foliar pattern. The additional ancillary tests did not yield any positive results.

Conclusion
Gross and histologic examination confirmed that this is a case of cerebellar vermis agenesis, also known as Dandy-Walker malformation. Only a few reports of Dandy-Walker malformation exist in veterinary medicine. This case presentation aims to highlight and present the gross and histologic findings of a rare CNS malformation.

217 | NASAL CONIDIOBOLOMYCOSIS WITH CENTRAL NERVOUS SYSTEM INVASION IN TWO SHEEP

E. De Luca [1, 2], M. Dorsch [1, 2], A. Martinez [3], C. Scully [3], J. Menk [1, 2]

[1] Department of Pathobiological Sciences, School of Veterinary Medicine, LSU, Baton Rouge, United States
[2] LADDL/LSU Diagnostics, School of Veterinary Medicine, LSU, Baton Rouge, United States
[3] Department of Veterinary Clinical Sciences, School of Veterinary Medicine, LSU , Baton Rouge, United States

Background
Conidiobolomycosis is an important disease of sheep caused by *Conidiobolus* spp., a fungus that induces granulomatous inflammation mainly in two forms: rhinopharyngeal and rhinofacial. While frequently diagnosed in tropical and subtropical regions of Brazil, documented cases in other countries are rare and likely underdiagnosed.

Materials & Methods
Two lambs were presented with epistaxis, respiratory distress, exophthalmos, and periocular swelling unresponsive to antimicrobial and anti-inflammatory therapy. Postmortem examination, histopathology with Gomori Methenamine-Silver (GMS) stain as well as fungal culture in one case, were performed.

Results
On gross examination, both animals had a yellow and firm mass within the caudal nasal cavity, extending to the ethmoid turbinates and focally effacing the left orbit, cribriform plate, and frontal sinus. The masses extended into the frontal cerebral lobes and retrobulbar muscles. Histologically, the nasal mucosa was expanded by granulomatous inflammation and areas of necrosis with numerous GMS-stained fungal hyphae surrounded by eosinophilic sleeves. Fungal morphology was consistent with *Conidiobolus* spp. The inflammation extended to the leptomeninges, frontal cerebral lobes and retrobulbar muscle with similar intralesional hyphae. Additional findings in the brain included lymphoplasmacytic perivascular cuffs, fibrin thrombi and fibrinoid necrosis. Fungal culture yielded growth of *Conidiobolus* spp.

Conclusion
These cases further highlight the invasive potential of *Conidiobolus* spp., which should be considered as potential differential diagnosis in neurologic diseases in small ruminants, particularly in endemic regions. In a diagnostic setting, a combination of morphological evaluation, fungal culture and/or molecular techniques is ideal to reach a final etiological diagnosis.

300 | URACHAL REMNANTS IN REGULARLY SLAUGHTERED SHEEP – ANATOMO-HISTOPATHOLOGICAL FINDINGS

S. Mignacca [1], S. Bagatella [2], B. Amato [3], M. Capucchio [2]

[1] Department of Agriculture, Food and the Marine - Veterinary Laboratories, Celbridge, Ireland
[2] Department of Veterinary Sciences, Torino University, Torino, Italy
[3] Veterinary School, University of Bristol, Bristol, United Kingdom

Background
Urachal remnants (URs), result from failed involution of the umbilical arteries and urachus after birth. Congenital URs seem infrequent in small ruminants, and a paucity of literature on their prevalence and morphological features exists.

Materials & Methods
A total of 1206 urinary bladders from slaughtered adult sheep were grossly examined and selected samples were collected for histopathological examination.

Results
Sixty-seven bladders (5.5%) had URs. 45 showed sessile or pedunculated, firm-elastic, pink-grey remnants. On cross section, 42 had a homogeneous appearance or, rarely, scattered brown-black pigmentation or fluid-filled cavitations. Histologically, they showed a variable number of cysts lined by occasionally metaplastic, transitional epithelium surrounded by abundant connective tissue. These were consistent with urachal cysts. Nineteen specimens consisted of filiform, fibrotic extensions up to 11 cm long, with a consistently lacerated free end and no apparent lumen on cross section. Histologically, cross sections proximal to the bladder's apex showed a central narrow lumen lined by multi-layered/pseudostratified epithelium surrounded by fibrovascular/collagenous stroma. Consecutive, slightly more distal sections showed replacement of the lumen and the epithelium by areas of bony metaplasia. In the more distal segments, these were substituted by irregularly arranged bundles of smooth musculature and a variable number of blood vessels. These structures were interpreted as urachal cordae. Three specimens, macroscopically classified as vesicourachal diverticula, showed an evident communication (1-2x5 mm) with the bladder's lumen.

Conclusion
This study shows that URs are relatively frequent in slaughtered sheep and provides new data on their, so far underreported, morphopathological characterization.

304 | PANTOTHENIC ACID DEFICIENCY IN NEWBORN AND WEANED PIGLETS ASSOCIATED WITH HIGH MORTALITY

L. Martino [1, 2, 3], J. Segalés [2, 5, 7], S. Loscertales [1, 3], M. Cid [1, 4, 5], A. Romagosa [6]

[1] Servei de Diagnòstic de Patologia Veterinària, Facultat de Veterinària, UAB, Bellaterra, Spain
[2] Departament de Sanitat i Anatomia Animals, Facultat de Veterinària, UAB, Bellaterra, Spain
[3] Universitat Autònoma de Barcelona (UAB), Bellaterra, Spain
[4] IRTA, Animal Health, Centre de Recerca en Sanitat Animal (CReSA), Bellaterra, Spain
[5] Unitat mixta d'investigació IRTA- UAB en Sanitat Animal, CReSA, Bellaterra, Spain
[6] Pig Improvement Company, Sant Cugat del Vallès, Spain
[7] WOAH Collaborating Centre for the Research and Control of Emerging and Re-emergi, Bellaterra, Spain

Background
Pantothenic acid (vitamin B5) deficiency is rarely described in pigs and results in peripheral neuropathy and necrosis of spinal cord neurons. We describe an outbreak affecting newborn suckling and nursery piglets.

Materials & Methods
Newborn piglets showed apathy, ataxia, and prostration on the second day of life, usually dying on the third day. Piglets were born healthy and consumed colostrum normally. Sows were unaffected. Two weeks later, recently weaned piglets exhibited similar clinical signs. Seven piglets, 5 affected (3 newborn and 2 weaned), and 2 non-affected (newborn), were necropsied. Samples from multiple organs, including skeletal muscle, brain, sciatic nerve, and spinal cord, were processed for histopathology.

Results
No gross lesions were found at necropsy. Histopathologically, cervical, thoracic and lumbar spinal cord neuronal bodies had variable degrees of central chromatolysis, cytoplasmic vacuolation and swelling, with displaced nuclei and occasional necrosis. Sciatic nerves had mild-to-intense axonal swelling and fragmentation. Lesions were more evident in weaned pigs and absent in non-affected pigs. A presumptive diagnosis of pantothenic acid deficiency was made. Supplementation of vitamin B complex to all newborn piglets prevented clinical signs from developing. Additionally, treatment of a small cohort of piglets developing clinical signs after missing their supplementation at birth resulted in reversal of signs. The origin of the deficiency has yet to be elucidated.

Conclusion
Pantothenic acid deficiency should be among the differential diagnoses of piglets with ataxia and muscle weakness. A fast diagnosis based on complete nervous system sampling is key to counteract this condition.

310 | ANTIBODY TESTING FOR THE DETECTION OF PNEUMOCYSTIS IN PORCINE LUNG TISSUES: A FOCUS ON IMMUNOHISTOCHEMISTRY

N. Gobbo Oliveira Erünlü [1], B. Blasi [1], H. Weissenböck [1], C. Weissenbacher-Lang [1]

[1] University of Veterinary Medicine, Vienna, Institute of Pathology, Vienna, Austria

Background
Pneumocystis spp. are atypical fungi, comprising several species, each exhibiting a high degree of host specificity. *Pneumocystis* is primarily an alveolar pathogen, attaching to type I pneumocytes. Lung injury and impaired gas exchange result predominantly from the host's inflammatory response rather than direct fungal damage. We aim to identify antibodies that cross-react with *Pneumocystis suis* and can be applied in other downstream methods.

Materials & Methods
Formalin-fixed paraffin-embedded (FFPE) lung tissue sections from *Pneumocystis*-positive pigs were used to evaluate commercially available anti-*Pneumocystis* antibodies by automated immunohistochemistry (IHC). Fourteen antibodies were tested; twelve were developed against *Pneumocystis jirovecii*, including eight antibodies of the 3F6 clone sourced from seven different brands. Five additional clones targeting *Pneumocystis* were also evaluated. One non-commercially available antibody was developed against *Pneumocystis carinii.* One commercially available antibody for detection of 1,3-β-glucan, a cyst wall component, was tested. Various antibody dilutions and antigen retrieval methods were tested to optimize staining quality while minimizing background and nonspecific staining.

Results
Five antibodies demonstrated specific staining of *Pneumocystis* organisms in FFPE pig lung sections. Nine antibodies produced questionable results with nonspecific staining patterns, and one antibody showed no staining. Significant variability in staining quality was observed among different brands of the 3F6 clone. Eight antibodies exhibited concomitant staining of macrophage-like structures alongside *Pneumocystis* organisms.

Conclusion
Only four commercially available antibodies provided satisfactory IHC results, free from significant background or nonspecific staining, when applied to pig lung tissues. Efforts are currently underway to develop our own antibody targeting *Pneumocystis suis.*

322 | CONCURRENT CUTANEOUS AND NASAL PHAEOHYPHOMYCOSIS CAUSED BY SETOPHAERIA ROSTRATA IN A GOAT

P. Pinczowski [1], A. Chamings [1], B. O'Rourke [1], M. Westman [1], S. Slattery [2], M. Gimeno [1, 3]

[1] Elizabeth Macarthur Agricultural Institute, NSW, DPIRD, Menangle, Australia
[2] Local Land Services, Narrabri, Australia
[3] The University of Sydney, Sydney, Australia

Background
*Setophaeria rostrata*is a dematiaceous, saprophytic fungus primarily associated with plants but increasingly recognised as an opportunistic pathogen in animals and humans. While isolated cases of nasal or cutaneous infection have been reported in ruminants and horses, concurrent lesions have not previously been documented. The affected goat originated from a meat enterprise in Wee Waa, NSW, Australia, a region that experienced continual flooding for three months, with livestock exposed to persistently wet conditions—favourable for fungal proliferation.

Materials & Methods
A two-year-old rangeland female goat presented with multifocal nodular skin lesions on the pinnae and limbs, nasal stertor, and poor body condition. Due to the severity of clinical signs, the goat was euthanised. Gross and histopathological examinations were performed. Fungal culture was conducted on Sabouraud agar, with identification by microscopy and ITS sequencing.

Results
Postmortem examination revealed abundant friable yellow material within the nasal cavity, effacing the conchae, and numerous exudative, crusting nodular skin lesions. Histopathology showed severe chronic-active pyogranulomatous rhinitis and dermatitis with abundant intralesional fungal elements, including pigmented 3–20 µm round to piriform yeast-like bodies and 3–4 µm septate hyphae. Findings were consistent with phaeohyphomycosis. Culture and sequencing confirmed *S. rostrata* the aetiologic agent.

Conclusion
This is the first report of concurrent nasal and cutaneous phaeohyphomycosis due to *S. rostrata* in a goat. Environmental exposure during prolonged flooding likely facilitated infection. This case highlights the organism's thermotolerance, pleomorphism, and cross-kingdom pathogenicity, and underscores the value of integrating histopathology with culture and molecular diagnostics.

324 | EFFECTS OF LIVE LARVAE OF TENEBRIO MOLITOR AS FEED INGREDIENTS ON POULTRY GUT HEALTH: HISTOMORPHOMETRICAL AND HISTOLOGICAL INVESTIGATIONS

S. Bagatella [1], M. Malik [1], T. Hassan [1], M. Arif [1], A. Sereno [1], V. Bongiorno [1], S. Bellezza Oddon [2], Z. Loiotine [2], I. Biasato [2], L. Gasco [2], M. Capucchio [1]

[1] Department of Veterinary Sciences, University of Turin, Grugliasco, Italy
[2] Department of Agricultural, Forest and Food Sciences, University of Turin, Grugliasco, Italy

Background
Insects are considered an emerging protein source in poultry nutrition, yet little is known about the effects of live insect larvae as feeding ingredients on gut health.

Materials & Methods
198, 40-week-old Bionda Piemontese hens were randomly allotted to 3 diets (6 replicate/pens; 11 animals/pen): basal diet, TM5 and TM10 groups, receiving no, 5% and 10% of live *Tenebrio monitor* (TM) larvae, respectively. After 84 days, 12 hens/group were slaughtered, and gut segments, liver and spleen were collected for histomorphometrical and histopathological evaluation. Jejunum sections were also stained with periodic-acid Schiff, Alcian Blue pH 2.5 and High-Iron Diamine to characterize mucins. The evaluated morphometric indices were villus height (Vh), villus width (Vw), crypt depth (Cd), Vh/Cd, mucosal (MT), submucosal (SmT) and muscularis (MuT) thickness. A semiquantitative scoring system was used to assess inflammation severity (gut segments, liver, spleen), pattern and type (gut segments), degeneration (liver), lymphoid hyperplasia and depletion (spleen), and the intensity of the histochemical stains in crypts and villi.

Results
Vh, Vw, Cd, Vh/Cd, MT and MuT differed significantly ($p<0.001$) among intestinal segments, with the first five being higher in duodenum and MuT in ileum. Inflammation was higher in ileum ($p<0.05$), while the base of the crypts showed a higher intensity ($p<0.001$) for all stainings performed. No significant differences were observed between other parameters or, relevantly, among dietary groups.

Conclusion
The administration of live TM larvae showed no negative impact on the morphopathological parameters analyzed herein, suggesting their potential use as safe feeding ingredients in poultry diets.

358 | OUTBREAKS OF CONTAGIOUS ECTHYMA IN A SHEEP FLOCK FROM SOUTHERN BRAZIL

S. Headley [1], G. Queiroz [1], S. Zuchieri [1], J. Jesus Lima [1], M. Marques [1], J. Fritzen [1], A. Alfieri [1]

[1] Universidade Estadual de Londrina, Londrina, Brazil

Background
Contagious ecthyma, CE (ORF, sore mouth) is a viral, zoonotic, infectious disease of sheep and goats caused by members of the genus *Parapoxvirus*, and family *Poxviridae*. Although CE is common worldwide, epidemiological data is scarce in Brazil, and most cases are diagnosed by histopathology. This report describes an outbreak of CE in a sheep flock from Southern Brazil

Materials & Methods
In June 2020, 6.7% (20/300) of sheep from a farm in southern Brazil developed mild clinical CE with spontaneous regression. Four years later, 2.2% (4/180) of adult ewes developed CE. 10 days thereafter, 76.1% (137/180) of this category were affected. 90% (54/56) of lambs that had contact with these ewes were also affected. Biopsy of the oral lesions were collecetd from eight ewes for histopathology and molecular analyses to amplify Orf virus.

Results
All lesions were restricted to the oral cavities of the affected animals. Histopathology revealed severe proliferative pustular glossitis with numerous eosinophilic inclusion bodies within degenerated keratinocytes. PCR amplified a 487 bp fragment of the RNA polymerase subunit gene of Orf virus from 50% (4/8) of the evaluated samples. Phylogenetic analyses revealed that the strain from this study clustered with similar strains of Orf virus from several countries.

Conclusion
The findings herein observed are consistent with previous descriptions of CE and add to the few studies that have investigated the molecular origin of CE in Brazil. The elevated morbidity may be due to contact of naïve sheep with animals from the initial outbreak.

359 | SPONTANEOUS OVINE GAMMAHERPESVIRUS 2 INFECTIONS IN TWO SHEEP FROM SOUTHERN BRAZIL

S. Headley [1], G. Queiroz [1], J. Jesus Lima [1], F. Silva [1], D. Silva [1], B. Machado [1], J. Fritzen [1], G. Yoshitani [1], S. Zuchieri [1], P. Pereira [1], A. Alfieri [1]

[1] Universidade Estadual de Londrina, Londrina, Brazil

Background
Ovine gammaherpesvirus 2 (OvHV-2) is a *Macavirus* that causes sheep-associated malignant catarrhal fever (SA-MCF), in which sheep are the asymptomatic carrier hosts and mammalians are susceptible hosts. *Macaviruses* associated with MCF are referred to as malignant catarrhal fever virus (MCFV) for sharing the 15A epitope. This report describes the findings observed in spontaneous infections of OvHV-2 in the asymptomatic hosts.

Materials & Methods
Two 1-year-old Texel ewes from a farm in Southern Brazil died suddenly and were submitted to post-mortem evaluations. Tissue fragments from the liver, lung, kidney, small intestine, lymph node, uterus, and brain were processed for histopathology and the immunohistochemical (IHC) detection of MCFV antigens and used in PCR assays to amplify OvHV-2 DNA.

Results
Histopathology revealed lymphocytic interstitial pneumonia, lymphocytic portal hepatitis, interstitial lymphocytic nephritis, and atrophic enteritis with widespread vasculitis in both animals. IHC revealed intracytoplasmic intralesional MCFV antigens within epithelial cells of the lungs, intestines, kidneys, and liver of both sheep. PCR amplified OvHV-2 DNA from the lung, liver, kidney, small intestine and mesenteric lymph node of both sheep, and from the uterus and brain of one animal. Direct sequencing confirmed the specificity of the amplicons.

Conclusion
The concomitant detection of MCFV antigens and OvHV-2 DNA from multiple organs of these animals with widespread vasculitis confirmed active infections by this *Macavirus*. This report represents one of the few documented cases worldwide to confirm OvHV-2 infections in the asymptomatic hosts. In these cases, the absence of typical clinical manifestations of SA-MCF indicates that these animals were subclinically infected.

363 | GENOMIC AND HISTOPATHOLOGICAL CHARACTERISTICS OF BOVINE E. COLI MASTITIS

S. Kromann [1], R. Heidemann Olsen [1], V. Krömker [1], H. Andreasen [2], A. Becker Saidenberg [3], M. Stegger [3], H. Elvang Jensen [1]

[1] University of Copenhagen, Frederiksberg, Denmark
[2] Vendsyssel Landdyrlæger, Tårs, Denmark
[3] Statens Serum Institut, København, Denmark

Background
Escherichia coli is a common aetiology in bovine mastitis, causing both mild, moderate, and severe infections. In fact, *E. coli* is a dominating pathogen in severe mastitis, often destining animals for slaughter if the acute infection is survived. Despite the prominent role of *E. coli* in mastitis, factors determining disease severity are unknown. The project aims to characterise the pathogen and host response to elucidate factors contributing to disease severity.

Materials & Methods
During 2024, *E. coli* isolated from cows (n=153) clinically suffering from mild, moderate or severe mastitis were subjected to whole-genome sequencing (WGS) with subsequent bioinformatic analyses determining phylogenetic relations and multi-locus sequence types (MLST). Furthermore, in a subset of samples, several *E. coli* colonies were subjected to multiple-locus variable number tandem analysis (MLVA) to investigate whether samples contained single or multiple strains. From cows that died, were euthanised or slaughtered, tissue for microbiology and histopathology was collected and characterised morphologically and immunohistochemically.

Results
WGS revealed ST10, ST58 and ST1125 as prominent sequence types, constituting 20.3%, 19% and 9.9%, respectively. Interestingly, cases of clonality were observed both within and between farms. MLVA-data indicated clonality within samples independent of clinical severity. Additionally, data obtained from slaughtered cows suggested prolonged survival of *E. coli* and extensive destruction of glandular tissue.

Conclusion
Bovine *E. coli* mastitis was determined to be a monotype infection, often leading to marked destruction of functional mammary gland tissue in cows surviving infection. Surprisingly, clonal lineages could, in some cases, be isolated from separate cows.

419 | HISTOPATHOLOGY AND DISTRIBUTION OF THE A/H3N1 AVIAN INFLUENZA VIRUS IN YOUNG HENS AND ROOSTERS COMPARED TO ADULT LAYING HENS.

A. Kycko [1], K. Śmietanka [1]

[1] National Veterinary Research Institute, Puławy, Poland

Background
In 2019, an avian influenza virus, the A/H3N1 strain, formally classified as low-pathogenic avian influenza (LPAI), caused an epidemic in poultry in Belgium, characterised by high mortality and a decrease in egg production in laying hens. The aim of this study was to compare histopathological changes and distribution of the virus in the tissues of young and adult hens and young roosters.

Materials & Methods
The tissues from brain, kidney, heart, lung, liver, oviduct and duodenum collected from 4-week-old hens and roosters and 30-week-old laying hens (each group: n=25) on days 2, 4, 7, 10 and 14 post inoculation with A/H3N1 virus, were subjected to histopathology and immunohistochemistry using monoclonal antibody against AIV virus.

Results
Histopathological lesions were observed in the brain, kidney and heart at 4, 7 and 10dpi in all the groups and involved moderate non-suppurative encephalitis and mild to moderate interstitial lymphohistiocytic infiltrations in the kidney and heart. In the oviduct, lymphohistiocytic infiltrations and fibrinous exudate were observed in adult hens at 10 and 14 dpi. The immunolabelling of the viral antigen was detected in the same cases in the neuronal cells in the brain, single epithelial cells in the oviduct, single cardiomyocytes, focally in the renal tubules and the inflammatory infiltrations.

Conclusion
The results indicated higher pathogenicity of the A/H3N1 virus in the adult hens compared to the young birds, at the same time demonstrating neuro-, nephro- and cardiotropism of the virus regardless of sex and age, supporting the necessity of surveillance of the virus in poultry.

426 | IMMUNOEXPRESSION OF 14-3-3 SIGMA IN NORMAL, HYPERPLASTIC, AND NEOPLASTIC LUNG TISSUE IN SHEEP

D. Hodor [1], A. Hîruța [1], A. Negoescu [1], C. Borfălău [1], I. Pană [1], C. Toma [1], C. Cătoi [1]

[1] University of Agricultural Science and Veterinary Medicine, Cluj-Napoca, Romania

Background
The 14-3-3σ protein is an epithelial marker commonly linked to carcinogenesis. This study aimed to evaluate the 14-3-3σ immunohistochemical expression in different sheep lung diseases and to assess its potential as a prognostic biomarker in ovine pulmonary adenocarcinoma (OPA).

Materials & Methods
Twenty-five lung samples (5 healthy lung tissues, 5 cases of parasitic pneumonia with associated epithelial hyperplasia and 15 OPA cases) and the corresponding 5/15 regional lymph nodes diagnosed with metastasis, were HE-stained, and immunolabeled for 14-3-3σ and Ki67. 14-3-3σ expression was assessed semi-quantitatively and quantitatively using ImageJ2. The Ki-67 proliferation index (Ki67-PI) was calculated by counting 1000 nuclei at x400. For statistical analysis, OPA samples were grouped by metastasis status and compared using t-tests and MANOVA.

Results
Both normal (3/5) and hyperplastic (2/5) tissues showed low(+) to moderate(++) 14-3-3σ labeling of bronchial/bronchiolar epithelium, type II pneumocytes, including type II pneumocyte hyperplasia in the hyperplastic tissue. In most (8/15) OPA cases, less than 15% of the neoplastic areas were immunolabeled with moderate(++) intensity. Median Ki-67 PI was 0.5 (range: 0–5). 14-3-3σ immunoexpression was detected in two lymph node metastases.
Lung tumors (n=5) with lymph node metastases showed significantly higher 14-3-3σ immunoexpression. Combined immunolabeling levels of 14-3-3σ and Ki-67PI differed significantly between metastatic and non-metastatic cases (p-value < 0.05), suggesting a potential role for these markers in tumor progression.

Conclusion
The increased expression of 14-3-3σ in metastatic compared to non-metastatic OPA cases suggests its potential utility as a prognostic biomarker.

434 | BLUETONGUE VIRUS SEROTYPE 3 OUTBREAK IN THE NETHERLANDS: MORPHOLOGICAL CHANGES IN ABORTED AND STILLBORN CALVES IN 2024/2025

R. Dijkman [1], L. Harkema [1], M. Delany [1], J. De Jong [1], K. Brink [1], N. Bruijn [1]

[1] Royal GD, Deventer, Netherlands

Background
In September 2023 the first case of Bluetongue virus Serotype 3 was reported in the Netherlands. The pathological changes, in cattle were mostly acute muscle degeneration, especially in oesophagus and rumen. From October 2024 up to February 2025 24 neonatal and 20 stillborn and aborted calves with hydrocephalus were submitted to Royal GD for necropsy.

Materials & Methods
The aborted and stillborn calves were examined via standard protocol, including bacterial culture of abomasal contents, histology of brain, lung, heart, liver and placenta, PCR for *Coxiella Burnetii* and ELISA testing for BVD virus. The neonatal calves were examined macroscopically and relevant tissues were sampled for histopathology, bacterial culture and/or PCR. Schmallenbergvirus and Bluetongue PCR were only performed on request.

Results
The pathological changes in the calves and aborted fetuses were characterized by hydrocephalus or hydranencephaly, with dystrophic changes, sometimes dystrophic mineralization, microangiopathy or sometimes a lymphocytic inflammation, most evident in brain, lungs and heart. Bluetongue PCR was performed and positive in 6 fetuses and 4 neonatal calves. Schmallenbergvirus PCR was performed en negative in 1 fetus and 4 calves. Two fetuses tested positive for *Coxiella burnetii* concordingly, 6 calves had pneumonia and 4 had enteritis due to other causes.

Conclusion
All necropsies were performed in a diagnostic setting, with restrictions on Bluetongue and Schmallenbergvirus PCR accessibility. Bluetongue PCR was performed and positive in 10 cases. The morphological changes were characteristic and therefore all cases mentioned were reported as suspected Bluetongue infections.

440 | SUDDEN DEATH IN PIGS CAUSED BY EMCV

A. Pegoraro Poor [1], P. Soster [1], B. Bracco Donatelli Muro [1], J. Callens [1], M. Nollet [1], F. Bahamonde [1], M. Englebienne [1], M. De Gussem [1]

[1] Poulpharm, Izegem, Belgium

Background
Encephalomyocarditis virus (EMCV) is an infrequent but impactful cause of sudden death and reproductive losses in swine. Rodents are the main reservoir, and outbreaks can lead to economic and welfare concerns. Diagnosis requires combining clinical, pathological, and molecular findings.

Materials & Methods
Three cases of sudden death in weaned and grower pigs from different farms were investigated. One farm reported clear signs of rodent infestation, while others had low or uncertain rodent activity. Recently deceased piglets were sent to a diagnostic laboratory. Standardized necropsies were performed, and heart tissue was sampled for histopathological examination using HE staining. In one case, myocardial tissue was analyzed for EMCV using a commercial real-time PCR kit performed in-house.

Results
Gross cardiac lesions included pale, soft, and slightly enlarged hearts. White spots were also observed in the myocardium. No relevant gross lesions were observed in other organs. Histology consistently showed myocardial necrosis, massive lymphocyte and macrophage infiltration, intracellular edema, myofiber disorganization, and calcium deposition. EMCV was confirmed by PCR in one case, with a Ct value of 23. Based on the clinical presentation of sudden death on the farm, gross and microscopic findings, and PCR confirmation, no further differential diagnosis was required.

Conclusion
These cases reinforce the importance of including EMCV in the differential diagnosis of sudden death in weaned and grower pigs, even when rodent exposure is not overt. A combined diagnostic approach of gross pathology, histology, and PCR proved essential for accurate identification and highlights the need for awareness and preventive measures.

455 | CORRELATION OF PENTRAXIN 3 AND INFLAMMATION IN LUNGS AND REGIONAL LYMPH NODES OF EQUIDS AND RUMINANTS

S. Vural [1], O. Demirtaş [1], K. Çetin Altun [2], O. Kutsal [1]

[1] Ankara University Faculty of Veterinary Medicine Department of Pathology, Ankara, Turkey
[2] Turkish Standards Institute, Ankara, Turkey

Background
Pentraxin 3 (PTX3) is a significant biomarker, particularly in inflammatory conditions such as respiratory tract infections. This study aimed to investigate the correlation between PTX3 expression and the severity of inflammation in paraffin-embedded lungs and mediastinal lymph nodes from equids and ruminants diagnosed with pneumonia, regardless of the etiological agent. Additionally, it searched to identify the cell types expressing PTX3.

Materials & Methods
Lungs and mediastinal lymph nodes were collected from equine (n: 20) and 20 cattle (n: 20), and controls (n:2, lung tissues from 1 horse and 1 cattle, which did not show any signs of pneumonia) fixed in 10% buffered formalin, embedded paraffin and examined histopathologically and immunohistochemically (ABC-P).

Results
Histopathological evaluation revealed pneumonias of varying types and severities. PTX3 positivity was observed in parallel with the severity of lesions. The positivity was evaluated as +1, +2, +3, semi-quantitatively. PTX3 positivity was found to be significantly higher in inflamed lungs compared to healthy ones. Cell types that showed PTX3 positivity did not differ among the species, or the type of pneumonia. Severe positivity of PTX3 was seen in bronchial/bronchiolar epithelial cells (cattle n:9, equidae n:8), exfoliated epithelial cells (cattle n:4, equidae n:6), alveolar macrophages (cattle n:3, equidae n:3), and exudate in the bronchi (cattle n:1, equidae n:2). Most severe positivities were seen in most severe cases such as broncho (n:5)/bronchointerstitial (n:3)/interstitial (n:9) pneumonia.

Conclusion
These findings highlight the need for further studies investigating PTX3 positivity in different systemic diseases, particularly those causing herd-level health problems.

457 | PERICARDITIS IN VACCINATED PIGS PROTECTED AGAINST AFRICAN SWINE FEVER VIRUS CHALLENGE

S. Vreman [1], N. Oreshkova [1], L. Peeters [1], N. Gerhards [1], K. Wiese [1], C. Dierickx [1], T. Ploegaert [1]

[1] Wageningen Bioveterinary Research, Wageningen University and Research, Lelystad, Netherlands

Background
Insights about the protective host responses after African swine fever virus (ASFV) infection are largely lacking. To address this question, pigs were vaccinated in two different studies with low doses of moderately virulent attenuated ASFV strains. A substantial number of vaccinated pigs (80%) survived ASFV challenge, however a pericarditis was observed in all survivors at necropsy two weeks post challenge. Here the pathogenesis of the observed pericarditis was studied.

Materials & Methods
From two studies (histo) pathology of immunized challenged survivors (IS, n=17), immunized challenged non-survivors (INS, n= 4), non-immunized challenged controls (positive control, n=4) and non-immunized and not challenged controls (negative control n=4) were compared with focus on heart tissue using HE stain and immunohistochemistry (e.g. CD3, CD21 and IBA-1). Immune related gene transcription analysis was performed on formalin fixed paraffine embedded heart tissue with the nCounter® platform.

Results
All IS pigs showed a pericarditis characterized by infiltrates of mainly round cells and no bacterial cause was identified. Other organs did not show macroscopic lesions at the end of the study. In contrast, ICNS showed clinical signs and ASFV related pathology during necropsy with occasionally acute myocardial hemorrhages, but no clear pericarditis. Gene analysis, histology and IHC of heart tissue indicated a prominent role for T-cells and plasma cells.

Conclusion
These preliminary findings suggest immune-mediated pericarditis after challenge of vaccinated pigs. Follow-up studies are needed to reveal the pathogenesis of the pericarditis and its relevance and association with the immune status of the vaccinated animals.

OTHERS INCL. AI

99 | BUILDING AN OPEN-SOURCE VETERINARY PATHOLOGY DATABASE POWERED BY LARGE LANGUAGE MODELS (LLMS)

A. Orlowska [1], L. Spica [1], L. Ressel [1]

[1] University of Liverpool, Liverpool, United Kingdom

Background
Veterinary pathology departments manage extensive archives often stored in diverse formats, creating challenges for efficient data retrieval. Migration to new software typically requires time-consuming, costly, and incomplete processes. We propose an open-source AI-powered database solution capable of extracting and structuring information from heterogeneous datasets.

Materials & Methods
Non-organized data (Word, PDF, Excel; >28K files) were collected. A Large Language Model (LLM) processed the data locally, extracting key information and structuring it into JSON format. LLM-generated vector embeddings were stored in a Chroma vector database. A custom graphical user interface (GUI) was developed, enabling semantic searches and AI-enhanced results through reranking and analysis. Features included an AI chat for conversational querying and built-in graphical analytics tools.

Results
The database handled a wide range of queries, from basic informational searches to complex, multi-faceted questions on pathological cases across species. The use of vector embeddings enabled accurate interpretation of nuanced medical terminology and context. Customizable GUIs were developed to tailor the system for specific reporting needs. The project will be available as open source.

Conclusion
This AI-enhanced database offers a powerful tool for veterinary pathology, automating information extraction and standardization from diverse sources. Hosting on a local server ensures privacy compliance. The system improves retrieval efficiency and enhances the usability of historical data repositories.

152 | ARTIFICIAL INTELLIGENCE APPLIED TO IMAGE ANALYSIS FOR THE PATHOLOGICAL EVALUATION OF INTERSTITIAL PNEUMONIA IN SMALL RUMINANT LENTIVIRUSES INFECTION

A. Gómez [1], I. Glaria [2], I. Moncayola [2], I. Echeverría [3], A. Rodríguez-Largo [4], L. Arriazu [5], A. Calero [6], E. Pérez [1], M. Pérez [1], A. De Diegp [7], L. Luján [1], R. Reina [2], R. De Miguel [8]

[1] Universidad de Zaragoza, Zaragoza, Spain
[2] Instituto de Agrobiotecnología (CSIC-Gobierno de Navarra), Mutilva, Spain
[3] Universidad Pública de Navarra, Pamplona, Spain
[4] Universitat Autònoma de Barcelona, Barcelona, Spain
[5] Laboratorio Agroalimentario de Navarra, Villava, Spain
[6] Recombina Biotech, Mutilva, Spain
[7] Centro de Investigación Biomédica de Aragón (CIBA), Zaragoza, Spain
[8] AnaPath Services GmbH, Liestal, Switzerland

Background
Small ruminant lentiviruses (SRLV) cause chronic interstitial pneumonia, characterized by thickening of the pulmonary septa, which develops years after infection. There are no vaccines available and their development require pathological evaluation in in vivo trials. However, the slow progression of interstitial pneumonia is a handicap for proper evaluation. Artificial intelligence (AI)-assisted image analysis has emerged as a key tool in assessing vaccine protection for infectious diseases and could be useful in SRLV studies.

Materials & Methods
This study evaluated the protective efficacy of a recombinant Sendai virus (SeV-GFP) expressing the SRLV *gag*-P25 protein (rSeV-GFP-P25) in 21 lambs divided into three groups (n = 7). Group 1 received three intranasal inoculations with cell culture medium only, while groups 2 and 3 were intranasally immunized with SeV-GFP and rSeV-GFP-p25, respectively. Sixteen weeks after the third immunization, lambs were intratracheally infected with homologous SRLV. After 25 weeks post-infection, lung lesions were evaluated through blind semiquantitative histopathological analysis and image analysis (QuPath 0.5.1). Using supervised machine learning, the software was trained to quantify the area occupied by alveolar septa and alveolar lumen. Results were expressed as percentage of pulmonary parenchyma (alveolar septa).

Results
Histopathological analysis showed no significant differences between groups due to the mild severity of lesions. However, image analysis revealed increased septal thickening in groups 1 ($p < 0.001$) and 2 ($p = 0.006$) compared to group 3, indicating that rSeV-GFP-P25 protected against SRLV interstitial pneumonia.

Conclusion
AI-based image analysis can improve pathologic assessment in vaccine trials for respiratory viruses.

287 | ANALYSIS OF TIGHT JUNCTION PROTEIN DYNAMICS BY IMMUNOHISTOCHEMISTRY IN PORCINE INTESTINAL EPITHELIAL CELLS (IPEC-J2) CELLS EXPOSED TO OSMOTIC STRESS

B. Passeri [1], P. Borghetti [1], M. Andrani [1], M. Di Pentima [1], A. Luppi [2], E. De Angelis [1], V. Cavalli [1], L. Ferrari [1], R. Saleri [1]

[1] University of Parma, Parma, Italy
[2] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Parma, Italy

Background
The intestinal epithelium is crucial for nutrient absorption, immune defense, and health. Constant osmotic stress challenges its barrier integrity, making osmotic balance essential. Epithelial cells adapt by regulating osmolyte transporters and tight junction proteins (TJp), ensuring permeability control and systemic stability in response to solute-specific changes.

Materials & Methods
The aim of this study was to investigate the regulation of tight junction proteins in intestinal epithelial cells exposed to osmotic changes, starting from two different conditions: an unstable monolayer simulating previous pathogen-induced damage and a stable monolayer mimicking a healthy state. IPEC-J2 cells from pre-colostral piglet jejunum were cultured in transwells and exposed to hyperosmotic environments using mannitol or sucrose (500 mOsm/L) for variable time (24 h and 4 days). The study evaluated changes in cell morphology, permeability of the monolayer (transepithelial electrical resistance, TEER), cell viability and TJp (Claudin-4, Occludin, ZO-1) localization.

Results
Data showed that mannitol-induced hyperosmolar exposure, regardless of the initial steady state, caused a decrease in cell viability and significant TEER reduction, starting from 24 hours, associated with a significant decrease in TJp levels. Conversely, sucrose-induced hyperosmolarity in both initial conditions preserved higher cell viability over time, increased TEER. Claudin-4, Occludin, and ZO-1 phenotypical expression was as well increased in the sucrose-induced hyperosmolar environment.

Conclusion
These results suggest that intestinal epithelial cells react differently depending on the osmotic agent and underline the crucial role of osmotic regulation in preserving the integrity of the epithelial barrier. Finally, sucrose was found to strengthen intestinal permeability.

346 | GALLERIA MELLONELLA AS AN ALTERNATIVE MODEL FOR ASSESSING PATHOGENICITY OF TOXIGENIC PENICILLIUM ROQUEFORTII ISOLATES FROM TRADITIONAL MOLDY CHEESES

N. Ertas Onmaz [1], K. Yagli [2], L. Çakir Bayram [2], G. Ekebas [2], S. Sugeçti [3], Y. Ozkaya [1]

[1] Erciyes University, Faculty of Veterinary, Food Hygiene and Veterinary Public He, Kayseri, Turkey
[2] Erciyes University, Faculty of Veterinary, Department of Pathology, Kayseri, Turkey
[3] Zonguldak Bülent Ecevit Universty, aycuma Vocational School of Food and Agricult, Zonguldak, Turkey

Background
Uncontrolled production of mold-ripened cheeses in Türkiye has led to the emergence of Penicillium roquefortii strains capable of producing potent mycotoxins such as roquefortine C and aflatoxin-like compounds. Assessing their pathogenic potential is crucial for public health and food safety.

Materials & Methods
Five toxigenic *P. roquefortii* isolates from naturally ripened cheeses in Türkiye were tested. *G. mellonella* larvae were injected with spore suspensions (10^6 spores/mL) and monitored for seven days. Larval hemolymph and tissues were collected for cytological (Cytospin Centrifuge) (PAS and Papanicolaou staining) and histopathological (Hematoxylin-Eosin and PAS staining) examinations. Molecular docking analysis was performed to assess the interaction between fungal toxins and host hormone-binding proteins.

Results
Infected larvae exhibited epithelial degeneration, fat body necrosis, hemocyte proliferation, and melanized nodule formation. Fungal hyphae and conidia were detected in tissues. Cytology revealed increased plasmatocyte and granulocyte phagocytosis, melanization, and fungal element presence in hemolymph. Molecular docking confirmed strong binding affinities between toxins and host proteins, supporting the in vivo findings.

Conclusion
The *Galleria mellonella* model effectively demonstrated the pathogenic effects of toxigenic P. roquefortii isolates. This integrated approach highlights its potential as a rapid and practical system for preliminary fungal pathogenicity assessment.

347 | RECONSTRUCTING THE CELL: A COMPARATIVE INSIGHT INTO CYTOLOGY AND CELL BLOCK METHOD IN VETERINARY EFFUSIONS

K. Yagli [1], G. Ekinci [2], G. Ekebas [1], M. Ay [2], V. Gunes [2], N. Ertas Onmaz [3], L. Çakir Bayram [1]

[1] Erciyes University, Faculty of Veterinary, Department of Pathology, Kayseri, Turkey
[2] Erciyes University, Faculty of Veterinary, Department of Internal Medicine, Kayseri, Turkey
[3] Erciyes University, Faculty of Veterinary, Food Hygiene and Veterinary Public He, Kayseri, Turkey

Background
Effusion cytology is a valuable diagnostic tool for identifying inflammatory processes in cats and dogs. Cytospin and cell block techniques enhance diagnostic sensitivity by preventing cell loss in serous and septic effusion samples. The cell block method, in particular, contributes significantly to diagnostic evaluation through increased cellularity and well-preserved cytomorphological details. Although widely used in human pathology, the cell block technique—which facilitates histological and immunohistochemical analyses as a form of micro-biopsy—remains underutilized in veterinary cytopathology.

Materials & Methods
Samples were collected from 12 small animals (8 cats, 4 dogs) diagnosed with pleural, peritoneal, or pericardial effusion. Each sample was processed in three ways:

1. Cytospin smears were prepared using a Cytospin 4 centrifuge and stained with Diff-Quick and Hematoxylin-Eosin.
2. Microbiological cultures and species identification were performed to detect potential pathogens.
3. Cell blocks were prepared using the Shandon Cytoblock Kit, and selected blocks were subjected to immunohistochemical staining.

Results
Cytospin smears allowed detailed observation of inflammatory cell morphology and facilitated early detection of bacteria in infected samples. Cell block sections preserved tissue architecture and enabled multiple immunohistochemical analyses from a single sample. Microbiological cultures yielded both Gram-positive cocci (*Staphylococcus aureus*) and Gram-negative bacilli (*Escherichia coli*). Cell block samples were successfully archived for future investigations.

Conclusion
The combined use of cytospin and cell block techniques improves diagnostic accuracy in veterinary effusion cytology. This approach enables safe histological and microbiological evaluation without the need for repeat sampling. Cell blocks function as micro-biopsies, offering long-term archiving and integrated diagnostic potential.

364 | PROTEOMIC INSIGHTS IN PATHOPHYSIOLOGIC AND BIOCHEMICAL MECHANISMS OF ADVANCED-PLATELET-RICH FIBRIN PLUS (A-PRF+)-INDUCED CELLULAR REGENERATION IN PRIMARY EQUINE FIBROBLASTS

D. De Biase [1], V. Vestuto [1], M. Miranda [1], V. Golino [1], G. Pepe [1], T. Ciaglia [1], A. Bertamino [1], P. Campiglia [1], E. Sommella [1], M. Pasolini [2], G. Piegari [2], O. Paciello [2]

[1] Department of Pharmacy, University of Salerno, Fisciano, Italy
[2] Department of Veterinary Medicine and Animal Production, University of Naples, Naples, Italy

Background
Wounds are common in equine practice and often lead to complications such as infections, delayed healing, and hypertrophic scarring. These complications are not always easily resolved and could harm the horse's health. Developing affordable and effective treatments has become an increasingly important focus in veterinary research. Equine A-PRF+ (advanced-platelet-rich fibrin plus) demonstrates regenerative properties comparable to its human counterpart, but cellular-level investigations exploring its molecular mechanisms remain limited. This study investigated the *in vitro*effects of equine A-PRF+ on primary fibroblast cell cultures.

Materials & Methods
Cellular proliferation, migration, metabolism and oxidative stress were evaluated in vitro on primary fibroblast cell cultures by immunofluorescence, PCR and ELISA. Moreover, proteomic analysis by nLC-HRMS/MS was performed to identify protein profiles involved in matrix remodelling, cell proliferation, and inflammation.

Results
Treatment with platelet concentrate resulted in increased cell proliferation, enhanced migration, and significant changes in cell cycle progression compared to control groups. Reactive oxygen species (ROS) production and organelles metabolism stimulation. Secretome analysis of A-PRF+ revealed a complex protein profile indicating active cellular responses and an increase in genes and proteins associated with cell proliferation and wound regeneration. Finally, proteomic analysis of treated fibroblasts confirmed the differential expression of key proteins associated with extracellular matrix dynamics and tissue regeneration processes.

Conclusion
This study provides new insights into the molecular mechanisms of A-PRF+, highlighting its potential to modulate equine fibroblast activity and promote tissue regeneration. These findings support further exploration of A-PRF+ in regenerative medicine applications.

391 | MOLECULAR AND CELLULAR CHARACTERIZATION OF EQUINE VANINS – PUTATIVE BIOMOLECULES IN EQUINE ASTHMA

K. Landmann [1], F. Bartenschlager [1], C. Schnabel [2], A. Zeyner [3], L. Mundhenk [1]

[1] Institute of Veterinary Pathology, Freie Universität Berlin, Berlin, Germany
[2] Emmy Noether Junior Research Group, Institute of Immunology, Leipzig University, Leipzig, Germany
[3] Institute of Agricultural and Nutritional Sciences, Martin Luther University, Halle, Germany

Background
Equine asthma (EA) is the most common chronic respiratory disease in horses, often associated with hypercrinia and mucus plugging. Recent proteomic data revealed an enrichment of Vanin-1 (VNN1) in the mucus of horses affected by severe EA. VNN1 is associated with chronic respiratory diseases in humans and encodes a pantetheinase, a branch of the Nitrilase superfamily, which can release cysteamine. Cysteamine as a pharmacological compound can prevent asthma development. However, Vanins in horses remain entirely uncharacterized.

Materials & Methods
Equine Vanin genes were identified *in silico* and analyzed for structural proteomic features using SOSUI, SignalP, PredGPI, and NetNGlyc. Tissue samples from various organs and airway regions of healthy horses were collected post-mortem for RT-qPCR (n = 5) and *in-situ hybridization* (n = 3) to analyze the tissue and cellular expression patterns. The cloned coding sequences were transfected into HEK293 cells to study the cellular transport of the proteins. Their post-translational glycosylation pattern was identified via Endo-H and PNGase-F treatment.

Results
In silico analysis identified three equine Vanin genes (eVNN1, eVNN2, eVNN3) clustered on chromosome 10, encoding canonical Nitrilase superfamily domains. All genes exhibited broad tissue expression, but distinct respiratory localization, with eVNN1 and eVNN2 in respiratory epithelium and eVNN3 in submucosal glands. The glycosylated eVNN1 and eVNN3 were secreted by the cells, while the glycosylated eVNN2 remained cell-associated.

Conclusion
The three equine Vanins seem to cover different functional niches in equine airways. This paves the way for future research into their putative role in EA.

398 | "GLOMERUL-AI": APPLYING CONVOLUTIONAL NEURAL NETWORKS IN CANINE GLOMERULAR DISEASES

V. Crocchianti [1], L. Aresu [2], L. Ressel [3]

[1] Service d'Anatomie Pathologique, VetAgro Sup, CampusVétérinaire, Marcy l'Etoile, France
[2] Department of Veterinary Sciences, University of Turin, Torino, Italy
[3] University of Liverpool, Liverpool, United Kingdom

Background
Distinguishing normal from pathological glomeruli and immune complex glomerulonephritis (ICGN) from non-ICGN remains challenging with standard histological stains. Convolutional neural networks (CNNs) offer a tool to enhance diagnostic precision. This study evaluates the application of CNNs in the histopathological assessment of canine glomerular diseases.

Materials & Methods
Haematoxylin and Eosin (HE) and Periodic Acid-Schiff (PAS) slides from normal kidneys (n=4), ICGN (n=64), non-ICGN (n=70), and amyloidosis (n=24) were obtained from the EVRPS database and classified using WSAVA gold standard criteria. Slides were scanned, and glomeruli were manually annotated by capturing tiles at 20× magnification for each slide used. Tiles number per category was balanced. These annotated glomerular regions served as ground truth for training a DenseNet201 convolutional neural network using a transfer learning approach. Two binary CNNs were trained: "Healthy vs Pathological" (ICGN + non-ICGN + Amyloidosis), and "ICGN vs non-ICGN", using both HE and PAS stains. Accuracy was assessed using 3- or 5-fold cross-validation. All slides originated from a single laboratory.

Results
The CNN distinguished healthy from pathological glomeruli with an average accuracy of 0.99 (SD 0.05 for HE, 0.08 for PAS) in 3-fold validation. For ICGN vs non-ICGN classification, accuracy was lower: 0.70 (SD 0.16) for HE and 0.75 (SD 0.12) for PAS in 5-fold validation.

Conclusion
The CNN showed high accuracy in detecting pathological glomeruli but performed moderately in distinguishing ICGN from non-ICGN. Expanding the dataset to external labs will follow this early-stage project. Tools such as Grad-CAM will help visualize the model's focus.

456 | MORPHOLOGY OF THE DIGESTIVE TRACT OF LARVAE OF GALLERIA MELLONELLA, AN INVERTEBRATE MODEL FOR INFECTIONS WITH ENTEROPATHOGENIC BACTERIA

M. Liebler-Tenorio [1], P.-A. Saenger [1], M. Fuchs [1]

[1] Friedrich-Loeffler-Institut, Jena, Germany

Background
Larvae of the Great Wax Moth, *Galleria mellonella,* are successfully used in systemic and oral experimental infections in particular with enteropathogenic bacteria. Melanization, response to touch, and microscopical monitoring of labelled bacteria predominate as read-out parameters. Here, the morphology of the larval digestive tract is described as basis for a more detailed assessment of host-pathogen interactions in intestinal infections.

Materials & Methods
Larvae of about 2 cm length were fixed in stretched position in Carnoy´s solution, enclosed in agarose, dissected along the body midline and embedded in paraffin. Paraffin sections were stained with HE, trichrome, PAS and Giemsa. In some larvae, the digestive tract was dissected, fixed in glutaraldehyde and embedded in araldite for transmission electron microscopy.

Results
The established embedding procedure allowed contiguous visualisation of all compartments of the digestive tract including foregut, midgut and hindgut. The stomadeal valve separated foregut from midgut, the proctodeal valve midgut from hindgut. Foregut and hindgut had cuticular epithelium with delicate spines in the foregut. Midgut was lined by columnar epithelial cells and goblet-like cells with large eosinophilic PAS-negative cytoplasmic inclusions. Ingesta was separated from epithelium by the peritrophic membrane. The variably thick epithelium formed occasionally villus-like protrusions. The epithelium cranial to the proctodeal valve was highly vacuolated.

Conclusion
Larvae of *Galleria mellonella* present an alternative to mammalian models to study bacterial or fungal pathogenicity and evaluate therapeutic interventions. Morphologic examination of the digestive tract allows further refinement of the model, thus reducing experiments with vertebrates and contributing to the 3R principle.

458 | VIRTUAL REALITY IN NECROPSY

S. Vural [1], O. Kutsal [1], E. Yarsan [2], S. Sevin [2], K. Çetin Altun [3], O. Demirtaş [1]

[1] Ankara University Faculty of Veterinary Medicine Department of Pathology, Ankara, Turkey
[2] Ankara University Faculty of Vet Medicine Dep of Pharmacology and Toxicology, Ankara, Turkey
[3] Turkish Standards Institute, Ankara, Turkey

Background
Virtual Reality (VR) is a creates a three-dimensional artificial environment, which has a wide use as technology continues to grow in various fields, including education.
In veterinary pathology, effective necropsy and proper sampling are crucial. We aimed to create a virtual environment to enable additional practice opportunities for undergraduate students, particularly when cadavers are limited. Students can perform necropsy, macroscopic examinations, and sampling virtually. This enables the students to see different species, and create a safe self-learning environment.

Materials & Methods
Models were created from fresh cadavers of 1 donkey, 1 horse and 1 cow. Using VR software, simulations were developed that to perform virtual necropsy with VR headsets and joysticks. Certain procedures that could not be fully simulated were supported with audio-visual instructional videos.

Results
The new VR simulation is started to be used for last-year students and international trials have started. The feedback from our students and colleagues have been positive, and it is planned to be used in the undergraduate necropsy classes the next term. Also, the feedbacks were taken to evaluate and discuss for future improvements.

Conclusion
This application of virtual reality to necropsy is one of the first of its kind globally, and first in our field in Türkiye. This project also serves as a model for other fields and may inspire future projects. The primary disadvantages include high cost and the inability to replicate sensory experiences. Also, the background work with the modelling, making videos and voice records to later combine took lots of effort and working hours.

SMALL ANIMALS OTHER THAN CANCER

82 | A RARE CASE OF FATAL CANINE DEMODICOSIS WITH ECTOPIC DISSEMINATION OF MITES INTO PERIPHERAL LYMPH NODES

A. Kim [1], J. Baek [1], N. Moon [1], B. Ku [1], K. Lee [1]

[1] Animal and Plant Quarantine Agency, Gimcheon, South Korea

Background
Demodex is a genus of tiny mites that normally live in or near hair follicles of mammals including dogs. It normally do es not evoke any clinical symptom, however, its abnormal overgrowth induces a disease called demodicosis. Among two forms of demodicosis, generalized form is more problematic than localized form, although it is usually confined to a skin disease. Herein, we introduce a rare case of fatal canine demodicosis with ectopic disseminat ion of mites into peripheral lymph nodes.

Materials & Methods
A carcass of mixed breed male dog was submitted for autopsy. Because it was a rescued dog, its age and clinical history were unknown. The rescuer initially suspected starvation as a cause of death. Pathological examination was done with dissection and gross observation of the carcass, followed by histopathological evaluation of formalin-fixed tissue of major organs and specific lesions.

Results
Grossly, severe multifocal skin lesions presenting scab formation with pyogenic exudate were noted. Peripheral lymph nodes were enlarged. Despite that the dog showed systemic wasting, gastric and intestinal contents were abundant. In addition to severe folliculitis by colonization of Demodex in the dermis, granulomatous lymphadenitis with fibrosis due to the dissemination of Demodex into the parenchyma of lymph nodes was remarked. A thrombus containing intralesional mites was also seen in the lumen of the subcapsular blood vessel.

Conclusion
Generalized demodicosis is usually not a life-threatening disease. However, if ectopic dissemination of the mites into the lymph nodes occurs, it can cause severe lymphadenitis, which can be fatal.

83 | CONGENITAL GALLBLADDER AGENESIS IN A DOG: AN INVISIBLE DRIVER OF HEPATIC AND PANCREATIC PATHOLOGY

M. Brys [1], K. Chiers [1]

[1] Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

Background
Gallbladder agenesis is an exceptionally rare congenital anomaly in dogs, typically identified only postmortem. While often asymptomatic, it may predispose to progressive hepatobiliary disease. In some cases, it is associated with ductal plate malformation, a developmental disorder of the intrahepatic bile ducts. This case describes a dog with acute hepatic failure, neurological signs, and pancreatitis in which gallbladder agenesis and associated hepatic anomalies were identified postmortem.

Materials & Methods
A 5-year-old Cocker Spaniel (10 kg) presented with diarrhoea, vomiting, lethargy, anaemia, abdominal effusion, melena, icterus, and abnormal neurological behaviour. Bloodwork revealed markedly elevated alkaline phosphatase (1936 IU/L), alanine transaminase (389 IU/L), ammonia (241 µmol/L), lactate (8.1 mmol/L), and bilirubin (124 µmol/L). Postmortem examination and histopathological evaluation of major organs were performed.

Results
Gross examination revealed macronodular hepatic cirrhosis with secondary icterus and acquired extrahepatic portosystemic shunts. The pancreas was diffusely swollen, and notably, the gallbladder was absent. Histologically, the liver showed hydropic degeneration of hepatocytes with cholestasis, fibrosis, and multifocal nodular regeneration. A ductal plate malformation was observed, confirmed by absent Ki-67 immunoreactivity. The pancreas exhibited acute peripheral necrosis.

Conclusion
This case highlights gallbladder agenesis as a rare congenital anomaly with significant clinical implications. The concurrent presence of ductal plate malformation, hepatic cirrhosis, and acquired extrahepatic portosystemic shunts suggests a congenital hepatobiliary defect progressing to multisystemic disease. These findings emphasise the importance of considering congenital abnormalities in dogs with unexplained hepatic and neurological signs, and contribute valuable insights to the limited literature on gallbladder agenesis in veterinary medicine.

108 | COINCIDENCE OR SUSPICION: TOXOPLASMA GONDII IN CASES OF FELINE NASAL DISEASE

S. Vieira de Carvalho [1, 2], C. Marques [3, 4], S. Sousa [3, 4], J. Costa [3, 4], R. Noiva [1, 2]

[1] CIISA, Interdisciplinary Centre of Research in Animal Health, Lisbon, Portugal
[2] Associated Laboratory AL4AnimalS, Lisbon, Portugal
[3] INSA, Instituto Nacional de Saúde Dr. Ricardo Jorge, Porto, Portugal
[4] CECA, Center for Study in Animal Science, Porto, Portugal

Background
B-cell lymphoma is the most common feline nasal cavity tumor while lymphoplasmacytic rhinitis represents a potentially equally destructive chronic lymphoid nasal disease, evolving in cycles of inflammation, tissue damage and repair.
Toxoplasmosis is a globally important zoonosis and several studies indicate that latent infection plays a role in the pathogenesis of diseases characterized by excessive inflammation in humans. A high prevalence of toxoplasmosis was observed in human patients with lymphoma and leukemia. We aim to investigate this possible connection in cats, since they act both as a definitive and intermediate host for Toxoplasma gondii.

Materials & Methods
Formalin-fixed paraffin-embedded biopsy tissues from 80 cases of chronic nasal disease (65 of lymphoplasmacytic rhinitis and 15 of lymphoma), diagnosed at the Pathology Laboratory of the Faculty of Veterinary Medicine, University of Lisbon between 2017 and 2023, were processed for conventional PCR and immunofluorescence to detect Toxoplasma DNA and antigens, respectively. All cases were tested by immunofluorescence, while all lymphoma samples and 24 randomly selected rhinitis samples were tested by PCR.

Results
Immunofluorescence yielded no clearly positive cases (10 were inconclusive and 70 negative), while PCR detected Toxoplasma DNA in 40% (6/15) of lymphomas and 8.3% (2/24) of rhinitis cases.

Conclusion
Our findings suggest that there is potential role for Toxoplasma in lymphoproliferative diseases, perhaps as a trigger of chronic lymphoid-mediated inflammatory disease or as a depressor of immunity.

116 | PATHOLOGICAL CHARACTERIZATION OF RENAL LESIONS IN FELINE CHRONIC KIDNEY DISEASE

M. Haralambie [1], A. Negoescu [1], O. Haralambie [1], H. Hodor [1], M. Bocoș-Bințințan [1]

[1] University of Agricultural Science and Veterinary Medicine, Cluj-Napoca, Romania

Background
This study aims to identify the main histopathological lesions in cats suspected of having CKD using special stains and classify them according to the affected area, type, frequency, and severity. We also aimed to identify foci of metastatic mineralisation in the aorta and whether these are associated with renal lesions.

Materials & Methods
For this study, we collected, following necropsy, kidneys (n=50) and thoracic aortic fragments from cats suspected of having CKD, other renal pathologies, and healthy cats, which we used as controls (n=2). The usual Hematoxylin and Eosin stain was used for histopathological examination, followed by three special stains: Trichrome Masson, Periodic Acid-Schiff (PAS), and Von Kossa stain. The parameters considered include the glomerular compartment, the interstitial compartment, the renal tubules, and the thoracic aorta lesions.

Results
The most important histological changes observed in the kidney were represented by glomerulosclerosis, thickening of Bowman's capsule, tubular mineralisation, Bowman's capsule mineralisation, interstitial fibrosis, and interstitial inflammation, which are irreversible changes. The predominant inflammatory infiltrate was lymphoplasmacytic. Two rare lesions were also identified: focal segmental glomerulosclerosis with synechiae formation and mineral deposits observed in the aorta, predominantly localised within the tunica media layer.

Conclusion
In conclusion, the histological lesions observed in the kidneys highlight the severe and irreversible nature of the condition. The glomeruli were the structures most affected by the kidney, followed by the interstitial space and the renal tubules. The von Kossa stain helped identify discrete areas of mineralisation in the aorta, which cannot usually be determined using only the usual Hematoxylin and Eosin stain.

149 | TEMPORAL CLUSTER OF FELINE TUBERCULOSIS IN THE UK CAUSED BY MYCOBACTERIUM CAPRAE, ASSOCIATED WITH RAW MEAT-BASED DIET CONSUMPTION

A. Schlachter [1], E. Bruno-McClung [1], C. Pritchard [1], J. Wheeler [1], I. Miteva [1], C. O'Halloran [2], R. Ellis [1], D. Jorge [1], D. Gunn-Moore [2], R. Rua-Domenech [1], A. Nunez [1]

[1] Animal and Plant Health Agency, Addlestone, United Kingdom
[2] Royal School of Veterinary Studies & Roslin Institute, University of Edinburgh, Edinburgh, United Kingdom

Background
Mycobacterium caprae, a member of the *Mycobacterium tuberculosis* complex, can cause tuberculosis (TB) in several mammalian species but is exotic to the UK. A temporal cluster of 16 suspected feline TB cases was identified in 2023-2024, in cats fed predominantly raw meat diets in England and Scotland. Nine were confirmed by bacteriological and molecular techniques to be caused by *M. caprae*. Five of these cases underwent pathologic analysis to characterise the disease.

Materials & Methods
15/16 cats fulfilled the case definition for feline *M. caprae* infection, based on history, clinical presentation, positive interferon gamma release assay testing and/or positive mycobacterial PCR or positive Ziehl-Neelsen staining of tissue aspirates or bronchoalveolar lavage. In seven cases, additional samples (7/7) and carcasses (5/7) were submitted for *M. caprae* specific PCR or culture and whole genome sequencing. Histopathology was performed on tissues collected at postmortem.

Results
Clinically, 14/15 cats had pulmonary disease, 8/15 cases had abdominal lesions; 13/15 were euthanised or died due to severity of disease. *M. caprae* was identified in 9/16 cases. In the five carcasses submitted to Animal and Plant Health Agency for pathology, necrotising and granulomatous lesions of varied severity and extension affected primarily the lymph nodes, lungs and liver.

Conclusion
The heterogeneous clinical picture seen highlights the variability in presentation of *M. caprae* infection in cats. While it remains difficult to characterise lesions without knowing the time of infection, the changes highlight particularities in the feline response to mycobacterial infections and provide evidence towards a speculative pathogenesis.

162 | FIRST FATAL HPAIV H5N1 (CLADE 2.3.4.4B) INFECTION IN A DOMESTIC CAT IN ITALY

D. D'Annunzio [1], M. Moreno [1], T. Tosi [1], B. Bassi [1], M. Muscatello [2], B. Bortolami [3], D. Drago [3], F. Fusaro [3], M. Monne [3], P. Palumbo [3], S. Savegnago [3], T. Terregino [3], M. Merialdi [1]

[1] Experimental Zooprophylactic Institute of Lombardia and Emilia-Romagna, Brescia, Italy
[2] Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Italy
[3] WOAH/EU and National Reference Laboratory for AI & ND, IZSVE, Legnaro, Italy

Background
The global distribution and evolutionary potential of H5Nx goose/Guangdong Eurasian lineage high pathogenicity avian influenza (HPAI) viruses pose a significant public health threat. The clade 2.3.4.4b caused the first HPAI panzootic and broadened its host range to numerous mammalian species. This study details the pathological findings from the first reported fatal high pathogenicity avian influenza virus (HPAIV) H5N1 infection in a domestic cat in Italy.

Materials & Methods
One cat from a backyard poultry farm experiencing an HPAI outbreak died shortly after the onset of respiratory signs. Tissue samples were collected for virus detection, genomic characterization and histopathological examination.

Results
In the affected cat, the main gross lesions included pulmonary edema and congestion, cerebral edema and hyperemia and scattered white-tan pinpoint lesions within the hepatic parenchyma. Histologically, non-suppurative meningoencephalitis, necrotizing bronchointerstitial pneumonia, and necrotizing hepatitis were observed. Lung and brain tested positive for H5N1 Clade 2.3.4.4b HPAI. Whole genome analysis classified the HPAI viruses from both the cat and birds within the EA-2024-DI genotype, the current genotype responsible for HPAI outbreaks in domestic birds in Italy, indicating that the cat was infected following exposure to infected poultry.

Conclusion
The fatal outcome in this cat underscores the pathogenic potential of H5N1 clade 2.3.4.4b in mammals. As demonstrated by the acquisition of host-specific adaptive mutations following spillover (here, one nonsynonymous mutation compared to the avian isolate, data not shown), these viruses pose an ongoing threat of host range expansion and increased pathogenicity, particularly regarding mammal-to-mammal transmission and potential zoonotic spillover events.

242 | NEUROPATHOLOGY OF AGEING IN DOGS

C. Lima [1], M. Pires [2, 3], L. Orge [4, 5], M. Pires [5]

[1] National Institute for Agricultural and Veterinary Research (INIAV), Vairão - Vila do Conde - Porto, Portugal
[2] Institute of Biomedical Sciences Abel Salazar of the University of Porto , Porto , Portugal
[3] Neuropathology Unit of University Hospital Center, Santo António, Porto, Portugal
[4] National Institute for Agricultural and Veterinary Research (INIAV), Oeiras - Lisboa, Portugal
[5] Animal and Veterinary Research Centre, University of Trás-os-Montes/Alto Douro, Vila Real, Portugal

Background
Advances in medicine and nutrition have provided an increase of dog's life span. The neuropathological changes associated with aging are described in various animal species, including the elderly dog which develops cognitive deficits and neuropathological changes similar to those seen in human aging and in patients suffering from Alzheimer disease, making these a good model to study the early stages of this condition.

Materials & Methods
Brain tissue of 70 dogs of different breeds, between 1 and 18 years old, were studied. The neuropathological study involved macroscopic examination and routine H&E staining. Immunohistochemical detection of β- amyloid protein, ubiquitin, astrocytic reactivity to glial fibrillary acid protein and immunoreactivity to tau protein was performed in the same cases. Additionally, an ultrastructural study was performed in 4 cases. Results were subjected to comparative analysis in accordance with the age group (1-7 years; 8 -12 years and 13 or more years), in accordance with dog's age relating to man's, depending on dog's weight (<65 and ≥ 65 years) and size (small, medium, large and giant).

Results
The main neuropathological age related-changes findings were: meningeal fibrosis (81%), ventricular enlargement (49%), vascular hyalinization (88,6%), cortical astrocytosis (80,6%), lipofuscin storage (86%), increased number of inclusions and ubiquitinated bodies (88,1%), vascular deposition of β-amyloid protein (46%) and amyloid plaques (31.4%). The ultrastructural study of four elderly dogs identified neurofibrillary tangles in neurons of temporal cortex.

Conclusion
The obtained results reinforce the theory that elderly dogs are a useful study model for Alzheimer disease and human aging.

244 | MICRORNA EXPRESSION PROFILING IN CANINE MYXOMATOUS MITRAL VALVE DISEASE HIGHLIGHTS POTENTIAL DIAGNOSTIC TOOL AND MOLECULAR PATHWAYS

E. Elvio [1], N. Santarelli [2], F. Valeri [1], D. Caivano [1], E. Lepri [1]

[1] University of Perugia, Perugia, Italy
[2] Clinique vétérinaire Univet Karukéra , Aix en Provence, France

Background
Myxomatous Mitral Valve Disease (MMVD) is the most common heart disease in dogs. While echocardiography is the gold standard for diagnosis, molecular biomarkers such as microRNAs (miRNAs) may support classification and prognosis. The aim of the study is to evaluate the expression of selected miRNAs in mitral valve tissues from dogs with MMVD at different disease grades.

Materials & Methods
Eight candidate miRNAs (let-7a, let-7b, let-7c, miR-21, miR-30b, miR-133b, miR-98, miR-103) were selected via KEGG and DIANA-mirPath bioinformatic tools. Expression was assessed by qPCR on FFPE samples collected during diagnostic necropsies from 15 dogs affected by MMVD, furtherly divided on the bases of severity of gross lesions into low-grade (8 cases) and high-grade (7 cases); 7 dogs dead for other diseases not involving the heart (intoxication or trauma) served as control cases. qPCR results were normalized to miR-29a. Gene target prediction (miRDB) and enrichment analysis (GO, PANTHER) were performed.

Results
All miRNAs, except miR-103 in low-grade samples, were upregulated in MMVD tissues compared to controls. Significant differences between low- and high-grade MMVD were observed for let-7a, miR-30b, and miR-103 ($p < 0.05$), and for let-7b, miR-21, and miR-133b ($p < 0.01$). Let-7c and miR-98 showed no significant differences between groups ($p > 0.05$). Enrichment analysis revealed associations with biological processes such as morphogenesis and cellular development, and with signaling pathways including PDGF, TGF-β, insulin/IGF, and angiogenesis ($p < 0.05$).

Conclusion
miRNA expression correlates with MMVD severity, highlighting diagnostic and prognostic potential. Their link to key pathways suggests a functional role in disease progression. Further validation is needed to suggest a possible diagnostic utility.

271 | PANOPHTHALMITIS WITH MULTINUCLEATED GIANT CELLS SECONDARY TO LEISHMANIA INFANTUM IN A CAT

E. Colombino [1, 2, 3], A. Oliver-Guimerá [1], E. Montero-Cortijo [1], D. Viana [1], A. Mayordomo-Febrer [2], M. López-Murcia [2], L. Mangas [3], J. Corpa [1], J. Ortega [1]

[1] Pathology Group, PASAPTA, Facultad Veterinaria, Universidad UCH-CEU, Alfara del Patriarca (Valencia), Spain
[2] Departamento Medicina-Cirugía Animal, Facultad Veterinaria, Universidad UCH-CEU, Alfara del Patriarca (Valencia), Spain
[3] Hospital Clínico Veterinario UCH-CEU, CEU Universities, Alfara del Patriarca (Valencia), Spain

Background
Leishmaniasis is caused by the protozoan *Leishmania infantum* and is an emerging disease in domestic cats. Ocular manifestations are rare compared to cutaneous or mucocutaneous forms. Thus, this report describes a case of panophthalmitis secondary to Leishmania infection in a cat.

Materials & Methods
An adult male stray European Shorthair cat was referred for unilateral enucleation due to severe buphthalmia, blepharoconjunctivitis, corneal perforation, and advanced uveitis. The cat tested negative for FIV, FeLV and toxoplasmosis but serology was positive for Leishmania. The enucleated eye, eyelid and surrounding periorbital tissues, were submitted for histopathological evaluation. Periodic acid-Schiff (PAS) and Grocott staining were performed to exclude fungal infection, while immunohistochemistry was used to detect Leishmania antigens.

Results
Histopathological analysis revealed severe inflammation affecting the cornea, iris, ciliary body, retina, anterior and posterior chambers, eyelid, and periorbital tissues. Interestingly, numerous giant multinucleated cells were observed associated with macrophages, occasional lymphocytes, and plasma cells. Many macrophages contained numerous 2–4 µm intracellular oval amastigotes with eccentric, round to ovoid basophilic nuclei and linear kinetoplasts (<1 µm). The inflammation led to both anterior and posterior synechiae. Retinal ganglion cell layer atrophy and retinal detachment were also observed. Additionally, there was fragmentation of lens fibres and focal corneal ulceration with full-thickness perforation. PAS and Grocott did not reveal fungal hyphae. Immunohistochemistry confirmed strong positivity for Leishmania antigens.

Conclusion
Based on the histopathological findings, a severe granulomatous panophthalmitis and dermatitis secondary to leishmaniasis was diagnosed. This represents a rare and atypical presentation of feline leishmaniasis.

321 | A CASE OF CANINE APOLIPOPROTEIN A-I AMYLOIDOSIS WITH SEVERE PULMONARY EMPHYSEMA

N. Sedghi Masoud [1], Y. Kawamura [2], K. Hamada [3], Y. Itoh [4], T. Murakami [1]

[1] Veterinary Toxicology Lab, Tokyo University of Agriculture and Technology, Tokyo, Japan
[2] YP Lab, Saitama, Japan
[3] KyotoAR Advanced Veterinary Medical Center, Kyoto, Japan
[4] Smart Core Facility Promotion Organization, Tokyo University of Agriculture and Technology, Tokyo, Japan

Background
Canine pulmonary amyloidosis is an age-related condition involving amyloid deposits derived from apolipoprotein A-I (ApoA-I) confined to pulmonary arteries and is usually asymptomatic. In this study, we report a case of canine ApoA-I amyloidosis with severe emphysema.

Materials & Methods
An eight-year-old neutered male mixed-breed dog presented with respiratory distress and died the same day. Thoracic radiographs, CT images and post-mortem examination revealed severe diffuse pulmonary emphysema. At necropsy, the lungs, oesophagus, and heart were collected, formalin fixed, paraffin-embedded, and subjected to histopathological analysis, immunohistochemistry, LC-MS/MS, and RT-PCR followed by Sanger sequencing.

Results
Histologically, marked dilatation of alveolar spaces and the destruction of alveolar walls were observed throughout the lung parenchyma. Severe amyloid deposition was found in the alveolar septa, pulmonary artery walls, and lamina propria of the oesophageal mucosa. LC-MS/MS identified ApoA-I as an amyloid protein in both the lung and oesophagus, with frequent detection of peptides from N-terminal region (~80 residues). IHC confirmed strong ApoA-I positivity on amyloid. RT-PCR and LC-MS/MS confirmed the absence of N-terminal mutations in ApoA-I.

Conclusion
This is the first reported case of severe pulmonary ApoA-I amyloidosis with esophageal involvement in a dog, suggesting possible systemic deposition. Unlike typical localised asymptomatic cases, this case showed symptomatic and possibly systemic disease. Given the low incidence (0.7%) in dogs under ten, factors beyond ageing may be involved. In humans, systemic ApoA-I amyloidosis may result from N- or C-terminal mutation of ApoA-I. Further analysis of the C-terminal region is required to detect any possible mutations.

326 | RETROSPECTIVE STUDY ON VIRUS-INDUCED ENCEPHALOMYELITIS IN DOGS BETWEEN 1962 AND 2022

I. Nägler [1, 2], A. Fayyad [1, 3], C. Puff [1], W. Baumgärtner [1, 2], P. Wohlsein [1]

[1] Department of Pathology, University of Veterinary Medicine Hannover, Hannover, Germany
[2] Center for Systems Neuroscience, University of Veterinary Medicine Hannover, Hannover, Germany
[3] Department of Veterinary Medicine, Faculty of Agriculture and Veterinary Medici, Nablus, Palestine

Background
Retrospective studies which cover a long time span and include large numbers of animals are of great value to obtain information about the temporal occurrence of diseases including host-pathogen interactions and transmission routes. This can contribute to a better understanding of the epidemiology of infectious agents.

Materials & Methods
The archived necropsy reports of all dogs examined at the Department of Pathology, University of Veterinary Medicine Hannover, between 1962 and 2022 were evaluated regarding the macroscopic and histologic findings in the central nervous system (CNS). Cases of non-suppurative inflammation were re-evaluated using haematoxylin and eosin-stained sections. Whenever a viral etiology was suspected, immunohistochemistry was performed for specific pathogens.

Results
During the investigated time period 20117 dogs were sent for necropsy, of which 2646 (13%) had neuropathological changes. In 776 cases, non-suppurative encephalitis and/or myelitis was found. 186 (24%) of these cases were linked to *Morbillivirus canis* (CDV) infections, an agent frequently detected until the early 2000s. *Varicellovirus suidalpha1* (SHV1) infection was diagnosed in 119 dogs (16%), mainly from the 1970s to the 1990s. Other infectious agents were detected sporadically. The remaining cases couldn´t be etiologically determined.

Conclusion
Distemper remains the most frequent cause of non-suppurative encephalitis in dogs. However, only sporadic cases were documented since 2005. After a transient high prevalence, SHV1 was rarely observed in the past 30 years. Summarized, this retrospective study shows the value of archived material for further pathogenetic investigations, although the number of unresolved cases demonstrates the challenges in etiologic clarification of non-suppurative encephalitis.

331 | MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERISATION OF SYNCYTIAL KNOT-LIKE LESIONS ASSOCIATED TO HYPOXIC CHANGES IN CANINE PLACENTA: A PROMISING TRANSLATIONAL PERSPECTIVE

C. Romanello [1], S. Dell'Aere [1], D. Groppetti [1], F. Riva [1], P. Roccabianca [1]

[1] University of Milan, Lodi, Italy

Background
Hypoxia's effects on the foetus are a significant concern and are linked to placental lesions and pregnancy-related disorders. Syncytial knot (SK), a large syncytium with transcriptionally inactive nuclei surrounded by proliferative tissue, is a marker of hypoxia in conditions like pre-eclampsia and intrauterine growth restriction in humans. This study is the first to describe the morphological and immunohistochemical characteristics of Syncytial knot-like (SKL) lesions in canine placentas.

Materials & Methods
In this study 7 canine placentas from caesarean sections were formalin-fixed, paraffin-embedded and stained with H&E. Immunohistochemistry targeting Ki-67 and RNA Polymerase II was performed and the results were analysed to assess proliferation and transcriptional activity, respectively.

Results
SKLs were described as dense nuclear aggregates with condensed chromatin, multifocally distributed within the labyrinth and surrounding necrotic areas in placentas related to neonatal death (3/7) and in one placenta from a dam affected by autoimmune disease (autoimmune polyarthritis), whereas were rare to absent in samples from healthy puppies (3/7). These nuclear aggregates showed no RNA Polymerase II expression, indicating transcriptional inactivity. Conversely, cytotrophoblasts adjacent to necrotic areas and to SKL aggregates exhibited strong Ki-67 expression, suggesting localised cell proliferation due to hypoxic conditions.

Conclusion
Our preliminary findings suggest that SKLs mirror human SKs in morphology and transcriptional inactivity and are associated with focal cytotrophoblast proliferation. Given the dog's naturally superficial placentation, resembling the shallow trophoblast invasion seen in conditions such as pre-eclampsia, these results suggest a potential role for the canine model in studying human placental pathologies.

385 | EHLERS-DANLOS-LIKE SYNDROME IN A YOUNG WHIPPET DOG

V. Castiglioni [1], S. Maffi [2], J. Van der Lugt [3]

[1] IDEXX Laboratories Italia S.r.l., Novara, Italy
[2] Clinica Veterinaria Dr. Sergio Maffi, Palazzolo sull'Oglio (BS), Italy
[3] IDEXX B.V., Netherlands

Background
Ehlers-Danlos syndrome (EDS) is a condition that encompasses many heritable, congenital defects of dermal connective tissue, leading to variable degrees of skin hyperextensibility and fragility. Described in several canine and feline breeds, its pathogenesis has not been fully elucidated, although multiple genetic defects involved either in collagen synthesis or in its post-translational modifications are suspected to occur. Here we describe EDS in a juvenile Whippet dog, a canine breed in which this disorder has not been reported so far.

Materials & Methods
A 3-month-old male Whippet was referred while suffering from cutaneous thinning, hyperextensibility, tearing and lacerations. The dog had joint hyperextensibility, abnormal gait, olecranon bursitis and bilateral corneal opacities. Clinical signs started developing at birth. His mother and siblings were asymptomatic. Cutaneous biopsies were histologically evaluated and genetic test for COL5A1 gene mutation was performed on a saliva swab.

Results
Histological evaluation revealed an abnormal and thin dermis. Collagen fibers were shortened, disarrayed, curled, uneven in size and width, with interstitial mucin. Abnormal collagen fibers had red cores, rather than blue with Masson's trichrome histochemical stain. The genetic test was negative for mutations of COL5A1 gene.

Conclusion
Clinical presentation, histopathological findings and histochemical stain were consistent with EDS in a canine breed in which this entity has not been reported so far. However, the only commercially available test did not confirm alteration in the COL5A1 gene. This supports the notion than other genes might be involved in the pathogenesis of this syndrome in this breed, warranting further studies.

386 | THE BUILDING OF A TRANSLATIONAL RESEARCH NETWORK FOR SYSTEMIC AMYLOIDOSIS IN ANIMALS: THE TERESA INITIATIVE

V. Moccia [1], C. Tucciarone [1], M. Milazzo [2], G. Martino [1], G. Westermark [3], M. Basset [4, 5], M. Mazza [6], C. Palizzotto [7], F. Ferri [1, 7], E. Zini [1, 7, 8], S. Ricagno [2], S. Ferro [1]

[1] Università degli Studi di Padova, Padova, Italy
[2] University of Milan, Milano, Italy
[3] Uppsala University, Uppsala, Sweden
[4] University of Pavia, Pavia, Italy
[5] Amyloidosis Research and Treatment Center, IRCCS, Policlinico San Matteo, Pavia, Italy
[6] Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy
[7] AniCura, Granozzo con Monticello, Italy
[8] Clinic for Small Animal Internal Medicine, Vetsuisse, University of Zurich, Zurich, Switzerland

Background
Preliminary investigations started in 2015 revealed an unexpectedly high prevalence of systemic AA amyloidosis in shelter cats in Italy. This new scenario highlighted the need for further research to gain a broader perspective on a previously underestimated disease, now emerging in a new natural animal model with significant implications for both veterinary and translational medicine.

Materials & Methods
multidisciplinary group of researchers initiated the formation of a collaborative network. Multiple in-person and virtual meetings were held to recruit members, define the objectives and outline future directions of the team to further study AA amyloidosis in animals.

Results
In 2024, the Translational Research Network for Systemic Amyloidosis named TeRESA was founded. TeRESA aims to advance and disseminate knowledge on AA amyloidosis in animals, promoting interdisciplinary collaboration, educational outreach, and enabling the integration of new collaborators who may contribute to advancing the network's research goals. Since its foundation, TeRESA members have published one scientific review, delivered one oral and two poster presentations at national and international congresses, organized a national conference and launched its official website. Further, one monthly meeting is scheduled to discuss the integrated research lines of TeRESA, currently including the monitoring of the prevalence of AA amyloidosis in cat shelters, the identification of novel diagnostic biomarkers, and structural and genetic investigations to elucidate disease pathogenesis.

Conclusion
International scientific networks like TeRESA offer valuable frameworks for the creation of multidisciplinary teams focused on the comprehensive study of diseases in animals, aligned with the One-Health approach.

396 | IMMUNOHISTOCHEMICAL CHARACTERIZATION OF THE INFLAMMATORY INFILTRATE DURING MYCOBACTERIUM AVIUM DISEASE IN THE DOG.

E. Elvio [1], E. Scorsi [1], A. Reginato [2], C. Eleni [3], A. Caprioli [3], F. Mira [4], M. Orlandi [5], M. Marenzoni [1], C. Tocchi [6], I. Porcellato [1]

[1] University of Perugia, Perugia, Italy
[2] CDVet research, Roma, Italy
[3] Istituto Zooprofilattico Sperimentale del Lazio e della Toscana “M. Aleandri”, Roma, Italy
[4] Istituto Zooprofilattico Sperimentale della Sicilia “A. Mirri”, Palermo, Italy
[5] Veterinary Laboratory "MyLav" , Passirana di Rho, Italy
[6] Private Practitioner - Veterinary Clinic “Etiopia“ , Roma, Italy

Background
Canine mycobacteriosis due to *Mycobacterium avium* complex (MAC) infections are rarely reported, but considered an emerging disease mostly for immunocompromised animals. The purpose of this study is to investigate immunophenotype of inflammatory cells in canine MAC disease.

Materials & Methods
Five dogs were necropsied after the diagnosis of MAC disease obtained by cytology or histology of incisional biopsies, confirmed by Fite-Faraco staining and specific PCR. Representative samples were submitted to immunohistochemical investigations using anti-CD3, -CD20, -Iba1, -MAC387, -CD163, -CD204 and -CD206 antibodies. For each case, neutrophilic infiltration, bacterial load, and extent of necrosis were also assessed.

Results
Histologically, granulomas were composed by macrophages with distinct morphology and immunohistochemical features: IBA-1, a pan-macrophage marker, was diffusely positive in all cases; MAC387 (M1 macrophage marker) showed weak immunoreactivity in 4 cases while it was higher in one case associated with widespread necrosis. CD163 and CD204 (targeting M2 macrophages) showed diffuse, moderate to strong, expression in all the cases. Finally, CD206, the reference marker for M2 macrophages, showed a diffuse but less intense positivity compared with the previous M2-oriented markers in 4/5 cases, and was negative in 1.

Conclusion
The immunophenotype of inflammatory cells during canine MAC disease is consistent with Th1 cellular response with mostly M2-oriented macrophages. These results are similar to findings in bovine paratuberculosis, in which M2-polarization seems to induce bacterial persistence and disease progression; however, in vivo studies show that the balance between M1- and M2-polarized macrophages is dynamic and may change during the course of the disease.

417 | HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL INVESTIGATIONS ON THE PRESENCE OF MAST CELLS IN FELINE LYMPH NODES

A. Barfuss [1], F. Seehusen [1], M. Nolff [2]

[1] Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
[2] Clinic for Small Animal Surgery, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

Background
The biological behavior of feline mast cell tumors (MCTs) less well understood than in dogs, and there are currently no standardized criteria for assessing mast cell metastasis in feline lymph nodes. This study aimed to evaluate whether the canine lymph node classification system developed by Weishaar et al. is applicable to cats.

Materials & Methods
Giemsa-stained lymph node sections from cats with inflammation (n=12), degenerative/hyperplastic changes (n=23), neoplasia (n=13), and MCT metastases (n=5; defined as concurrent MCT and nodular mast cell aggregates in nodes) were examined and compared to controls (n=8). Mast cells were counted per mm² using image analysis software on scanned histological slides. Their distribution across nodal compartments — sinuses, cortex, paracortex, and medullary cords — was recorded. Mast cells were assessed as single cells or clusters (>3 cells). CD117 (c-KIT) immunohistochemistry was used to support mast cell identification.

Results
Mast cell counts varied between different types of lymph node lesions. High mast cell densities were observed not only in mast cell tumor cases but also in controls and those from cats with other tumors, suggesting a possible association between neoplasia and mast cell presence. CD117 staining generally revealed fewer mast cells than Giemsa staining.

Conclusion
The classification system by Weishaar et al for canine nodal MCT metastasis cannot be directly applied to cats. Due to differences in baseline mast cell distribution and potential neoplastic influences, feline-specific criteria are needed to avoid misclassification of mast cell presence in feline lymph node tissue.

428 | LATE ONSET NEURONAL CEROID LIPOFUSCINOSIS IN A KORAT CAT

G. Vadori [1], S. Ferraresso [1], V. Vadori [2], E. Grisan [2], M. Patruno [1], L. Melotti [1], S. Ferro [1], A. Valentini [1], M. Bernardini [1], G. Brocca [3], M. Castagnaro [1]

[1] University of Padua, Legnaro, Italy
[2] London South Bank University, London, United Kingdom
[3] University of Prince Edward Island, Charlottetown, Canada

Background
Lysosomal Storage Diseases, a large group of inherited or acquired disorders caused by defects in lysosomal metabolism affecting both mammals and birds, are mostly caused by mutations in the genes encoding lysosomal acid hydrolases.

We report here a suspected novel adult-onset form of Neuronal Ceroid Lipofuscinosis (NCL) in a Korat cat.

Materials & Methods
A 4.5-year-old female Korat cat was referred to the Veterinary Teaching Hospital of Padua University for progressive neurological signs (marked ataxia and intermittent tremors) and underwent a complete neurological examination, brain MRI and electromyography (EMG).

After euthanasia, due to clinical deterioration, pedigree evaluation, gross, histological, ultrastructural, genetic and deep learning analysis (DLA) were performed.

Results
Pedigree analysis indicated high inbreeding and a likely autosomal recessive trait. Post-mortem examination revealed grossly acute enteritis and mild splenomegaly and hepatomegaly. Brain MRI showed mild cerebellar atrophy whereas EMG indicated no muscle pathology. CNS histopathology showed widespread intraneuronal vacuolation containing autofluorescent, PAS- and Ziehl-Neelsen-positive material. DLA revealed a marked reduction in cerebellar neuronal density. TEM analysis of neurons showed enlarged lysosomes, lipofuscin accumulation, and lamellar bodies, amorphous densities, and granular osmophilic deposits inclusions, consistent with NCL. Whole Genome Sequencing of case's and maternal blood samples DNA identified 1,100 events of non-synonymous loss of heterozygosity within exonic areas. Functional analysis related to lysosomal activity narrowed down the potential genetic cause to the Progranulin gene.

Conclusion
The analyses completed thus far suggest the presence of a novel disease, not yet described in cats, consistent with an adult form of NCL11.

433 | A CASE OF INTRAHEPATIC SPLENOSIS IN A DOG WITH CLINICAL PROGRESSION AND RELAPSING HAEMOABDOMEN

S. Gasparini [1], N. Pinto da Cunha [2], J. Martins [3], V. Baldassarre [2]

[1] Clinica Veterinaria e Laboratorio San Marco, Veggiano, Italy
[2] Vedis laboratory, Porto, Portugal
[3] AniCura Atlântico Hospital Veterinário, Mafra, Portugal

Background
Splenosis is a condition rarely reported in dogs and may be encountered as incidental finding at necropsy or during diagnostic imaging. It is the result of implant of splenic tissue onto serosal surfaces or other abdominal organs, such as liver, following splenectomy or splenic rupture. This report describes a case of intrahepatic splenosis with clinical progression and relapsing hemoabdomen, caused by increased size of ectopic splenic tissue and haemorrages.

Materials & Methods
A 9-years-old female neutered German shorthaired pointer dog previously splenectomised in 2021, with an histologic diagnosis of a benign lesions, was referred because of abdominal distension and vomiting in March 2024. CT scan revealed multiple hepatic lesions and hepatic cytology and histology were performed. In October 2024 the dog was referred for haemoabdomen, and CT scan showed again multiple liver masses. One of those was removed and analysed on histology. In April 2025 the dog is still alive, despite recurrent episodes of haemoabdomen.

Results
Cytology of the liver showed the presence of extramedullary haematopoiesis. In both cases histology revealed the presence of variably sized hematic lacunae lined by flattened endothelium and associated with haemorrhages, hematopoietic precursors and rare small lymphoid aggregates. These lesions were interpreted as ectopic splenic tissue. Splenosis within the mesentery was also observed.

Conclusion
Splenosis should be kept in mind as part of differential diagnosis of liver masses in patients with a history of splenic trauma or splenectomy**,** and it can cause recurrent haemoabdomen**.** Splenosis should be also included in the differential diagnoses for cytologic findings of hepatic extramedullary hemopoiesis.

453 | ASTROGLIAL DYSFUNCTION, DEMYELINATION, AND NODULAR INFLAMMATION IN NECROTIZING MENINGOENCEPHALITIS (NME)

M. Church [1], S. Hernandez [1], C. Assenmacher [1], J. Alvarez [1]

[1] University of Pennsylvania, Philadelphia, United States

Background
Necrotizing Meningoencephalitis (NME), a form of Meningoencephalitis of Unknown Origin (MUO), is a progressive neuroinflammatory disease. Due to limited understanding of its pathophysiology, definitive ante-mortem diagnosis is unfeasible. Dogs with NME are grouped under the broader MUO category, and treated with similar therapeutics as other MUOs. Our long-term objective is to identify distinct disease mechanisms within each MUO subtype to improve diagnostic accuracy, therapeutic approaches, and prognostic outcomes. We therefore analyzed inflammatory patterns as they relate to neuropathologic changes in NME and Granulomatous Meningoencephalomyelitis (GME), including: immune cell infiltration, demyelination, and glial characteristics.

Materials & Methods
Formalin-fixed paraffin embedded (FFPE) blocks from the cerebrum of NME (*n* = 11), GME (*n* = 11), and control (*n* = 5) cases were stained for immune, myelin, and glial markers. Grading schemes for immune and glial cell involvement were developed. Myelin stained slides were scanned and analyzed to assess demyelination.

Results
In the leptomeninges, NME was characterized by mild immune cell infiltration, in contrast to prominent, B cell-rich aggregates in GME. In the neuroparenchyma, both diseases exhibited lymphocyte infiltration; however, demyelination was more pronounced in NME, particularly within the subcortical white matter. Notably, areas of the brain affected by NME display a reduction in expression of aquaporin-4 (AQP4). Additionally, we found that AQP4 expression levels correlate with the extent of microglial and macrophage activation.

Conclusion
These findings suggest that astrocyte dysfunction in regions of microglial inflammation is a driver of NME and that adaptive immune responses likely play a supportive role.

467 | NEU5GC EXPRESSION IN CANINE INTESTINAL BIOPSIES AND ITS CORRELATION WITH COLITIS SEVERITY, MICROBIOMA AND CELL APOPTOSIS VIA LYSOSOMAL PATHWAY ACTIVATION

G. Rossi [1], D. De Bellis [1], S. Ricci [2], C. Carnevali [3], G. Baldan [1], A. Gavazza [1], M. Muollo [1], L. Biagini [1]

[1] School of Biosciences and Veterinary Medicine, University of Camerino, Matelica, Italy
[2] Christian Doppler Laboratory for Innovative Gut Health Concepts of Livestock, Vienna, Austria
[3] Italian National Agency for New Technologies, Energy and Sustainable Development, Casaccia Research Center, Rome, Italy

Background
Sialic acids are key components of cell membranes glycocalyx. The evolutionary loss of the cytidine-5'-monophospho-N acetylneuraminic acid hydroxylase (CMAH) gene prevents the conversion of sialic acid Neu5Ac, present in all mammals, into Neu5Gc. As a result, antibodies to Neu5Gc-glycans can develop. Prolonged uptake of Neu5Gc by CMAH- dogs from dietary sources of CMAH+ mammals can lead to its incorporation into tissues (xenosialization) promoting inflammation (xenosialitis) and lysosomal accumulation, triggering apoptosis and enteric barrier dysfunction. Objective is to investigate the relationship between Neu5Gc incorporation, gut microbiota composition, and intestinal inflammation in CMAH-negative dogs.

Materials & Methods
Biopsies from 105 dogs with clinical history and endoscopic findings consistent with inflammatory bowel disease (IBD) were analysed via immunohistochemistry (IHC) to detect Neu5Gc expression (Creative Diagnostic, DMABH-C003). Expression intensity was scored digitally and correlated with colitis severity. Apoptosis was measured by TUNEL method and lysosomes quantified by IHC (LAMP2, ThermoFischer, PA1-655). Fecal microbiota from 294 dogs (healthy and enteropathic) was analyzed. Distribution of desialising bacteria was performed using two sequencing techniques for different regions of the 16S rRNA gene.

Results
Neu5Gc expression was significantly increased in the colonic mucosa of enteropathic dogs ($p<0.005$), alongside a higher prevalence of Clostridiales and Bacteroidales ($p<0.001$). These dogs also exhibited increased apoptosis and LAMP2 expression.

Conclusion
Dysbiosis may promote Neu5Gc incorporation and xenosialization in CMAH- dogs, along with increasing apoptosis and barrier dysfunction. Increased Neu5GC may lead to greater uptake of xeno-sialo-antigens by enteropathic dogs since *Bifidobacteria*, known for their cross-feeding of sialic acids activity, did not differ between healthy and enteropathic dogs.

CANCER

68 | GOSSYPIBOMA-DERIVED LETHAL SARCOMA IN A DOG

A. Rigillo [1, 2], G. Giulia [2] M. Gambini [1], R. Magnoni [2], C. Perri [1], S. Remoli [1]

[1] I-Vet Diagnostics srl , Cellatica, Italy
[2] Clinica Veterinaria "I Ronchi" , Gallarate, Italy

Background
The term "Gossypiboma" refers to the granulomatous reaction induced by a retained surgical sponge. Animals and people with this condition might be asymptomatic for years, and neoplastic evolution is reported.

Materials & Methods
A 5 year-old Cavalier King Charles Spaniel was referred to a veterinary hospital for a suspected abdominal mass. Computed tomography confirmed the presence of a mass between pancreas and stomach. Cytological smears of the mass revealed non-atypical round cells with multinucleated giant cells, favoring a diagnosis of granuloma. At surgery, a 10 cm surgical sponge was macroscopically recognizable.

Results
The mass was difficult to trim being mainly composed by cotton tissue associated with mineralized areas. At histology, sheets of non-atypical round cells and many multinucleated giant cells surrounding birefringent elongated foreign material were present. Osseous trabeculae and scant mitoses were also observed, and a diagnosis of gossypiboma with suspected areas of neoplastic evolution was provided. After three months without follow-up due to owner's will, the dog returned with hemoabdomen and was humanely euthanized during surgery due to multiple masses involving all the abdominal organs. Histology of these masses was consistent with a neoplasm composed by more atypical cells embedded within scant amorphous eosinophilic matrix, and with numerous mitotic figures. At immunohistochemistry neoplastic cells were negative to Iba-1 and Vimentine-positive, supporting a final diagnostic suspicion of extraskeletal osteosarcoma.

Conclusion
Gossypiboma-related tumors, mainly osteosarcoma, are poorly reported in veterinary medicine, but still an ongoing malpractice problem to take into account in a routine diagnostic setting.
Definitive diagnosis as osteosarcoma would have to include marker analyses such as Runx2, SATB2 or ALP, which was not yet performed in this case.

84 | PLATELET-DERIVED GROWTH FACTOR RECEPTOR BETA EXPRESSION IN PRIMARY AND METASTATIC CANINE ANAL SAC ADENOCARCINOMA AS A POTENTIAL THERAPEUTIC TARGET

A. Verdi [1], S. Dell'Aere [1], C. Zamboni [1], D. Stefanello [1], R. Ferrari [1], E. Gariboldi [1], L. Maniscalco [2], S. Iussich [2], C. Romanello [1], V. Grieco [1], C. Giudice [1], C. Recordati [1], P. Roccabianca [1]

[1] University of Milan, Lodi, Italy
[2] University of Turin, Grugliasco, Italy

Background
Apocrine gland anal sac adenocarcinoma (AGASAC) is a malignant and invasive tumour frequently developing regional nodal metastases prior to diagnosis. Apart from surgical excision, therapeutic options are limited. Platelet-derived growth factor receptor beta (PDGFRβ) is a tyrosine kinase receptor involved in tumour growth, invasion, metastasis and angiogenesis. Although tyrosine kinase inhibitors (TKIs) have shown promising efficacy for AGASAC treatment, studies on their PDGFRβ expression are limited. This study aims to evaluate PDGFRβ expression in canine primary AGASACs from 5 different histotypes and in their regional nodal metastasis to provide evidence supporting the therapeutic use of TKIs for AGASACs.

Materials & Methods
Samples from primary AGASACs and, when present, of their nodal metastases were morphologically assessed and classified as solid, tubular/rosette, neuroendocrine or mixed histotypes. Immunohistochemistry on tumours and metastases was performed with a polyclonal anti-Human PDGFRβ antibody made in rabbit cross-reactive with dog. PDGFRβ expression and intensity were scored semi-quantitatively.

Results
Fifty-one AGASACs and 33 nodal metastases were included. Of these, 42/51 AGASACs (82%) expressed PDGFRβ (range: 5-100% of neoplastic cells), and 25/42 had ≥50% of positive cells. Positivity by tumor histotype was: 18/26 mixed-solid, 10/11 solid, 9/9 mixed-tubular/rosette, 4/4 tubular and 1/1 neuroendocrine. PDGFRβ was expressed in 29/33 nodal metastasis with reduced (15/29), equal (12/29) or increased (2/29) expression compared to that of the corresponding primary tumour. All four negative metastases corresponded to PDGFRβ-negative primary tumours.

Conclusion
PDGFRβ was commonly expressed by AGASACs and their metastases further supporting PDGFRβ-targeting TKIs as adjuvant therapies in selected canine AGASACs.

100 | TUMOUR-ASSOCIATED MACROPHAGES IN CANINE SOFT TISSUE SARCOMA

G. Avallone [1], E. Brigandì [1], A. Rigillo [2], B. Bacci [1], P. Roccabianca [3]

[1] DIMEVET-Università di Bologna, Ozzano dell'Emilia, Italy
[2] I-Vet, Flero (BS), Italy
[3] University of Milano, Lodi, Italy

Background
Canine soft tissue sarcomas (STS) are a heterogeneous group of neoplasms often treated as a single biological entity. However, recent studies suggest that behaviour and tumor immune microenvironment (TIME) may vary according to STS histotype. This study aims to characterize and quantify tumor-associated macrophages (TAMs) in canine STS.

Materials & Methods
Eighty-five STS were included based on availability of FFPE tissue and confirmed histological/immunohistochemical diagnosis: 18 fibrosarcomas, 13 leiomyosarcomas, 17 liposarcomas, 15 myxosarcomas, and 22 perivascular wall tumours (PWTs). Histological grade, Iba-1 (generic macrophage marker) and CD204 (M2 macrophage marker) expressions were assessed. Digital image analysis quantified positivity as Iba-1 and CD204 scores (positive area/total area), and Iba-1/CD204 ratio in the entire tumor section. Statistical analysis was performed.

Results
Iba-1, CD204, and Iba-1/CD204 scores varied significantly across histotypes. Iba-1 expression was higher in PWTs than in myxosarcomas; CD204 expression was lower and the Iba-1/CD204 ratio higher in PWTs than in myxosarcomas, leiomyosarcomas, and fibrosarcomas. No significant association was found between TAMs scores and tumor grade overall. However, in myxosarcomas, higher Iba-1 and CD204 scores correlated with higher grade; a similar but non-statistically significant trend was noted in PWTs.

Conclusion
TAMs varied across STS histotypes. CD204+ TAMs, often linked to poor prognosis, were less frequent in PWTs and more abundant in higher-grade myxosarcomas. These findings align with previous reports on tumor-infiltrating lymphocytes, suggesting a more favourable TIME in PWTs compared to other STS types.

139 | TRANSCRIPTOMIC CHANGES OF CANINE HISTIOCYTIC SARCOMA CELLS PERSISTENTLY INFECTED WITH CANINE DISTEMPER VIRUS MAINTAINED UNDER DIFFERENT CONDITIONS

T. Asawapattanakul [1, 2], K. Schughart [3, 4], M. Von Köckritz-Blickwede [1, 5], F. Armando [6], W. Baumgärtner [1, 2], C. Puff [1]

[1] University of Veterinary Medicine Hannover, Hannover, Germany
[2] Center for Systems Neuroscience, Hannover, Germany
[3] University of Münster, Münster, Germany
[4] Department of Microbiology, Immunology and Biochemistry, University of Tennessee, Memphis, Tennessee, United States
[5] Research Center for Emerging Infections and Zoonoses (RIZ), Hannover, Germany
[6] University of Parma, Parma, Italy

Background
Canine distemper virus (Onderstepoort strain; CDVOnd) has potential oncolytic properties. Therefore, understanding the virus-induced transcriptomic changes of neoplastic cells can reveal mechanisms of tumor metabolism within different zones of a solid neoplasm after oncolytic virotherapy. This study comparatively evaluated the gene expression in CDVOnd-infected canine histiocytic sarcoma cells (DH82) cultivated under hypoxia and starvation for different time frames to simulate various tumor zones.

Materials & Methods
Persistently CDVOnd-infected DH82 cells were cultured under 1% hypoxia and starvation, harvested on day 1 (short-term) and day 3 (prolonged exposure) and compared to standard conditions (controls). RNA sequencing was performed with data processing using FastQC, STAR alignment, and DESeq2 for differentially expressed gene (DEG) analysis. Functional enrichment was assessed via Gene Ontology.

Results
CDVOnd-infected DH82 cells cultured under hypoxia and starvation showed altered DEGs compared to controls at all investigated time points. Infected DH82 cells cultured under hypoxic conditions mainly showed downregulation of immune-related genes, while upregulated genes were related to development and angiogenesis in short-term exposure, and to cell cycle and apoptosis in prolonged exposure. Conversely, short-term starvation activated genes associated with the immune system and chemotaxis, but suppressed genes linked to development, particularly circulatory system, and proliferation. Prolonged starvation upregulated genes associated with the cell cycle while downregulated genes were linked to development and homeostasis.

Conclusion
Hypoxia and starvation, mimicking central zones of a solid tumor, lead to dynamic changes of gene expression in CDVOnd-infected DH82 cells. This phenotype might affect tumor behavior and thereby represent new therapeutic targets.

157 | TRANSITIONAL MENINGIOMA IN A FREE-RANGING MANTLED HOWLER MONKEY (ALOUATTA PALLIATA) FROM COSTA RICA

M. Romero-Vega [1, 2], K. Gregor [1], I. Hagnauer [3], A. Alfaro-Alarcón [2, 4], A. Beineke [1]

[1] Department of Pathology, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany
[2] Department of Pathology, School of Veterinary Medicine, National University, Heredia, Costa Rica
[3] Rescate Wildlife Rescue Center, Fundacion Restauracion de la Naturaleza, Alajuela, Costa Rica
[4] Institute of Virology, Charité-Universitätsmedizin Berlin, Berlin, Germany

Background
Spontaneous central nervous system tumors are rare in captive non-human primates and exceedingly uncommon in free-ranging individuals, with only a few cases documented. Meningiomas has been reported in non-human primate research colonies, but remains poorly characterized in wild populations.

Materials & Methods
An adult, non-gravid, free-ranging female mantled howler monkey (*Alouatta palliata*) was found recumbent in a fragmented forest patch in Tambor, Puntarenas, Costa Rica. The animal was transferred to a wildlife rescue center, where it was unresponsive to external stimuli and exhibited severe vulvar and perianal myiasis. Euthanasia was performed, followed by a full post-mortem examination. Necropsy revealed poor body condition and four firm, expansile, granular masses (0.5–1 cm in diameter) originating from the dura mater on the inner surface of the temporal bone, causing compression atrophy of the adjacent temporal lobe.

Results
Histologically, the masses were well-circumscribed, encapsulated, and expansile, exhibiting a characteristic whorled pattern supported by a prominent fibrovascular stroma. Neoplastic cells were elongated, with eosinophilic cytoplasm and centrally located nuclei containing condensed chromatin. Mild anisocytosis and anisokaryosis were observed, and occasional psammoma bodies were present. Immunohistochemically, the tumor cells expressed vimentin, pancytokeratin, E-cadherin, and S100.

Conclusion
Based on the histological features and immunohistochemical profile (S100 positive), the neoplasm was diagnosed as a transitional meningioma. This case contributes to the limited knowledge of neoplasia in wild non-human primates and highlights the need for further surveillance in free-ranging populations.

176 | GLYCOSYLATION PATTERNS IN CANINE HYALINIZING PANCREATIC ADENOCARCINOMA

S. Semzenisi [1] , R. Romelia [1], H. Hodor [1], T. Tabaran [1]

[1] Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterina, Cluj-Napoca, Romania

Background
Hyalinizing pancreatic adenocarcinoma is well known in humans but rarely described in dogs. The presence of hyaline material in these tumors gives them a distinctive appearance and raises questions about their origin and role. In some cases, hyaline may represent a type of extracellular matrix component, possibly composed of glycoproteins, non-amyloid fibrillary proteins, or by-products of stromal changes. Although once thought to be amyloid, recent studies suggest it may consist of altered glycoproteins or fibrotic deposits. Identifying its composition may clarify pancreatic disease progression in dogs.

Materials & Methods
Pancreatic tissue samples from dogs with neoplastic lesions were processed and stained with HE. A diagnosis of hyalinizing pancreatic adenocarcinoma was made based on histological evaluation. Lectin histochemistry was performed using Sambucus nigra agglutinin (SNA), Phaseolus vulgaris leucoagglutinin (PHA-L), and Erythrina cristagalli lectin (ECL) to detect carbohydrate structures. Fluorescent lectin binding was assessed using confocal laser scanning microscopy.

Results
Each case showed a unique lectin-binding pattern. Case 1 exhibited strong SNA (+++) and weak ECL (+) binding, with no reactivity for PHA-L. Case 2 showed weak binding only for PHA-L (+). Case 3 showed moderate SNA reactivity (++), with no binding for PHA-L or ECL. These differences suggest variability in sialic acid and galactose content, indicating biochemical diversity in hyaline composition.

Conclusion
Lectin histochemistry revealed altered glycosylation in pancreatic hyaline. The findings support a non-amyloid, heterogeneous origin and highlight lectins as valuable tools for investigating pancreatic pathology in dogs.

181 | CD44 SPLICING DYNAMICS IN CANINE OSTEOSARCOMA CELLS

N. Massimini [1], N. Di Domenico [2], C. Rubin [3], L. Mincarelli [2], N. De Maria [4], N. Romanucci [1], N. Cammà [2], N. Della Salda [1]

[1] University of Teramo, Teramo, Italy
[2] Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy
[3] Uppsala University, Uppsala, Sweden
[4] University of Turin, Turin, Italy

Background
CD44 is a widely expressed cancer stem cell marker whose overexpression in osteosarcoma (OSA) is linked to metastasis and treatment resistance, although prognostic value remains unclear. Splicing may contribute to its variability, as different isoforms are associated with tumor progression. The gene includes constitutive and variable exons (v1–v10, exons 6–15), whose different combinations generate multiple isoforms. This study investigates the association between CD44 isoforms and invasive behavior of canine OSA cells.

Materials & Methods
D17 and Penny cell lines were grown under standard conditions or embedded in type I collagen (3D) in short-term and long-term cultures, modeling cancer invasion. CD44 cDNA was amplified and sequenced using Oxford Nanopore Technologies (ONT). Reads were basecalled (SUP), filtered (Q-score <10), and analyzed using Flair software with CanFam3 as reference.

Results
The mean read length (including primers, barcodes, and target) was 560 bp, with a total of 11.9 million reads. Qualitative results indicated that six mRNA isoforms were unique to the metastatic D17 cell line, composing by the following exon variants: 1) v7-v8, 2) v4-v5-v7, 3) v4, 4) v4-v5, 5) v10, 6) v4-v5-v8. Five isoforms were only present in long-term culture of the D17 metastatic cell line, as follows: 1) v3-v4-v5; 2) v8, 3) v1; 4) v4-v5-v6; 5) v6.

Conclusion
Long-read sequencing provides new insights into CD44 splicing in dogs. Identified isoforms may enhance invasion binding tumour extracellular domain and they align with findings in human OSA. Further analysis in canine tissues is planned.

192 | WHEN DO CATS DEVELOP CANCER? AGE AT TUMOR DIAGNOSIS IN 4,594 FELINE CASES FROM TWO PATHOLOGY-BASED ANIMAL CANCER REGISTRIES, ITALY

N. Fonti [1], A. Carnio [2], C. Cocumelli [2], E. Dhein [3], C. Eleni [2], V. Galietta [2], F. Guscetti [3], A. Lachi [4], F. Parisi [5], A. Poli [5], P. Scaramozzino [2], F. Millanta [5]

[1] University of Padova, Legnaro, Italy
[2] Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Rome, Italy
[3] University of Zurich, Zurich, Switzerland
[4] Saint Camillus International University of Health and Medical Sciences, Rome, Italy
[5] University of Pisa, Pisa, Italy

Background
Cancer in cats often progresses silently, with clinical signs typically emerging at advanced stages. Lymphoma, squamous cell carcinoma, adenocarcinoma, and fibrosarcoma are among the most common malignancies. Although early diagnosis improves outcomes and screening tests are being introduced, specific guidelines for cancer screening in pets are still lacking. This multicenter retrospective study evaluated the age at tumor diagnosis and influencing factors in feline oncology patients.

Materials & Methods
A total of 4,594 feline cases with histologically confirmed neoplasia were coded using a modified Vet-ICD-O-canine-1 system and analyzed by tumor behavior, sex, neuter status, breed, and tumor type. Age distribution differences were assessed using non-parametric tests, and an Event History Analysis model evaluated the impact of these parameters on timing of first cancer diagnosis.

Results
The median age at malignant tumor diagnosis was 11 years, one year later than for benign tumors. Males ($p < 0.001$) and non-purebred cats ($p < 0.001$) were diagnosed at a younger age, whereas Persian ($p < 0.01$) and Siamese ($p < 0.05$) cats were diagnosed significantly later. Lymphomas had the earliest median age at diagnosis. Multivariate analysis confirmed that male sex (Time Ratio = 0.96, $p < 0.05$) and non-purebred status (Time Ratio = 0.92, $p < 0.001$) were associated with an accelerated time to malignancy diagnosis.

Conclusion
These findings highlight the need for species-specific cancer screening approaches in cats and underscore the role of pathology-based data in developing evidence-based screening protocols in feline oncology.

194 | FIRST REPORT OF ORAL AMELANOTIC MELANOMA IN A YELLOW-LEGGED GULL (LARUS MICHAHELLIS)

N. Fonti [1], F. Parisi [2], M. Pacini [3], R. Ceccherelli [3], V. Moccia [1], S. Ferro [1], A. Poli [2], F. Millanta [2]

[1] University of Padova, Legnaro, Italy
[2] University of Pisa, Pisa, Italy
[3] Lipu Bird Life Italia, Parma, Italy

Background
An adult, free-ranging male yellow-legged gull (*Larus michahellis*) was admitted to the Marine and Aquatic Bird Recovery Center (CRUMA), managed by Lipu, in Livorno, Italy, exhibiting marked weakness and a non-resectable oral mass occupying most of the oral cavity. Due to the poor prognosis and compromised quality of life, the animal was humanely euthanized.

Materials & Methods
A complete necropsy was conducted, and samples were submitted for further histopathological and immunohistochemical analyses at the Department of Veterinary Sciences, University of Pisa.

Results
Post-mortem examination revealed a well-defined, firm, white mass (4 x 5 cm), originating from the upper beak, lateral to the choanal cleft. No significant alterations were observed in other organs, and no evidence of metastasis was found. Histologically, the lesion consisted of a densely cellular, non-encapsulated proliferation of poorly differentiated epithelioid to spindle-shaped cells. These cells displayed marked pleomorphism with indistinct eosinophilic cytoplasm, round to oval nuclei, and prominent nucleoli. Severe anisocytosis and anisokaryosis were present, with a mitotic count of 18 in 2.37 mm². Immunohistochemically, neoplastic cells exhibited moderate cytoplasmic immunolabeling for vimentin, PNL-2, and melan-A.

Conclusion
Based on gross, histopathological, and immunohistochemical features, a diagnosis of oral amelanotic melanoma was made. While melanomas have been reported in various wild and domestic animal species—many of which share oncogenic mechanisms with humans—this case represents the first documented occurrence in *Larus michahellis*. This finding contributes to the limited literature on avian melanocytic tumors and may enhance our understanding of their presentation and biology in birds.

202 | DRUG-INDUCED POLYPLOID GIANT CANCER CELLS IN A PRECLINICAL MODEL OF HIGH GRADE SEROUS OVARIAN CANCER

L. Bertola [1, 2] , L. Sala [1, 2], P. Roccabianca [1], P. Riccaboni [1], A. Capuano [3], S. Muzzin [3], I. Pellarin [3], F. Basso-Valentina [3], G. Baldassarre [3], C. Lenardi [4], C. Perego [5], E. Scanziani [1, 2], C. Recordati [1, 2]

[1] Department of Veterinary Medicine and Animal Sciences (DIVAS)University of Milan, Lodi, Italy
[2] Mouse & Animal Pathology Laboratory (MAPLab), Fondazione Unimi, Milan, Milan, Italy
[3] Centro di Riferimento Oncologico Aviano (CRO), Molecular Oncology Unit, Aviano, Italy
[4] Department of Physics, University of Milan, Milan, Italy
[5] Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy

Background
Chemotherapy remains the standard treatment for high-grade serous ovarian cancer (HGSOC), the most common subtype of human ovarian tumours. However, it has been linked to the induction of polyploid giant cancer cells (PGCCs), which contribute to recurrence and progression. Despite limited characterization, PGCCs may serve as prognostic markers and therapeutic targets. This study aimed to retrospectively characterize PGCCs and their association with chemotherapy in a murine HGSOC model.

Materials & Methods
FFPE tumor samples from 46 C57BL/6 female mice injected intraperitoneally with 2×10^6 ID8 $p53^{-/-}/PTEN^{-/-}$ HGSOC cells were analysed. Mice received intraperitoneal treatment with either PBS (n=23) or carboplatin (n=23) and were sacrificed 21 days post-treatment. Tissues were stained with H&E, digitized, and assessed using a novel semiquantitative grading system evaluating tumor burden, growth pattern, necrosis, inflammation, aberrant mitoses, and PGCC presence. Hotspots were captured at 20x magnification (276 images total), and PGCCs were manually counted. ImageJ software was used to quantify neoplastic cells by size and validated based on the manual counts.

Results
Carboplatin significantly reduced tumor burden but increased PGCC numbers and neoplastic cell size. PGCCs were more abundant and larger in treated mice, particularly at tumor periphery. Tumor necrosis, inflammation, and mitotic abnormalities showed no significant group differences. Manual and automated PGCC counts were well correlated.

Conclusion
We developed and validated a grading system and an automated method for PGCC quantification in HGSOC. While carboplatin reduced tumour mass, it enhanced PGCC formation, especially at tumor periphery, highlighting a potential resistance mechanism.

206 | ATYPICAL TESTICULAR NEUROMA IN A HORSE: EXPANDING THE DIFFERENTIAL DIAGNOSIS

R. Marica [1], A. Hota [1], F. Tabaran [1], A. Negoescu [1]

[1] Faculty of Veterinary Medicine, Cluj-Napoca, Romania

Background
Testicular neuromas are typically benign proliferations of neuronal tissue that commonly develop at castration sites as a response to nerve trauma. Their occurrence in intact animals is extremely rare. This case report documents a testicular neuroma in a non-castrated horse.

Materials & Methods
A 2-year-old mixed-breed rescue horse was presented for clinical examination and elective castration. Upon gross examination, one testis appeared enlarged and exhibited a firm consistency. Tissue samples were collected for microscopic evaluation. Histological analysis was performed using haematoxylin and eosin, as well as Masson's trichrome staining. Immunohistochemical evaluation was carried out using antibodies against neuron specific enolase (NSE) and S100.

Results
Histopathological examination revealed that over 80% of the testicular parenchyma had been distended and replaced by an abnormal, non-neoplastic proliferation of neuronal tissue with an irregular distribution. Numerous peripheral nerve bundles, composed of neurofilaments and Schwann cells of varying sizes, were present along with extensive diffuse fibrosis and moderate multifocal inflammatory infiltrates, primarily consisting of macrophages. The remaining testicular parenchyma was compressed, and adjacent seminiferous tubules showed minimal degenerative changes. Immunohistochemistry demonstrated strong NSE and S100 positivity within the lesion, confirming the diagnosis of a testicular neuroma associated with fibrosis and granulomatous orchitis.

Conclusion
In the absence of a known medical history, it is suspected that the neuroma developed following a previous traumatic event. Although rare in non-castrated animals, testicular neuroma should be considered in the differential diagnosis of testicular masses in horses.

213 | IMMUNOHISTOCHEMICAL EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR AND PLATELET-DERIVED GROWTH FACTOR RECEPTOR IN FELINE MAMMARY CARCINOMAS

W. Łopuszyński [1], M. Szczubiał [1], K. Bulak [1], A. Śmiech [1], M. Marcinowska [2]

[1] University of Life Sciences, Lublin, Poland
[2] University of Agriculture, Krakow, Poland

Background
The vast majority of feline mammary carcinoma (FMCs) have a high metastatic potential. It is estimated that approximately 25% of female cats have regional or distal micrometastases at the time of FMCs diagnosis. Surgical treatment supported by chemotherapy is insufficient, and the average survival time after removal of FMCs is 8–12 months. Tyrosine kinase inhibitors may offer an additional terapeutic approach. Vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor (PDGFR) have become key targets for cancer therapy. This study aimed to evaluate the immunohistochemical expression of VEGFR and PDGFR in FMCs.

Materials & Methods
Twenty samples of formalin-fixed paraffin-embedded FMCs were immunohistochemically labeled with anti-VEGFR and PDGFR antibodies. The number of positive cells and the intensity of the reaction were taken into account in order to assess their immunoexpression.

Results
VEGFR and PDGFR were expressed in 16 (80%) and 14 (70%) of cases respectively. The predominant expression pattern was cytoplasmic, although differences in the number of positive cells and reaction intensity were demonstrated. A positive correlation was noted between VEGFR and PDGFR.

Conclusion
Most FMCs in the conducted studies showed immunohistochemical expression of VEGFR and PDGFR. Therefore, it can be hypothesized that these tumours may be susceptible to therapy with specific tyrosine kinase inhibitors; immunohistochemistry may be used as a predictive indicator of tyrosine kinase inhibitor therapy, which requires confirmation in clinical trials.

225 | COMPARATIVE HISTOLOGICAL AND STEROID-RECEPTOR ASSESSMENT OF INFLAMMATORY VS NON-INFLAMMATORY GRADE-III CANINE MAMMARY CANCERS

G. Valdivia [1], D. Calabuig [1], A. Alonso-Diez [1], V. Hidalgo [1], M. Pérez-Alenza [1], L. Peña [1]

[1] Complutense University of Madrid, Madrid, Spain

Background
Inflammatory mammary carcinoma (IMC) and grade III non-IMC mammary carcinomas are the most malignant types of canine mammary cancer (CMC). IMC behaves as a distinct, more aggressive entity with a poorer prognosis. Little is known about the expression of steroid hormone receptors, and effective treatment options are lacking. Their histological patterns and steroid hormone receptor profiles were compared, following the updated ASCO/CAP guidelines for human breast cancer, which state that even low-positive tumours (cut-off ≥1%) may benefit from targeted anti-hormonal therapy.

Materials & Methods
Tumours from 46 bitches (16 IMC, 30 non-IMC) were histologically classified and immunostained for oestrogen receptor (ER), progesterone receptor (PR), and androgen receptor (AR). Receptor status was recorded as the percentage of positive nuclei and interpreted using the updated ASCO/CAP cut-off (≥1%), previous criteria (>10%), and other semiquantitative methods.

Results
None of the IMC tumours showed myoepithelial proliferation. Anaplastic and invasive micropapillary carcinomas were over-represented in IMC ($p = 0.045$). ER-positive nuclear percentages were higher in non-IMC (mean 12%) than in IMC (mean 7%; $p = 0.027$). PR positivity was similar between groups (80% non-IMC vs 81% IMC); however, PR+ non-IMC cases showed longer median survival ($p = 0.049$). AR+ tumours were more frequent in non-IMC (67%) than in IMC (19%; $p = 0.002$).

Conclusion
IMC and grade III non-IMC tumours differ in morphology and receptor expression. PR is a favourable prognostic marker in non-IMC, while AR immunolabelling identifies canine subgroups that could benefit from anti-androgen therapies, supported by existing veterinary-approved drugs.

234 | SOX10 IN EQUINE NORMAL SKIN AND MESENCHYMAL TUMOURS: AN IMMUNOHISTOCHEMICAL STUDY

E. Vazquez-Fernandez [1], E. Ricci [1], G. Rocchigiani [1]

[1] University of Liverpool, Liverpool, United Kingdom

Background
SOX10 plays a crucial role in neural crest embryonic development, cancer progression, tumour microenvironment, and metastasis. SOX10 has been widely studied in human medicine, but little research has been conducted in the veterinary field. The main aim of the study was to characterise SOX10 immunolabelling in equine normal skin and mesenchymal skin tumours. To the authors' knowledge, this is the first description of SOX10 immunolabelling in equids.

Materials & Methods
Cases of equine haired skin were retrieved from the University of Liverpool database, diagnosed as dermal sarcomas with nerve sheath tumour morphology (sNST) or equine sarcoids. IHC against SOX10 was performed in all cases and examined in the tumour and unaffected skin.

Results
Thirty biopsies (three donkeys and twenty-seven horses) were retrieved from the archive, twenty sNST and ten sarcoids. Normal skin consistently revealed nuclear immunolabeling of Schwann cells of nerves, melanocytes, myoepithelium of both sebaceous and sudoriferous glands, dermal papillae and outer root sheath of hair follicles. Cytoplasmic and nuclear immunolabelling was multifocally seen in the secretory cells of sudoriferous glands. Sarcoids (100%) and sNST (95%) were mostly negative, apart from a weak granular labelling in one sNST (5%).

Conclusion
Although further research is needed, this study offers new insights into the potential use of SOX10 as a marker in equine dermatopathology, particularly for neuroectodermal-derived tumours (e.g. melanomas or adnexal neoplasm), as its specificity is similar to previously described in human and veterinary medicine. However, despite its widespread use, SOX10 is not recommended for the diagnosis of sNST.

250 | CHONDROSARCOMA OF THE URINARY BLADDER IN A DOG

A. Reginato [1], A. Campanile [2], E. Scorsi [1], F. Scarpa [1], I. Porcellato [3], E. Elvio [3]

[1] CDVet research, Roma, Italy
[2] Private practitioner - ENDOVET Group, Benevento, Italy
[3] University of Perugia, Perugia, Italy

Background
Extraskeletal chondrosarcomas are rare in dogs; reported sites are adrenal gland, eye, gastric ligament, ileum, kidney, liver, spleen, testicle, and vagina.

Materials & Methods
A 16 months-old female Golden Retriever with long lasting haematuria, strangury and dysuria underwent complete urine analysis with bacteriological culture and antibiogram that allowed the isolation of *Serratia liquefaciens*. Clinical symptoms didn't improve after appropriate antibiotic therapy, and abdominal ultrasound examination revealed a 7 cm mass with irregular borders, located in the trigonous region of the urinary bladder. Urethrocystoscopy showed a reddish and irregular-shaped mass with partial occlusion of urinary bladder lumen. Biopsies were collected for histological examination.

Results
Histologically, the tumor was composed by atypical spindle to round-shaped cells in the lamina propria, multifocally embedded in lightly basophilic material consistent with chondroid matrix. Neoplastic cells showed marked atypia (swollen nuclei, prominent nucleoli), occasional mitoses and apoptosis. A morphological diagnosis of extraskeletal well-differentiated chondrosarcoma was made. Immunohistochemistry was performed, showing strong and diffuse nuclear immunostaining for Runx-2, slight and focal nuclear and cytoplasmic positivity to S100, strong and diffuse cytoplasmic positivity to Vimentin, while Desmin was completely negative. Interestingly, also aggregates of spindle to polygonal cells in the lamina propria had strong nuclear positivity to Runx2. Due to the worsening of clinical condition the dog was euthanized soon after histological diagnosis.

Conclusion
The presented case is a unique case of extraskeletal chondrosarcoma arising in the urinary bladder of a young dog, which has not been previously reported in the literature.

276 | FIBROBLASTIC SARCOID ASSOCIATED WITH BPV 1 AND 2 IN A HARTMANN ZEBRA

E. Colombino [1, 2, 3], A. Oliver-Guimerá [1], A. Barragán [1], D. Viana [1], L. Carbonell [4], J. Martínez-Gimenez [4], F. Uzal [5], P. Pesavento [5], J. Corpa [1], J. Ortega [1], E. Montero-Cortijo [1]

[1] Pathology Group, PASAPTA, Facultad Veterinaria, Universidad UCH-CEU, Alfara del Patriarca (Valencia), Spain
[2] Departamento Medicina-Cirugía Animal, Facultad Veterinaria, Universidad UCH-CEU, Alfara del Patriarca (Valencia), Spain
[3] Hospital Clínico Veterinario UCH-CEU, CEU Universities, Alfara del Patriarca (Valencia), Spain
[4] Bioparc Valencia, Valencia, Spain
[5] CAHFS and School of Veterinary Medicine, UC DAVIS, San Bernardino, California, United States

Background
Sarcoids in horses are often linked to bovine papillomavirus (BPV), and similar lesions have occasionally been reported in zebras. However, BPV detection in zebras has been inconsistent. This study describes a sarcoid associated with BPV 1 and 2 in a Hartmann zebra.

Materials & Methods
A 10-year-old female captive Hartmann zebra was humanely euthanized because of a mass in the right pectoral region, which had regrown two months after surgery and partial resection with incomplete healing. A complete necropsy was conducted, and samples of the main organs were collected for histopathological analysis. In situ hybridization (ISH) for BPV was performed on the mass.

Results
A 15 cm multinodular, white and firm mass, was observed infiltrating the subcutaneous tissue of the right pectoral muscle. The mass was ulcerated but not attached to the sternum, and no metastasis were observed in the rest of the carcass. Histologically, in the superficial and deep dermis there was an encapsulated, infiltrative multinodular, neoplasm composed of spindle cells arranged in bundles within a moderate collagen matrix. Mild, multifocal epithelial hyperplasia was observed. Lymphoplasmacytic infiltrate and granulation tissue were associated with the neoplasm. The mitotic count was 2 per 2.37 mm^2. ISH was strongly positive for BPV 1 and 2 in the spindle neoplastic cells, but negative in the epithelium.

Conclusion
The tumour was classified as a fibroblastic sarcoid associated with BPV 1 and 2. This is the first description of a sarcoid associated with BVP 1 and 2 detected by ISH in a Hartmann zebra.

297 | A SURVEY TO STUDY THE CORRELATION BETWEEN THE ENVIRONMENT AND CANCERS IN DOGS AND HUMANS IN ITALY

E. Di Napoli [1], V. Russo [1], D. De Biase [2], G. Piegari [1], S. Papparella [1], R.S. Magalhaes [3], K. Owada [3], G. Rosato [4], A. Budillon [5], C. Palmieri [3], O. Paciello [1]

[1] University of Naples Federico II, Naples, Italy
[2] University of Salerno, Salerno, Italy
[3] The University of Queensland, Gatton , Australia
[4] ASL Napoli 1 Centro - CRIUV Regione Campania, Naples, Italy
[5] Istituto Nazionale Tumori Fondazione G. Pascale - IRCCS, Naples, Italy

Background
Neoplastic disease in dogs and humans, when sharing spaces, environments, and exposures, is characterised by the same clinical manifestation, metastatic potential, and genomic instability. The main difference lies in the latency, being shorter in the dog, making it a sentinel of an environmental alert. Surveys aim to gather data on environmental risk factors influencing cancer occurrence in both species.

Materials & Methods
The QR code survey was distributed in the waiting rooms of human hospitals and veterinary clinics. The questionnaire was composed of 57 multiple-choice and completion questions. The questions concerned general information about the owner and the animal, eating habits, and places where the owners and their dogs lived.

Results
A total of 350 survey responses were collected. After excluding 65 incomplete responses, 285 were analysed. Fifty-nine canine tumours were reported, primarily concentrated in Naples and surrounding municipalities. Of these, 13 dogs lived in high-risk areas, three near industrial sites, and 5 in territories characterised by environmental threats. The reported tumour types in dogs involved the skin, mammary gland, stomach, liver, testicles, bone, pancreas, gums, thyroid, intestines, and blood. In 33 out of 59 cases, tumours were recorded in the dog and its human owner.

Conclusion
The information from the survey allowed us to identify the main risk factors, such as environmental pollutants, that may predispose dogs and humans to cancer and implement better prevention strategies to reduce cancer incidence and mortality in both species.

298 | PRIMARY MAMMARY GLAND SQUAMOUS CELL CARCINOMA WITH MULTIPLE METASTASES IN A YOUNG EWE

S. Mignacca [1], S. Bagatella [2], F. Spada [2], L. Zarucco [2], M. Capucchio [2]

[1] Department of Agriculture, Food and the Marine - Veterinary Laboratories, Celbridge, Ireland
[2] Department of Veterinary Sciences, Torino University, Torino, Italy

Background
Mammary tumours in sheep are exceedingly rare. Here, we describe for the first time a metastatic squamous cell carcinoma (SCC) arising from the mammary parenchyma in an ewe.

Materials & Methods
The excised udder of a 3,5-year-old ewe with a previous history of chronic bacterial mastitis and severe swelling of the udder was submitted for pathological investigation. The animal was euthanised 6 weeks after surgery due to poor prognosis and the carcass was submitted for necropsy.

Results
The surgically removed udder (weighing 14 kg) showed intact skin, was severely swollen and was connected with two cord-like masses that extended into the subcutis of the right hindlimb and into the pelvic cavity. On cut section, the udder showed diffuse, severe parenchymal necrosis with firm, pale, disorganized tissue delimitating large cavitations filled with abundant, thick, brown-pink fluid. At necropsy, the non-resected portion of the cord-like masses extended into the pelvic cavity and was accompanied by multifocal, firm nodules within the mammary lymph-nodes, caudal mesentery, large intestine, caudal parietal peritoneum, diaphragm, heart, and pulmonary parenchyma. Histologically, all mammary and extramammary masses were infiltrative and consisted of neoplastic squamous epithelial cells (immunopositive for pancytokeratin) organized in variably-sized clusters, with keratin covering their surfaces sometimes organized in concentric layers (keratin pearls). A diagnosis of primary mammary SCC with multiple metastases was made.

Conclusion
This case highlights the importance of a thorough pathological investigation to identify previously unreported neoplasms in sheep, and may provide useful informations for the comparative pathology of mammary SCCs in animals.

311 | ALP AND RUNX-2 EXPRESSION IN CANINE OSTEOSARCOMA ARE ASSOCIATED WITH HISTOLOGICAL GRADE AND NODAL STATUS.

G. Avallone [1], E. Brigandì [1], R. Bianchi [2], G. Sarli [1], T. Franceschini [3], A. Grillini [3], M. Fiorentino [3], A. Verdi [2], F. Millanta [4], P. Roccabianca [2]

[1] DIMEVET-Università di Bologna, Ozzano dell'Emilia, Italy
[2] University of Milano, Lodi, Italy
[3] DIMEC - University of Bologna, Bologna, Italy
[4] Department of Veterinary Sciences, University of Pisa, Pisa, Italy

Background
Alkaline phosphatase (ALP) and Runx-2 have been proposed as useful diagnostic markers in canine osteosarcoma. This study aimed to evaluate their co-expression and investigate associations with clinicopathological features.

Materials & Methods
Cases were retrospectively selected based on the availability of paraffin-embedded tissue. Histological slides were reviewed, diagnoses confirmed, and tumors graded according to Loukopoulos et al. Nodal status was recorded and double immunohistochemistry for ALP and Runx-2 performed. Staining and co-expression were semi-quantitatively scored based on the percentage of positive tumor cells: score 1 (1–10%), score 2 (11–50%), score 3 (51–90%), and score 4 (>90%). Statistical analyses were performed.

Results
Thirty osteosarcomas were included: 13 were grade 1, 8 grade 2, and 9 grade 3. Lymph node was negative in 18 cases, positive in 4, and unavailable in 8. ALP expression scores were: 1 in 5 cases, 2 in 5, 3 in 7, and 4 in 5; absent in 8 cases. Runx-2 was scored 1 in 3 cases, 2 in 3, 3 in 11, 4 in 3, and absent in 10. Four cases were negative for both. Grade 3 tumors showed higher Runx-2 expression ($p = 0.008$). Nodal metastases were associated with lower ALP expression ($p = 0.036$). Co-expression was detected in 15 cases, more frequent in axial than appendicular osteosarcomas ($p = 0.044$).

Conclusion
These findings confirm ALP and Runx-2 as useful diagnostic markers for canine osteosarcoma, with potential prognostic relevance related to grade and nodal status.

312 | RENAL DYSPLASIA ASSOCIATED WITH PTGS2 GENE MUTATION IN A BORDER COLLIE PUPPY

J. Fiedorowicz [1], K. Paździor-Czapula [1], I. Otrocka-Domagała [1]

[1] University of Warmia and Mazury, Olsztyn, Poland

Background
Renal dysplasia in puppies can be of hereditary or of infectious origin and diagnosis is based on histopathological and clinical findings. PTGS2 gene mutation is one of the possible causes of familial renal dysplasia in dogs and has been reported in several breeds.

Materials & Methods
Three of six Border Collie puppies from a single litter died at the age of a few months with signs of progressive kidney failure. All the pregnancy-associated infections were excluded. Both parents were tested for mutation of PTGS2 gene by PCR, and they turned out to be heterozygous. Kidney of the one of the affected puppies was sent for histopathology, processed and stained routinely. Additionally, periodic acid-Schiff (PAS), Masson's trichrome, von Kossa and immunohistochemical staining (smooth muscle actin, CD31) were performed.

Results
Macroscopically, multiple cysts were observed in the kidney. Microscopically the medulla and cortex were diffusely disorganized. Collecting tubules were irregularly formed and often ectatic, with the presence of immature tubules. Bowman's spaces of glomeruli were often dilated with atrophy of glomerular tufts and occasional thickening of Bowman's capsule. Proteinaceous fluid and sometimes basophilic material were observed in the lumen of tubules and glomeruli. Proliferation of connective tissue and small arterioles were present with mild infiltration of inflammatory cells. The medulla was partially occupied by large cysts.

Conclusion
This case suggests an association between mutations in the PTGS2 gene and renal dysplasia in Border collies. Thus, genetic screening should be considered in this breed in planning future litters.

320 | INTRATUMOURAL CPMV IMMUNOTHERAPY IN A CASE OF CANINE INFLAMMATORY MAMMARY CANCER

G. Valdivia [1], A. Alonso-Diez [1], L. Barreno [1], J. Affonso de Oliveira [2], G. Ortiz-Diez [1], M. Suarez [1], P. García-San José [1], N. Steinmetz [2], S. Fiering [3], M. Pérez-Alenza [1], L. Peña [1]

[1] Complutense University of Madrid, Madrid, Spain
[2] University of California San Diego, California, United States
[3] Geisel School of Medicine at Dartmouth, New Hampshire, United States

Background
In dogs with mammary cancer, intratumoural immunotherapy using empty CPMV (eCPMV, Cowpea Mosaic Virus, devoid of RNA) has demonstrated substantial clinical benefits. Full CPMV nanoparticles exert a more potent effect in murine cancer models. The pathological and clinical effects of full CPMV in a dog with inflammatory mammary cancer (IMC) are reported for the first time.

Materials & Methods
A 12-year-old spayed mixed-breed bitch with IMC received four weekly intratumoural CPMV alongside the institutional standard of care (oral firocoxib, toceranib phosphate, and metronomic cyclophosphamide). H&E, PAS, Masson's trichrome, and immunohistochemistry for phenotype markers, MPO, CD3, CD20, Iba-1, MUM-1 and FOXP3 were performed on surgical samples. CPMV treatment was continued for a total of 7 doses.

Results
The clinical signs of IMC (pain, oedema, and erythema) resolved after the second injection, and by the fourth, tumour volume had diminished so markedly that radical surgery became feasible. Histology revealed extensive, multifocal maturing fibrosis leading to cancer cell death: neoplastic tubules were encased by collagen-producing myofibroblasts with basement membrane thickening, which progressed to dense, acellular collagen masses. These changes were not seen in IMC cases treated with standard-of-care therapy alone (controls). A lymphoplasmacytic infiltrate, with moderate numbers of Iba-1+ macrophages and MPO+ neutrophils, was observed. Recurrence appeared four months later. Overall survival was 292 days, compared to 52 days in controls. Treatment was well tolerated; quality of life remained excellent.

Conclusion
CPMV immunotherapy seems to induce/enhance marked fibrosis. More treated patients are needed to confirm histopathological changes and assess its clinical benefits.

333 | CASE REPORT: RENAL ADENOCARCINOMA IN ARABIAN BUSTARD (ARDEOTIS ARABS)

M. Carrasco [1], A. Perez de Vargas [1], V. Herder [2]

[1] Reneco International Wildlife Consultants LTD, Abu Dhabi, United Arab Emirates
[2] MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom

Background
At the National Avian Research Center in Abu Dhabi, a captive breeding program has been developed for the Arabian Bustard. A 13-year-old male was presented with unspecific clinical symptoms and progressive clinical deterioration. An intraabdominal mass associated with the kidney was detected by palpation and confirmed by laparotomy, followed by euthanasia, as the surgical removal of the mass was not possible. At necropsy, a unilateral, non-encapsulated mass was found attached to the cranial pole of the left kidney. The mass was compromising and displacing the surrounding internal organs.

Materials & Methods
Samples from the mass, kidneys, and other internal organs were collected in 10% formalin, submitted for histopathology analysis and stained with HE and Masson's trichrome.

Results
The enlarged kidney showed a partially encapsulated, densely cellular, well-differentiated, epithelial neoplasm composed of medium-sized polygonal cells arranged in tubules. In one area, there was focal infiltration into adjacent connective tissue; the mitotic count was less than 0.63 mitoses per mm^2. Multifocally and randomly throughout the mass urate tophi were observed, occasionally surrounded by small numbers of viable and degenerate heterophils, macrophages, and multinucleated giant cells. The microscopic study described renal adenocarcinoma with intratumoral gout. No remarkable microscopic lesions were found in other organs.

Conclusion
Renal neoplasia is infrequently described in birds, and although this is a unique case report of an individual condition, very little scientific information has been published to date in this species. This highlights the importance of specialized veterinary care, diagnostic support, and further research in wildlife conservation breeding and additional studies and publications.

337 | NEUROLOGICAL SIGNS ASSOCIATED WITH MICROCYSTIC MENINGIOMA ACCOMPANIED BY NASOPHARYNGEAL MYIASIS IN A FREE-RANGING DEER

P. Carvalho [1], P. Mendonça [1], V. Silva [1], P. Faísca [2, 3, 4], M. Orge [1, 2]

[1] INIAV, Oeiras, Portugal
[2] University of Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal
[3] Universidade Lusófona, Centro Universitário de Lisboa, Lisboa, Portugal
[4] Dnatech, Lisboa, Portugal

Background
Despite the neuropathological studies following Chronic Wasting Disease (CWD) surveillance, which has contributed to the knowledge of neuropathological profiles in cervids, tumours are still rarely reported. This is due to the fact that relatively few animals in the wild live long enough to develop tumours, which are usually ageing-associated. On the contrary, myiasis in cervids, particularly nasopharyngeal, is a common parasitic infection that is expanding in Europe.
We report a rare meningioma in a free-ranging deer heavily affected by nasopharyngeal myiasis.

Materials & Methods
Under CWD and epizootic hemorrhagic disease (EHD) surveillance, an adult male deer (*Cervus elaphus*) showing circling movements and apathy was slaughtered and tested. Brain tissue samples were collected for histopathology and immunohistochemistry (IHC) for AE1/AE3, Vimentin, CD31, VWB and NSE was performed.

Results
Gross examination revealed numerous *Oestridae*-like dipteran larvae within the nasopharynx and nasal turbinates, along with an extra-axial greyish mass compressing the left piriform lobe and basal nuclei, extending into the left thalamus causing axis and hypophysis deviation. Histologically, consisted of a non-encapsulated cellular proliferation arranged in trabeculae with empty spaces containing clusters of cells with pale, round nuclei, and occasional foci of cells with elongated nuclei. Adenohypophysis was focally invaded. Neoplastic cells showed positive immunolabelling only for vimentin and AE1/AE3. CWD and EHD resulted negative.

Conclusion
Histomorphological and IHC findings support the diagnosis of a microcystic meningioma, a rare subtype of grade I meningioma (WHO classification).

340 | PERICYTE MICROVASCULAR COVERAGE IN CANINE MAMMARY CARCINOMAS

G. Vicente [1, 2], R. Strefezzi [1], R. Vasconcelos [3]

[1] Laboratory of Comparative and Translational Oncology, FZEA-USP, Pirassununga, Brazil
[2] Department of Pathology, School of Veterinary Medicine and Animal Science, São Paulo, Brazil
[3] Veterinary Pathology Service, FCAV-UNESP, Jaboticabal, Brazil

Background
Canine mammary carcinomas (CMCs) are highly prevalent neoplasms in female dogs. Pericytes are specialised cells that support the basement membrane and the endothelial cells of blood vessels. The proteoglycan NG2 plays a crucial role in the recruitment, activation, proliferation, and motility of pericytes and is considered a marker for this cell type. Pericytes also interact with various components of the cancer microenvironment and influence vascular permeability, potentially enhancing angiogenesis, tumour growth, and metastasis formation. Damage to pericytes can compromise tissue vascularisation, leading to hypoxia and inflammation, which may also contribute to cancer progression. This study aimed to characterise the pericyte microvascular coverage as a prognostic marker in CMCs.

Materials & Methods
Twenty cases of CMCs were subjected to immunohistochemistry and double-stained with anti-NG2 and anti-vWF antibodies. Small blood vessels (diameter <50 µm), with and without pericyte coverage, were counted in an intratumoral area of 0.4 mm^2. The microvessel counts were compared between high-malignancy (solid, anaplastic and comedocarcinoma) and low-malignancy (simple, complex and mixed-types, maximum grade II) CMCs, as well as post-surgical survival.

Results
High-malignancy CMCs exhibited higher counts of NG2-positive microvessels and lower counts of NG2-negative microvessels ($p<0.01$) than low-malignancy CMCs. Using ROC curve analysis, a cutoff of 11 microvessels was determined. Cases with more than eleven NG2-positive microvessels in 0.4 mm^2 had a significantly shorter survival ($p<0.01$; median survival = 260 days).

Conclusion
We suggest that pericyte microvascular coverage is a prognostic marker for CMCs and that the role of pericytes in the tumour microenvironment requires further investigation.

341 | TUMOUR-INFILTRATING LYMPHOCYTES IN CANINE CUTANEOUS MAST CELL TUMOURS

F. Ramos [1, 2], R. Strefezzi [1]

[1] Laboratory of Comparative and Translational Oncology, FZEA-USP, Pirassununga, Brazil
[2] Department of Pathology, School of Veterinary Medicine and Animal Science, São Paulo, Brazil

Background
Mast cell tumour (MCT) is a frequent malignant skin neoplasm in dogs. CD30, a member of the TNFR family, is variably expressed by canine mast cells and activated lymphocytes and has emerged as a therapeutic target in human mast cell disorders. CD30 expression in TILs remains unexplored in canine MCTs. The present study aimed to quantify tumour-infiltrating T, B and CD30+ lymphocytes to explore their association with prognosis.

Materials & Methods
Fifty-five cutaneous MCT (35 low-grade, 20 high-grade), treated with wide surgical resection with curative intent, were subjected to immunohistochemistry for CD3, PAX5 and CD30. Positive intratumoral lymphocytes were counted in random fields in a total area of 0.4 mm^2. The numbers of CD3+, PAX5+, and CD30+ tumour-infiltrating lymphocytes (TILs) were compared with histological grade and post-surgical survival.

Results
The average CD30+ lymphocyte count was 17 (range: 0–79). A cut-off value of 46 TILs was calculated using a ROC curve. Dogs with MCTs that presented more than 46 CD30+ TILs in 0.4 mm^2 showed shorter post-surgical survival ($p=0.0217$). The numbers of CD3+ and PAX5+ TILs were not significantly associated with MCT histological grade, Ki67 index, mitotic count or survival. However, positive correlations were observed between CD30+ and PAX5+ lymphocytes ($p=0.0049$) and CD3+ and PAX5+ cells ($p<0.0001$).

Conclusion
Our findings suggest that CD30+ TILs are associated with shorter post-surgical survival in dogs with MCTs. Further investigation is needed to identify the specific subset of CD30+ TILs (B or T cells) and clarify their role within the tumour microenvironment.

342 | REEVALUATING MAST CELLS IN CANINE LYMPH NODES: METASTASIS OR NOT?

K. Bittencourt [1, 2], R. Strefezzi [1], A. Nishiya [3], J. Cirillo [4], K. Cadrobbi [4], R. Ubukata [3, 4], A. Voges [3, 4], P. Jark [5], C. Pires [6], D. Sanches [6], F. Sueiro [7]

[1] Laboratory of Comparative and Translational Oncology, FZEA-USP, Pirassununga, Brazil
[2] Department of Pathology, School of Veterinary Medicine and Animal Science, São Paulo, Brazil
[3] Veros Hospital Veterinário, São Paulo, Brazil
[4] E+ Especialidades Veterinárias, São Paulo, Brazil
[5] Oncospes Oncologia Veterinária, Ribeirão Preto, Brazil
[6] Centro Veterinário de Anatomia Patológica (CVAP), São Paulo, Brazil
[7] TECSA Laboratory, Campinas, Brazil

Background
Evaluation of lymph node (LN) status using the Weishaar system is considered the gold standard for detecting mast cell tumour (MCT) metastasis. However, it lacks an objective comparison of mast cell counts in normal LNs to support the criteria for the HN1 ("pre-metastatic") and HN2 ("early metastasis") classes. Moreover, most of the LNs assessed were not the sentinel LNs (SLNs), and Toluidine Blue staining was not performed in the majority of cases. This study aimed to propose a standardised method for the analysis of SLNs in dogs with MCTs.

Materials & Methods
Twelve LNs from healthy dogs and nineteen SLNs from dogs with MCTs (9 low-grade, 6 high-grade and 4 subcutaneous) were analysed. The number of mast cells per 0.237 mm^2 was determined in 5 hotspots in Toluidine Blue-stained sections and compared with post-surgical survival.

Results
The average follow-up was 330 days. All LNs from healthy dogs were classified as MC≤1/field, whereas SLNs from dogs with MCTs showed a range from no mast cells to more than one MC/field, isolated or in small groups (MC>1/field), or large clusters of mast cells that clearly distorted the LN architecture ("evident metastasis"). No significant difference in survival was found between cases with MC≤1/field and MC>1/field ($p>0.05$). Cases with "evident metastasis" had significantly shorter survival ($p=0.0110$; median survival = 88 days).

Conclusion
The presence of a considerable number of mast cells in SLNs from dogs with MCTs may not impact survival, suggesting that the current criteria for defining LN metastasis should be reassessed.

343 | EVALUATION OF THE LYMPHATIC VASCULATURE IN CANINE CUTANEOUS MAST CELL TUMOURS

K. Bittencourt [1, 2], R. Strefezzi [1], A. Nishiya [3], J. Cirillo [4], K. Cadrobbi [4], R. Ubukata [3, 4], A. Voges [3, 4], P. Jark [5], C. Pires [6], D. Sanches [6], F. Sueiro [7]

[1] Laboratory of Comparative and Translational Oncology, FZEA-USP, Pirassununga, Brazil
[2] Department of Pathology, School of Veterinary Medicine and Animal Science, São Paulo, Brazil
[3] Veros Hospital Veterinário, São Paulo, Brazil
[4] E+ Especialidades Veterinárias, São Paulo, Brazil
[5] Oncospes Oncologia Veterinária, Ribeirão Preto, Brazil
[6] Centro Veterinário de Anatomia Patológica (CVAP) , São Paulo, Brazil
[7] TECSA Laboratory, Campinas, Brazil

Background
The lymphatic system plays a crucial role in draining fluids, proteins and cells from tissues, thereby regulating homeostasis. In the context of cancer, its function is contrasting: while it helps reduce interstitial pressure within the tumour, it also facilitates the dissemination of cancer cells, promoting tumour progression and the development of metastases. The present study aimed to characterise intra- and peritumoral lymphatic vasculature and to assess its prognostic value in canine cutaneous mast cell tumours (MCTs).

Materials & Methods
Twenty-five low-grade and fifteen high-grade cases of canine cutaneous MCTs were evaluated. Lymphatic vessels were identified by immunohistochemistry using an anti-LYVE-1 antibody and quantified in five hotspot fields (0.08 mm² each) within intra- and peritumoral regions. Any positively stained cell or cell cluster, with or without a lumen, was considered a countable microvessel. The peritumoral area was defined as the fields immediately adjacent to the tumour margin. The results were compared with disease-related mortality and post-surgical survival.

Results
The number of intratumoral lymphatic vessels was not an indicator of disease-related mortality. Dogs that died due to the MCT exhibited a higher number of peritumoral lymphatic vessels (p=0.0204). A cutoff value of 119 peritumoral lymphatic vessels was established using ROC curve analysis. Dogs with more than 119 peritumoral lymphatic vessels had significantly shorter post-surgical survival (p=0.0189; median survival = 162 days).

Conclusion
The number of peritumoral lymphatic vessels is a prognostic indicator of mortality and post-surgical survival in canine cutaneous MCTs.

344 | HER2 EXPRESSION IN INFLAMMATORY AND NON-INFLAMMATORY CANINE MAMMARY CARCINOMAS

I. Silva [1, 2], R. Strefezzi [1], B. Gouveia [1, 2], R. Vasconcelos [3], J. Xavier [4], A. Gama [5, 6]

[1] Laboratory of Comparative and Translational Oncology, FZEA-USP, Pirassununga, Brazil
[2] Department of Pathology, School of Veterinary Medicine and Animal Science, São Paulo, Brazil
[3] Veterinary Pathology Service, FCAV-UNESP, Jaboticabal, Brazil
[4] Paulista University (UNIP), São Paulo, Brazil
[5] Animal and Veterinary Research Centre (CECAV), UTAD, Vila Real, Portugal
[6] Associate Laboratory for Animal and Veterinary Science (AL4Animals), Vila Real, Portugal

Background
Mammary tumours are common neoplasms in female dogs, with up to 85% of cases being malignant. Among these, inflammatory mammary carcinomas (IMCs) stand out as the most aggressive form, characterised by rapid clinical progression and poor prognosis. The human epidermal growth factor receptor 2 (HER2) has been widely demonstrated as an important tumour marker. However, there is still no consensus in the literature regarding its expression in IMCs. The purpose of the present study was to compare HER2 expression between IMCs and non-inflammatory mammary carcinomas (non-IMCs).

Materials & Methods
Twenty-nine canine IMC and thirty non-IMC samples were histologically and immunohistochemically classified according to the 2019 Davis-Thompson Foundation proposal. HER2 expression was evaluated by immunohistochemistry using an anti-HER2 antibody (A0485, Dako), and the samples were scored on a scale from 0 to 3+, with cases scoring 2+ or 3+ considered positive.

Results
IMCs exhibited higher HER2 scores compared with non-IMCs (p=0.0006). The majority of the IMCs (63.3%) were HER2-positive, whereas the majority of the non-IMCs (75.7%) were negative (p=0.0025). No differences were found in HER2 positivity regarding histological grades and clinical outcome.

Conclusion
Canine IMCs exhibit higher HER2 expression compared with non-IMCs, suggesting a potential role for this protein in the malignant behaviour of these neoplasms.

353 | FIBROBLAST ACTIVATION PROTEIN (FAP) AS A POSSIBLE THERAPEUTIC TARGET IN SELECTED CANINE MALIGNANT TUMOURS

S. Dell'Aere [1], D. Stefanello [1], E. Gariboldi [1], A. Ubiali [1], R. Ferrari [1], L. Auletta [1], M. Bocci [2], A. Galbiati [2], S. Cazzamalli [2], E. Gilardoni [2], V. Balbi [1], G. Bianchi [1], A. Verdi [1], D. Neri [3], P. Roccabianca [1]

[1] Department of Veterinary Medicine and Animal Sciences, University of Milan, Lodi, Italy
[2] Philochem AG, R&D Department, Otelfingen, Switzerland
[3] Philogen S.p.A., Siena, Italy

Background
Fibroblast Activation Protein (FAP) is a serine protease overexpressed by cancer-associated fibroblasts (CAFs) and neoplastic cells in diverse human tumours. Despite its diagnostic and therapeutic potentials demonstrated in humans, limited data exist for canine tumours. This study aims to characterise FAP immunohistochemical expression in canine tumours to assess its potential therapeutic relevance.

Materials & Methods
FAP expression was assessed by immunohistochemistry on microarrays of normal tissues, positive controls and six different canine tumour types, using polyclonal (AbCam, ab53066) and monoclonal (AbCam, ab207178, clone EPR20021) anti-FAP antibodies, validated for dogs. Expression was semi-quantitatively scored for percentage (scale 0-4) and intensity (scale 0-3) in neoplastic cells, stromal CAFs, vessels, and stroma, according to published methods.

Results
Sixty-six tumours examined were ten per type: apocrine gland anal sac adenocarcinomas (AGASAC), soft tissue sarcomas (STS), mast cell tumours (MCT), hemangiosarcomas (HSA), osteosarcomas (OSA), lymphomas, and six melanomas (4 skin/digit, 3 oral). FAP was variably expressed in neoplastic cells (58/66 cases), CAFs (58/66), and vasculature (60/66); expression was intense in >50% of neoplastic cells in AGASACs, STSs, and MCTs, moderate in 11-50% in HSA, OSA and lymphomas, and weak <50% in 2/6 melanomas. Stromal positivity was inconsistent. The polyclonal antibody produced stronger overall staining, whereas the monoclonal antibody had greater specificity.

Conclusion
According with the results, FAP represents a promising molecule for targeted diagnostic and therapeutic applications. Differences between monoclonal and polyclonal stains likely reflect the presence of multiple FAP isoforms; the combined use of both markers may improve patients' enrolment for preclinical studies.

361 | IMMUNOHISTOCHEMICAL PROFILING OF B7-H3 EXPRESSION IN CANINE SOLID AND LIQUID NEOPLASMS

L. Cesar Conti [1], S. Capellero [1], S. Divari [1], L. Maniscalco [1], E. Mazzone [1], M. Olimpo [1], L. Aresu [1], R. De Maria [1]

[1] University of Turin, Grugliasco, Italy

Background
B7-H3 (CD276) is overexpressed in human cancers and correlates with unfavourable outcomes. Previous investigations in canine oncology have focused on sarcomas. This study evaluates the immunohistochemical expression of B7-H3 in a diverse spectrum of canine neoplasms and investigates associations with the histological grade.

Materials & Methods
Immunohistochemistry was performed on 101 canine tumours (melanoma, osteosarcoma, soft tissue sarcoma, hemangiosarcoma, and lymphomas) and 6 normal tissues using an anti-human B7-H3 antibody (Clone RBT-B7H3, BioSB) on the Ventana platform. Protein expression was assessed using a validated scoring system combining cytoplasmic positivity and staining intensity (each scored 0–3), with total scores (0–9) categorised as negative (0), low (1–3), or high (4–9) expression (PMID: 33387703). Statistical analysis of associations between B7-H3 expression and tumour grade was conducted using Fisher's exact test.

Results
High B7-H3 expression appeared in 73% of solid tumours: soft tissue sarcomas (93%), melanomas (71%), osteosarcomas (68%), and hemangiosarcomas (62%). Low expression occurred in 21% of melanomas, 27% of osteosarcomas, 7% of soft tissue sarcomas, and 19% of hemangiosarcomas. Only 7% of melanomas, 5% of osteosarcomas, 18% of hemangiosarcomas were negative. Conversely, 90% of B cell and 70% of T-cell lymphomas showed negative expression. Normal tissues displayed negative to low expression. No significant correlation emerged between B7-H3 expression and tumour grade.

Conclusion
These results reveal robust B7-H3 expression across canine solid tumours with limited expression in lymphoid neoplasms and normal tissues. This tumour-specific pattern positions B7-H3 as a promising candidate for targeted immunotherapeutic strategies, particularly CAR cell-based approaches.

369 | THYMIC SARCOMATOID CARCINOMA WITH METASTASIS IN A RABBIT

V. Weiss [1], F. Künzel [1], J. Schindler [1], T. Donovan [2], M. Kiupel [3], C. Bertram [1]

[1] University of Veterinary Medicine, Vienna, Austria
[2] Schwarzman Animal Medical Center, New York, United States
[3] Michigan State University, East Lansing, United States

Background
Thymic tumours are frequent in rabbits, with almost exclusive reports of thymomas. Here, we report the first documented case of thymic sarcomatoid carcinoma in a rabbit, a tumour type that has been previously described in humans.

Materials & Methods
A 5-year-old female neutered rabbit was presented with bilateral recurrent prolapse of the third eyelid due to bilateral exophthalmos. Thoracic radiographs and a CT-scan revealed a cranial mediastinal mass along with numerous nodules in both lungs. Due to progressive respiratory distress, the rabbit was euthanised 119 days after initial presentation.

Results
Necropsy revealed a 2x4x6 cm large firm mass cranial to the heart, as well as multiple pulmonary nodules measuring 1-5 mm in diameter. Histological examination showed a moderately cellular neoplasm composed of spindle cells arranged in interwoven bundles or haphazard patterns. The tumour cells were embedded in an abundant collagenous stroma. Tumour cells exhibited moderate nuclear pleomorphism, but low mitotic activity. Scattered small aggregates of small lymphocytes were admixed between tumour cells, along with widespread necrosis, multifocal calcification, and numerous multinucleated giant cells. Immunohistochemistry showed diffuse immunopositivity of the spindle cell population for vimentin and multifocal labelling for cytokeratin AE1/3, cytokeratin 5/6, and cytokeratin 7. The pulmonary masses showed the same histological findings as the thymic tumour.

Conclusion
Histological and immunohistochemical findings of the thymic tumour are consistent with a sarcomatoid carcinoma. A carcinoma is diagnosed based on the positive labelling of some tumour cells for epithelial markers and the presence of pulmonary metastasis.

373 | IN VITRO INVESTIGATION OF THE ONCOLYTIC POTENTIAL OF TWO BOVINE HERPESVIRUSES IN CULTURED CANINE OSTEOSARCOMA AND HISTIOCYTIC SARCOMA CELLS

M. Di Pentima [1], S. Minesso [1], V. Franceschi [1], R. Di Lecce [1], A. Corradi [1], G. Donofrio [1], E. Razzuoli [2], W. Baumgärtner [3], R. Vescovini [1], B. Passeri [1], F. Armando [1]

[1] University of Parma, Parma, Italy
[2] Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle D'Aosta, Genova, Italy
[3] University of Veterinary Medicine Hannover, Hannover, Germany

Background
Canine osteosarcoma (c-OSA) and histiocytic sarcoma (c-HS) are characterized by aggressive behavior. This is due to frequent metastasis, recurrence and limited response to conventional treatments. Therefore, viral oncolysis represent an interesting alternative therapeutic approach. Bovine herpesvirus type-4 (BoHV-4) and type-1 (BoHV-1) could represent potential candidates due to their oncolytic features reported in other species cell lines. This study aims to evaluate the oncolytic potential of BoHVs in these relevant canine tumors.

Materials & Methods
Canine cell lines—D17 and OSA2 (c-OSA), DH82 (c-HS), and cutaneous fibroblasts—were infected with BoHV-4-EGFPΔTK and BoHV-1 at MOI ranging from 0.1-4 (BoHV-4) and 0.01-1 (BoHV-1). Infection rates were assessed by counting positive cells (five 10x fields). Flow cytometry was used at 48 and 96 hours to quantify infected (GFP^+), dead ($7AAD^+$), and double-positive cells. Immunofluorescence was used to investigate cell death pathways. Particularly, apoptosis (cleaved-caspase-3) and pyroptosis (Gasdermin-D).

Results
BoHV-4 at 1 MOI infected c-OSA and c-HS cell lines, inducing cytopathic effects at 48 and 96 hours, with infectious rate ranging from 90-100% infected cells at the later time-point, along with, and decrease of cell viability (DH82: 63%, OSA2: 68%). In contrast fibroblasts show a limited infection (21%) and high viability (~90%). BoHV-1 infected at 1MOI all cell lines with a higher percentage of infected cells at 48h (ranging from), particularly D17, inducing a cytopathic effect and complete resolution of the infection at 96h.

Conclusion
These findings support BoHVs as promising oncolytic viruses for canine tumors, warranting further investigation into their therapeutic potential.

374 | ENVIRONMENTAL ANTHRACOSIS AS A RISK FACTOR FOR CANINE PULMONARY ADENOCARCINOMA

M. Amalia [1], C. Palmieri [1], R. Magalhaes [1], B. Brunetti [2], B. Bacci [2], M. Anjomanibenisi [2]

[1] The University of Queensland, Gatton, Australia
[2] The University of Bologna, Bologna, Italy

Background
Environmental air pollution, particularly fine particulate matter (PM2.5), is a known carcinogen, with carbonaceous particles playing a crucial role in pulmonary anthracosis and lung cancer. While this association is well established in human medicine, evidence remains limited in dogs. Thus, this study investigates the potential link between carbon particle accumulation and pulmonary adenocarcinoma in dogs.

Materials & Methods
A total of 183 canine lung tissue samples, with and without primary lung tumours and anthracosis, were collected from archives of the University of Queensland and the University of Bologna. The samples were divided into four groups: (1) Primary lung tumour with anthracosis; (2) Primary lung tumour without anthracosis; (3) Non-neoplastic with anthracosis; (4) non-neoplastic without anthracosis. Carbon particles were quantified by manual and automated image analysis (QuPath), and the association with lung tumour was analysed using logistic regression, with significance at $p<0.05$.

Results
Of the 183 samples, 62.30% (N=114) had lung tumour with anthracosis, 27.87% (N=51) non-neoplastic with anthracosis, 6.01% (N=11) lung tumour without anthracosis, and 3.83% (N=7) non-neoplastic without anthracosis. Significant associations were found between the number of particles ($p=0.002$) and age ($p<.001$) with pulmonary adenocarcinoma. In contrast, no significant associations were found between sex ($p=0.876$) and breed (*1.00*), and lung cancer.

Conclusion
Chronic carbon accumulation in the lungs may increase the risk of pulmonary adenocarcinoma in dogs, highlighting the relevance of air pollution as a potential risk factor in lung cancer pathogenesis. Potential confounding by age and limited sample sizes in certain groups warrant cautious interpretation and further research to substantiate these findings.

377 | CASE REPORT OF METASTASIS OF PHEOCHROMOCYTOMA IN GUINEA PIG (CAVIA PORCELLUS)

S. Nešić [1], I. Vučićević [1], M. Vučićević [1], S. Aleksić Kovačević [1]

[1] Faculty of veterinary medicine, University of Belgrade, Belgrade, Serbia

Background
Pheochromocytoma is a rare neuroendocrine tumor originating from chromaffin cells of the adrenal medulla. While well-documented in humans and several domestic animal species, its occurrence in guinea pig is exceedingly uncommon and poorly described in literature. This report presents a detailed histopathological evaluation of pheochromocytoma in a guinea pig, aiming to contribute to the limited knowledge of endocrine neoplasms in exotic pets.

Materials & Methods
In this case, clinical examination and radiographic imaging were performed in a 4-year-old guinea pig, which was initially considered male before autopsy but was later determined to be a hermaphrodite. Post-mortem tissue samples were processed and stained using the standard hematoxylin-eosin method.

Results
A guinea pig in terminal condition was presented with lethargy, tachypnea, and tachycardia. Hypopyon with visible blood and fibrin in the anterior chamber of the left eye was observed during clinical examination. Radiographic imaging revealed extensive periosteal proliferation of the right coxofemoral joint, consistent with advanced coxarthrosis. Despite supportive care, the animal's condition rapidly deteriorated. Postmortem examination revealed a pheochromocytoma in the adrenal gland, liver, and lungs. Histopathological analysis confirmed that the primary tumor was a pheochromocytoma in adrenal gland. Histopathological evaluation also revealed metastases of tumor cells in the liver, lungs, kidney, brain, and eye.

Conclusion
This case highlights the importance of considering adrenal tumors in the differential diagnosis of cardiovascular and systemic signs in guinea pigs. Also it underscores the need for heightened awareness and diagnostic capabilities in exotic animal medicine.

382 | DRUG REPURPOSING IDENTIFIED BORTEZOMIB AND DIGOXIN AS EFFECTIVE IN VITRO AND IN A MOUSE MODEL OF CANINE NEPHROBLASTOMA.

Y. Machida [1], A. Ishihara [1], R. Nakahira [1], T. Nagashima [1], M. Michishita [1]

[1] Nippon Veterinary and Life Science University, Tokyo, Japan

Background
Canine nephroblastoma is a rare embryonal mixed tumour derived from metanephric blastemal cells, predominantly affecting young dogs. Approximately 50% of cases progress to metastatic disease, resulting in a poor prognosis. This study aimed to identify novel therapeutic candidates using an FDA-approved drug library.

Materials & Methods
A nephroblastoma from the left kidney of a 10-year-old neutered male Miniature Dachshund was used for primary culture. Tumour tissue was minced and enzymatically digested to isolate single cells, which were cultured in DMEM/10% FBS and passed over 50 generations to establish the NVLCN cell line. NVLCN cells were screened *in vitro* with 765 FDA-approved compounds, and cell viability was evaluated. NVLCN cells were also subcutaneously transplanted into female SCID Beige mice, and candidate compounds were administered. A control group of mice received vehicle treatment. The formed tumours were examined histologically and immunohistochemically. Nuclear NF-κB expression following treatment was evaluated by Western blotting.

Results
High-throughput screening identified bortezomib and digoxin as potent candidates, with IC_{50} values of 43 nM and 0.62 μM, respectively. Xenograft tumours exhibited tubular, papillary, and partially solid epithelial proliferation, resembling the primary tumour. Cytoplasm of tumour cells had vimentin and CK AE1/AE3 immunolabeling. *In vivo*, both agents suppressed tumour growth, with bortezomib exerting more pronounced anti-tumour effects than digoxin. Western blotting of NVLCN cells showed decreased nuclear NF-κB expression after bortezomib treatment.

Conclusion
Bortezomib and digoxin exert anti-tumour effects against canine nephroblastoma. Bortezomib may act by inhibiting NF-κB nuclear translocation and represents a promising therapeutic candidate. These findings were demonstrated both *in vitro* and experimentally.

384 | EXTRACELLULAR VESICLE-ASSOCIATED MIR-222-3P AND MIR-186-5P AS HYPOXIC MARKERS IN CANINE OSTEOSARCOMA: AN IN VITRO STUDY

R. De Maria [1], L. Maniscalco [1], M. Poncina [1], S. Divari [1], L. Parisi [1], S. Sonia [1], L. Cesar Conti [1], E. Mazzone [1], L. Aresu [1]

[1] University of Turin, Grugliasco, Italy

Background
The hypoxic microenvironment plays a critical role in the progression of canine osteosarcoma (cOSA) by promoting different cellular responses, including the release of extracellular vesicle (EVs). Given the high clinical aggressiveness of cOSA, the aim of this study was to evaluate the miRNAome profile in EVs released in vitro by four canine osteosarcoma cell lines following hypoxic conditions.

Materials & Methods
D17, D22, Penny, and Wall cell lines were cultured under normal and hypoxic conditions (200uM $ClCo_2$) for 24 hours confirmed by HIF-1α. EVs were isolated by size-exclusion chromatography and characterized by Nanoparticle Tracking and Western Blotting. miRNAs extracted from EVs were then sequenced and analyzed bioinformatically. The most representative miRNAs were identified and validated by q-PCR using the miRCURY LNA miRNA PCR assay.

Results
MiRNome profiling identified 233 miRNAs differentially expressed in EVs from all analyzed cell lines. 94 miRNAs were exclusively expressed under hypoxic condition. After bioinformatic analysis, 41 miRNA in Wall and D22 cell lines were selected for further validation by q-PCR that revealed that miR-222-3p and miR-186-5p were significantly downregulated in Wall cell line under hypoxic conditions ($p \leq 0.005$).

Conclusion
This preliminary study provides evidence that in vitro hypoxia is accompanied by a statistical downregulation of miR-222-3p and miR-186-5p in Wall cell line. Although in human osteosarcoma, low serum levels of miR-222-3p are associated with poor prognosis and miR-186-5p is recognized as a key hypoxia-responsive miRNA, further investigations need to be assessed to identify EV-associated miRNAs as biomarkers in cOSA.

389 | OCULAR CHROMATOPHOROMA IN A TEGU (SALVATOR MERIANAE)

S. Catoi [1], P. Pop [1], L. Bel [1], T. Tabaran [1]

[1] University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Cluj-Napoca, Romania

Background
Chromatophoromas are neoplasms composed of pigment-producing and light-reflecting cells that affect reptiles, fish and amphibians. Typically, chromatophoromas originate in the skin of the head and body, with reported sizes ranging from 0.2 to 2.0 cm in diameter. This report details the clinical and histological presentation of a rare ocular chromatophoroma in a tegu (*Salvator merianae*).

Materials & Methods
A six-year-old male Argentine black and white tegu (*Salvator merianae*) was euthanized and submitted for autopsy due to a progressively enlarging ocular mass. Following a complete autopsy the mass was sampled and routinely processed for cytology and histology.

Results
The animal presented a well-demarcated, irregular, white to pale pink, multilobulated mass measuring up to 2.5 cm in diameter located at the medial canthus of the eye. The mass was compressing the ocular globe, causing visible indentation and mild displacement of the eye within the orbit. Cytology examination revealed round to oval cells, with indistinct borders, abundant, finely granular cytoplasm and large nuclei. Histopathological examination revealed a well-demarcated mass composed of densely packed, pigmented iridophore-like cells arranged in sheets and nests within a vascular stroma. The neoplastic cells had abundant cytoplasm with iridescent pigment granules, round to oval nuclei, fine chromatin, with 1-2 nucleoli. Mild to moderate anisocytosis and anisokaryosis were present, with rare mitoses.

Conclusion
To the authors knowledge this is the first documented case of chromatophoroma in a tegu (*Salvator merianae*), underscoring the importance for greater awareness and precision in reptile tumor diagnosis.

392 | DISTRIBUTION OF MAMMARY GLAND TUMORS IN DOGS ACCORDING TO HISTOLOGICAL TYPE

A. Hîruța [1], Z. GÁL [1], A. Pop [1], A. Irimie [1], M. Taulescu [1], V. Bocăneț [2], C. Gal [3], E. Gagniuc [4], C. Cătoi [1]

[1] University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania
[2] Technical University, Cluj-Napoca, Romania
[3] Synevovet Laboratory, Chiajna, Romania
[4] University of Agriculture and Veterinary Medicine, Bucharest, Romania

Background
Mammary tumors are common in dogs, typically treated via surgical excision; the histological tumor type and grade are valuable prognostic markers. This study explores the correlation between tumor location and histological tumor type to aid in earlier, more accurate prognostic assessments.

Materials & Methods
This study examined 250 female dogs with spontaneous mammary tumors, analyzing 361 nodules by routine histology and classified according to current literature. Out of 250 female dogs, 47.2% were castrated and 52.8% intact or of unspecified reproductive status. Tumor type distribution across ten anatomical sites (thoracic, abdominal cranial and caudal, inguinal-left and right) was evaluated using the Chi-square test.

Results
The mean age of the patients was 9.36 years. Among 46 breeds, mixed breeds (n=57), Bichons (n=45), and German Shepherds were most affected. A significant association was found between tumor presence and mammary gland region ($p < 0.001$), with the left and right inguinal and caudal abdominal glands most frequently affected. A total of 25 histological canine mammary gland tumor types were identified. Complex carcinoma (n=87) was the most common histological type predominantly present on the right caudal abdominal segment (p=0.003; 16/87), followed by intraductal papillary carcinoma (n=54) on the left caudal abdominal segment (12/54), tubular carcinoma (n= 38), and mixed carcinomas (n= 38) on the left caudal abdominal segment (3/38; 4/38).

Conclusion
The anatomical location of mammary gland tumors shows a strong correlation with histological type, highlighting its relevance as an important marker in guiding treatment strategies.

413 | A CASE OF ORAL SPINDLE CELL SARCOMA WITH INTRACYTOPLASMIC INCLUSION BODIES IN A DOG.

N. Meertens [1], K. Dam [2], T. Veenendaal [3]

[1] Royal GD Animal Health, Deventer, Netherlands
[2] Evidensia Dierenziekenhuis Arnhem, Arnhem, Netherlands
[3] Center for Molecular Medicine, University Medical Center Utrecht, Utrecht, Netherlands

Background
Oral spindle cell sarcomas are relatively common in dogs and include low-grade sarcomas which can be a challenge for diagnosis and therapy. This case report describes a case of low-grade soft tissue sarcoma with intracytoplasmic inclusion bodies in the gingiva of a 9-year old dog.

Materials & Methods
A 9-year old dog was presented with an oral gingival mandibular mass, removed by partial mandibulectomy. Histopathology, immunohistochemistry for vimentin, desmin, alpha-smooth muscle actin, and muscle-specific actin were performed, as well as transmission electron microscopy (TEM).

Results
The mass consisted of spindle-shaped cells in interlacing bundles. The cells had a tapered to blunt-ended nucleus, and often contained round hyaline eosinophilic inclusion bodies, sometimes adjacent to the nucleus. The cells showed mild anisocytosis and anisokaryosis, 2 mitotic figures in 2,37 mm². The neoplastic cells stained positive for vimentin and alpha-smooth muscle actin (SMA). Most cells were negative for muscle-specific actin (MSA), except few cells in the centre of the mass. The cells were mostly negative for desmin. The inclusion bodies did not react to any of the antibodies used. TEM showed abundant rough endoplasmatic reticulum, thin filaments and macropinocytotic vesicles within the neoplastic cells. The inclusion bodies consisted of large cytoplasmic globular non-membrane bound clusters of thin filaments.

Conclusion
This case report is to our knowledge the first case of a canine spindle cell sarcoma with intracytoplasmic inclusion bodies. The histopathological, immunohistochemical and electron microscopical findings are very similar to low-grade myofibroblastic sarcomas with intracytoplasmic hyaline inclusion bodies described in humans.

415 | EPIDEMIOLOGICAL EVALUATION OF NEOPLASTIC PATHOLOGIES IN DOGS FROM A CENTRAL ROMANIAN REGION

A. Hota [1], G. Toma [1], R. Pop [1], R. Marica [1], A. Tabaran [1]

[1] University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Cluj-Napoca, Romania

Background
Neoplastic diseases are among the most frequently diagnosed conditions in dogs. Epidemiological studies of canine tumors contribute to the broader understanding of neoplasms and provide valuable statistical data for veterinary clinicians.
This study aimed to statistically evaluate the epidemiological characteristics of spontaneous tumors in pet dogs from a central region of Romania. The analysis was conducted at the Department of Pathology, Faculty of Veterinary Medicine, Cluj-Napoca and included canine neoplasia cases recorded between 2020 and 2023.

Materials & Methods
Samples were selected from the department's database, with data including sample type (cytology, biopsy, cadaver), details about the animal (species, age, sex, breed), anamnesis and microscopic diagnosis.

Results
From 963 tumors identified, 54% of them (n = 520) were malignant, 38% (n = 369) were benign and the biological behavior could not be determined in 74 tumors (8%). The most common tumor sites were the skin (45.79%, n = 441), mammary gland (15.16%, n = 146), and digestive tract (7.89%, n = 76). Tumors were more frequently diagnosed in female dogs (49.83%, n =440) than in males (44.96%, n = 397). Over three-quarters of mammary gland tumors were malignant, representing 75.34% (n = 110) of all mammary neoplasms. The most affected breeds were Bichons, Labrador Retrievers, German Shepherd Dogs, French Bulldogs and Golden Retrievers.

Conclusion
In conclusion, malignant tumors were more prevalent in the sample of dogs, especially in adult and geriatric populations. The skin was the most frequent tumor site, while the mammary gland harbored the most malignant forms.

424 | PRIMARY PAPILLARY MALIGNANT MESOTHELIOMA OF THE TUNICA VAGINALIS IN A DOG WITH PERITONEAL METASTASIS: A RARE BUT CRITICAL DIFFERENTIAL DIAGNOSIS

R. Pop [1], D. Hodor [1], A. Tăbăran [2]

[1] Pathology Department, Faculty of Veterinary Medicine, Cluj-Napoca, Romania
[2] Pathology, Cluj-Napoca, Romania

Background
Tumours of the testicle are frequent in older dogs, however, malignant mesothelioma of the tunica vaginalis is a rare and often overlooked differential diagnosis in cases of scrotal or testicular enlargement. Mesotheliomas arise from serous membranes such as the pleura, peritoneum, and tunica vaginalis, and may present with non-specific clinical signs including hydrocele or abdominal effusion. Their aggressive biological behavior and tendency for metastasis, make early and accurate diagnosis critical.

Materials & Methods
An 8-year-old male Caucasian Shepherd dog presented with scrotal enlargement and ultrasonographic evidence of peritoneal masses. Following castration, the left testis, gastro-splenic ligament, gastric serosa, and peritoneum were submitted for pathological examination. Tissues were processed routinely, stained with HE, and analyzed immunohistochemically for vimentin and multicytokeratin.

Results
Grossly, multiple firm, white nodular masses projected from the serosal surfaces of the testis and associated tissues were observed. Tumour cells formed papillary projections with stratified polygonal layers, moderate anisokaryosis, and occasional mitoses. Immunohistochemically, most tumour cells were vimentin-positive, while superficial cells showed cytokeratin positivity, confirming their mesothelial origin. Infiltration into surrounding tissues and evidence of peritoneal metastases were observed. Histologically, a malignant mesothelioma with papillary architecture was diagnosed.

Conclusion
This case highlights a rare presentation of primary papillary malignant mesothelioma of the tunica vaginalis with peritoneal spread in a dog. Despite its rarity, such tumours should be included in the differential diagnosis for scrotal or testicular masses, especially when accompanied by abdominal effusion or peritoneal nodules. Early recognition is critical due to its aggressive behaviour and poor prognosis.

427 | CYSTIC PANCREATIC LESIONS IN CATS – BENIGN PROCESS OR PRECURSORS OF PANCREATIC CARCINOMA?

P. Grest [1], S. Blatter [2], F. Seehusen [1], S. Soto [2], M. Wartenberg [3]

[1] Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
[2] Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Bern, Switzerland
[3] Institute of Tissue Medicine and Pathology, University of Bern, Bern, Switzerland

Background
Cystic pancreatic lesions are diagnostically challenging in animals, where their rarity make accurate classification particularly difficult. In humans, such lesions may represent pseudocysts, non-neoplastic pancreatic cysts, or cystic neoplasms like serous cystic adenomas, intraductal papillary mucinous neoplasms (IPMN), mucinous cystic neoplasms (MCN) or pancreatic ductal adenocarcinoma with large-duct growth pattern. In cats cystic pancreatic tumors have been only sporadically reported, and cystic pancreatic neoplasms are not included in the WHO classification of tumours of domestic animals.

Materials & Methods
In this retrospective study, 8 cases of cats aged 5-11 years, diagnosed with pancreatic cysts, cystic adenoma, or cystic adenocarcinoma were included. Diagnostic imaging revealed uni- or multilocular cystic lesions involving the pancreas/pancreatic ductal system, which were surgically excised. Histologic analysis, including special stains to classify the epithelial cells, was performed and follow-up was collected.

Results
Histopathology revealed epithelial cystic proliferations from the exocrine pancreas, typically displaying papillary and/or tubular architecture. Cellular atypia was minimal, mitoses were rare, and no evidence of infiltrative growth was observed, supporting a diagnosis of pancreatic cystadenoma. Follow-up revealed that most cats were alive more than one year after diagnosis, although some showed persistent cystic pancreatic changes on imaging.

Conclusion
This case series highlights the need for greater awareness in veterinary medicine for benign cystic pancreatic tumors in cats, as comprehensive pathological descriptions of these lesions are currently lacking in the literature. Unlike human IPMN and MCN, which can progress to carcinoma, here, the lack of progression points toward a favorable prognosis.

429 | THREE-DIMENSIONAL CELL CULTURES OF CANINE HEMANGIOSARCOMA RETAIN PHENOTYPIC FEATURES OF PRIMARY TUMORS BUT EXHIBIT DISTINCT OXIDATIVE METABOLIC PROFILE

C. Tugnoli [1], F. Borrelli [1], C. Algieri [1], S. Coluccelli [2], G. Avallone [1], L. Muscatello [1], R. Terragni [3], L. Marconato [1], L. Ciammaichella [1], O. Candini [4], S. Frabetti [4], P. Roccabianca [5], F. Millanta [6], F. Trombetti [1], S. Nesci [1], D. De Biase [1], M. Lauriola [1], G. Sarli [1]

[1] University of Bologna, Bologna, Italy
[2] Solid Tumor Molecular Pathology Laboratory - IRCCS Bologna, Bologna, Italy
[3] Pet Care, Bologna, Italy
[4] Evotec (Modena) srl, Medolla, Modena, Italy
[5] University "La Statale", Milan, Italy
[6] University of Pisa, Pisa, Italy

Background
There is increasing interest in using three-dimensional growths (3DGs) as *ex vivo* model to investigate spontaneous tumours (STs), offering an alternative to laboratory animals.

Materials & Methods
We studied phenotypic, metabolic and genetic traits of canine hemangiosarcomas and related 3DGs. In mitochondria isolated from tumours and 3DGs (2µg protein, respectively), were assessed NADH-, Succinate-, and TMPD/ascorbate-oxidoreductase activities by measuring oxygen consumption. FFPE tissues were processed for histopathology. To generate 3DGs, fresh tumours were dissociated, seeded into a bioreactor (VITVO®, Rigenerand, Italy), and cultured for 7 days at 37°C in 5% CO_2. Fresh 3DGs were analyzed for oxidative activity; FFPE 3DGs were evaluated morphologically and scored for cellularity (scale based on cells per 0.237 mm^2: 1: <20, 2: 20–39, 3: 40–59, 4: 60–79, 5: >80). Next-generation sequencing (NGS) was performed using a laboratory-developed NGS panel (CanFam3.1) on FFPE samples from both STs and 3DGs, calling only variants detected in >10% of reads (>200x) and both strands.

Results
Among 10 hemangiosarcomas, 3DGs were generated in 7, with growth scores of 2 (n=3) or 3 (n=4). Necrosis and haemorrhage prevented growth in 3 cases (two without growth, one scored 1). STs and 3DGs shared morphology and immunophenotype, though CD31 was more frequently expressed than FVIII-RA. Oxidoreductases activity was significantly higher in 3DGs than in STs. NGS comparison (n=5), showed 4 concordant and 1 discordant profile.

Conclusion
3DGs replicate the phenotypic and genetic features of STs, but show enhanced oxidative metabolism, likely due to more favourable environmental conditions.

430 | GASTRIC KAPOSIFORM-LIKE HEMANGIOENDOTHELIOMA IN A CAT: PATHOLOGICAL FINDINGS AND FOLLOW-UP

C. Borfălău [1], A. Negoescu [2], R. Marica [2], A. Bărbulescu [3], K. Aharoni [4], C. Gal [5], M. Taulescu [2]

[1] University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania
[2] Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania
[3] A&A Medical Vet, Bucharest, Romania
[4] Koret School of Veterinary Medicine, The Hebrew University, Rehovot, Israel
[5] Synevovet, Bucharest, Romania

Background
Kaposiform hemangioendothelioma (KHE) is a rare vascular neoplasm that primarily affects humans, especially children, and has limited documentation in veterinary medicine.

Materials & Methods
This report describes the clinicopathological findings of an unusual gastric Kaposiform-like hemangioendothelioma in a 2-year-old male cat with a history of recurrent vomiting. Endoscopy revealed complete pyloric obstruction caused by an infiltrative mass, which, together with the enlarged regional lymph nodes, was surgically excised and submitted for pathological evaluation. Histologically, the samples were routinely processed and stained using Hematoxylin and Eosin, Masson's Trichrome, and Periodic acid-Schiff (PAS). Anti-Erythroblastosis Transformation-Specific Regulated Gene 1 (ERG, clone EPR3864) and α-SMA (ab5694) antibodies were used for immunohistochemistry to determine the vascular origin of the neoplastic cells.

Results
Microscopically, the gastric wall was transmurally infiltrated by a poorly delimited mass composed of spindle cells arranged in short bundles with slit-like vascular lumina and a swirling growth pattern giving a glomeruloid appearance, features characteristic of Kaposiform hemangioendothelioma. Immunohistochemistry revealed a positive labeling for ERG of endothelial cells surrounded by α-SMA-positive cells, further supporting the morphological diagnosis. Concurrently, the gastric lymph nodes displayed diffuse sclerosis with lymphoid tissue atrophy. No recurrence or metastatic lesions were observed five months after surgical resection.

Conclusion
This is the first documented case of a gastric KHE in cats. Histological and immunohistochemical evaluations are essential for differentiating KHE from other vascular lesions such as Kaposi sarcoma, spindle/epithelioid cell hemangioendothelioma, and reactive angioendotheliomatosis. Associated lymph node sclerosis may reflect a paraneoplastic fibrotic response, offering new insights into tumor–host interactions in feline vascular neoplasms.

432 | CONCOMITANT ERYTHEMA MULTIFORME IN A DOG WITH CUTANEOUS EPITHELIOTROPIC T-CELL LYMPHOMA

S. Gasparini [1], C. Carolina [1], M. Michela [1]

[1] Clinica Veterinaria e Laboratorio San Marco, Veggiano, Italy

Background
Canine erythema multiforme (cEM) is a T-cell mediated hypersensitivity reaction that can be triggered by drugs, infections or food, although most cases are idiopathic. A paraneoplastic origin has been described in human, but appears to be exceedingly rare in dogs: in the two reported cases it was associated with an intestinal lymphoma and a thymoma.

Materials & Methods
A 9-year-old, neutered male Border collie dog was referred for a 2-year history of severe, diffuse, scaling and ulcerative dermatitis with pruritus. The main clinical differential diagnoses included cutaneous epitheliotropic lymphoma, cEM and cutaneous lupus erythematosus. Cytology of cutaneous lesions, popliteal and retromandibular lymph nodes were performed. Skin biopsies were taken for histopathology and immunohistochemistry.

Results
Skin and lymph nodes cytology was suggestive of cutaneous lymphoma with possible nodal involvement. On histology, superficial dermis was altered by sheets of atypical medium to large lymphoid cells forming aggregates within both epidermal and follicular epithelium. Atypical cells were CD3 and Granzyme B positive and CD20 negative. The combination of morphologic atypia, architectural features, and phenotype was compatible with epitheliotropic T- cell lymphoma, of LGL-type. In addition, numerous, occasionally confluent, apoptotic keratinocytes with lymphocytic satellitosis were observed in the epidermis and hair follicles at all levels, suggesting the concomitant presence of cEM.

Conclusion
To the authors' knowledge, this is the first report of cEM and a neoplasm observed simultaneously in the same tissue. In this case, a paraneoplastic origin of the cEM can be hypothesized, although a definite association between the two entities cannot be established.

437 | PROTOCOL FOR TRANSLATIONAL TUMOUR MODELLING ON ORGANOTYPIC BRAIN SLICE CULTURES WITH TUMOUR CELL LINES.

S. Kohale [1, 2], G. Beythien [1, 2], W. Baumgärtner [1, 2], C. Puff [1]

[1] University of Veterinary Medicine, Hannover, Germany
[2] Centre for Systems Neuroscience, Hannover, Germany

Background
In canines, brain tumours are associated with poor prognosis after conventional therapy and low survival rates, highlighting the need to study their behaviour. Beside classical approaches including scratch wound assays, organotypic brain slices (OSCs), used in neuroscientific fields including electrophysiology, investigating viral tropism, and pharmacotherapy, represent a unique 3D environment to study canine tumour cell behaviour *in vitro*.

Materials & Methods
A Krumdiek tissue slicer was used to obtain fresh OSCs from deceased dogs without neurological history. 40,000 cells of well-defined canine tumour cell lines, including DH82 cells, were injected in OSCs using a stereotactic injector. OSCs were fixed in 10% neutrally buffered formalin after 1, 3 and 7 days of co-culture and different embedding strategies were tested to assess the migratory behaviour of injected cells in multiple planes. Serial sections were stained with H&E and used for immunohistochemistry.

Results
Expanding tumour cell populations were observed after 3 and 7 days of co-culture. Initial formalin fixation for 45 min preserved co-culture integrity and allowed fast handling of multiple OSCs. Trimming of OSCs prior to paraffin embedment-maintained structure better than post-embedment trimming and allowed better assessment of the migratory behaviour.

Conclusion
Canine OSCs were successfully used to co-culture tumour cells allowing the investigation of tumour cell migratory behaviour in a 3D environment that closely resembles the *in vivo* situation. This setup is also a potential platform to study the behaviour of other tumour cells (e. g. malignant lymphoma and canine glioblastoma cells) broadening possible applications of this technique.

438 | EVALUATION OF 14-3-3Σ PROTEIN EXPRESSION AND ITS RELEVANCE IN CANINE INTESTINAL EPITHELIAL TUMORS

A. Negoescu [1], B. Borfălău [1], D. Hodor [1], A. Hîruța [1], C. Gal [2], C. Cătoi [1], M. Taulescu [1]

[1] Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterina, Cluj-Napoca, Romania
[2] Synevovet, Bucharest, Romania

Background
The 14-3-3σ protein is involved in the regulation of the cell cycle, apoptosis, and DNA repair, and is linked to tumorigenesis, though its role in the development of intestinal carcinomas in animals remains unclear.

Materials & Methods
This study included 38 canine intestinal samples: 16 adenomas, 19 adenocarcinomas, and 3 normal mucosa, which were routinely processed and stained with H&E and 14-3-3σ antibody. Histological classification was performed per WHO criteria, and staged using the TNM system. Immunohistochemical analysis was conducted using a semiquantitative method; for quantitative image analysis, ImageJ software was used.

Results
Cytoplasmic immunolabeling for 14-3-3σ was observed in 56.25% adenomas and 57.89% adenocarcinomas. No immunoexpression was identified in normal intestinal mucosa. Adenomas exhibited mild (44.44%) immunoexpression, whereas adenocarcinomas showed moderate (54.54%) and high (36.36%) intensity. Nuclear immunolabeling was detected in 6 adenocarcinomas, 3 of which were associated with mesenteric lymph node metastases. Metastatic neoplastic cells also showed moderate 14-3-3σ expression. Furthermore, in the case of the T staging for these 6 adenocarcinoma cases, three were classified as T3, two as T4, and one case was designated as T1 due to its representation by a small-sized biopsy specimen. In emboli, clusters of neoplastic cells were negative for 14-3-3σ, while individual tumor cells exhibited high cytoplasmic labeling.

Conclusion
Protein 14-3-3σ may contribute to canine intestinal carcinoma development and progression. Further research is needed to link its expression to survival outcomes.

444 | INVESTIGATION OF TUMOR IMMUNE MICROENVIRONMENT (TIME) IN FELINE NASAL PLANUM CUTANEOUS SQUAMOUS CELL CARCINOMAS (CSCC)

L. De Paolis [1, 2], F. Armando [3], F. Dell'Anno [1, 2], M. Di Pentima [3], I. Porcellato [2], C. Brachelente [2], B. Passeri [3], K. Cappelli [2], L. Mechelli [2], S. Mecocci [2], G. Burrai [4], L. Gibelli [5], F. Fruscione [1], M. Ciurkiewicz [6], W. Baumgärtner [6], E. Lepri [2], E. Razzuoli [1]

[1] National Reference Center of Veterinary and Comparative Oncology, IZSPLV, Genoa, Italy
[2] Department of Veterinary Medicine, University of Perugia, Perugia, Italy
[3] Department of Veterinary Medicine, University of Parma, Parma, Italy
[4] Department of Veterinary Medicine, University of Sassari, Sassari, Italy
[5] IZSLER, Milano, Italy
[6] University of Veterinary Medicine of Hannover, Hannover, Italy

Background
Cutaneous squamous cell carcinoma (cSCC) is the most common malignant skin tumor in cats, accounting for 15–48% of feline cutaneous neoplasms. While chronic UV exposure is a well-established etiological factor, increasing evidence supports the involvement of feline papillomavirus (FCaPV) in a subset of cases. Despite multiple therapeutic efforts, no universally effective treatment exists. This study explores the tumor immune microenvironment (TIME) of feline cSCC, aiming to identify potential prognostic markers for therapeutic approaches.

Materials & Methods
Forty-five fresh nasal planum cSCC samples from necropsies or diagnostic biopsies were analysed. PCR for FcaPV DNA and RT-qPCR for E6/E7 oncogene and immune-related markers gene expression was performed. Additionally, 57 FFPE were retrospectively selected and histologically evaluated. CD3 and CD20 expression was semi-quantitatively assessed by IHC.

Results
FCaPV-2, -3, -4, and -5 DNA was detected in 11/45, 7/45, 6/45, and 2/45 cases, respectively. Four cases displayed FcaPV-2/3, -3/-4, -3/-5 and -4/-5 coinfection. All FCaPV$^+$ samples expressed E6/E7 oncogenes. Gene expression analysis revealed significant upregulation of CXCL8, TNF-α, IL-17, and IL-23 in FCaPV$^+$ samples compared to non-tumoral control tissues. Comparing the intratumoral and extratumoral compartment, IHC revealed a higher density of CD3+ cells intratumorally, while CD20+ cells were distributed extratumorally. No significant correlation was found between FCaPV status or coinfections and immune infiltrates.

Conclusion
Data obtained by gene expression analysis suggest a proinflammatory TIME in feline cSCC. The presence of intratumoral CD3+ T-cell infiltrates warrants further investigation using additional immune markers to better characterise the immune landascape and ultimately suggest therapeutic strategies.

446 | NGF EXPRESSION IN MAMMARY GLAND CARCINOMAS AND ADENOMAS IN BITCHES

J. Bubak [1], I. Janus-Ziółkowska [1], R. Ciaputa [1], M. Nowak [1], Z. Marzec [1], A. Piotrowska [2], P. Dzięgiel [2]

[1] Wroclaw University of Environmental and Life Sciences, Wrocław, Poland
[2] Wroclaw Medical University, Wrocław, Poland

Background
Canine mammary cancer (CMC) is a prevalent malignant tumor in female dogs.The expression of nerve growth factor (NGF) in CMC remains underexplored. In human breast cancer, NGF promotes cell proliferation, angiogenesis, and metastasis. This study aims to investigate NGF expression CMC cancerogenesis.

Materials & Methods
Fourty-eight canine mammary gland tissue samples were analyzed: 29 carcinomas, 9 adenomas , and 9 normal tissues. Samples were stained with H&E for histological evaluation. Immunohistochemistry assessed the expression of Ki-67, HER2, and NGF. Ki-67 proliferation index was categorized as low (<20%) or high (≥20%). HER2 positivity was defined by strong, complete staining in >30% of tumor cells. NGF expression was semi-quantified based on distribution and intensity.

Results
NGF-positive cytoplasmic staining was observed in epithelial cells (75.8% carcinomas, 77.7% adenomas, 44.4% normal tissue), myoepithelial cells (24% carcinomas, 44.4% adenomas, 44.4% normal tissue), and cancer-associated fibroblasts (CAFs) (20% carcinomas, 0% adenomas, 11% normal tissue). A strong positive correlation was found between staining intensity and the percentage of reactive cells in both tumor cells ($p<0.05$; $r=0.78$) and CAFs ($p<0.05$; $r=0.99$). High intensity and percentage of reactive CAFs were observed in samples with high mitotic activity (>20% Ki-67-positive cells) compared to those with low mitotic activity (<20% Ki-67-positive cells).

Conclusion
The results indicate that NGF expression is elevated in tumor cells and cancer-associated fibroblasts (CAFs) in CMC, particularly in samples with high proliferative activity. The strong correlation between NGF staining intensity and the percentage of reactive cells supports its potential role as a cellular marker associated with tumor progression and cancerogenesis in CMC.

449 | MITOTIC COUNT AND PROLIFERATIVE INDEX IN 24 CASES OF CANINE ORAL AMELANOTIC MELANOMA: TISSUE MICROARRAY CORES AND WHOLE-SECTION TISSUE ANALYSIS

K. Bulak [1], K. Wankiewicz [1], W. Łopuszyński [1]

[1] University of Life Sciences in Lublin, Lublin, Poland

Background
The tissue microarray (TMA) is used for microscopic evaluation of a large number of formalin-fixed, paraffin-embedded tissue cores, simultaneously applied to an acceptor paraffin block. Mitotic count (MC) is used to determine the histological grade of tumours. The Ki-67 marker is widely used in oncology to assess the proliferative index (PI).

Materials & Methods
Twenty-four cases of oral amelanotic melanoma in dogs aged 7–18 years were selected for the study and grouped according to the Smedley criteria into low (n=12) and high (n=12) histological malignancy grade. TMA cores of 2 mm in diameter were analyzed after extraction from donor paraffin blocks. The MC was compared between TMA cores (2 TMA cores per case) and whole tissue sections in low- and high-grade tumours after visualization of mitotic figures by H&E staining. The PI was compared between low- and high-grade tumours in TMA cores (2 TMA cores per case) after assessment of Ki-67 protein immunoreactivity. Microscopic slides were evaluated using 3DHistech software. Statistical analysis was performed using IBM SPSS Statistics 25.

Results
There were no significant differences in MC values between TMA cores and whole tissue sections in low- and high-grade melanomas. No significant differences were observed between low- and high-grade tumours and PI in TMA cores.

Conclusion
TMA cores of 2-mm in diameter provide similar MC results compared to those obtained from whole tissue sections. This study indicates that PI consistent with growth rate is similar in low- and high-grade canine oral amelanotic melanomas.

459 | CANINE MAMMARY ANAPLASTIC CARCINOMAS: RESULTS FROM A PORTUGUESE MULTICENTRIC STUDY

D. Rebelo [1] L. Silva [1], A. Santos [2], F. Seixas [3], M. Pires [3], A. Alves [3], C. Marrinhas [4], H. Vilhena [5], F. Ferreira [6], J. Santos [7], P. Patrícia [8], J. Correia [6], P. Faísca [9], A. Gama [3]

[1] Dep. Vet. Sciences, Univ. Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal
[2] ICBAS-Univ. Porto, Centro Est. Ciência Animal (CECA), AL4Animals Assoc. Lab., Porto, Portugal
[3] Dep. Vet. Sci., UTAD, Animal and Vet. Res. Cent.-CECAV, AL4Animals Assoc. Lab, Vila Real, Portugal
[4] Onevetgroup - Hosp. Veterinário do Baixo Vouga, Aveiro, Portugal
[5] ICBAS-UP; Cent. Inv. Vasco Gama (CIVG - EUVG); CECAV; AL4Animals Assoc. Lab, Porto, Portugal
[6] F.Med.Vet-ULisboa; Center Interd. Res. Anim. Health-CIISA, AL4Animals Assoc. Lab, Lisboa, Portugal
[7] DNAtech Veterinary Laboratory, Lisboa, Portugal
[8] ICBAS-UP; LAQV, REQUIMTE, Univ. Porto, Porto, Portugal
[9] DNAtech Vet. Laboratory; CECAV; Al4Animals; FMV-ULusófona, Centro Univ. Lisboa, Lisboa, Portugal

Background
Canine mammary anaplastic carcinoma (MAC) is an uncommon but highly aggressive mammary tumor subtype. The aim of this multicentric study was to gather and characterize a consistent number of MAC from four Portuguese institutions.

Materials & Methods
Canine mammary tumors (CMT) submitted for histopathology between 2001 and 2022 were retrospectively assessed from the laboratory databases. CMT with a diagnosis compatible with MAC were reviewed and histologically graded (Zappulli et al., 2019). Immunohistochemistry was performed in a subset of MAC for molecular phenotype classification, using antibodies for estrogen/progesterone receptors (ER/PR), HER-2 and Ki-67, according to Goldhirsch et al. (2013) criteria for human breast cancer.

Results
Seventy cases were diagnosed as MAC, being characterized by the proliferation of anaplastic epithelial cells that invade the surrounding normal or hyperplasic/dysplastic mammary gland and periglandular tissue. Dogs were mainly mixed breed (31/62; 50%), with a mean age of 9.8years. Most MAC were high-grade (n=56;80%) and most cases with available lymph nodes presented metastases at diagnosis (22/34;64,7%). Eleven cases (15.7%) presented characteristics of an inflammatory mammary carcinoma. Molecular phenotype was investigated in 38 cases, with most MAC being classified as Luminal B-like HER2 negative (ER and/or PR+; HER2-;Ki67≥20%) (n=22;57.9%) and Triple Negative (TN) (ER and PR-; HER2-) (n=10;26.3%) phenotypes.

Conclusion
MAC were generally high-grade carcinomas, with LN involvement at diagnosis and classified as Luminal B-like (highly proliferative) or TN immunophenotypes, both classically associated with poor outcome. Further research is required to identify novel therapeutic targets for MAC, fostered by the participation of collaborative multicenter networks.

462 | INTRAVASCULAR T-CELL LYMPHOMA WITH PREDOMINANT RENAL INVOLVEMENT IN TWO DOGS: HISTOPATHOLOGIC AND IMMUNOHISTOCHEMICAL CHARACTERIZATION

E. De Luca [1, 2], M. Carossino [1, 3], F. Del Piero [1, 3]

[1] Department of Pathobiological Sciences, School of Veterinary Medicine, LSU, Baton Rouge, United States
[2] LADDL/LSU Diagnostics, School of Veterinary Medicine, LSU, Baton Rouge, United States
[3] LSU Diagnostics, SVM, Louisiana State University, Baton Rouge, United States

Background
Intravascular lymphoma (IVL) is a rare angiotropic neoplasm characterized by an intravascular proliferation of neoplastic lymphocytes in the absence of a primary extravascular mass. In dogs, IVL is uncommon, and most of the patients reported nervous clinical signs with brain and spinal cord involvement. In this study we described two cases of IVL with a predominant renal involvement and the immunophenotype of the neoplastic lymphocytes.

Materials & Methods
Azotemia due to elevated BUN and creatinine was identified in two 5-year-old dogs, leading to a diagnosis of acute kidney disease. Postmortem examination, histopathology and immunohistochemistry were performed to characterize the neoplastic population in the kidney.

Results
Necropsy revealed a severe bilateral nephromegaly and hemorrhage. Histologically, glomerular and interstitial blood vessels were occluded by sheets of neoplastic round and large cells with marked anisocytosis and anisokaryosis. A similar neoplastic population was also noted in the blood vessels of the lung, cerebral cortex, meninges, and eye in one case. The staining pattern was compatible with a diagnosis of T-cell IVL (CD3+, granzyme B+), with scattered CD20+ B-cell aggregates interpreted as inflammatory foci. A moderate number of IBA1+ monocytic cells was also observed intravascularly.

Conclusion
These are two cases of T-cell IVL with a systemic distribution, characterized by a predominant renal involvement, followed by respiratory and central nervous system, highlighting the invasive potential of IVL. These findings underscore the importance of postmortem evaluation and IHC to accurately identify such cases of “atypical lymphoma”.

466 | SQUAMOUS CELL CARCINOMA AND APOCRINE ADENOCARCINOMA ARISING FROM MULTIPLE SWEAT GLAND HAMARTOMAS IN A CAT

V. Muller [1], E. Reyes-Gomez [2], D. Prata [3], M. Coste [1]

[1] Antech Laboratories, Massy, France
[2] Ecole nationale vétérinaire d'Alfort , Maisons-Alfort, France
[3] IDEXX Laboratories, Saint-Denis, France

Background
Introduction : In cats, sweat glands tumors are well described, but sweat gland nevi or hamartomas had only been rarely reported. They are considered benign although they may locally recur after excision.

Materials & Methods
Materials and Methods : A female domestic shorthair cat exhibited progressive skin lesions since birth. They presented as multiple raised, well-circumscribed, alopecic, hyperpigmented plaques, ranging from ovoid to linear in shape and affecting the left forelimb (extending from the nailbed to the shoulder), the left thorax, and the lumbar area. Histopathological examination was performed on samples obtained at 9 months of age. Seven years later, further sampling was prompted by the extension and development of deep ulceration of the limb lesions.

Results
Results : Initial biopsies harvested at several locations showed multiple cystic and severely hyperplastic apocrine sweat glands compatible with congenital harmatomas. Seven years later, identical lesions were identified on the limb with occurrence of concurrent but spatially distinct squamous cell carcinoma and apocrine adenocarcinoma.

Conclusion
Conclusion : This is the first report of multiple sweat gland hamartomas in a cat that progressed towards two different malignancies that appeared concomitantly. This example encourages to closely monitor this type of lesion in cats.

FORENSIC

30 | COMMON FINDINGS OF FORENSIC POSTMORTEM EXAMINATIONS OF EQUIDS

J. Coltart [1]

[1] University of Liverpool, Neston, United Kingdom

Background
Equine welfare is an area of growing concern, with an increasing demand for forensic postmortem examinations (PMEs) to assess cases of suspected neglect or abuse. This study aims to identify the most frequent diagnoses encountered in forensic equine PMEs, thereby highlighting the key challenges faced by veterinary pathologists in welfare-related investigations.

Materials & Methods
Data were collected from forensic equine PME reports submitted to the University of Liverpool Veterinary Pathology Service between October 2017 and September 2023. Diagnoses and descriptive case details were extracted from the database, categorised, summarised, and subjected to logistic regression analysis where applicable.

Results
A total of 93 forensic cases were analysed. Cobs were the most commonly represented breed. The predominant findings included starvation (37%), poor general management (24%), severe hoof overgrowth (7%), ragwort toxicity (7%), infections (5%), masses/tumours (5%), blunt trauma and/or fractures (5%), and severe wounds (4%). Compared with non-welfare PMEs conducted over the same period, forensic cases were more likely to involve female horses or stallions, animals at vulnerable ages (very young or old), individuals with low body condition scores, and those that had been euthanised.

Conclusion
This study provides valuable insights into the common pathological findings in equine welfare cases and underscores trends that may aid in future investigations. Given the limited published literature on this topic in the UK, these findings contribute important data to support further research in veterinary forensic science and equine welfare.

34 | THE PORCINE CLAW IN FORENSIC PATHOLOGY: OPPORTUNITIES AND LIMITATIONS

G. Rosato [1], F. Seehusen [1]

[1] University of Zurich, Zurich, Switzerland

Background
The pathological examination of porcine claws is a valuable diagnostic tool in both individual and herd health assessments. It allows detailed diagnosis of claw diseases—such as abscesses, ulcers, and structural changes—and identification of underlying causes like bacterial infections. Trauma-related lesions linked to inadequate housing can also be detected, offering insights into animal welfare and management improvements.

Materials & Methods
At the Institute of Veterinary Pathology Zurich (IVPZ), claw examinations are mostly performed for forensic purposes. The process includes reviewing case history, macroscopic examination with photographic documentation, and sample collection. Claw measurements are compared with reference standards. Dehorning allows assessment of the dermis, while deeper structures—bones, joints, and ligaments—are evaluated by sectioning the claw along the median plane.

Results
Between 2019 and 2024, the IVPZ performed 505 forensic necropsies, with 90 cases (18%) involving pigs. Among these, 42 (47%) were full necropsies and 48 (53%) partial or organ submissions. The cases included 34 adult pigs, 21 fattening pigs, and others such as abortions or piglets. Limb-related conditions, including claw disorders, were identified in 59 cases (66%). Frequent diagnoses included horn overgrowth, abscesses, septic arthritis, tendinitis, and osteomyelitis. Most cases were referred by veterinary offices.

Conclusion
Claw examination plays a vital role in herd health evaluation. By combining diagnostic techniques and clinical data, disease patterns can be identified and welfare strategies developed. However, post-mortem delays, hard tissue processing, and financial limitations may affect outcomes. Small lesions may require advanced imaging, which is resource-intensive.

67 | EXPRESSION OF CELL SURFACE MARKERS CD34, CD45 AND CD105 IN GRANULATION TISSUE DURING PORCINE SKIN WOUND HEALING

C. Bækgård [1], L. Andresen [1], M. Hansen [1], K. Freude [1], K. Barington [1]

[1] University of Copenhagen, Frederiksberg, Denmark

Background
During skin wound healing, circulating bone marrow-derived cells are attracted to the wound site. Part of these cells express CD34, CD45, and CD105. This study is aimed to determine if selected cell surface markers are expressed in a time-dependent manner in granulation tissue (GT), which potentially could be used for assessing wound age in veterinary forensic pathology.

Materials & Methods
Experimental wounds were surgically incised on the backs of 47 pigs. Wounds were left to heal by second intention until pigs were euthanized on day 5, 10, 15, 20, 25, 30, or 35, and GT was sampled. Levels of CD34 and CD105 positive cells in GT were examined using flow cytometry and cell sorting. Moreover, levels of CD45 positive cells were examined using immunohistochemical staining of the GT. One-way ANOVA with multiple comparisons was performed to discover differences between the groups ($P>0.05$).

Results
A total of 32, 35, and 39 wounds were included in the evaluation of CD34, CD105, and CD45 positive cells, respectively. CD34 positive cells ranged from 7% to 24%, and no differences were seen between groups. The percentage of CD105 positive cells ranged from 1% to 12%, and the highest expression were found in wounds of 15 days of age. The percentage of CD45 positive cells ranged from 15% to 70%, and the number of positive cells decreased with increasing wound age.

Conclusion
CD45 and CD105 positive cells in GT showed a time-dependent pattern that may potentially be useful for obtaining objective age assessments of wounds.

81 | POST-MORTEM INTERVAL DETERMINATION USING RNA DEGRADATION ASSESSMENT TECHNIQUES IN CAT (FELIS CATUS)

L. Botta [1], S. Divari [1], P. Pregel [1], E. Ricci [2], L. Ressel [2], F. Scaglione [1]

[1] Dept. Veterinary Sciences, University of Turin, Grugliasco, Italy
[2] Dept. Veterinary Anatomy Phisiology and Pathology, University of Liverpool, Liverpool, United Kingdom

Background
Estimating the time since death, or Post-Mortem Interval (PMI), is a crucial aspect of forensic veterinary pathology. Although numerous factors are implicated in decomposition timescale, environmental temperature and its variation strongly affect its progression and the accuracy of PMI estimates, respectively. Accumulated Degree Days (ADDs) assist PMI determination, by adding daily average environmental temperature. Biomolecular techniques, particularly RNA-based analyses, offer promising applications due to the intrinsic instability of RNA after death.

Materials & Methods
Fifty-eight deceased cats, collected from veterinary clinics, underwent forensic autopsy. Six cats served as controls and were necropsied within one hour post-mortem. The others were exposed to either an outdoor environment ("field") or stored at +4°C ("refrigerated") for 1, 3, 14 and 28 days (6 cats each). ADDs associated with each cat were then calculated. Microfluidic electrophoresis was performed on liver samples to obtain RNA Integrity Number (RIN) and DV200 values, alongside qPCR and digital droplet PCR to analyse the degradation of target reference gene mRNAs (RPL17, GAPDH, HMBS). Group differences were assessed by Kruskal-Wallis test.

Results
A statistically significant reduction ($p<0.05$) of RIN, DV200 and RPL17-mRNA levels was observed in "field" cats within the 51-200 ADDs range, compared to controls. In the "refrigerated" group similar degradation patterns were found between 14- and 28-day cats compared to controls (RIN, DV200 and qPCR results on GAPDH-mRNA, $p<0.01$).

Conclusion
RIN and DV200 seem promising biomarkers for PMI estimation, particularly for differentiating recent deaths from those exceeding 50 ADDs. Further validation on larger sample sizes is recommended.

379 | PATHOLOGICAL FINDINGS OF STARVATION IN HORSES

H. Crosby-Durrani [1], J. Coltart [1]

[1] University of Liverpool, Neston, United Kingdom

Background
The demand for equine forensic postmortem examinations (PMEs) is increasing, with many welfare cases being submitted as potentially starved due to emaciated body condition score (BSC). Diagnosing starvation requires all other disease processes to be excluded. While characteristics of starvation are available for humans and dogs, these are not entirely applicable to equids, as hindgut fermenters whose gastrointestinal tract (GIT) is rarely empty.

Materials & Methods
A retrospective study was conducted using the University of Liverpool Veterinary Pathology database, sampling cases submitted for forensic equine PME between October 2017 -September 2023. Extracted data were categorised, described, and subjected to logistic regression analysis.

Results
Eighty-three equine forensic cases were included, with 34 suspected starvation and 49 other neglect cases without suspicion of starvation. Starvation was classified when no other disease process could explain the emaciated BSC. Suspected starvation cases comprised 25 ponies and 9 horses. The non-starvation neglect cases comprised 39 ponies and 10 horses. Common findings in starvation cases included reduced muscle mass (Odd's ratio (OR): 200, p <0.01), absence of fat stores (OR: 11.58, p <0.01), serous atrophy of fat (OR: 26.07, p <0.01), atrophy of the liver, and ulceration of the stomach. Horses were more likely to be starved with no other disease processes present than ponies (OR: 5.0, p 0.05).

Conclusion
Starvation as the sole cause of death is unusual in equines, which typically have concurrent exacerbating conditions. Horses are more likely to present with starvation alone than ponies, which commonly have additional disease processes.

409 | CORTICOTROPIN-RELEASING FACTOR (CRF) AS A BIOMARKER FOR AGONAL STRESS IN FELINE FORENSIC CASES

W. Huang [1], C. Lin [1]

[1] National Taiwan University, Taipei, Taiwan

Background
Understanding the extent of suffering experienced by animals before death is a key concern in veterinary forensic science.

Materials & Methods
To address the lack of reliable biomarkers for this purpose, this study investigates corticotropin-releasing factor (CRF) as an indicator of the agonal phase in cats. A total of 79 forensic necropsy cases were retrospectively analyzed using immunohistochemistry (IHC), with 12 cases additionally examined via in-situ hybridization (ISH).

Results
A total of 79 forensic necropsy cases were retrospectively analyzed using immunohistochemistry (IHC), with 12 cases additionally examined via in-situ hybridization (ISH). CRF mRNA was primarily detected in the hypothalamus and thalamus, while CRF peptides were observed in multiple brain regions, including the neocortex, hippocampus, and amygdala. ISH signals were markedly reduced in tissues subjected to freeze-thaw cycles and postmortem delays. Western blot analysis confirmed CRF expression consistent with the expected precursor peptide size. Statistical analysis revealed significantly higher CRF expression in refrigerated tissues and in animals with body condition scores (BCS) of 6–9. Notably, CRF peptide levels were elevated in cases with short agonal periods and in deaths involving severe distress.
These findings suggest that CRF expression levels may correlate with agonal stress and indicate the potential of CRF as a biomarker for evaluating the agonal phase in veterinary forensic investigations.

Conclusion
To our knowledge, this is the first study to comprehensively assess CRF expression in feline forensic cases across varied agonal conditions.

HORSES

153 | SUDDEN AND UNEXPECTED DEATH IN HORSES: A RETROSPECTIVE CASE STUDY

N. Borel [1], M. Bänziger [1], A. Fürst [2], C. Schwarzwald [3]

[1] Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
[2] Equine Department, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
[3] Clinic for Equine Internal Medicine, Equine Department, Swiss Equine Cardiology, Zurich, Switzerland

Background
Sudden death (SD) and unexpected death (UD) are instances of unpredictable death events in horses. They are distinguished by the presence (SD) and absence (UD) of clinical signs prior to death. While these events have mostly been studied in racehorses, there is a lack of data concerning the general equine population, especially in Europe. In previous reports, the cause of death remained unexplained in 22-68% of cases. The aim of this study was to retrospectively evaluate similar cases in Switzerland.

Materials & Methods
At the Institute of Veterinary Pathology in Zurich, a total of 86 cases were identified over the last 20 years that could be assigned to the categories SD or UD.

Results
The main causes of death identified in the pathological examination were trauma, cardiovascular disease and gastrointestinal problems, while 25.6 % of cases remained unexplained. Our study further found that the average age at the time death was higher (11 years) compared to available data from the literature focusing on thoroughbred racehorses (4 years). Finally, we identified only 25% of cases associated with exercise as cause of death, despite it being almost always cited as the primary cause of death in racehorses.

Conclusion
This study found that unpredictable death events occur in older horses and are less commonly associated to exercise than existing literature on racehorses. These findings illustrate a need to more thoroughly investigate risk factors for SD and UD in the general horse population.

360 | OVINE GAMMAHERPESVIRUS 2-RELATED INFECTIONS IN EQUIDS FROM SOUTHERN BRAZIL

S. Headley [1], J. Carrizo [1], I. Santos [1], G. Yoshitani [1], J. Jesus Lima [1], J. Fritzen [1], F. Silva [1], I. Frederico [1], S. Zuchieri [1], A. Alfieri [1], J. Lisbôa [1], P. Pereira [1]

[1] Universidade Estadual de Londrina, Londrina, Brazil

Background
Ovine gammaherpesvirus 2 (OvGHV2) causes sheep-associated malignant catarrhal fever (SA-MCF) in Brazil, where subclinical infections due to OvGHV2 are more frequent than clinical SA-MCF. Descriptions of OvGHV2-related infections in equids worldwide are limited; all documented cases were associated with clinical outbreaks of SA-MCF. This report presents the findings of OvGHV2-infections in equids without typical manifestations of SA-MCF.

Materials & Methods
Two horses that died with acute neurological syndromes and a mule were submitted for necropsy. Additionally, organs of the two horses and the mule and nasal samples from five horses that cohabited on the same farm were submitted for the molecular detection of OvGHV2.

Results
Both horses had cerebral edema and necrotizing hepatitis with lymphoplasmacytic enteritis in one of these. The mule had widespread lymphadenitis, interstitial pneumonia, with necrotizing and lymphocytic enteritis. PCR detected OvGHV2 DNA in multiple tissues of the two horses and the mule as well as in two of the nasal samples of the horses. Equine herpesvirus and *Sarcoscystidae* members were not detected by PCR.

Conclusion
The detection of OvGHV2 DNA in the organs of the horses that died as well as in the nasal samples of two horses from the same farm and the mule indicated the participation of this virus in the development of the syndromes herein described. These findings add to the occurrence of OvGHV2-related infections in mammalians from Southern Brazil without classical manifestations of SA-MCF. Additionally, a lamb from the farm where the horses were maintained was subclinically infected by OvGHv2.

LABORATORY ANIMALS

36 | UNEXPECTED ENDOCRINE PANCREATIC HYPERPLASIA AFTER SINGLE INTRAVENOUS ADMINISTRATION OF AT-211 IN ICR MICE

S. Do [1], Y. Koo [1], Y. Choi [2], H. Chung [2]

[1] Konkuk University, Seoul, South Korea
[2] Korea Institute of Radiological and Medical Sciences, Seoul, South Korea

Background
Astatine-211 (At-211) is an alpha-emitting radionuclide under development in Korea for the treatment of intractable cancers and infectious endocarditis. While its manufacturing, biodistribution, and toxicity have been investigated, most studies focus on organs with high radionuclide uptake, such as the liver, kidneys, and bone marrow. Pancreatic effects of At-211 remain poorly characterized.

Materials & Methods
At-211 was intravenously administered to 6-week-old male ICR mice at various doses. Histopathological examination of major organs and immunohistochemistry for pancreatic markers were performed. In a separate study, CBC and blood chemistry were analyzed at multiple points following At-211 administration.

Results
At-211-treated mice exhibited pancreatic endocrine hyperplasia with enlarged islets as confirmed by strong immunoreactivity insulin and glucagon-like peptide-1 receptor (GLP-1R). At-211-treated mice revealed significantly decreased serum glucose levels compared to untreated controls, suggesting possible increase in insulin production.

Conclusion
Although pancreatic uptake of At-211 is presumed to be low, pancreatic endocrine islet hyperplasia and reduced glucose level were observed. These findings suggest the inclusion of pancreatic evaluation in the safety assessment of therapeutic radionuclides, even when direct tissue exposure appears minimal as observed with At-211.

42 | DEEP LEARNING ALGORITHM FOR IDENTIFYING AND QUANTIFYING GLOMERULOSCLEROSIS IN A RENIN-AAV DB/DB UNX MOUSE MODEL OF DIABETIC NEPHROPATHY

J. Schoening [1], A. Balsamo [2], E. Huynh Cong [3], J. Gerdes [3]

[1] Evotec SE, Hamburg, Germany
[2] Evotec France SAS, Toulouse, France
[3] Evotec International GmbH, Goettingen, Germany

Background
Artificial intelligence (AI) and deep learning are revolutionizing the segmentation and quantification of complex histopathological tissues. AI-assisted whole slide image (WSI) analysis can improve efficiency for both research and diagnostic purposes compared to traditional microscopic assessment. We generated a mouse model of chronic kidney disease harbouring hypertension, obesity and diabetes comorbidities. These mice develop heavy proteinuria and glomerulosclerosis, similar to advanced type 2 diabetes in humans.
We present a deep learning algorithm to identify and quantify glomerulosclerosis in the mouse kidney.

Materials & Methods
Mice homozygous for the leptin receptor mutation (db/db) underwent unilateral nephrectomy and received either LacZ-AAV, or renin-AAV to induce hypertension. Half of the cohort received lisinopril as a treatment control. After termination, formalin-fixed paraffin-embedded kidney sections were PAS-stained and digitized. Supervised iterative algorithm training was performed on WSIs with Visiopharm® software to distinguish glomeruli from background tissue, and to identify sclerotic tissue within the non-sclerotic mesangial matrix of each glomerulus. Glomeruli were scored based on glomerulosclerosis area percentage, and a glomerulosclerosis index (GI) was calculated for each animal.

Results
The algorithm effectively detected glomeruli and sclerosis within glomeruli, highlighting differences between treatment groups. The percentage of severely sclerotic glomeruli and GI were significantly increased in hypertensive groups, compared to LacZ-AAV controls and lisinopril treated groups. Results further correlated with renal functional parameters.

Conclusion
We successfully developed an AI algorithm for glomerulosclerosis detection and demonstrate its capabilities in an *in vivo* mouse model. In the future, we aim to extend this tool to further nephropathy models.

85 | INTEGRATING MORPHOLOGICAL AND TRANSCRIPTIONAL CHANGES IN THE BRAIN FOLLOWING SARS-COV-2 NEURONAL INFECTION IN THE K18-HACE2 MURINE MODEL

S. De Neck [1], R. Penrice-Randal [1, 2], U. Hetzel [1], P. Sharma [3], E. Bentley [3], A. Kirby [3], G. Balistreri [4, 5], J. Stewart [3], A. Kipar [1, 3]

[1] LAMP, Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
[2] Department Biochemistry, Cell & Systems Biology, University of Liverpool, Liverpool, United Kingdom
[3] Department of Infection Biology and Microbiomes, University of Liverpool, Liverpool, United Kingdom
[4] Department of Virology, Faculty of Medicine, University of Helsinki, Helsinki, Finland
[5] School of Biomedical Sciences, The University of Queensland, Brisbane, Australia

Background
SARS-CoV-2, the causative agent of COVID-19, frequently affects the central nervous system clinically, with symptoms reported both in acute COVID-19 and Long COVID. The pathomechanisms underlying the neurological impairments remain incompletely understood, in particular since SARS-CoV-2 is only rarely detected in patient brains. Using the K18-hACE2 murine model, the present study investigated morphological and molecular changes associated with neuronal infection, mimicking a potential worst-case scenario.

Materials & Methods
The brains of K18-hACE2 mice, infected intranasally with SARS-CoV-2 Delta or mock infected, were examined by light and transmission electron microscopy, including immune-EM for viral NP, to investigate the effect and outcome of neuronal infection. Bulk RNA sequencing, followed by differential gene expression, pathway enrichment, and deconvolution approaches served to investigate transcriptomic changes associated with the infection, with a focus on neurons.

Results
Ultrastructural examination of infected neurons revealed the presence of SARS-CoV-2 at various stages of replication, confirming that the virus can complete its replication cycle in neurons. Infection of the neurons was not associated with overt pathological changes. However, infected brains showed a distinct transcriptomic profile. In addition to the positive enrichment of pathways associated with inflammation, immune and antiviral responses, we observed negative enrichment of pathways associated with synapses, axon and neuron projections, and certain behaviours.

Conclusion
The results showed that *in vivo*, neurons are permissive for SARS-CoV-2 in K18-hACE2 mice, allowing completion of the viral replication. While not associated with overt cytopathic effects, neuronal infection may induce functional changes that could potentially be transient.

154 | SPONTANEOUS LESIONS OF THE GLANDULAR STOMACH IN CBYB6F1-TG(HRAS)2JIC MICE IN 26-WEEK CARCINOGENICITY STUDIES

M. Levi [1], V. Mowat [1], R. Wakefield [1]

[1] Labcorp, Huntingdon, United Kingdom

Background
The CByB6F1-Tg(HRAs)2Jic mouse (rasH2® mouse strain) is employed in 26-week carcinogenicity studies of pharmaceuticals. The incidence of spontaneous lesions of the glandular stomach was determined and their morphology and significance were discussed.

Materials & Methods
Historical control data were provided for 422 males and 320 females from nine 26-week carcinogenicity studies conducted between 2015 and 2022 at Labcorp, Huntingdon/Eye, UK. The administration route included 6 oral gavage, 3 subcutaneous and 1 intravenous studies. Animals came from two suppliers.

Results
Findings commonly observed and associated within the same tissue included mucosal inflammatory cells (neutrophilic/mixed) infiltrates [31/422 total incidence (TI), 0-24% range of incidence (RI) in males; 41/320 TI, 0-28% RI in females], hypertrophy of mucous cells [31/422 TI, 0-33% RI in males; 16/320 TI, 0-28% RI in females], hyperplasia of the glandular mucosa [16/422 TI, 0-16% RI in males; 32/320 TI, 0-28% RI in females]. The incidence of erosion/ulcers was low [10/422 TI, 0-13% RI in males; 8/320 TI, 0-10% RI in females]. Correlated macroscopic observations comprised masses, raised areas/thickening and depressions of the glandular mucosa. No association of findings with the dosing procedure was seen. In males only, mucous cell hypertrophy appeared more common in animals from Supplier B than Supplier A (12% vs 0.5% TI, respectively, $p < 0.01$).

Conclusion
High variability in the incidence of findings was observed, particularly in males. Hyperplastic and hypertrophic findings occurred mostly in association with inflammatory changes, likely indicating a response to a chronic stimulus and are possibly a sequel to erosions/ulcers.

197 | ROLE OF EPITHELIAL-MESENCHYMAL TRANSITION IN SUNITINIB-INDUCED METASTATIC DISSEMINATION IN A XENOGRAFT ORTHOTOPIC TRIPLE-NEGATIVE BREAST CANCER MOUSE MODEL

L. Sala [1, 2, 3], G. Dellavedova [3, 4], L. Bertola [1, 2], G. Magani [1, 2], A. Mangoni [5], S. Tommasi [5], J. Hulin [5], C. Ghilardi [4], C. Recordati [1, 2]

[1] Department of Veterinary Medicine and Animal Sciences (DIVAS), University of Mil, Milan, Italy
[2] Mouse & Animal Pathology Laboratory (MAPLab), Unimi Foundation, Milano, Italy, Milan, Italy
[3] These authors contributed equally to this work, Milan, Italy
[4] Department of Experimental Oncology, IRCCS Istituto di Ricerche Farmacologiche M, Milan, Italy
[5] Flinders Health and Medical Research Institute, Flinders University, Adelaide, A, Adelaide, Australia

Background
Sunitinib is an anticancer agent that suppresses tumour growth by inhibiting angiogenesis but has shown poor efficacy in Triple-Negative Breast Cancer (TNBC). Previous evidence suggests that Sunitinib may paradoxically favour metastatic dissemination through endothelial cell senescence or increase in cancer stem cells, as well as promote tumor aggressiveness through vasculogenic mimicry (VM). This study aimed to investigate the mechanisms underpinning Sunitinib-induced metastatic dissemination in a xenograft orthotopic TNBC mouse model.

Materials & Methods
Human MDA-MB-231 cells were implanted into the mammary fat pad of SCID mice. Mice were treated with Vehicle or Sunitinib (40 mg/kg orally) for 5 weeks and sacrificed at the end of treatment (interim endpoint) or at terminal endpoint (n = 4-8/group). Tumors and lungs were formalin-fixed and paraffin embedded. Tumors were stained with PAS and CD31 to assess VM and angiogenesis, and lungs were stained with MHCI to quantify metastases. Expression of Epithelial-Mesenchymal Transition (EMT) markers (CDH2, SNAI1, SNAI2, TWIST1, VIM) was assessed by RT-qPCR on frozen tumor samples.

Results
Sunitinib significantly reduced tumor volume, but increased lung MHCI+ metastases, ultimately resulting in no survival benefit. Intratumoral CD31+ area was significantly reduced at interim endpoint in treated tumors. No VM structures were observed. Expression of CDH2 and SNAI2 was significantly upregulated in mice treated with Sunitinib at both endpoints.

Conclusion
While Sunitinib effectively reduces tumor growth and angiogenesis, it concurrently promotes lung metastasis, potentially through EMT rather than VM. Further investigation is warranted to confirm the role of EMT in Sunitinib-induced metastatic dissemination in TNBC.

208 | QUANTITATIVE EVALUATION OF SARS-COV-2 INFECTION ASSOCIATED LESIONS IN THE BRAINS OF K18-HACE2 MICE

M. Rosiak [1, 2, 3], K. Hülskötter [1], K. Gregor [1], E. Leitzen [1], G. Beythien [1], S. Clever [4, 5], M. Ciurkiewicz [1], L. Allnoch [1], C. Meyer Zu Natrup [4, 5], T. Tuchel [1], A. Volz [4, 5], W. Baumgärtner [1]

[1] Department of Pathology, University of Veterinary Medicine Hannover, Hannover, Germany
[2] Center for Systems Neuroscience, Hannover, Germany
[3] Research Training Group Virus detection, pathogenesis and intervention VIPER, Hannover, Germany
[4] Research Center for Emerging Infections and Zoonoses, Hannover, Germany
[5] Institute of Virology, University of Veterinary Medicine Hannover, Hannover, Germany

Background
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of coronavirus disease 2019 (COVID-19). Although respiratory symptoms are the most known feature of COVID-19, neurological manifestations also occur frequently in patients with reported incidence ranging from 36% to 100%. The specific mechanism of central nervous system invasion and neurological injury following SARS-CoV-2 infection are still unclear and require further investigation.

Materials & Methods
K18-hACE2 mice were intranasally infected ($5\times10^4 TCID_{50}$, Germany/BavPat1/2020 strain) with SARS-CoV-2 and sacrificed 3, 6, 7 or 8 days post infection (dpi). Brains were collected and histology and immunohistochemistry were performed.

Results
Brains of infected animals showed mild to moderate, lymphohistiocytic, perivascular, meningeal and parenchymal infiltration, as well as vacuolization of the neuropil and neurons. Inflammatory changes and detection of SARS-CoV-2 antigen were mainly observed in animals sacrificed after 6, 7 and 8 dpi. Lesions were evenly distributed throughout the cerebrum and brain stem, while being absent or minimal in the cerebellum. Additionally, a moderate microgliosis with associated phenotypical changes was observed.

Conclusion
SARS-CoV-2 infection associated lesions in K18-hACE2 mice were most prominent at 6 dpi and include mild to moderate, lymphohistiocytic meningoencephalitis with microgliosis, as well as viral antigen spread.

222 | CLINICAL PATHOLOGY STANDARD PARAMETERS IN NUDE RATS.

A. Bendrimia [1], A. Bidaut [1], V. Jacquemet [1], M. Defontis [2]

[1] ERBC, Baugy, France
[2] Tox and Diag, Bully, France

Background
Long term pharmacology and safety evaluation of innovative human-derived cell therapy products require the use of immunocompromised animals to avoid immune-mediated destruction of injected human-derived cells. Athymic Nude (RH-FoxN1rnu) rats represent an interesting alternative to broadly used Nude mice, particularly when implantation surgery is required and animals of larger size are preferred. Clinical pathology data obtained from this rat strain remain sparse in the literature.

Materials & Methods
We sampled 34 untreated Nude male (N=16) and female (N=18) rats and analyzed a standard panel of hematology (Sysmex XN-1000V), biochemistry (Pentra C400) and coagulation (STA-Satellite Max) parameters routinely used in toxicology studies. Blood smears were prepared and examined. Data were qualitatively reviewed and compared with data obtained from conventional rat strains.

Results
Whilst most biochemistry and coagulation parameters were overall unchanged, in hematology, white blood cell differential counts strongly differed from those recorded in other rat strains. Alterations in blood smear examination data were present and matched with hematology analytical findings.

Conclusion
Full clinical pathology profiles of nude rats including blood smear examination enabled to characterize the Nude rat strain and to produce first reference intervals. Specific hematology changes were observed and consistent with the T-cell deficient phenotype of this rat strain. Those results should be taken into consideration when interpreting study results.

235 | SHORT-CHAIN PFAS PERINATAL EXPOSURE INDUCES COGNITIVE DYSFUNCTION AND GLIAL ACTIVATION IN ADULT RATS

G. Foiani [1], M. Moretti [2], C. Zanardello [1], M. Sannia [3], E. Melchiotti [1], A. Vitola [4], F. Gallocchio [1], L. Lorenzini [2], L. Giardino [2,3], F. Mutinelli [1], L. Calzà [3,4], M. Vascellari [1]

[1] Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padua, Italy
[2] Department of Veterinary Medical Science, University of Bologna, Ozzano dell'Emilia, Bologna, Italy
[3] IRET Foundation, Ozzano dell'Emilia, Bologna, Italy
[4] Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy

Background
Short-chain per- and polyfluoroalkyl substances (PFAS) are emerging environmental pollutants characterized by bioaccumulative properties. This study investigates the effects of perinatal exposure to GenX and PFBA on cognitive functions in adult rats and explores their potential association with neuroinflammation and neuronal impairment.

Materials & Methods
Sprague-Dawley female rats were exposed to GenX and PFBA (both at 0.5 and 5.0 mg/kg body-weight/day) via food pellets, from 30 days before mating, through gestation and lactation. Cognitive performance was evaluated in adult offspring using Morris Water Maze (MWM). Coronal FFPE hemibrains (Bregma -2.60) from female offspring of dams exposed to 5.0 mg/kg body-weight/day were analysed for the percentage of immunoreactivity (%IR) to Iba1, GFAP, and MAP2 with NIS-Elements software.

Results
In adult rats of both sexes, PFAS exposure impaired performance in the MWM probe test compared to controls (one-way ANOVA, Dunn's test: males – PFBA 0.5 mg/kg, p=0.0147; PFBA 5.0 mg/kg, p=0.0046; GenX 5.0 mg/kg, p=0.00425; females – PFBA 5.0 mg/kg, p=0.0241; GenX 5.0 mg/kg, p<0.0001). In PFBA-exposed female offspring, Iba1 %IR was increased in the hippocampus (p=0.0094) and the hypothalamus (p=0.0204). GFAP %IR was higher in the hippocampus for both GenX (p=0.0248) and PFBA (p=0.0422), and in the parietal cortex for PFBA-exposed animals (p=0.0058). No changes were observed for MAP2 %IR.

Conclusion
Perinatal exposure to short-chain PFAS impairs spatial learning and memory in adult rats, with a more pronounced effect of GenX in females. The observed increase in glial markers %IR suggests a role for glial activation and neuroinflammation in PFAS-induced cognitive dysfunction.

280 | ACUTE AND SUBACUTE ORAL TOXICITY ASSESSMENT OF KINKELIBA (COM-BRETUM MICRANTHUM G. DON) ETHANOLIC EXTRACT IN BALB/C MICE

S. Sall [1], A. Alexandru [1], R. Romelia [1], E. Ecaterina [1], D. Dragoş [1], A. Haşaş [1], A. Amiali [2], M. Meriem [3]

[1] University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania, Romania
[2] Laboratory of Food Technology and Humain Nutrition at National Higher School of , Alger, Algeria
[3] University of Bejaia, Laboratory of Plant Biotechnology and Ethnobotany, Bejaia, Algeria

Background
Combretum micranthum G. Don is a medicinal plant traditionally employed in West Africa for its diuretic and gastrointestinal therapeutic properties. Despite its extensive ethnomedicinal use, comprehensive toxicological assessments are still lacking.

Materials & Methods
For the acute toxicity assessment, female mice received oral doses of 50, 300, and 2000 mg/kg of extract for 14 days. In the subacute study, female and male mice were administered daily doses at the same concentrations over 28 days. Throughout both protocols, clinical signs, body weight, and food and water consumption were regularly monitored. At the end of each study, hematological, biochemical, and histopathological parameters were analyzed, in accordance with OECD Guideline 420.

Results
In the acute toxicity study, no mortality or significant clinical manifestations were observed at any administered dose; however, significant variations were noted in platelet counts and amylase activity. In the subacute model, slight but non-critical alterations in hepatic and renal biomarkers were detected, without any signs of systemic toxicity. Histopathological examination revealed similar lesions in both acute and subacute phases, including multifocal mixed inflammatory infiltrates in the periportal area of the liver, minimal bacterial overgrowth in the superficial layer of the gastric mucosa, minimal medullary mineralization and mononuclear inflammatory infiltrate cells in the kidneys accompanied by mononuclear inflammatory infiltrates, and minimal to moderate vacuolization in the pancreatic acini.

Conclusion
These results indicate that *C. micranthum ethanolic* extract is relatively safe at the tested doses, reinforcing its traditional use and supporting further research into its pharmacological potential.

316 | EARLY DETERMINATION OF THE OUTCOME FOLLOWING USUTU VIRUS INFECTION OF WT 129/SV MICE

M. Gerardy [1], M. Garigliany [1]

[1] University of Liège, Liège (Sart Tilman), Belgium

Background
Usutu virus (USUV) is an emerging mosquito-borne *Orthoflavivirus* closely related to West Nile Virus. Both are currently co-circulating all over Europe, causing seasonal episodes of mass mortalities in birds as well as sporadic neurological diseases in mammals, including humans. The host-pathogen interactions involved in these occurrences have not been described yet, and the growing number of *Orthoflavivirus* infections reported in Europe recently undoubtedly represents a serious threat for public health. Considering their occasional susceptibility, mammals used to study *Orthoflaviviruses* pathogenicity are mostly immunocompromised. However, the need to develop a relevant, immunocompetent mammalian model to study USUV pathogenesis has become crucial.

Materials & Methods
We first developed our own model based on the dramatically different phenotypes observed in 9 (susceptible) and 15 day-old (resistant) pups following footpad infection. We then compared the viral dissemination between these two groups over time using both qualitative (immunohistochemistry, viral isolation) and quantitative (RT-qPCR) methods, to identify the main immune barrier(s) involved in these contrasting outcomes. We later characterized the host-pathogen interactions in the identified interfaces using scRNA-seq and proteomic assays.

Results
Interestingly, even though the quantitative differences seemed minimal in the early stages of dissemination, major qualitative dissimilarities (presence/absence of infectious viral particles) were observed as soon as 24h post injection.

Conclusion
The precocity and severity of the differences spotted between our two murine models suggested a very early determination of the phenotype following the viral inoculation and a significant role of the skin in the control of the infection, which we eventually confirmed.

328 | SPONTANEOUS CUTANEOUS PAPILLOMAS IN TWO ATHYMIC NUDE MICE

L. Bertola [1, 2], G. Milite [3], G. Marsella [4], E. Scanziani [1, 2], C. Recordati [1, 2]

[1] Department of Veterinary Medicine and Animal Sciences (DIVAS)University of Milan, Lodi, Italy
[2] Mouse & Animal Pathology Laboratory (MAPLab), Fondazione Unimi, Milan, Milan, Italy
[3] Scientific Consultant, Udine, Italy
[4] IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy

Background
Mouse papillomas are exceedingly rare benign epithelial tumors associated with infection by *Mus musculus* papillomavirus (MmuPV1). Despite being extensively studied as models for viral oncogenesis, immune responses, and malignant transformation under experimental conditions, spontaneous outbreaks of murine papillomas are exceptionally rare and have been only infrequently reported.

Materials & Methods
Two Hsd:Athymic Nude-Foxn1^{nu} mice (Envigo)—one subcutaneously inoculated with an ovarian carcinoma patient-derived xenograft (PDX) and the other with a human lung carcinoma cell line—developed raised cutaneous lesions and subsequently underwent complete necropsy. Organs were processed routinely for histopathological evaluation, and immunohistochemistry for papillomavirus L1 antigen detection was performed on the cutaneous lesions.

Results
Both mice developed focal, exophytic skin lesions on the dorsal side near the interscapular region, distinct from the PDX inoculation site on the flank. One of the lesions was ulcerated. Histological examination revealed hyperplastic epithelial fronds supported by thick fibrovascular stalks. Hypergranulosis and multifocal intranuclear inclusions were observed within the epithelium, although classic koilocytotic changes were absent. Immunohistochemistry for papillomavirus L1 antigen was negative. Additionally, both mice demonstrated multifocal renal tubular degeneration and regeneration with tubular intranuclear viral inclusions, and in one case, a concurrent multicentric lymphoma was identified.

Conclusion
This report describes 2 cases of cutaneous papilloma with concurrent mouse kidney parvoviral inclusions bodies nephropathy. Further investigations are required to conclusively determine the involvement of MmuPV1 in the development of these papillomas.

375 | A FULLY ATTENUATED INTRANASAL SARS-COV-2 LIVE VACCINE INDUCES A STRONG LOCAL MACROPHAGE AND T-CELL MEDIATED IMMUNE RESPONSE

T. Britzke [1], J. Schön [1], N. Halwe [1], L. Ulrich [1], D. Hoffmann [1], M. Beer [1], A. Breithaupt [1]

[1] Friedrich-Loeffler-Institut, Greifswald - Insel Riems, Germany

Background
The SARS-CoV-2 pandemic led to the rapid development of new vaccine candidates, including live attenuated vaccines (LAV). Intranasally administered LAV mimic the natural infection and therefore induce local mucosal immunity, representing a promising alternative vaccination strategy to mRNA vaccines. We evaluated the attenuation of two genetically modified SARS-CoV-2 LAVs (OTS206, OTS228) and the morphological correlates for the nasal immune response in the Syrian hamster model.

Materials & Methods
After intranasal inoculation of OTS206, OTS228, SARS-CoV-2 wildtype virus (WT), or non-infectious cell culture medium, five hamsters each were examined two and five days post inoculation (dpi). Three levels of the nasal cavity were used for histopathological examination of the respiratory (RE) and olfactory epithelium (OE). Following immunohistochemistry, digital image analysis was applied for quantification of virus infection (SARS-CoV-Nucleoprotein), immune cell infiltrates (Iba1, CD3) and damage of OE (Beta-3-Tubulin) and RE (Cytokeratin-18).

Results
WT and OTS206 caused moderate to severe virus associated tissue damage with widespread loss of the OE. The number of macrophages increased significantly at two dpi but slightly decreased already at five dpi. In clear contrast, OTS228 infection led to only scattered infected cells and no tissue damage. Increased numbers of macrophages were detected only after 5 dpi. All infection groups showed increased numbers of T-cells at 5 dpi, but both OTS vaccines induced twice as high numbers as after WT infection.

Conclusion
We saw that the intranasal LAV OTS228 is fully attenuated. Despite the limited infection, OTS228 induces a strong macrophage and T-cell mediated immune response.

463 | WHAT'S GOING ON IN THE LIVERS IN MY STUDY? TEST-ITEM RELATED TOXICITY OR SPONTANEOUS FINDING?

M. Rezaee [1], P. Hall [1], S. Rombouts [1], F. Poosti [1], L. Ratsamy [1], J. Ling [1], R. Fleurance [1]

[1] UCB, Braine L'Alleud, Belgium

Background
Histopathology is essential in preclinical studies to identify toxicity and biodistribution of test items. However, spontaneous lesions may complicate interpretation of such studies. This study highlights hepatic lesions and their negative impact on IHC data integrity.

Materials & Methods
Mice from a biodistribution study were used to assess viral vector tissue distribution and reporter gene expression. The reporter gene, mCherry—indicating the presence of the viral vector—was evaluated following IV administration at three dose levels. A total of 47 animals were divided into 10 groups: vehicle (Group 1); Test Item 1 (Groups 2–4; low, mid, high dose respectively, same for others); Test Item 2 (Groups 5–7); and Test Item 3 (Groups 8–10). Liver was stained with H&E and IHC for mCherry. Histologic findings and mCherry expression were scored from 0 to 5.

Results
Histopathological analysis revealed multifocal hepatic necrosis, degeneration, and mononuclear cell inflammation in 17 of 47 animals (36.17%). Lesions were observed in 1 vehicle animal, 4 from Group 4, 3 from Group 5, 1 from Group 6, 3 from Group 7, 2 from Group 8, 2 from Group 9, and 1 from Group 10. Lesion severity and mCherry expression were inversely correlated. A dose-dependent increase in mCherry expression was observed in all animals, except those with lesions. One vehicle animal had severe lesions, suggesting findings were not test-item-related.

Conclusion
Hepatic lesions reduced mCherry expression, complicating interpretation. This underscores the importance of histopathological evaluation in biodistribution studies, which is often overlooked and not requested.