IBERIAN
Veterinary Pathology
Meeting 2026

JUNE

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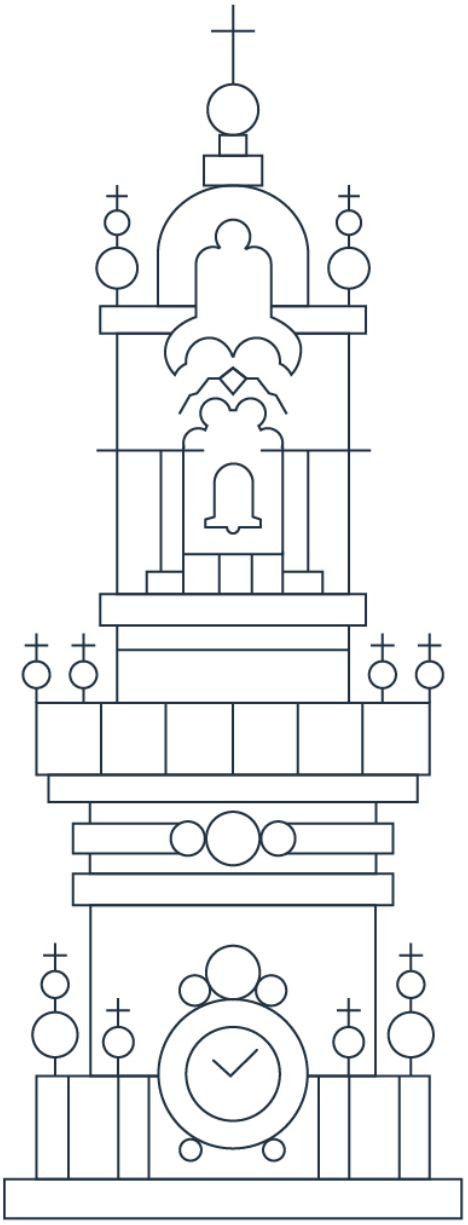

Oporto, June 16 - 19, 2026

Abstract Book

IBERIAN Veterinary Pathology Meeting 2026
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June 18–19, 2026 · Joint Meeting of SPPA & SEAPV

The Iberian Veterinary Pathology Meeting 2026 is a joint scientific event organized by the Portuguese Society of Veterinary Pathology (SPPA) and the Spanish Society of Veterinary Pathology (SEAPV), in collaboration with ICBAS – University of Porto, SPCV, and INIAV.

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- Sandra Branco – Univ. Évora (SPPA)
- Leonor Orge – INIAV (SPCV)
- Maria dos Anjos Pires – UTAD (SPCV)
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- Adelina Gama – UTAD (SPPA)
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- Ester Blasco Ortega – SEAPV
- Antonio Rodríguez Bertos – SEAPV
- Ayoze Castro Alonso – SEAPV

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Program

Scientific Programme

Wednesday, 17 June

Time	Activity	Location / Room
16:00 – 18:30	Poster set-up	Foyer of tICBAS / FFUP
19:00 – 20:30	**Welcome Reception**	*Shared entrance area Ion Floor 4 of Edifício A (Main Building),Rua Jorge Viterbo Ferreira campus, Porto*

Thursday, 18 June – Day 1

Time	Session	Title	Speaker
08:00 - 09:00	**Registration and Poster setup**		
09:00 - 09:15	**Opening Session**	Welcome Address	Organising Committee
09:15 - 10:30	**High Pathogenicity Avian Influenza (HPAI)** *Moderators: Miguel Fernández and Ana Losada*	**Recent Epidemiology and Interspecies Pathological Presentation**	**Margarida Henriques (INIAV) & Javier Asín Ros (UC Davis)**
10:30 - 11:00	Coffee Break & Poster Viewing / Poster Presentations		
11:00 - 11:10	**Oral Communications I — Infectious Diseases / Diagnostic Innovation** *Moderators: Javier Asín Ros and Adelina Gama*	O-1 Pathological characterization of the highly virulent PRRSV virus Rosalia strain in pregnant sows under experimental conditions.	Paula Curto (U Lleida)
11:10 - 11:20		O-2 Deep learning-assisted spatial quantification of PRRSV-1 by RNAscope in situ hybridization.	Giuditta Capaldi (U Córdoba)
11:20 - 11:30		O-3 Differential diagnosis of bovine tuberculosis in slaughterhouse samples with suspected lesions: optimization of histopathology and immunohistochemistry	Néstor Porras González (UCMadrid-VISAVET)
11:30 - 11:40		O-4 Developing an AI-based model to support TB surveillance at the slaughterhouse	Enric Vidal (IRTA–CReSA)
11:40 - 11:50		O-5 In vivo characterization of neutrophil extracellular traps during early Mycobacterium avium subsp. paratuberculosis infection in a sheep model.	Noive Arteche-Villasol (U León)
11:50 - 12:00		O-6 Clinicopathological Study of Mycobacterium avium subsp. hominissuis in domestic cats.	Dante Jose Rocca (UABarcelona)
12:00 - 12:10		O-7 Morphological and immunohistochemical insights into Mycobacterium pseudoshottsii infection in European sea bass	Blanca Chinchilla (UCMadrid)
12:10 - 12:20		O-8 Mass mortality event in juvenile barbels (Barbus barbus) linked to Sanguinicola sp. (Aporocotylidae)	Céline Melanie Möckli (U Bern)
12:30 - 14:00	Lunch Break & Poster Viewing / Poster Presentations		
14:00 - 14:10	**Oral Communications II — Clinical, Forensic and Digital Pathology** *Moderators: Joaquín Ortega and Ángela Alonso*	O-9 Advancing Veterinary Forensic Pathology: Diatom Identification in Formalin-Fixed Specimens for Retrospective Drowning Diagnosis.	Nicolás Aradilla (UCMadrid-VISAVET)
14:10 - 14:20		O-10 Beyond plug-and-play: AI-assisted and digital image analysis in investigative pathology	Tânia Carvalho (Champalimaud Foundation)
14:20 - 14:30		O-11 Catheter-associated venous air embolism in a hospitalized horse	Silvia Guil Luna (U Córdoba)
14:30 - 14:40		O-12 Paraprobiotic supplementation modulates intestinal immune populations in weaned piglets	Carmen Álvarez-Delgado (U Córdoba)
14:40 - 14:50		O-13 Methanobrevibacter smithii-Induced Mucosal Immune Modulation in Early-Weaned Piglets Revealed by Deep Learning-Based Histopathology	José María Sánchez Carvajal (U Córdoba)
14:50 - 15:00		O-14 Pulmonary arteriopathy associated to acute pulmonary hypertension in a young bitch following closure of left-to-right patent ductus arteriosus	Ana Garcia-Galán Pérez (U Murcia)
15:00 - 15:10		O-15 From primary tumour to metastasis: Molecular subtype changes in canine mammary tumours	Adelina Gama (UTAD)
15:10 - 15:20		O-16 Pathological findings in the 2025–2026 Foot-and-Mouth Disease outbreak in Cyprus	George Nikolaou (U Nicosia)
15:30 - 16:00	Coffee Break & Poster Viewing / Poster Presentations		
16:00 - 17:00	**Spatial Omics in Pathology** *Moderators: Tânia Carvalho and Irina Amorim*	**Unlocking Tissue Molecular Information with Mass Spectrometry Imaging**	**Eduardo Chicano Gálvez (IMIBIC)**
17:00 - 18:30	**General Assembly**		**SEAPV & SPPA**

Friday, 19 June – Day 2

Time	Session	Title	Speaker
09:00 - 10:30	**African Swine Fever (ASF)** *Moderators: Miguel Fernandez and Carlos Manuel Martinez*	**African Swine Fever in Domestic Pigs and Wild Boar: Past Lessons and Present Challenges**	**Librado Carrasco Otero (UCO) & Antonio Rodríguez Bertos (UCM–VISAVET)**
10:30 - 11:00	Coffee Break & Poster Viewing / Poster Presentations		
11:00 - 11:10	**Oral Communications III — Neoplasia and Comparative Pathology** *Moderators: Elena Mozos Mora and Ana Rodríguez Largo*	O-17 Histopathological and immunohistochemical study of integrins expression in canine gastric carcinoma	Adrián Arribas Mercado (UCMadrid-VISAVET)
11:10 - 11:20		O-18 Beyond Mammary Neoplasia: Reproductive Lesions in Queens	Maria dos Anjos Pires (UTAD)
11:20 - 11:30		O-19 Differences in histopathology and local immune response in steady and progressive transmissible venereal tumors in Mexican Dogs	Diego Pérez Maroto (U Léon)
11:30 - 11:40		O-20 Changing the Odds of Canine Lymphoma: From Characterization to Antibody-drug Conjugate Development	Rita da Silva O'Neill Pedrosa (U Lisboa)
11:40 - 11:50		O-21 Pretenders among lymphomas – don't be betrayed by the eye	Nuno Renato Amorim de Lima (IPSN-CESPU)
11:50 - 12:00		O-22 Five- year pathological monitoring of highly pathogenic avian influenza H5N1 in naturally infected poultry and wild birds in Portugal	Francisco Furtado (INIAV)
12:00 - 12:10		O-23 Characterization of tumor-associated stroma and fibrosis in feline mammary carcinomas	Joana Jesus (ICBAS)
12:10 - 12:20		O-24 Comparative proteomic profiling of tuberculous granulomas at different stages of lesion development in cattle and pigs using MALDI-imaging mass spectrometry	Jaime Gómez Laguna (U Córdoba)
12:30 - 14:00	Lunch Break & Poster Viewing / Poster Presentations		
14:00 - 14:10	**Oral Communications IV — Teaching, Fish Pathology and Zoo/Wildlife** *Moderators: Ana Losada and Maria A. Pires*	O-25 Gamification, game-based learning and veterinary pathology education	Estefanía Montero (U Cardenal Herrera)
14:10 - 14:20		O-26 Comparative epidemiological analysis of tumors of the digestive system in dogs and cats	Diana Araújo (ICBAS)
14:20 - 14:30		O-27 Unrecognized manifestations of chronic furunculosis in turbot (Scophthalmus maximus): deep muscular lesions and pericarditis associated with *Aeromonas salmonicida*	Xoel Souto Guitián (U Santiago de Compostela)
14:30 - 14:40		O-28 Disseminated fatal toxoplasmosis in captive ring-tailed lemurs (*Lemur catta*): a case series	Javier Mª De Pablo-Moreno (UCMadrid-VISAVET)
14:40 - 14:50		O-29 Immunohistochemical study of pleural mesothelioma in an angolan lion (*Panthera leo bleyenberghi*)	Cristina Alabajos Báguena (U Cardenal Herrera)
14:50 - 15:00		O-30 Arthritis and osteomyelitis caused by *Enterococcus* spp. in periurban sparrows	Ana Isabel Abad Fau (Universidad de Zaragoza)
15:00 - 15:10		O-31 Granulomatous pneumonia in sea turtles	Laia Fabra Dalmau (U Cardenal Herrera)
15:10 - 15:20		O-32 Development of a pilot model to estimate *post-mortem* interval (PMI) in elasmobranchs	Ayoze Castro Alonso (U Las Palmas de Gran Canaria)
15:30 - 16:00	Coffee Break & Poster Viewing / Poster Presentations		
16:00 - 17:00	**Lumpy Skin Disease** *Moderators: Jaime Gómez Laguna and Sandra Branco*	**Lumpy skin disease outbreak in Catalonia**	**Enric Vidal Barba** (IRTA–CReSA)
17:00 - 17:30	**Final Scientific Session**	**Awards, Session Chairs Summary & Open Discussion**	**Tânia Carvalho and Jaime Gómez Laguna**
17:30 - 18:00	Poster removal		
19:30	**Conference Dinner at Casa da Música, Porto**		

Poster Presentation/Discussion Schedule

Group A

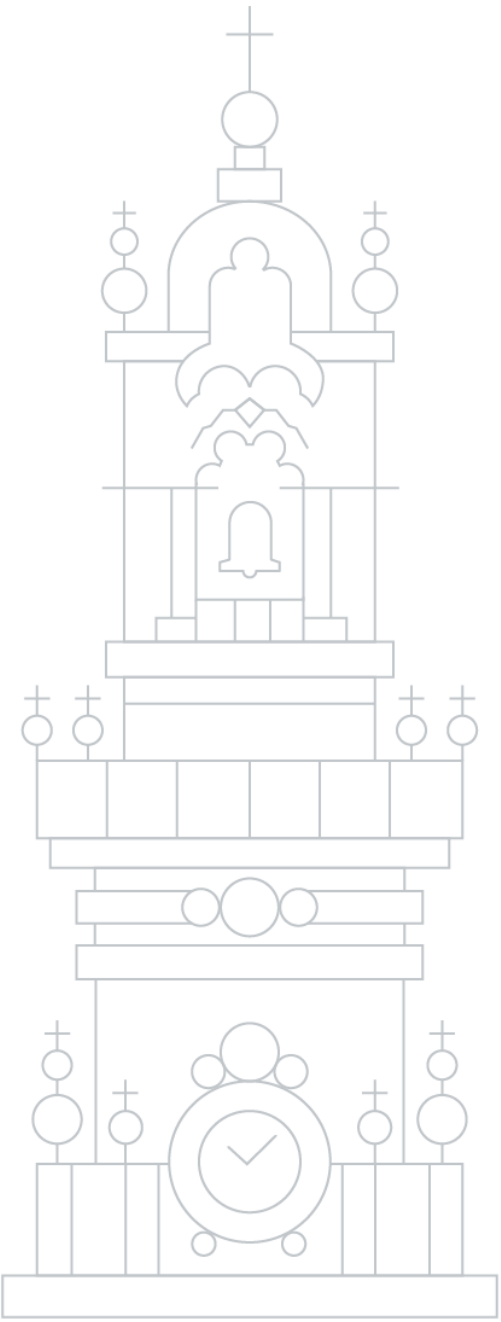
Thursday, June 18th	Friday, June 19th	Title	Presenter
10:35 - 10:38	12:45 - 12:48	PO-1 Bridging morphology and omics: a histology-guided approach to chronic cutaneous furunculosis in turbot	Xoel Souto Guitián (U Santiago Compostela)
10:38 - 10:41	12:48 - 12:51	PO-2 Quince extract modulates early HPV-associated lesions in K14HPV16 mice	Helena Vala Correia (IPV/CERNAS)
10:41 - 10:44	12:51 - 12:54	PO-4 Atypic tetraparesis induced by embolic disease associated to aortic valvular bacterial endocarditis in a dog	Carlos Cáceres (Facultad Veterinaria de Murcia)
10:44 - 10:47	12:54 - 12:57	PO-5 Periocular extraskeletal telangiectatic osteosarcoma in a dog	Marta Silva Samos (U León)
10:47 - 10:50	12:57 - 13:00	PO-9 Fibrinous pleuropneumonia in the South African porcupine (*Hystrix africaeaustralis*)	Sandra Núñez Pallarés (U CEU Cardenal Herrera)
10:50 - 10:53	13:01 - 13:04	PO-16 Detection of TNF-α by RNAscope™ in turbot tissues (*Scophthalmus maximus*): contributions to the anatomical-pathological study of the vaccine-induced	Beatriz Ordóñez Gómez (U Santiago de Compostela)

Group B

Thursday, June 18th	Friday, June 19th	Title	Presenter
12:45 - 12:48	15:35 - 15:38	PO-6 Floating in mucin: immunohistochemical decoding of a mucinous mammary carcinoma	Elena Mozos Mora (U Córdoba)
12:48 - 12:51	15:38 - 15:41	PO-8 *Brucella* spp. in stranded cetaceans of Galicia	Raquel Puig-Lozano (U Las Palmas de Gran Canaria)
12:51 - 12:54	15:41 - 15:44	PO-10 Reconstructing Host-Like Conditions in vitro to Trigger the L3-L4 Transition in Anisakis simplex and Capture Stage-Specific Molecular Signatures	Paula Ramos (IPMA)
12:54 - 12:57	15:44 - 15:47	PO-12 Histopathological characterization of neurobrucellosis in stranded cetaceans in the valencian community (2022–2026)	Carmen García-Romero Moreno (U CEU Cardenal
12:57 - 13:00	15:47 - 15:50	PO-14 Osseous metaplasia secondary to sialocele in a dog	Fernanda AG de Seixas Travassos (UTAD)

Group C

Thursday, June 18th	Friday, June 19th	Title	Presenter
15:35 - 15:38	10:35 - 10:39	PO-7 Spinal cellular hemangioblastoma in a dog: a rare entity	Jéssica Molin (U Lleida)
15:38 - 15:41	10:38 - 10:42	PO-11 Intensity, Morphological and Molecular Identification of *Stephanostomum baccatum* (*Trematoda: Acanthocolpidae*) Metacercariae in Yellowtail Flounder, *Limanda ferruginea* commercialized in Portuguese Supermarkets	Paula Ramos (IPMA)
15:41 - 15:44	10:41 - 10:45	PO-13 Morphologic staging of cutaneous lesions in chronic *Aeromonas salmonicida* infection	Xoel Souto Guitián (U Santiago Compostela)
15:44 - 15:47	10:44 - 10:48	PO-15 Oral neuroma in dogs: a rare entity	Fernanda AG de Seixas Travassos (UTAD)
15:47 - 15:50	10:47 - 10:51	PO-17 Use of feather pulp in the diagnosis of Marek's disease: viral replication and immunopathological characterization	Natalia Garcia (U Leon)



INVITED SPEAKERS

Margarida Henriques (INIAV)

Margarida Henriques Mourão is a senior researcher with a PhD in Biotechnology and over 20 years of experience in veterinary virology and molecular diagnostics. Based at the National Reference Laboratory (INIAV), she works on diagnosis, surveillance, molecular characterization, and phylogenetic analysis of animal and zoonotic viruses, within national and European disease monitoring programs.

ABSTRACT

Avian influenza (AI), commonly known as bird flu, is a viral disease of major global concern due to its impact on animal health, poultry production, wildlife conservation, and, in certain circumstances, public health. It is caused by influenza A viruses of the family Orthomyxoviridae, which are notable for their remarkable genetic diversity and rapid evolutionary dynamics. These viruses are classified into subtypes based on the hemagglutinin (H) and neuraminidase (N) surface proteins, resulting in a wide range of combinations with varying biological and epidemiological characteristics.

Wild aquatic birds, particularly those belonging to the orders Anseriformes and Charadriiformes, constitute the natural reservoir of avian influenza viruses. In these hosts, infection is often asymptomatic, facilitating silent circulation and long-distance dissemination through migratory movements. Transmission occurs through direct contact with infected birds or indirectly via contaminated environments, including water, feed, and fomites. The introduction of the virus into domestic poultry populations can lead to outbreaks with significant economic impact, particularly when highly pathogenic avian influenza (HPAI) strains are involved. These strains are associated with severe clinical disease, high mortality, and the implementation of strict control measures such as culling and movement restrictions.

Over the past decades, the global distribution of avian influenza viruses has expanded considerably, driven in part by the emergence and spread of highly pathogenic strains, notably those of the H5 lineage. Europe has experienced successive epidemic waves, especially since 2020, affecting both wild and domestic bird populations. These dynamics underscore the adaptability of the virus and the importance of coordinated surveillance and response strategies across countries.

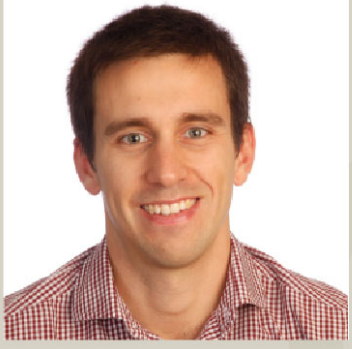
In Portugal, the epidemiology of avian influenza has evolved in parallel with broader European trends. While historically considered at relatively lower risk, the country has reported an increasing number of detections in recent years, particularly in wild birds. Coastal regions and wetlands play a key role in this context, as they serve as important habitats for migratory species that may introduce the virus. The detection of highly pathogenic strains in these populations has heightened concerns regarding the risk of transmission to domestic poultry.

National surveillance systems have been essential in monitoring the presence and spread of avian influenza. Passive surveillance, based on the investigation of dead or clinically affected birds, together with active surveillance in both wild and domestic populations, has provided valuable insights into virus circulation. In recent years, outbreaks in domestic flocks have been reported, often linked to environmental exposure and proximity to wild bird habitats. These events have required the implementation of control measures in accordance with European Union regulations, including the establishment of protection and surveillance zones.

Temporal patterns of virus detection in Portugal suggest a seasonal trend, with increased activity during autumn and winter, coinciding with the arrival of migratory birds from northern and central Europe. However, more recent observations indicate that virus circulation may extend beyond these periods, reflecting possible changes in ecological and epidemiological dynamics. The diversity of affected species, ranging from wild seabirds to backyard poultry, highlights the complexity of the current situation.

This presentation will provide a concise introduction to avian influenza virus biology and the general features of the disease, followed by an overview of recent epidemiological trends in Portugal. By focusing on the national context within a broader international framework, it aims to support a clearer understanding of the evolving epidemiology of avian influenza and the ongoing need for effective surveillance, biosecurity, and preparedness measures.

High Pathogenicity Avian Influenza (HPAI)
Recent Epidemiology and Interspecies Pathological Presentation



Javier Asín Ros (UC Davis)

Dr. Javier Asín Ros received his Veterinary Medicine Degree and PhD from the University of Zaragoza, Spain, and is a diplomate of the European College of Veterinary Pathologists. He is currently an Associate Professor at the University of California, Davis. He primarily serves as a diagnostic pathologist at the San Bernardino branch of the California Animal Health and Food Safety Laboratory System, the state's veterinary diagnostic laboratory network.

ABSTRACT

Highly pathogenic avian influenza virus (HPAIV) goose/Guangdong lineage H5N1 clade 2.3.4.4b was detected for the first time in North America in December 2021 in migratory birds and has since severely impacted the poultry industry. In the United States, multiple states have been affected, with cases reported in a broad variety of avian and mammalian species. Most notably, HPAIV H5N1 spilled over from wild birds into dairy cattle at the end of 2023, and the first detections were reported in Texas in 2024. It then rapidly spread to multiple other states, with evidence supporting both interstate movement and cow-to-cow transmission as principal contributors to dissemination. California has been one of the most severely affected states, with two of its main food industries, dairy cattle and poultry, impacted simultaneously over several months between 2024 and 2025. This situation posed a challenge to veterinary diagnostic laboratories and placed pathologists and other diagnosticians at the forefront of the outbreak response.

The goal of this presentation is to describe the main lesions and diagnostic challenges in different species affected by HPAIV H5Nx diagnosed at the California Animal Health and Food Safety Laboratory System, the state's veterinary diagnostic laboratory network, between 2022 and 2026.

Affected wild avian species primarily include migratory waterfowl, birds of prey, seabirds, and shorebirds. The main lesions observed in these species are pancreatic necrosis; necrosis in other organs such as the heart, liver, and spleen; and necrotizing to lymphoplasmacytic encephalitis. Diagnosis is confirmed via RT-qPCR from oropharyngeal/choanal or cloacal swabs. Complementary

tests include immunohistochemistry and in situ hybridization, which are useful techniques to visualize viral antigens or RNA, respectively, within tissue sections.

Cases in commercial and backyard poultry often present as a sharp increase in mortality. In addition to the lesions described in other avian species, chickens often exhibit comb and wattle cyanosis and hemorrhages on the proventricular glands. However, some cases can be subtle grossly. Similar diagnostic tests to those used in wild birds are applied.

The syndrome caused by HPAIV H5N1 in dairy cattle may be clinically nonspecific and includes lethargy, fever, decreased ruminal motility and feed intake, tacky feces, and reduced milk production. Mortality is low, and most affected animals recover. The mammary gland is the main target organ, with patchy necrotizing mastitis reported as the principal lesion. The virus is shed in milk, which is the sample of choice for testing via RT-qPCR. Testing of bulk tank milk is prioritized over testing of individual animals for surveillance and herd-level screening.

Other affected mammalian species include multiple wild terrestrial and marine mammals, as well as domestic and zoo felids. In most of these species, the main lesion is a necrotizing and lymphoplasmacytic encephalitis, with necrosis also observed in other organs. Pasteurization inactivates HPAIV; however, mortality events have occurred in cats fed raw colostrum or milk from affected cows. As the brain often contains high viral loads, it is the tissue of choice for testing via RT-qPCR in these species.

A diagnosis of HPAI triggers a rapid response to minimize spread. In commercial poultry, confirmation leads to depopulation of the affected flock, whereas affected dairies are placed under quarantine with movement restrictions and enhanced biosecurity measures. The impact of this outbreak in California has been unprecedented. As of February 2025, the California Department of Food and Agriculture reported that 75% of the state’s dairies had been infected and that more than 15 million commercial birds had been depopulated, including over 70% of the state’s layers.

Eduardo Chicano Gálvez (IMIBIC)

Eduardo Chicano Gálvez, PhD, is Head of the Mass Spectrometry and Molecular Imaging Unit at IMIBIC (Spain). His work focuses on spatial omics using mass spectrometry imaging (MS-Imaging) to map the molecular composition of tissues with high spatial resolution. His research bridges analytical chemistry, biochemistry, pathology, and bioinformatics, enabling the visualization of proteins, lipids, and metabolites directly within histological sections.

ABSTRACT

What if you could see not just what molecules are present in tissue, but exactly where they are located? Mass spectrometry imaging (MSI) answers this question by transforming biological tissues into detailed maps of molecular distribution, revealing the spatial architecture of disease, drug action, and biological function with unprecedented clarity. This presentation takes you on a journey through the technical foundations and practical applications of MSI, demonstrating how this transformative technology is reshaping pathology and biomedical research.

At its core, MSI asks a fundamental question: which molecules exist at each specific location within a tissue sample? Answering this requires orchestrating four interconnected domains: thoughtful sample preparation that preserves both tissue structure and chemical information, carefully selected instrumentation that defines the resolution and chemical specificity of our spatial maps, intelligent workflows that transform raw data into biological insight, and deep data analysis that converts molecular signals into biological narratives.

Sample preparation is where MSI begins—and where many analyses succeed or fail. We will explore how tissue sectioning protocols, matrix deposition techniques, and ionization source selection fundamentally shape what information we can extract. Different tissues, different analytes, and different biological questions demand different preparation strategies. The IMSMI unit has developed optimized protocols for diverse applications: flash-frozen tissues for comprehensive lipid mapping, formalin-fixed tissue analysis for seamless integration into biomedical and clinical workflows, and innovative sampling approaches for challenging substrates where conventional methods fail. These

evidence-based protocols emerge from years of systematic optimization and meticulous troubleshooting, transforming preparation from an art into a rigorous science.

Instrumentation determines our analytical vocabulary—the chemical specificity and spatial resolution defining what we can discover. High-resolution mass spectrometers provide the chemical selectivity to identify unknowns in complex tissue mixtures. Ion mobility coupling adds an additional dimension for structural differentiation and isomer separation. Understanding these platforms—their individual strengths, inherent limitations, and most appropriate applications—is essential for designing experiments that genuinely answer biological questions rather than simply generating data.

Workflows bridge instrumentation and insight. From laser parameter optimization and spatial resolution decisions to sophisticated data processing pipelines and statistical validation, each choice shapes the final result. The IMSMI unit has developed standardized, rigorously validated workflows that balance analytical rigor with practical efficiency—methods that work not just in principle but consistently in practice, day after day, yielding reproducible, interpretable results.

But methodology alone does not justify MSI's fundamental importance. The true power emerges in biological applications. We will present concrete case studies demonstrating MSI's transformative impact: discovery of tumor microarchitecture invisible to conventional pathology in oral cancer, revealing unexpected spatial heterogeneity within lesions; characterization of toxin distribution patterns that identify unexpected metabolic hotspots; classification of chemotherapy-responsive versus non-responsive colon cancer patients based on their proteomics signatures; and lipid profiling in dopaminergic neurons from Parkinson's disease mouse models revealing disease-specific molecular alterations. These are not theoretical possibilities—they represent results we have systematically achieved through rigorous methodology and strategic thinking.

Mass spectrometry imaging is ultimately about seeing biology differently—and through that new vision, understanding it more profoundly. Whether you are developing MSI capabilities in your institution, considering adoption of this technology, or seeking to optimize existing implementations, this presentation will provide both the conceptual frameworks and practical knowledge necessary to advance your work in spatial omics analysis.

Librado Carrasco Otero (UCO)

A graduate and PhD in Veterinary Medicine from the University of Córdoba, he completed his training at various institutions, including the Institute for Pathology at the Animal Medical School Hannover (Germany), the Institute for Animal Health (Pirbright, UK), and the College of Veterinary Medicine at Kansas State University (USA).

ABSTRACT

ASF in Domestic Pigs

African Swine Fever (ASF) is a hemorrhagic viral disease that affects pigs and wild board, of all age groups, caused by the African swine fever virus (ASFV) a large and complex DNA virus (170-190 kb) that it is the only member of family Asfaviridae. The ASFV replicates in the cytoplasm of cells, with monocytes/macrophages being its main target cell, and it has a very complex structure and high genetic variability. This virus, which does not induce the production of neutralizing antibodies and for which there is no effective vaccine, is very resistant in the environment, as it can remain infectious for long periods of time, especially in organic matter and in meat products. One of the characteristics of the ASFV is to produce hemoadsorption in vivo and to adhere to the membrane of red blood cells, which allows to use soft ticks from the genus Ornithodoros as vectors. Although ASF is a less transmissible disease than others such as Foot-and-Mouth Disease, CSF, or PRRS, the fact that the virus circulates in the body bound to red blood cells and its great resistance to the environment makes it a disease with a great capacity for expansion.

Described for the first time by Montgomery in Kenya in 1921, it leaves the African continent for the first time in 1957, reaching Portugal and, subsequently, Spain (1960), countries from which it will spread throughout Europe (1964), Brasil (1978) and different Caribbean countries (1971-1978), from which it will be eradicated, (except for Sardinia, Italy) after years of struggle and control. After years in which the disease was thought to be only in Africa and Sardinia, in 2007 it reappeared in Georgia, from where it would spread through the Caucasus region and Russia. This led to the emergence, in 2014, of the disease in countries bordering Russia, such as Lithuania, Latvia, Estonia, and Poland, from where it has expanded to other European countries such as Belgium (2018) or Italy (2022). But ASF

not only spreads to Europe, but in 2018 the virus is detected in China, from which it will spread throughout Southeast Asia. In 2021, a new expansion of the ASFV occurs in the Caribbean, which is quickly controlled (2023). And in 2025, the virus is detected in wild boars in Cerdanyola del Vallès (Barcelona, Spain), an ASF outbreak that is being attempted to be controlled and that so far has not affected the farms in the area.

The clinical presentation and the gross pathological lesions of ASF may vary depending on the virulence of the virus isolate, the route, and dose of infection and host characteristics. ASFV isolates can be classified as highly virulent, moderately virulent, and low virulent. Highly virulent strains are responsible for the peracute course, that are characterized by a high fever (up to 42°C), anorexia, lethargy, respiratory distress or sudden death without signs of disease and usually no gross lesions are found at the postmortem examination.

The acute form of ASF is caused by highly or moderately virulent isolates. This form is characterized by high fever(40–42°C), lethargy, anorexia and respiratory distress. The animals show cyanosis of the ears, limbs, abdomen, tail, and perianal area, as well as petechial hemorrhages or ecchymosis in the skin. The mortality rates may reach up to 100% in the first week. At the postmortem examination, the most characteristic lesion is a hemorrhagic splenomegaly, which is accompanied by hemorrhagic lymphadenitis, in which the lymph nodes can appear from a marbled appearance to the appearance of a blood clot, with the gastrohepatic and renal lymph nodes being the most severely affected. Petechial hemorrhages are often observed in the kidney surface as well as in the mucosa or the serosa of other organs, as the urinary bladder.

This subacute form is due to the infection by moderately virulent isolates. Affected animals show moderate to high fever and the mortality rate ranges from 30 to 70%, dying the animals at 7–20 after infection. In this form, vascular changes (hemorrhages and edema) are much more intense than in the acute forms, due to the onset of thrombocytopenia and, subsequently, to erythrodiapedesis caused by an intense vasodilation. At the postmortem examination, the animals show edema in different cavities, and a characteristic edema in the wall of the gall bladder and in the perirenal area. Other lesions are a partial splenomegaly, with patches of spleen affected and other areas unaffected, hemorrhagic lymphadenitis and petechial hemorrhages in the kidney.

Antonio Rodríguez-Bertos (UCM–VISAVET)

He graduated and obtained his PhD from the Veterinary School (Complutense University of Madrid). He is currently a professor in the Department of Animal Medicine and Surgery (VS-UCM) and Head of the Animal Pathology and Forensic Veterinary Service at the VISAVET-UCM center.

ABSTRACT

ASF in Wild Boar

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ASF in Eurasian wild boar has gained major epidemiological relevance since the introduction of genotype II ASFV into Europe in 2007. Although domestic pigs and wild boar belong to the same species (*Sus scrofa*) and develop largely similar pathological lesions, experimental and field studies have demonstrated higher susceptibility in wild boar, with relevant differences in disease kinetics, viral dissemination, and time to clinical endpoint.

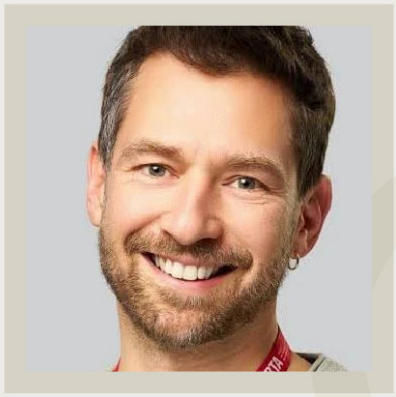
A key difference lies in the kinetics of infection. Wild boar generally exhibits a shorter incubation period and an earlier onset of clinical signs compared to domestic pigs. Likewise, viral replication and systemic dissemination occur earlier in wild boar, reflected by earlier detection of viremia and viral shedding. In contrast, domestic pigs tend to show a more delayed progression of infection. Overall, wild boar reaches the terminal stage more rapidly, resulting in a shorter time to humane endpoint.

Macroscopically, ASF is characterized in both species by a severe systemic haemorrhagic disease affecting primarily the lymphoid and vascular systems. The predominant acute/subacute lesions include hydrothorax, hydropericardium, ascites, pulmonary oedema, hyperaemic splenomegaly, and multifocal haemorrhages affecting mainly lymph nodes and kidneys, and less frequently the heart,

thymus, gastrointestinal tract, urinary bladder, pancreas and brain. Although lesion distribution is broadly comparable between subspecies, differences in kinetics are evident. In wild boar, gross lesions may appear earlier and tend to reflect a rapid and widespread involvement of lymphoid tissues, whereas in domestic pigs lesions often become progressively more severe as the disease advances, resulting in more extensive pathology at the terminal stage. A relevant practical limitation is that wild boar carcasses are frequently found in the field in varying degrees of autolysis, scavenging, or environmental degradation, which may hinder lesion interpretation.

Microscopically, no single lesion is considered diagnostic for ASF, and a comprehensive and complete histopathological evaluation is required. Regarding acute/subacute forms in wild boar, the most relevant findings include marked lymphoid depletion and lymphocytolysis in tonsils, spleen, bone marrow, and lymph nodes. Prominent vascular alterations, including hyperemia/congestion, multifocal haemorrhages, vasculitis, and microthrombi formation, are observed, particularly in gastrohepatic and renal lymph nodes, spleen, lungs, liver, kidneys, and brain, especially the choroid plexus. In addition, mononuclear inflammatory infiltrates are frequently detected in lungs, liver, and brain, reflecting the systemic nature of the disease.

Overall, wild boar tends to show earlier systemic dissemination and faster progression to severe disease, whereas domestic pigs may develop more extensive multisystemic involvement when the clinical course is prolonged, more frequently affecting secondary organs such as heart, urinary bladder, intestine, adrenal glands, and pancreas. However, these lesions are not specific enough to distinguish ASF from other haemorrhagic diseases, particularly classical swine fever (CSF), and definitive diagnosis requires laboratory confirmation by PCR, virus isolation, or antigen detection.



Enric Vidal Barba (IRTA–CReSA)

Dr. Enric Vidal (Barcelona, 1977), DVM, PhD. He completed his veterinary studies at the Autonomous University of Barcelona in 2000 and completed his doctoral thesis on the study of the pathogenesis of BSE at the same faculty in 2006. Since 2002, he has been head of the PRIOCAT laboratory (the reference laboratory for animal prion diseases in Catalonia) located at IRTA-CReSA (Animal Health Research Centre) a BSL3 facility where he also leads a research line on the neuropathology of TSEs. The line focuses on the study of the mechanisms of interspecies prion transmission barrier, the etiology of idiopathic prion diseases, the variability of prion disease phenotypes in nature and, more recently also gene-therapy based therapeutic approaches. Since 2007, he manages the Slaughterhouse Support Service (SESC) on behalf of the Health Department of the Generalitat de Catalunya (www.sesc.cat). This is a service to help official veterinary inspectors obtain a definitive diagnosis of slaughterhouse findings. He also participates in the anatomopathological studies of other CReSA research lines: tuberculosis, African swine fever, Classica swine fever, arbviruses, zoonotic coronaviruses, etc. and has an active participation in science divulgation activities (blogs, social networks, talks...). Since October 2025 he coordinates the scientific committee on Lumpy Skin Disease to support the government's management of the current LSD outbreak in Catalonia.

ABSTRACT

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Lumpy skin disease (LSD) is caused by *lumpy skin disease virus* (LSDV) or *Capripoxvirus lumpyskinpox*, a member of the family *Poxviridae*, which infects cattle and water buffalo, as well as a few wildlife species in African and Asian endemic regions including springbok, eland, Arabian oryx, gemsbok, gaur,

Sumatran serow, banteng, camel and giraffe. Wildlife in Europe has not been described to be susceptible to LSDV. The disease is characterised by fever, depression, enlarged superficial lymph nodes, ocular, nasal and oral discharges, reduced milk production, infertility or abortion, and, most notably, a generalised nodular dermatitis that may evolve to necrosis and ulceration.

Transmission occurs mainly through mechanical vectors, particularly hematophagous arthropods such as the stable fly (*Stomoxys calcitrans*) over short distances, other insects including mosquitoes and ticks have also been described as possible vectors. Long-distance spread is primarily associated with the movement of live infected animals. LSD has been classified in the European Union as a category A disease, requiring immediate eradication measures upon detection in previously free regions.

In October 2025, LSD was diagnosed in Spain for the first time. Outbreaks reported earlier that year in North Africa and Southern Europe, including Tunisia, France and Italy, likely increased the regional risk of incursion. The first Spanish outbreak was detected through passive surveillance in northeastern Catalonia (Alt Empordà). Subsequently, up to 16 additional outbreaks were confirmed in cattle farms, most of them located within a 20 km radius of the first cases during the following two months.

Control measures, including stamping-out of affected herds, strict animal movement restrictions and emergency vaccination, were rapidly implemented to contain the outbreak and prevent further spread. These interventions had a substantial social and economic impact on the regional cattle sector. Additional outbreaks were detected in late 2025 and again in 2026 in France, as well as in Spain, where they affected unvaccinated animals in holdings located within compulsory vaccination zones, including one outbreak in Catalonia (adding up to a total of 18 outbreaks) o and two in Aragon, thereby indicating ongoing viral circulation.

Skin samples were collected from all outbreaks detected in Catalonia, together with a range of tissues obtained from three complete necropsies, in order to characterise the pathological phenotype of the disease. Viral presence was confirmed through qPCR and lesion chronicity was staged anatomopathologically, and viral replication was assessed using *in situ* hybridisation techniques. Furthermore, vaccination response was evaluated in two vaccinated farms.

Estimation of lesion chronicity based on anatomopathological features suggested that LSDV had been circulating in Catalonia since at least early August 2025. Even though the precise origin and route of introduction of the virus remains undetermined, the most likely hypothesis is the import of live animals from LSD-free areas in France that where later diagnosed with the disease.

Macroscopic and microscopic findings from these outbreaks consisted of the characteristic proliferative and nodular dermatitis, with detection of viral RNA in epidermis, follicular epithelium, adnexal glands, piloerector muscle, blood vessels and dermal cells (mostly consistent with macrophages and fibroblasts). RNA detection was reduced in more chronic lesions. Moreover, other lesions that are not typically reported in cases of LSD were observed, such as interstitial emphysematous pneumonia and proliferative pleuritis, which were associated with high amounts of viral replication. These lesions will be presented to facilitate recognition of the characteristic lesions associated with this emerging disease.

Further studies are required to understand the impact of this emerging disease on our territory, including the role of local vectors and potential domestic and wild reservoirs in its maintenance and spread.

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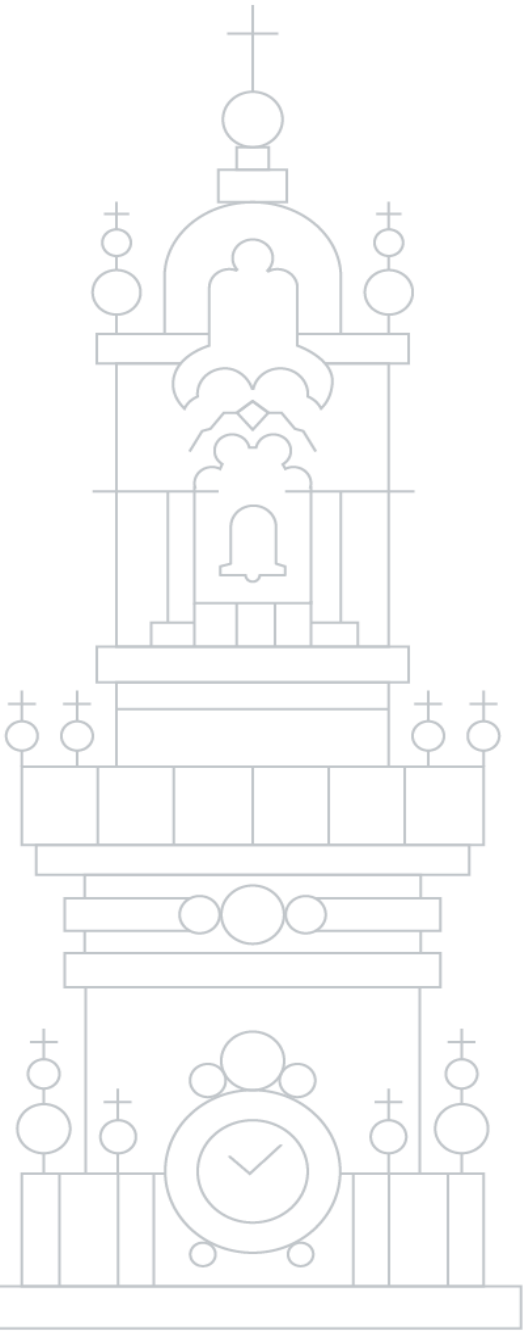

ORAL COMMUNICATIONS I

Infectious diseases/Diagnostic Innovation

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O1

Pathological characterization of the highly virulent porcine reproductive and respiratory syndrome virus Rosalia strain in pregnant sows under experimental conditions.

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Background/Objectives: Porcine reproductive and respiratory syndrome virus (PRRSV) remains one of the most economically significant infectious diseases in the swine industry. Highly virulent strains have been associated with severe reproductive failures under field conditions. This study aimed to pathologically characterize the highly pathogenic PRRSV Rosalia strain in experimentally inoculated pregnant sows.

Methods: Eight PRRSV-naïve pregnant sows (90±2 days of gestation) were intranasally inoculated with the PRRSV Lleida 029_22 Rosalia strain ($1x10^5$ $TCID_{50}$/ml), while three sows remained as non-infected controls. Sows were allowed to farrow naturally unless gestation exceeded 117 days, where farrowing was induced with PGF2a analogs. Clinical monitoring was performed until weaning. Offspring were classified at birth as born-alive or non-viable (stillborn, decomposed, autolytic or mummified). Necropsies were conducted on stillborn piglets at farrowing and on sows and surviving piglets at 21 days post-farrowing.

Results: Most sows showed no major clinical signs; however, one developed severe disease and was euthanized at 14 days post-infection (DPI). Piglet losses were substantial, reaching 93.2% at delivery and 98.1% by weaning in inoculated sows, compared to 13.3% and 15.6% in the control group, respectively. Infected sows showed interstitial pneumonia and mild-to-moderate lymphoid depletion with hyperplasia. Placental lesions, including mineralization, degenerative changes, and mononuclear vasculitis, suggested transplacental viral involvement. Uterine lesions, such as multifocal perivascular infiltrates in the myometrium and endometrium, were detected in only a few infected sows. Brain lesions included multifocal perivascular mononuclear infiltrates, perivascular edema and diffuse mild-to-moderate gliosis. These lesions were generally subtle but were more pronounced in the sow that developed acute disease and was euthanized at 14 DPI.

Conclusion: Experimental inoculation with the PRRSV Rosalia strain in late-gestation sows caused severe reproductive failure and multisystemic microscopic lesions, confirming its high pathogenicity and suggesting that highly virulent PRRSV strains may exhibit a broader tissue tropism.

Keywords: PRRSV, highly pathogenic strain, reproductive failure, experimental infection

Ethical approval: ☐ Not applicable; ☒ Approved

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O2

Deep learning-assisted spatial quantification of PRRSV-1 in tissues by RNAscope *in situ* hybridization.

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Background/Objectives: Precise characterization of the spatial tissue distribution of porcine reproductive and respiratory syndrome virus (PRRSV) is essential for understanding its pathogenesis. While immunohistochemistry is commonly used for this purpose, its diagnostic performance is limited by its low signal sensitivity. In contrast, RNAscope *in situ* hybridization (ISH) has proven highly sensitive for detecting PRRSV RNA. Furthermore, digital pathology enables detailed spatial analysis within tissues. Therefore, we implemented a deep learning (DL)-based digital pathology approach combined with RNAscope ISH to characterize and quantify PRRSV-1 distribution in target organs.
Methods: Lungs, tonsils, and tracheobronchial lymph nodes (TBLN) were collected from five 4-week-old piglets intranasally inoculated with the PRRSV-1 strain 3249 and euthanized at 14 days post-infection. Tissues were formalin-fixed and routinely processed for histopathological examination. RNAscope ISH was performed to detect viral RNA. A commercial software powered with artificial intelligence (Visiopharm) was applied in whole-slide images to quantify viral RNA signal density and assess spatial distribution within histological regions of interest.
Results: Positive intracellular ISH signals appeared as multifocal red clusters and dots predominantly associated with macrophages and/or histiocytic cells. In the lung, viral RNA was mainly detected in areas of severe interstitial pneumonia and bronchopneumonia. In tonsils and TBLN, the highest viral density was observed in lymphoid follicles, particularly within germinal centers. Marked differences in tissue distribution were identified, with the highest viral burden detected in tonsils and lungs, followed by TBLN.
Conclusions: The DL-driven digital pathology assessment of RNAscope ISH for PRRSV enabled a detailed quantitative and spatial characterization of PRRSV-1 distribution in target tissues, revealing associations with lesion severity, regional heterogeneity within organs and differences between organs. This integrated approach enhances objectivity and reproducibility in the evaluation of viral tropism and provides a valuable tool for advancing research on PRRSV pathogenesis.

Keywords: Porcine Reproductive and Respiratory Syndrome (PRSSV-1), RNAscope in situ hybridization, Spatial Analysis, Digital Pathology.

Ethical approval: ☐ Not applicable; ☒ Approved

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O3

Differential diagnosis of bovine tuberculosis in slaughterhouse samples with suspected lesions: optimization of histopathology and immunohistochemistry

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Bovine tuberculosis (bTB), caused by members of the *Mycobacterium tuberculosis* complex (MTC), remains a major challenge for animal health and control programs, requiring diagnostic approaches that are both accurate and applicable under routine conditions. In this context, pathology not only enables detection of lesions compatible with MTC infection but also plays a key role in the differential diagnosis of tuberculosis-like lesions.

This study evaluated the diagnostic performance of histopathology and immunohistochemistry (IHC), applied individually and in combination, for the detection of bTB, using molecular (PCR) and microbiological culture methods as reference standards. A total of 756 bovine tissue samples with suspected tuberculosis lesions were collected from slaughterhouses in Spain.

When combined (hematoxylin and eosin (H&E), Ziehl–Neelsen (ZN), and IHC), histopathological techniques showed high sensitivity (97.4%) and specificity (88.5%) relative to PCR. In discordant cases (PCR-negative but histopathology-positive), culture confirmed MTC infection in 33 out of 39 samples, increasing the estimated specificity to 98% when culture was included as a complementary reference. Individually, H&E staining showed a sensitivity of 96% and a specificity of 88.8%. ZN staining showed a sensitivity of 90.2% and a specificity of 90%, whereas IHC showed high sensitivity (97%) but lower specificity (63.3%), mainly due to cross-reactivity with non-target pathogens.

To improve diagnostic accuracy, cases with strong IHC labeling but negative ZN results were reclassified as non-compatible with bTB, reducing false-positive results. All reclassified cases (n = 111) were PCR-negative for MTC, and 35 were confirmed as *Rhodococcus equi* infections by PCR. This refinement of interpretation criteria significantly improved overall specificity.

In conclusion, the integrated use of histopathology and immunohistochemistry, supported by optimized interpretation criteria, provides a reliable approach for the differential diagnosis of bovine tuberculosis and represents a valuable complement to molecular techniques in routine surveillance and control programs.

Keywords: Bovine tuberculosis, histopathology, immunohistochemistry, differential diagnosis, Mycobacterium tuberculosis complex

Ethical approval: ☒ Not applicable; ☐ Approved

O4

Developing an AI-based model to support TB surveillance at the slaughterhouse

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Catalonia has been officially bovine tuberculosis (TB)-free since 2023. Nevertheless, domestic and wildlife reservoirs continue to threaten this status. Within eradication programmes, slaughterhouse surveillance is a key tool alongside the testing and culling strategy for early outbreak detection. The Catalan slaughterhouse support network (SESC; www.SESC.cat) coordinates the analysis of lesions compatible with TB (i.e. granulomas). However, granuloma submission rates often remain below optimal levels for effective passive surveillance and depend heavily on the ability of meat inspectors and assistants to recognise suspect lesions.

To address this limitation, we propose an AI-based tool to classify lesions into those macroscopically compatible with TB (TB-risk), requiring laboratory confirmation, and those that can be discarded (Non-TB). A second-stage classification targeting specific granuloma aetiologies is also envisaged. For this, 1000 images and its diagnostic metadata were collected from SESC's archives and manually annotated.

Still, a major challenge was the scarcity of well-annotated image datasets, particularly from certain less common categories of granulomas. To overcome this, we developed a hybrid training strategy combining synthetic and real data. Synthetic images were generated using a 3D blending pipeline in which annotated lesions were embedded into tissue backgrounds, followed by augmentation to enhance variability and realism. The model was subsequently fine-tuned on approximately 1000 real images representative of routine inspection conditions.

When evaluated on an independent set of 104 images, the model achieved 90.7% sensitivity and 60.6% specificity in distinguishing TB-risk granulomas from Non-TB lesions and normal tissues (~81% overall accuracy).

These findings demonstrate that integrating limited real data with synthetic data can yield robust performance. This approach provides a solid foundation for developing an AI-assisted tool to support meat inspectors, so far improving granuloma detection towards a level comparable to trained pathologists.

Keywords: Tuberculosis, granuloma, slaughterhouse, deep learning, AI

Ethical approval: ☒ Not applicable; ☐ Approved

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O5

In vivo characterization of neutrophil extracellular traps during early Mycobacterium avium subsp. paratuberculosis infection in a sheep model

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Mycobacterium avium subsp. *paratuberculosis* (Map) is the causative agent of paratuberculosis, a chronic granulomatous enteritis of ruminants. Increasing evidence highlights a central role for neutrophils extracellular traps (NETs) in the early response to Map infection, as they mediate in bacterial trapping and killing, but also may contribute to the initiation of granulomatous lesions.

Here, NET formation and the associated immune response were characterized in an ovine jejunal gut-loop model following Map infection. Sixteen Assaf lambs underwent surgery to isolate three intestinal loops, that were inoculated with PBS, sheep-type Map, or cattle-type Map. Animals were humanly euthanised at 8 hours post-infection and 2 days post-infection and gut-loops were routinely processed for histology (H&E and Ziehl-Neelsen) and immunochemistry (Map, H3Cit, neutrophil elastase, TLR2, IL-8, calprotectin, and IFN-γ). Samples showing the presence of Map and NETs were selected for transmission electron microscopy evaluation.

At 8 hours, Map-infected loops showed a marked neutrophilic infiltrate with fewer macrophages associated with Map, especially within lacteals and submucosal lymphatics. By 2 days Map burden was reduced and predominantly confined to lymphatic vessels, where it was associated with increased macrophages recruitment and persistent neutrophil presence. In contrasts, PBS-control loops exhibited minimal neutrophilic infiltration. H3Cit immunolabelling revealed web-like NETs predominantly at 8 hours and rounded aggregated NETs mainly at 2 days, both associated with Map. At both times post-infection, significantly higher expression of TLR2, IL-8, calprotectin and IFN-γ was observed in mainly neutrophils surrounding Map compared to non-infected controls. No differences were found between Map strains. Ultrastructurally, extracellular fibrillar scaffolds with electron-dense granules were associated with degenerated Map, consistent with NET formation.

This is the first study to characterized NETs formation *in vivo* in a paratuberculosis gut-loop model. Results support a key role of NETs in early Map infection, contributing to bacteria containment and strong innate pro-inflammatory immune response.

Keywords: Paratuberculosis, NETs, gut-loop, neutrophils

Ethical approval: ☐ Not applicable; ☒ Approved

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O6

Clinicopathological Study of *Mycobacterium avium subsp. hominissuis* in domestic cats.

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Background: Several mycobacterial species cause diverse clinical syndromes in cats. *Mycobacterium avium* subsp. *hominissuis* (MAH) is an opportunistic and zoonotic pathogen, widely distributed in the environment. Disseminated mycobacteriosis caused by MAH has emerged as a clinical entity in cats. However, the current literature on feline MAH infections is limited and lacks a detailed characterization. The aim of this work is to address this gap by describing the pathological findings in cats.

Methods: Two castrated male European cats, aged 8 and 2 years, from different owners were referred to the Veterinary Teaching Hospital over a 10-month period. The 2-year-old presented hyporexia, weight loss and a multilobulated abdominal mass. In the 8-year-old, abdominal lymphadenopathy was identified. Lymph node cytology showed macrophagic inflammation with bacilli compatible with mycobacteria. Complete post-mortem examinations were performed, and formalin-fixed, paraffin-embedded tissues were processed for histopathology. ZN staining, mycobacterial culture and 16S rRNA gene sequencing were performed on mesenteric lymph nodes.

Results: Grossly, both cases showed severe generalized granulomatous lymphadenitis, mainly affecting the mesenteric and ileocecal lymph nodes, and a segmental transmural granulomatous enteritis. Similar multifocal granulomatous lesions were found in the liver, spleen and lungs. Ziehl-Neelsen identified an extremely high burden of acid-fast bacilli within foamy macrophages. Mycobacterial culture followed by 16S rRNA gene sequencing identified the isolates as MAH in both cats.

Conclusion: This study provides a pathological and microbiological characterization of disseminated MAH in cats. The severity and distribution of lesions suggest an oral infection route and a primary intestinal disease caused by MAH in domestic felids. These findings suggest that MAH may be an underdiagnosed pathogen in feline intestinal and systemic disease.

Keywords: *Mycobacterium avium subsp. hominissuis*, nontuberculous mycobacteria, intestinal mycobacteriosis, granulomatous enteritis, disseminated infection

Ethical approval: ☒ Not applicable; ☐ Approved

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O7

Morphological and immunohistochemical insights into *Mycobacterium pseudoshottsii* infection in European sea bass

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Background/Objectives: Fish mycobacteriosis, caused by non-tuberculous mycobacteria (NTM), is a major constraint in aquaculture and a concern within the One Health framework. *Mycobacterium pseudoshottsii,* an emerging pathogen in European aquaculture, is closely related to zoonotic species, highlighting potential risks for human health. This study aimed to characterize granuloma development and explore the associated immune response in European sea bass (*Dicentrarchus labrax*).

Methods: Eight naturally infected fish were examined using histopathology and immunohistochemistry. Granulomas were classified into four developmental stages based on necrosis, cellular composition, and fibrosis. Bacillary distribution was assessed, and the immune response was evaluated through inducible nitric oxide synthase (iNOS) expression, among other markers.

Results: Four granuloma types were identified: Type I (early), with loosely organized macrophages and epithelioid cells; Type II (intermediate), showing central necrosis; Type III (advanced), with necrosis, foamy macrophages, and a thin fibrotic capsule; and Type IV (late), characterized by extensive necrosis and a thick capsule. Bacillary load increased with lesion progression, localizing mainly in necrotic areas and macrophages. Immunohistochemical results showed iNOS expression restricted to macrophages and lymphocytes, while epithelioid cells remained negative.

Conclusions: These findings provide insights into granuloma dynamics and host–pathogen interactions in *M. pseudoshottsii* infection. Ongoing immunohistochemical analyses will further elucidate immune mechanisms involved. This work contributes to improved understanding of mycobacteriosis progression in fish, supporting future diagnostic and control strategies in aquaculture within a One Health context.

Keywords: *Mycobacterium pseudoshottsii*, European sea bass, fish mycobacteriosis, aquaculture, pathology

Ethical approval: ☒ Not applicable; ☐ Approved

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O8

Mass mortality event in juvenile barbels (*Barbus barbus*) linked to *Sanguinicola sp.* (*Aporocotylidae*)

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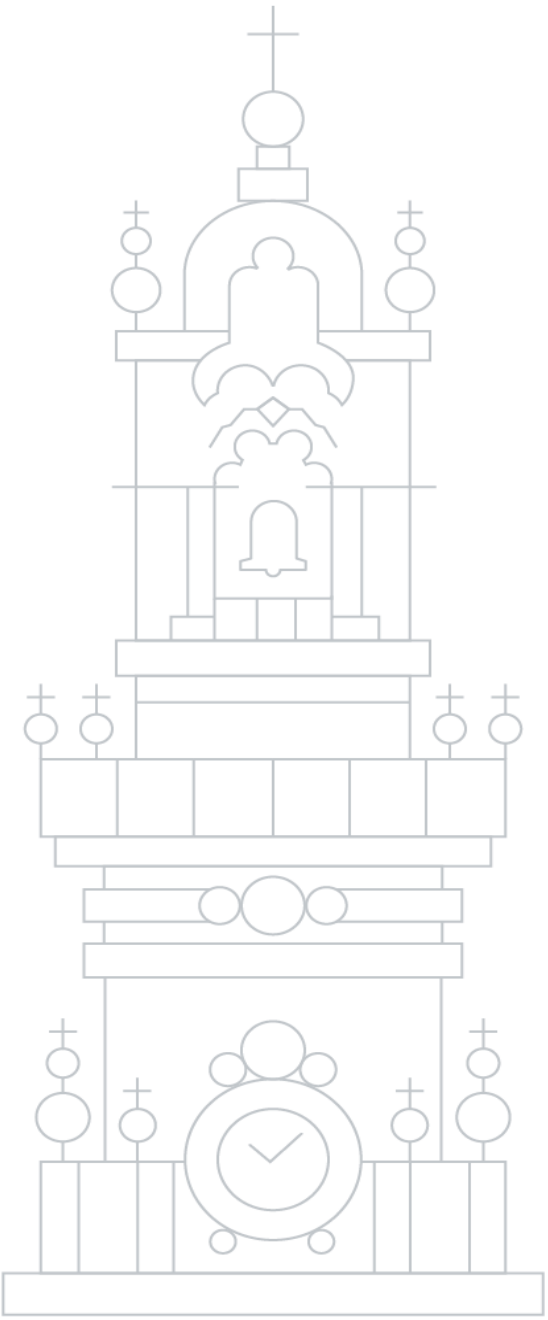
In August 2025, a mass mortality event affecting juvenile barbels (*Barbus barbus*) was reported in the river Wiese, Switzerland. Specimens were submitted for parasitological, bacteriological, histological, and molecular investigation. Histological analysis revealed severe infection with intravascular adult trematodes consistent with members of the family Aporocotylidae (*Sanguinicola spp.*), causing vascular obstruction and granulomatous inflammation, particularly in gills, heart, and kidney. Molecular characterization, based on partial 28S rDNA and mitochondrial *cox1* sequences confirmed affiliation with *Sanguinicolidae*.

However, the sequence demonstrated no close genetic similarity to currently described species, suggesting the presence of an only morphologically described species or a novel lineage. The severity and distribution of pathological lesions strongly suggest that the sanguinicolid infection was a major contributing factor to the observed mortality event.

To our knowledge, this represents the first report of a *Sanguinicola*-associated mass mortality event in wild juvenile barbels in Switzerland. The findings highlight the potential ecological relevance of sanguinicoliasis in natural fish populations. Given the limited molecular data available for European sanguinicolids, further research integrating morphological and molecular approaches are essential to improve species identification and to better understand the epidemiology and ecological impact of these parasites.

Keywords: Sanguinicoliasis, vascular parasite, blood fluke, histopathology, molecular identification

Ethical approval: ☒ Not applicable; ☐ Approved



ORAL COMMUNICATIONS II

Clinical, Forensic and Digital Pathology

IBERIAN Veterinary Pathology Meeting 2026 JUNE PORTO | PORTUGAL

O9

Advancing Veterinary Forensic Pathology: Diatom Identification in Formalin-Fixed Specimens for Retrospective Drowning Diagnosis.

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Background / Objectives: The diatom test is a well-established ancillary method in human forensic pathology for the supportive diagnosis of drowning; however, its implementation in veterinary forensic pathology remains limited, restricting the diagnosis of drowning in animals. The objective of this study is to retrospectively evaluate veterinary forensic cases involving suspected drowning, using diatom analysis of formalin-fixed tissues as a diagnostic method to determine the conventional consistency between the type and cause of death based on pathological findings.
Methods: A retrospective study of 11 animal carcasses recovered from aquatic environments and submitted for forensic pathological examination was conducted. In 7 cases, diatom analysis was conducted on available formalin-fixed organs. Pathology findings, initial forensic interpretations, and diatom test results were systematically reviewed. Diagnostic concordance between conventional pathology and diatom analysis was assessed.
Results: Conventional gross and histopathological examination demonstrated limited diagnostic specificity for drowning in carcasses recovered from aquatic environments, although pulmonary vascular leukocyte margination showed a statistically significant difference ($p = 0,0083$). Lesions suggestive of physical abuse (54.5%) and sexual abuse (9%) were also frequently observed, indicating a possible association between aquatic recovery and cases of animal abuse. Diatoms were detected in formalin-fixed tissues stored for up to 670 days post-fixation. The diatom test supported the presumptive forensic diagnosis in 6 of 7 cases and enabled the establishment of drowning as the cause of death in one additional case. Diagnostic concordance between diatom analysis and initial forensic assessment was high.
Conclusions: Diatom analysis in formalin-fixed tissues represents a methodologically robust and diagnostically valuable ancillary technique in veterinary forensic pathology. Its retrospective applicability enhances forensic diagnostic accuracy and enables the recovery of critical forensic evidence from archived material. Integration of this methodology into routine veterinary forensic pathology workflows may improve diagnostic certainty in aquatic-related cases.

Keywords: Diatoms, Drowning, Animal Abuse, Forensic Veterinary Pathology.
Ethical approval: ☒ Not applicable; ☐ Approved

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O10

Beyond plug-and-play: AI-assisted and digital image analysis in investigative pathology

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Introduction: AI-assisted and digital image analysis workflows are increasingly used in research pathology, but their practical value in experimental pathology remains uneven. We reviewed quantitative histopathology workflows implemented across 25 investigative pathology projects over two years, involving 707 whole-slide images from multiple tissues, stains, scanners and biological models. The aim was to evaluate the applicability, efficiency and limitations of different digital pathology approaches for experimental data quantification.
Methods: Whole-slide images from multiple scanners were analysed using QuPath-based workflows. Projects were grouped into six methodological categories: machine-learning or threshold-based pixel classification for lesion area segmentation; StarDist-assisted nuclear detection with machine-learning or threshold-based refinement for cell counting/IHC quantification; manual annotation; and mixed workflows. Operational variables included biological application, implementation burden and validation strategy. Semi-quantitative scores captured staining variability and lesion heterogeneity. Method distribution and workflow transferability were summarised descriptively. Implementation times were compared using appropriate tests, and associations with complexity metrics/annotation burden were assessed by Spearman correlation.
Results: The most frequent workflows were threshold-based approaches with (32%) or without StarDist nuclei segmentation (28%). These required less implementation time than machine-learning workflows (80 versus 120 min/slide), where annotation burden was often substantial. Faster implementation occurred when targets had clear morphologic or chromatic contrast, including tumour/parenchyma interfaces, trichrome-positive fibrosis, nuclear IHC markers and adipocyte/vacuole measurements. Fibrosis workflows were particularly efficient (~37 min/slide). Implementation time increased with lesion heterogeneity (ρ=0.66, p=0.037), particularly with necrosis/haemorrhage, emphysema-like spaces, and cytoplasmic or membranous markers. Workflow modules and custom-built scripts were reusable across methodological categories, but thresholds, classifiers and output metrics required project-specific fine-tuning for staining, scanner, tissue and lesion variability.
Conclusions: AI-assisted and digital image analysis were most effective as pathologist-guided assistive frameworks rather than universal plug-and-play solutions. Workflow modules were reusable, but final classifiers remained project-specific and tissue-complexity dependent.

Keywords: Digital Pathology, Image analysis, Investigative pathology, Quantitative histopathology, QuPath
Ethical approval: ☒ Not applicable; ☐ Approved

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O11

Catheter-associated venous air embolism in a hospitalized horse

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Background/Objectives: Venous air embolism is an uncommon but recognized fatal complication in hospitalized horses, frequently associated with intravenous catheter use. The aim of this report is to describe the clinicopathological and neuropathological findings of a catheter-associated air embolism in a young horse.
Methods: A 2-year-old purebred Spanish stallion underwent surgical removal of an odontoma. Shortly after surgery, following rupture of a three-way stopcock connected to a jugular catheter, the horse developed acute clinical signs including marked hyper-reactivity, head tilt, blindness, arrhythmia, heart murmur and muscle fasciculations. Supportive treatment was initiated, however, the animal progressively deteriorated and died following cardiac arrest. A complete necropsy, including gross, ultraviolet (Wood's lamp), and histopathological examination, was performed.
Results: Gross findings included pulmonary congestion and edema, mild subepicardial hemorrhages, and the presence of multiple intravascular gas bubbles within leptomeningeal vessels. Multifocal areas of encephalomalacia, particularly in the frontal and occipital cortex, were identified and further highlighted by ultraviolet light. Histologically, the cerebral cortex showed multifocal lacunar infarcts characterized by acute neuronal necrosis (red neurons), spongiosis, perivascular oedema, occasional mild inflammatory infiltrates and multifocal microhemorrhages. Similar but less prominent changes were also multifocally present within the brainstem and cerebellar cortex. Pulmonary edema, congestion and multifocal subepicardial hemorrhages were confirmed microscopically.
Conclusions: These findings support a diagnosis of catheter-associated venous air embolism with prominent neurological involvement. This case emphasizes the importance of appropriate catheter management in horses and highlights the diagnostic value of combining gross examination, ultraviolet imaging, and histopathology.

Keywords: Air embolism, horse, catheter
Ethical approval: ☒ Not applicable; ☐ Approved

O12

Paraprobiotic supplementation modulates T cell, B cell and macrophage populations in the small intestine of weaned piglets

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Background/Objectives: Antibiotic restrictions in livestock have driven the need for alternative strategies to reduce the impact of postweaning diarrhea and re-emerging pathogens, highlighting the interest in feed additives. As the gut is central to mucosal immunity via innate and adaptative responses, and heat-killed *Mycolicibacterium manresensis* (hkMm) has been shown to modulate T cell responses, this study aimed to further characterize its immunomodulatory effects on cellular, humoral, and innate immunity in weaned piglets.
Methods: Four-week-old piglets were randomly allocated into three groups (n=5): control or diets supplemented with 10 or 50 ppm of hkMm. At day 70, duodenal and jejunal samples were analyzed by immunohistochemistry using markers for T cells (CD3, FoxP3, IFN-γ, TCR-δ), humoral immunity (CD20, IgA), and macrophages (CD163). Statistical analyses were performed using GraphPad Prism.
Results: The intestinal mucosa was predominantly composed of intraepithelial $CD3^+$ cells, whereas $CD20^+$ and $CD163^+$ cells were mainly localized in the crypts. hkMm supplementation increased $CD3^+$ cells, particularly TCR-δ^+ and $FoxP3^+$ subsets, resulting in higher TCR-δ/CD3 and FoxP3/CD3 ratios, especially in the duodenum. IFN-γ^+ cells moderately increased at 10 ppm in the jejunum, accompanied by a higher IFN-γ/CD3 ratio. $CD20^+$ and $CD163^+$ cells showed slight increases with supplementation, while their ratios relative to $CD3^+$ remained stable. IgA staining was prominent in crypts and lamina propria, particularly in the duodenum. Correlation analysis revealed positive associations between $CD3^+$ and TCR-δ^+ cells in both intestinal segments (d: $r = 0.65$; j: $r = 0.53$), and between TCR-δ^+ and $CD163^+$ cells in the jejunum ($r = 0.70$), whereas $CD163^+$ cells were negatively correlated with IFN-γ^+ cells in the duodenum ($r = -0.53$).
Conclusion: hkMm supplementation modulated the intestinal immune response in a dose-dependent manner, promoting a regulated mucosal immune environment with increased TCR-δ^+, $FoxP3^+$, and $CD163^+$ cells. Further functional studies are required to clarify the underlying mechanisms.

Keywords: Piglet, paraprobiotic, immunity, intestine, immunohistochemistry
Ethical approval: ☐ Not applicable; ☒ Approved

O13

Methanobrevibacter smithii-Induced Mucosal Immune Modulation in Early-Weaned Piglets Revealed by Deep Learning-Based Histopathology

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Background/Objectives: Early weaning is a major stressor in swine production, disrupting intestinal immune homeostasis and mucosal development in piglets. Methanobrevibacter smithii, a dominant methanogenic archaeon of the gut microbiota, has been proposed as a potential regulator of intestinal health. This study evaluated the mucosal immunomodulatory effects of M. smithii supplementation in early-weaned piglets using a deep learning-driven digital histopathology approach.

Methods: Piglets were randomly assigned to three groups (n=9 each): unweaned, weaned with milk replacer (WMR) and WMR supplemented with M. smithii. Ileum, colon, and mesenteric lymph node (MLN) samples were collected for histopathological analysis including PAS-Alcian blue staining and immunohistochemistry for immune cells populations. Machine learning-assisted tissue segmentation and quantitative image analysis (Visiopharm) were applied to whole-slide images to assess morphometric parameters and to quantify the densities of IBA1$^+$, CD3$^+$, FoxP3$^+$, PAX5$^+$, IgM$^+$, IgA$^+$ and goblet cell populations across defined histological regions of interest.

Results: Quantitative analysis of IBA1$^+$ cells revealed no significant differences among groups, supporting the absence of inflammatory histopathological changes. In contrast, compared with the WMR group, M. smithii supplementation significantly increased CD3$^+$ intraepithelial lymphocytes and lamina propria lymphocyte populations in the ileum and colon, but also in MLN. FoxP3$^+$ cells were increased in the ileal and colonic lamina propria of M. smithii-supplemented piglets, indicating enhanced mucosal immune regulation. In MLN, M. smithii supplementation increased PAX5$^+$ B cells and lymphoid follicle numbers. IgM$^+$ cells were significantly increased in both intestinal segments compared with WMR piglets, whereas IgA$^+$ cells showed higher numbers in the ileum and MLN compared with unweaned piglets. Increased goblet cell abundance was observed in the ileum and colon of WMR and M. smithii-supplemented piglets compared with unweaned animals.

Conclusions: These results indicate that M. smithii supplementation promotes mucosal immune modulations that are predicted to be beneficial in early-weaned piglets. Deep learning-based digital histopathology enabled objective quantitative assessment of intestinal immune responses,

supporting its value for translational gut health research.

Keywords: Deep-learning, digital histopathology, mucosal immunity, piglets, *M. smithii*

Ethical approval: ☐ Not applicable; ☒ Approved

O14

Pulmonary arteriopathy associated to acute pulmonary hypertension in a young bitch following closure of left-to-right patent ductus arteriosus

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Pulmonary arteriopathy (PA), is an uncommon cause of pulmonary hypertension (PH) in dogs, characterized by diffuse constrictive and/or multifocal complex pulmonary arterial lesions, causing irreversible vascular obliteration, PH and right-sided heart failure. An acute variant of PA has been described in young dogs, appearing either alone or in conjunction with patent ductus arteriosus (PDA) and peritoneopericardial diaphragmatic hernia. We present the case of a 3.5- months-old, mixed-breed female dog, in which a left-to right PDA was surgically corrected, with an initially favorable recovery. At 36 hours post-surgery, the patient developed acute PH that progressed to right-sided congestive heart failure in 2 weeks despite treatment and was euthanized. Macroscopically, the main pulmonary artery and its two branches appeared dilated, and the ligation of the PDA was correctly positioned in the postmortem examination. Histopathology revealed constrictive lesions consisting of medial and intimal thickening of large-, medium-, and small- pulmonary arteries, with obliteration of the lumen of the arterioles. Medial thickening involved smooth muscle fibers hypertrophy and hyperplasia, and intimal thickening was characterized by endothelial hypertrophy and/or hyperplasia, which in some vessels was accompanied by subendothelial degeneration extending into the underlying media and by fibrinoid necrosis in some arterioles. Additionally, complex plexogenic lesions were observed in muscular arteries, consisting of a dilated vessel with dense, rounded aggregates of proliferating vascular cells arranged in a whorling pattern. Ancillary lesions included exudative alveolitis, thrombosis and calcification of the wall of a large artery, and some areas of hemorrhage and hyalinized fibrin in alveolar lumens. These findings are consistent with PA. Although PH was not clinically diagnosed before PDA closure and a causal relationship cannot be confirmed, a contributory role cannot be excluded. In any case, PA should be included in the differential diagnosis of acute PH in young dogs, with or without PDA.

Keywords: Pulmonary arteriopathy; pulmonary hypertension; patent ductus arteriosus; histopathology

Ethical approval: ☒ Not applicable; ☐ Approved

O15

From primary tumour to metastasis: Molecular subtype changes in canine mammary tumours

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Canine mammary carcinomas (CMCs) are the most prevalent neoplasms in intact female dogs, with up to 50% being malignant and capable of metastasizing to regional lymph nodes. In human breast cancer, molecular subtyping is critical to support therapeutic decisions, and changes in molecular subtype and receptor status can occur during tumor progression with implications for prognosis. This study aimed to evaluate hormone receptor discordance and molecular subtype changes between primary mammary tumours (PTs) and paired synchronous lymph node metastases (LNMs) in female dogs. Thirty canine mammary carcinoma cases with confirmed LNMs were retrospectively selected from the Histopathology Laboratory of UTAD (Vila Real, Portugal). Immunohistochemical analysis was performed to assess oestrogen receptor (ER), progesterone receptor (PR), HER2 status, and Ki-67 expression. Molecular subtypes were classified as luminal A-like, luminal B-like HER2-negative, luminal B-like HER2-positive, HER2-overexpressing or triple-negative. Discordance rates between PTs and LNMs were 11.1% for ER, 26.9% for PR, and 11.5% for Ki-67. All samples were HER2-negative and molecular subtypes was successfully determined in 27 of the 30 cases. Overall molecular subtype discordance was observed in 40.7% of cases. Among subtypes, luminal A-like showed the highest discordance rate (44.4%), followed by triple-negative (42.8%) and luminal B-like (36.4%). Of the discordant cases, five transitioned to subtypes associated with poorer prognosis, while six shifted toward subtypes associated with more favourable outcomes. Similarly to human breast cancer, molecular expression patterns in CMCs may not be preserved between PTs and synchronous LNMs. Further research is required to better understand the biological mechanisms underlying subtype discordance and to determine ideal treatment strategies based on these phenotypic changes, reinforcing the importance of a dual-site molecular approach for accurate prognosis and targeted therapy selection in CMCs.

Keywords: Dog, neoplasia, metastasis, molecular profile, subtype change

Ethical approval: ☒ Not applicable; ☐ Approved

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O16

Pathological findings in the 2025–2026 Foot-and-Mouth Disease outbreak in Cyprus

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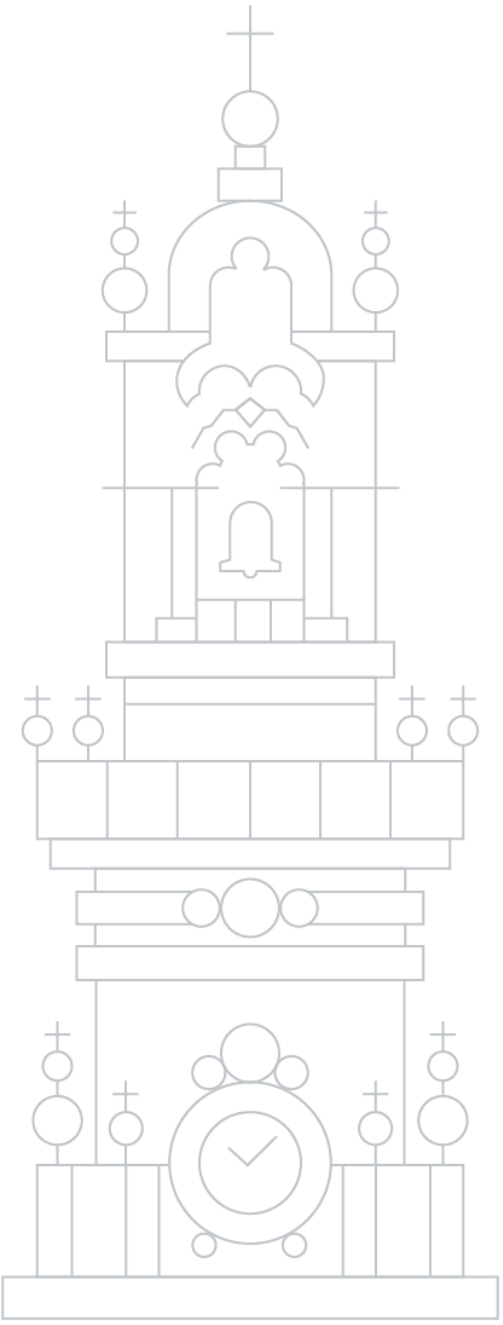
Background: An outbreak of Foot-and-Mouth Disease (FMD) was confirmed in Cyprus in late 2025, with continued cases into early 2026 involving cattle, sheep and goats. Molecular testing and phylogenetic analysis identified a SAT1 serotype, representing an atypical introduction for the Eastern Mediterranean. Because early detection relies heavily on lesion recognition, characterization of macro- and microscopic pathology was prioritized during field investigations.
Objective: To document and describe the principal gross and microscopic lesions observed in domestic ruminants affected during the 2025–2026 FMD outbreak in Cyprus, emphasizing features useful for diagnosis and differentiation from other vesicular diseases.
Methods: Clinical examinations, farm-level inspections and targeted sampling was performed on affected cattle, sheep and goats between December 2025 and March 2026. Tissue samples from oral mucosa, coronary band, and myocardium (especially from juvenile mortalities) were processed for routine histopathology. Selected specimens underwent RT-PCR and phylogenetic analysis.
Results: Gross pathology consisted mainly of vesiculo-erosive lesions of the tongue, dental pad, gingiva and coronary bands. Vesicles commonly progressed to coalescing erosions with fibrinous exudate. Small ruminants showed more subtle oral lesions, whereas young animals exhibited characteristic pale streaking of the myocardium. Microscopically, the oral mucosa displayed intraepithelial vesicles with ballooning degeneration, intercellular edema, and loss of cohesion among keratinocytes. Ulcerated areas exhibited variable fibrinopurulent exudates and granulation tissue. Myocardial samples from juvenile cases demonstrated multifocal myofiber necrosis characterized by myofibrilar hypereosinophilia, nuclear pyknosis and mononuclear interstitial infiltrates.
Conclusions: The Cyprus outbreak showed classical FMD pathology with species-dependent severity and notable myocarditis in young stock. The lesion spectrum aligns with SAT1 infection and supports rapid field recognition. Detailed pathological characterization strengthened differential diagnosis and enhanced early outbreak detection during an unusual regional introduction of FMD.

Keywords: Foot-and-Mouth, Macroscopic lesions, microscopic lesions, small ruminants, large ruminants

Ethical approval: ☒ Not applicable; ☐ Approved



ORAL COMMUNICATIONS III

Neoplasia and Comparative Pathology

O17

Histopathological and immunohistochemical study of integrins expression in canine gastric carcinoma

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Gastric carcinoma accounts for 60-70% of gastric neoplasms in dogs. Malignancy is associated with cellular features such as pleomorphism, anisocytosis, anisokaryosis, and high mitotic index; but undoubtedly, migration, infiltration, and metastasis are key determinants of tumor aggressiveness. These processes can be assessed through immunohistochemical analysis of integrins, heterodimeric transmembrane proteins composed of α and β subunits that mediates cell adhesion to the extracellular matrix. Aberrant integrin expression has been linked to metastatic progression in both human and veterinary oncology, including gastric tumors. In this study, gastric biopsies from 25 dogs were processed and histopathologically classified according to WHO criteria for domestic animals. The most frequent tumor type was signet ring cell carcinoma (52%, n=13), followed by undifferentiated (28%, n=7) and tubular carcinomas (24%, n=6). Immunohistochemistry was performed to evaluate the intensity of α1, α3 and β1 subunits immunoexpression using a semiquantitative scoring system (0–3). For all three subunits, superficial tumor cells showed lower immunoreactivity compared to normal epithelium and non-neoplastic glands. By contrast, highly infiltrative and metastatic cells exhibited increased integrin expression that was similar to or higher than that observed in epithelial cells. Among tumor types, signet ring cell carcinoma displayed the lowest immunoexpression, whilst undifferentiated and tubular carcinomas showed higher levels. Stroma mesenchymal cells demonstrated mild immunoreactivity, with an increased expression observed in tumor-associated fibroblasts. These results provide important information about the involvement of α1, α3 and β1 subunits in the morphology and biological behavior of each type of gastric carcinoma in the dog, as well as in the phenomenon of metastasis.

Keywords: Canine gastric carcinoma, histopathology, immunohistochemistry, integrin, metastasis
Ethical approval: ☒ Not applicable; ☐ Approved

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O18

Beyond Mammary Neoplasia: Reproductive Lesions in Queens

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Background / Objectives: Mammary tumors are among the most common neoplasia in queens. Mastectomy and concurrent ovariohysterectomy are frequently performed, but reproductive tract tissues are not routinely submitted for histopathological evaluation unless gross abnormalities are observed. As concurrent mammary and reproductive tract lesions remain poorly described in cats, this study aimed to investigate coexisting ovarian and uterine lesions in queens presented with mammary masses.

Materials and Methods: One hundred and four intact female queens (97 Domestic Shorthair, 7 Persians; 6-21 years, mean 10.2±3.0), with mammary lesions submitted to simultaneous mastectomy and ovariohysterectomy, were included. All tissues were submitted to histopathology evaluation.

Results: Malignant mammary tumors were identified in 81/104 (77.9%) queens. The most frequent malignant histological subtypes were tubular, tubulopapillary, and solid carcinomas. Uncommon tumor types were also identified, including carcinosarcoma (n=4), inflammatory (n=1), anaplastic (n=1), adenosquamous (n=3), and micropapillary (n=3) carcinomas. Among benign mammary lesions (23/104; 22.1%), adenomas, fibroadenomatous changes, and lobular hyperplasia predominated. Only 6/104 queens showed no reproductive tract lesions. Concurrent malignant mammary and uterine tumors were identified in 32 queens, mainly feline endometrial adenocarcinomas (FEA) (n=31; 29.8%), including in situ (n=7), papillary serous (n=22), and papillary serous with clear cells (n=2) histotypes; one queen presented uterine leiomyosarcoma. 9 queens had uterine adenomas. Pyometra (n=8) and cystic endometrial hyperplasia (n=50) were also frequent. Ovarian lesions were less common, with ovarian cysts predominating. Two queens presented ovarian metastases of mammary tumors, one of which also had uterine metastasis.

Conclusions: Feline concurrent mammary and reproductive tract lesions appear to be common. The frequent coexistence of FEA and mammary adenocarcinomas may suggest shared carcinogenic pathways. Additionally, atypical metastasis of mammary carcinomas to reproductive tract organs were observed. These lesions may have important clinical implications and could remain underdiagnosed if reproductive tissues are not routinely submitted for histopathological evaluation.

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Keywords: Feline, Mammary Tumors, Oncology, Ovary, Uterus
Ethical approval: ☒ Not applicable; ☐ Approved

O19

Differences in histopathology and local immune response in steady and progressive transmissible venereal tumors in Mexican Dogs

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The canine transmissible venereal tumor (TVT) is a neoplasm affecting the external genitalia of dogs and is recognized as one of the four naturally occurring contagious tumors reported in animals. TVTs can present in different stages, including steady, progressive, and regressive forms. In certain regions of Mexico, where TVT prevalence is high (5.15%), two main morphological types are commonly identified: a steady, pedunculated, strawberry-like form (Type A) and a progressive, multilobulated, cauliflower-like form (Type B). This study aimed to characterize the histopathological features and inflammatory infiltrate patterns in eight female stray dogs naturally affected by both TVT morphologies (n = 4 per type), in order to identify possible differences between them. Histopathological and immunohistochemical techniques were applied to tumor samples to evaluate the interaction between pathological morphology and the following cell markers: ionized calcium-binding adaptor molecule 1 (IBA1) for activated macrophages (including resident macrophages), inducible nitric oxide synthase (iNOS) for M1 macrophages, CD163 for M2 macrophages, CD3 for T lymphocytes, CD20 for B lymphocytes, and lambda light chain for plasma cells. Immunopositive cells were counted using the QuPath image processing software. For each sample, five random areas at 4x magnification were quantified. Statistical analyses were performed to assess the relation between inflammatory infiltrate and morphological types. The results showed a greater inflammatory infiltrate in Type A tumors than in Type B ones, with a parallel increase in activated macrophages and B lymphocytes. The presence of M1 and M2 macrophages was scarce in both types of tumors, and T lymphocytes were almost absent. This study reveals a stronger and more balanced local immune response in dogs with Type A TVTs compared with Type B tumors, which may underlie differences in tumor characteristics, although individual tumor heterogeneity should be considered.

Keywords: TVT, immunohistochemistry, macrophages, lymphocytes
Ethical approval: ☒ Not applicable; ☐ Approved

O20

Changing the Odds of Canine Lymphoma: From Characterization to Antibody-drug Conjugate Development

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Background/ Objectives: Immunotherapies are an emerging class of cancer therapies that are improving outcomes beyond conventional approaches. Among these, antibody-drug conjugates (ADCs) are particularly promising, combining antibody specificity with potent cytotoxic payloads, enabling targeted elimination of neoplastic cells within a precision medicine framework.
Successful ADC development depends on accurate tumour characterization, particularly tumour-associated antigens expressed on the cell membrane. Biobanks provide access to well-preserved biological samples essential for cancer research and novel therapies development. Canine lymphoma is the most prevalent hematopoietic neoplasm with diffuse large B-cell lymphoma (DLBCL) being the predominant subtype and a strong comparative model for human non-Hodgkin lymphoma. This study aimed to establish a canine lymphoma biobank, characterize tumour antigen expression, develop an antibody targeting tumour-associated antigens, and identify cytotoxic payloads for future ADC development.
Methods: A canine lymphoma biobank was established using samples from dogs diagnosed with lymphoma and followed at the Veterinary Teaching Hospital from the Faculty of Veterinary Medicine – University of Lisbon. Samples underwent histopathological and immunophenotypic characterization to identify potential immunotherapeutic targets. Following target identification, a recombinant antibody was developed through rabbit immunization and phage display selection of the best candidate. Additionally, eight candidate payloads were tested in two canine lymphoma cell lines (CLBL-1 and 17-71) to determine their cytotoxicity.
Results: The biobank included samples from twenty-two canine lymphoma patients, with DLBCL being the most prevalent subtype. CD20 was identified as a therapeutic target as well as another antigen that is currently being characterize. A highly selective anti-CD20 antibody with strong binding properties was successfully developed. Among the tested compounds, MMAE, DM1 and ARV-825 showed the highest cytotoxic activity against canine lymphoma cells.
Conclusions: This work highlights the central role of pathology in precision medicine approaches in veterinary oncology and establishes a platform for the development of novel immunotherapies for canine lymphoma.

Keywords: Canine Lymphoma, Immunotherapy, Biobank, ADC, Cytotoxicity
Ethical approval: ☐ Not applicable; ☒ Approved

O21

Pretenders among lymphomas – don't be betrayed by the eye

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Background/Objectives: Round cell tumours in dogs, such as lymphomas, cutaneous histiocytomas, mast cell tumours, plasma cell tumours, and amelanotic melanomas, present a diagnostic challenge due to their morphological similarity. These difficulties can lead to inaccurate classifications, directly impacting treatment decisions and patient prognosis. In routine diagnostic settings, classification may still rely predominantly on morphological evaluation, potentially leading to diagnostic discrepancies. This study aimed to assess the diagnostic impact of immunophenotyping in a large retrospective series of canine cases initially classified as lymphoma based on morphological criteria.

Methods: A retrospective study was performed on 201 archived cases (1995–2024) from the Histology and Anatomical Pathology Laboratory, University of Trás-os-Montes e Alto Douro. These cases, previously identified as lymphomas based on morphological criteria, underwent immunophenotyping using specific antibodies including anti-PAX-5, anti-CD3, anti-IBA1, and anti-MUM1.

Results: Definitive diagnoses were established for 161/201 (80.1%) cases, with 66 (32.8%) identified as B-cell lymphomas and 74 (36.8%) as T-cell lymphomas. Additionally, 16 cases (8.0%) were identified as histiocytic tumours and 5 (2.5%) as plasma cell tumours. The remaining 40 cases (19.9%) showed inconclusive immunophenotypic profiles and could not be definitively classified.

Conclusions: This study highlights the diagnostic value of immunophenotyping in differentiating lymphomas from other round cell tumours in dogs. The application of specific antibodies significantly enhanced diagnostic accuracy, as evidenced by the reclassification of 10.5% of cases initially classified as lymphomas into histiocytic or plasma cell tumours, while 19.9% showed inconclusive immunophenotypic profiles. These findings reinforce the limitations of morphology-based diagnosis in routine veterinary pathology.

Funding: Supported by Foundation for Science and Technology (FCT) projects UIDB/00772/2020 (doi:10.54499/UIDB/00772/2020) and Nuno R. Lima's doctoral scholarship (2023.02669.BD).

Keywords: Canine, lymphoma, immunophenotyping, round cell tumours, diagnostic discrepancies

Ethical approval: ☒ Not applicable; ☐ Approved

O22

Five- year pathological monitoring of highly pathogenic avian influenza H5N1 in naturally infected poultry and wild birds in Portugal

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Introduction: The first outbreak of highly pathogenic avian influenza (HPAI) H5N1 in Portugal was diagnosed in a backyard poultry flock in November 2021. This highly contagious disease subsequently spread to other regions of the country, affecting several intensive poultry farms and causing high mortality rates and severe economic losses.

Materials and Methods: Between November 2021 and December 2025, under the Avian Influenza Contingency Plan, between, 483 avian specimens (429 poultry and 54 wild birds) infected with HPAI were necropsied at the Nacional Reference Laboratory for Animal Diseases of Portugal (INIAV I.P.). Samples from the encephalon, heart, respiratory tract, liver, spleen, pancreas, kidney and gastrointestinal tract were collected from 30 specimens for histopathology.

Results: The most common lesions were caused by viral localization in endothelial and epithelial cells. This led to congestive-hemorrhagic vascular changes and inflammatory lesions in mucous membranes, which particularly affected the respiratory system. Some specimens exhibited serofibrinous exudate and fibrinous deposition in serous membranes. The severity and location of microscopic lesions varied, and they mainly consisted of vascular changes, necrotic foci, and lymphoplasmacytic inflammatory response, which was most evident in the trachea, lungs, spleen, and pancreas. Due to viral neurotropism, lesions indicative of non-suppurative encephalitis were commonly observed.

Conclusions: Necropsies revealed different lesion patterns, resulting from variable viral tropism within the same species. These patterns were consistent with previously described findings, except for reduced kidney involvement in chickens and the hydropericardium in Portuguese turkey specimens. The lesions reflected the widespread viral distribution of the virus throughout the body. However, in acute cases, lesion expression was less evident. Continuous monitoring of the lesion profiles is essential to identifying deviations in the virus's tropism and pathogenesis.

Keywords: HPAI, H5N1, poultry, wild birds, pathology

Ethical approval: ☒ Not applicable; ☐ Approved

O23

Characterization of tumor-associated stroma and fibrosis in feline mammary carcinomas

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Although tumor stroma is a key component of the tumor microenvironment and an established prognostic factor in human breast cancer, studies on the stroma of feline mammary carcinomas (FMCs) remain scarce. This study aimed to characterize the tumor stroma of 129 queens with 189 FMCs using semi-quantitative and quantitative approaches.

Tumor stroma percentage was estimated in a single microscopic field (10x magnification) showing the highest stromal content in Picrosirius red-stained cross-sections, following the tumor-stroma ratio (TSR) method described for human breast cancer by Hagenaars and colleagues (2022). A photomicrograph of the selected field (4x magnification) was then captured for digital image analysis in ImageJ software using two thresholds: Th135, representing total stroma, comprised all Picrosirius red-labelled structures, including thin collagen fibers; Th90, representing fibrosis, encompassed predominantly thicker collagen fibers. Additionally, the fibrosis-to-stroma ratio was calculated as Th90/Th135*100.

In the semi-quantitative assessment, most tumors had stromal percentages of ≤10% (62/189, 32.8%), 11-20% (40/189, 21.1%) and 21-30% (22/189, 11.6%). In the digital analysis, the median (IQR, range) percentage of total stroma – Th135 – was 17.8 (24.4, 0.01-79.17), while the median (IQR, range) percentage of fibrosis – Th90 – was 9.2 (16.2, 0.00-69.42). The median (IQR, range) fibrosis-to-stroma was 54.6 (22.6, 4.42-87.69). Cohen's kappa showed a poor agreement between the semi-quantitative assessment and Th135 (k = 0.193), with concordance in 34.9% (66/189) of tumors. Still, the majority of tumors displayed ≤30% stroma and fibrosis across methods, whilst the fibrosis-to-stroma ratio surpassed 50% in most tumors.

These findings suggest that FMCs generally exhibit low stromal content, with fibrosis comprising a substantial proportion of the stromal compartment. Further studies are needed to determine the biological and prognostic significance of stroma amount in FMCs.

Keywords: Feline mammary carcinoma, fibrosis, stroma, tumor microenvironment
Ethical approval: ☐ Not applicable; ☒ Approved

O24

Comparative proteomic profiling of tuberculous granulomas at different stages of lesion development in cattle and pigs using MALDI-imaging mass spectrometry

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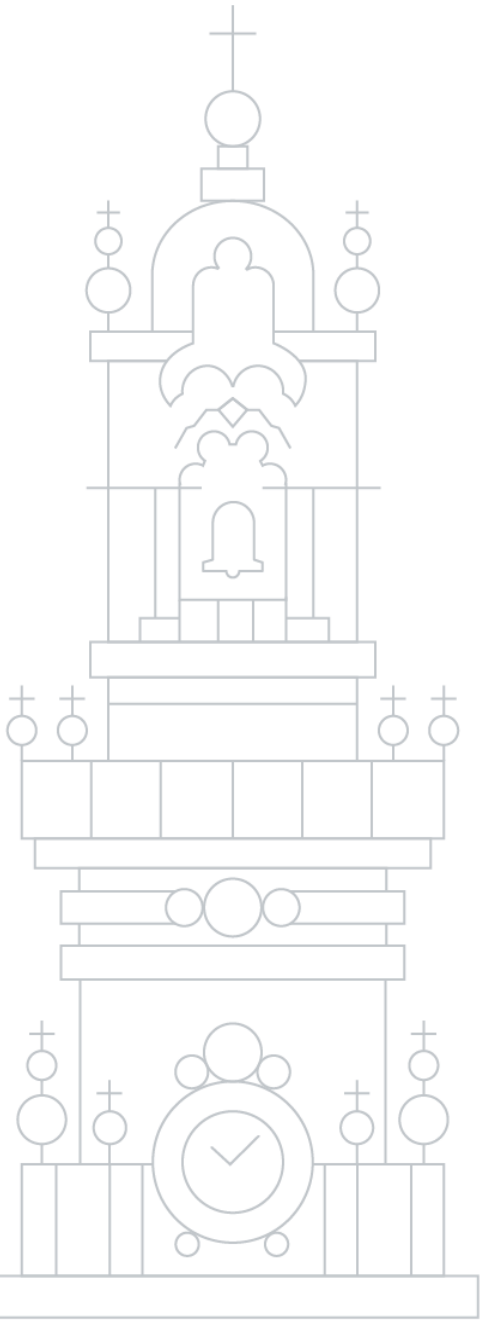
Background/Objectives: Tuberculosis remains a significant zoonotic disease characterised by the formation of granulomas. It is essential to understand its molecular composition across species and lesion stages, to elucidate the pathogenesis of this disease. Thus, the aim of this study was to characterise and compare the proteomic profile of tuberculous granulomas at different developmental stages in cattle and pigs using MALDI-MSI.

Methods: Lymph node samples from naturally infected cattle (n=220 granulomas) and pigs (n=78 granulomas) were analysed. Granulomas were classified histologically into four stages (I–IV) using H&E and Ziehl-Neelsen staining. MALDI-MSI was applied to identify m/z features, followed by bioinformatic and Gene Ontology (GO) enrichment analyses.

Results: Early-stage granulomas (I–II) in both species were associated with metabolic activation, controlled proteolysis, platelet aggregation, apoptosis regulation, and protein synthesis pathways. In cattle, these stages highlighted pathways such as protease regulation and focal adhesion, whereas in pigs, glycolysis and translational processes predominated. Advanced stages (III–IV) showed increased metabolic demand, cytoskeletal reorganisation, and immune activation. Notably, pigs exhibited enhanced pathways related to glycosylation and immune effector mechanisms, while cattle showed increased apoptosis and structural stabilisation. Consistent pathways across species included regulation of proteolytic activity, ECM-receptor interaction, and protein localisation, indicating conserved mechanisms during granuloma progression. Interestingly, complement-associated pathways were activated in the early-stage bovine granulomas, but appeared in the late-stage porcine granulomas. Additionally, the formation of neutrophil extracellular traps was characteristic only in early-stage granulomas in pigs.

Conclusions: Tuberculous granulomas exhibit a dynamic shift in their proteome from the early stages of metabolic adaptation and survival to the late stages of structural remodeling and immune amplification. Despite species-specific differences, shared pathways indicate that there are common mechanisms in granuloma maturation. This provides insight into host-pathogen interactions and highlight potential biomarkers of interest.

Keywords: MALDI-MSI, tuberculosis, stage granulomas, ontology
Ethical approval: ☒ Not applicable; ☐ Approved



ORAL COMMUNICATIONS IV

Teaching, Fish Pathology and Zoo/Wildlife

O25

Gamification, game-based learning, veterinary education, student engagement

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Background: Teaching Veterinary Pathology involves a large volume of theoretical content, which can make it difficult for students to understand, retain, and integrate key concepts. These challenges become more evident in advanced courses, particularly when students need to apply theorethical concepts to lesion interpretation and solve practical cases. Based on the available literature in other disciplines, previous studies support the effectiveness of game-based learning in visually oriented subjects increase students overall learning engagement. For this reason, the objective of the Path-satiempos project is to implement gamification in the learning of Veterinary pathology from the first to the fifth year of the Veterinary degree.
Materials and Methods: The *Path-satiempos* project is based on game-based learning and is currently in a pilot phase (academic year 2025–2026). It includes crosswords, word search puzzles, matching exercises, visual challenges, bingos and clinical riddles, all adapted to the different learning levels of each course of the Veterinary degree. These activities are being tested in a pilot group of students, with plans to expand them to the entire class in the following academic year. They will be integrated into seminars, workshops, and practical sessions. A test–retest design will be applied using an initial and final activity, along with eight additional exercises distributed across thematic blocks. Each evaluation will include performance analysis (accuracy and completion time) and a satisfaction survey.
Results and Discussion: Improvements in knowledge acquisition and concept retention are expected through gamification, as reflected by higher accuracy and reduced completion time in the retest. This is expected to be accompanied by increased student motivation, engagement, and meaningful learning. Preliminary results showed a good engagement from the pilot group during the activities.
Conclusions: Gamification through *Path-satiempos* is proposed as a complementary teaching tool with potential to enhance active learning, content integration, and student motivation.

Keywords: *Path-satiempos*: learning pathology through gamification
Ethical approval: ☒ Not applicable; ☐ Approved

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O26

Comparative epidemiological analysis of tumors of the digestive system in dogs and cats

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Introduction: Gastrointestinal (GI) disorders are a leading reason for veterinary care.
Methods: This study analyzed digestive tract tumors in dogs and cats in Portugal using data from the Vet-OncoNet database, focusing on frequency, risk factors, and geographic distribution.
Results and discussion: A total of 1,213 cases were included: 617 dogs (50.9%) and 596 cats (49.1%), with a higher proportion of males (54.9%) than females (45.1%). The most affected organs overall were the small intestine (26.5%) and liver/intrahepatic bile ducts (16.7%). Lymphoma was the most common tumor type in both species (42.2%), followed by adenocarcinoma (19.0%). Among dogs, mixed breeds, Labrador Retrievers, German Shepherds, and French Bulldogs were most affected. In cats, Common European, mixed-breed, and Norwegian Forest cats predominated. The incidence rate (IR) of digestive tumors was 3.5 times higher in cats than dogs. Male cats had a 1.5 times higher IR than females. Cats also had 16 times higher risk for GI lymphoma and twice the risk for adenocarcinoma compared to dogs. Certain dog breeds, including West Highland White Terrier, Siberian Husky, and Golden Retriever, showed higher tumor incidence. Spatial analysis revealed concentration in urbanized areas, particularly around Porto and Lisbon.
Conclusion: These findings highlight notable species-specific differences in digestive tract tumors, suggesting distinct genetic predispositions and possible environmental influences.

Keywords: Epidemiology, Digestive Tract Neoplasms, Comparative Oncology, Dogs, Cats, Vet-OncoNet
Ethical approval: ☐ Not applicable; ☒ Approved

O27

Unrecognized manifestations of chronic furunculosis in turbot (*Scophthalmus maximus*): deep muscular lesions and pericarditis associated with *Aeromonas salmonicida*

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Furunculosis, caused by *Aeromonas salmonicida*, is a major bacterial disease in aquaculture. In turbot (*Scophthalmus maximus*), chronic infection has been primarily associated with superficial granulomatous dermatitis, in contrast to salmonids, where deeper tissue involvement is recognized. Field observations, however, suggest that the lesion spectrum in turbot may be broader than currently described.

During on-farm monitoring of natural outbreaks in Galicia (Spain), fish displaying atypical lesions were identified and examined using bacteriological culture, specific qPCR, full necropsy, histopathology, and immunohistochemistry.

Two previously unrecognized patterns were identified. First, six fish presented deep, fluctuant muscular lesions characterized by cavitated areas containing viscous exudate. Histologically, these corresponded to extensive liquefactive myonecrosis with cavity formation and abundant granulocytic infiltration, with bacterial colonies located at lesion margins. *A. salmonicida* was consistently detected by immunohistochemistry and isolated from lesion exudates. Second, five additional fish showed pericardial involvement characterized by inflammatory lesions associated with bacterial presence in cardiac tissues. In both patterns, *A. salmonicida* was confirmed by qPCR and/or immunohistochemistry. These findings expand the recognized tissue tropism of *A. salmonicida* in turbot and indicate that chronic furunculosis may involve deeper and systemic compartments beyond the skin.

The presence of viable bacteria within muscular cavities suggests apotential role as persistent infection foci, while cardiac involvement points to previously underappreciated dissemination pathways. Recognition of these manifestations is relevant for diagnosis and may have implications for disease persistence and transmission under farm conditions.

Keywords: Turbot, Aeromonas salmonicida, furunculosis, muscle lesions, pericarditis

Ethical approval: ☒ Not applicable; ☐ Approved

O28

Disseminated fatal toxoplasmosis in captive ring-tailed lemurs (*Lemur catta*): a case series

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Lemur catta are strepsirrhine primates native to Madagascar, making them one of the most evolutionarily unique primate lineages and highly valuable for conservation since
94% of the 112 species of lemurs are endangered. We describe an acute outbreak of toxoplasmosis originating at a zoo that affected a group of lemurs, consisting of 8 adults and 4 juveniles.
Initially, affected animals exhibited clinical signs of apathy, fever and anorexia, with suspicion of intoxication. The first adult succumbed on day one, and antimicrobial therapy was subsequently administered to the group. On the second day, necropsy findings indicated severe, multifocal, subacute necrohemorrhagic hepatitis, initially suggestive of a hepatotropic agent etiology, thereby raising concerns regarding biosecurity and potential zoonotic risks. Samples were collected for histopathological examination (10% neutral buffered formalin), microbiological, and molecular analysis. On the same day, two additional animals (one adult and one juvenile) deceased. Necropsy findings corroborated previous observations, with the addition of severe ulcerative enterocolitis.
Microbiological cultures yielded negative results and antibiotic therapy was stopped thereafter. The remaining animals died over the following days. Histopathological examination on day five revealed granulomatous encephalitis and severe necrohemorrhagic splenitis, hepatitis, and myocarditis, with numerous amphophilic protozoal structures (~5µm) that were consistent with tachyzoites.
Infection with *Toxoplasma gondii* was definitively confirmed by PCR in multiple organs, supporting a diagnosis of acute disseminated toxoplasmosis. Additionally, ulcerative enterocolitis with fungal hyphae was observed, likely secondary to antibiotic-induced dysbiosis. The presence of cats in the area was later confirmed.
These findings emphasize the marked susceptibility of *Lemur catta* to acute disseminated toxoplasmosis, often associated with high mortality. This case series underscores the diagnostic value of histopathology, the necessity of strict biosecurity and necropsies protocols in zoological environments, and the importance of understanding species-specific susceptibility in the management of captive exotic collections.

Keywords: *Lemur catta*, *Toxoplasma gondii*, Histopathology, Zoo pathology, Biosecurity
Ethical approval: ☒ Not applicable; ☐ Approved

O29

Immunohistochemical study of pleural mesothelioma in an angolan lion (P*anthera leo bleyenberghi*)

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We present a case of a 20-year-old adult female Angolan lion (*Panthera leo bleyenberghi*) with a clinical history of exocrine pancreatic insufficiency, accompanied by hyporexia, vomiting, and chronic weight loss of more than 20 kg over the preceding months. Due to the deterioration of the animal's health status, humane euthanasia was performed.

During necropsy, carried out at the Pathology Service of the Veterinary Teaching Hospital (CEU Cardenal Herrera University), upon opening the thoracic cavity, a large amount of clear amber fluid (approximately 4 liters) was observed occupying both hemithoraces. The thoracic wall, the thoracic surface of diaphragm, and pulmonary surface showed abundant whitish multinodular lesions with a multifocal and coalescing distribution, ranging in size from 0.3 to 1 cm. These lesions were always superficial and did not infiltrate deeper structures either in the lungs or in the muscles.

Histologically, proliferation of epithelial cells forming papillary structures arising from the surface of the costal and pulmonary pleura was observed. The papillae were lined by cuboidal epithelium with large, oval nuclei and showed a fibrovascular stroma. Immunohistochemical studies were performed to better characterize the tumor. Neoplastic cells showed positive immunoreactivity for pan-cytokeratin AE1/AE3, vimentin, and Wilms' tumor suppressor gene 1 (WT1), confirming the diagnosis of mesothelioma.

Although this tumor has been described in other large felids, this represents the first reported case of pleural mesothelioma in the Angolan lion.

Funding: This study was supported by projects IDOC-25-09 and INDI 25/12 from CEU Cardenal Herrera University.

Keywords: Mesothelioma, immunohistochemistry, Angolan lion, zoo

Ethical approval: ☒ Not applicable; ☐ Approved

O30

Arthritis and osteomyelitis caused by E*nterococcus* spp. in periurban sparrows

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Musculoskeletal pathology is a common and economical problem in poultry. Bacterial chondronecrosis with osteomyelitis is relevant, and its aetiology is considered multibacterial. *Enterococcus cecorum* is considered the main cause of vertebral osteomyelitis in poultry; however, other bacteria such as *Staphylococcus* spp., *E. coli,* and *Yersinia pseudotuberculosis*, or virus such as reovirus, have been associated with skeletal problems in broilers. Sporadic cases have been reported in wild birds, especially birds of prey. This communication describes an outbreak of arthritis and osteomyelitis in sparrows caused by *Enterococcus* spp. In a short period of time, six sparrows from distant geographical origins were received at the La Alfranca Wildlife Recovery Center in Zaragoza that presented locomotor signs. The necropsy revealed purulent arthritis in several joints, particularly in the thoracic and lumbar vertebrae. Microscopically, necrotic osteomyelitis was observed. Microbiological analysis using classical methods and VITEK and MALDI-TOF techniques identified the isolate as *Enteroccocus* spp. This study presents the first reported outbreak of enterococcal osteomyelitis in sparrows. The epidemiology of these cases was surprising, giving the distant origin of the birds and the short period of time in which they were received. In addition, it could be a health risk, since sparrows are considered synanthropic birds. *Enterococcus* spp. are zoonotic bacteria and important carriers of antibiotic resistances. Currently, additional work is being carried out for its genetic characterization using molecular methods.

Keywords: Sparrows, Synanthropic, Microbiology, Enterococcus, Zoonosis
Ethical approval: ☒ Not applicable; ☐ Approved

IBERIAN Veterinary Pathology Meeting 2026
JUNE
PORTO | PORTUGAL

O31

Granulomatous pneumonia in sea turtles

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The loggerhead turtle (*Caretta caretta*) is the most common Chelonian species in the Mediterranean Sea, which represents a critical area for their feeding and development. This study describes three cases of female *C. caretta* from a head-starting program, necropsied at the facility and submitted for histopathological examination.

Macroscopically, pulmonary firm white nodules of around 1 mm were observed in two cases. Microscopically, granulomatous pneumonia was diagnosed in all three cases. Distinct organisms were associated with the granulomas in each case: non-pigmented hyphae, pigmented hyphae, and acid-fast bacteria compatible with *Mycobacterium* spp. Pulmonary granulomas were characterized by a necrotic core, surrounded by lymphocytes, giant cells and a fibrous capsule. A possible immunosuppressed state was suspected, potentially facilitating infection by opportunistic agents and promoting granulomatous pneumonia of different etiologies. In chelonians, fungal pneumonia is commonly associated with non-pigmented fungi such as *Aspergillus*, *Paecilomyces*, and *Penicillium* spp. Specifically, *Aspergillus* spp. affects the lower respiratory tract. Regarding bacterial granulomatous pneumonia in aquatic chelonians, *Mycobacterium* spp. infections are one of the most common causes, as these bacteria are ubiquitous in aquatic environments. *M. marinum* and *M. chelonae*, both of zoonotic concern, are the most frequently reported species, particularly in animals that were previously free-living and later incorporated into managed care or rehabilitation facilities. Histologically, granulomas associated with fungal infections and mycobacteriosis may show similar features, as observed in these cases, requiring special stains or PCR for etiological diagnosis.
In conclusion, the evaluation of these cases highlights the need for comprehensive diagnostic studies, as the similarity of the lesions may complicate an accurate diagnosis. Furthermore, pigmented fungal hyphae were identified in association with granulomatous pneumonia in loggerhead turtles for the first time, a finding that broadens the clinical relevance of these fungal agents.

Work funded by the CEU Cardenal Herrera University Research and Teaching Project IDOC25-09

Keywords: Mycobacteriosis, fungal, pigmented hyphae.
Ethical approval: ☒ Not applicable; ☐ Approved

O32

Development of a pilot model to estimate *post-mortem* interval (PMI) in elasmobranchs

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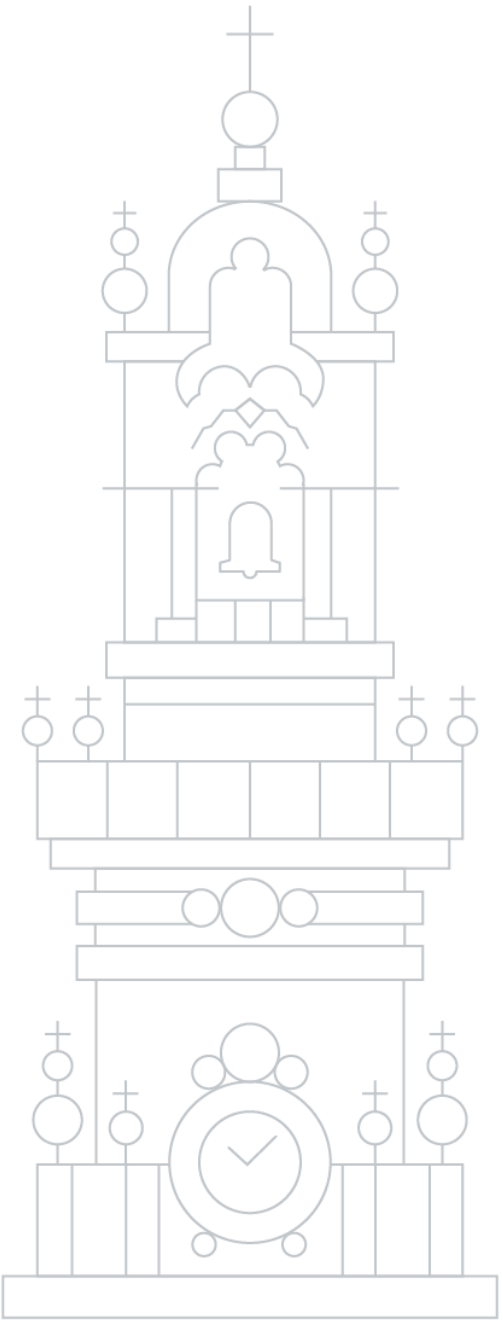
This study presents a pilot model to estimate the post-mortem interval (PMI) in elasmobranchs, using *Squalus megalops* as a reference species, and explores its applicability to two commercially relevant species, *Scyliorhinus stellaris* (nursehound) and *Galeorhinus galeus* (tope shark). The research was conducted within the framework of the Atlantic Whale Deal and SHARK-RAY Map projects, both focused on marine conservation and sustainable fisheries management.

Two *S. megalops* specimens were examined at nine predefined post-mortem intervals (0, 3, 6, 12, 24, 36, 48, 96, and 168 hours post-mortem). Histological and immunohistochemical analyses were performed using antibodies against key muscle proteins (actin, desmin, and dystrophin) and were complemented by digital image analysis in QuPath. In addition, ten specimens with unknown time of death (five *S. stellaris* and five *G. galeus*), obtained from artisanal fisheries in the Cantabrian Sea, were analysed. Statistical evaluation included parameter weighting through ranking, construction of a synthetic S-index, and PMI estimation using Theil–Sen robust regression.

The most reliable PMI indicators were myofibrillar disintegration, bacterial infiltration, and loss of anti-desmin immunoreactivity. Marked histological changes were observed from 6 h post-mortem onward, with advanced tissue degradation after 96 h. Desmin immunoreactivity declined early (3–6 h), whereas actin remained relatively stable until approximately 36 h, followed by a sharp decrease after 48 h.

The proposed model successfully estimated PMI in test specimens, with most predictions ranging between 18 and 32 h post-mortem. *S. stellaris* showed more homogeneous patterns, while *G. galeus* exhibited greater variability, including outliers. Although Theil–Sen regression provided robust calibration of the S-index, wide confidence intervals reflected both biological variability and the exploratory nature of the dataset.

Keywords: *Post-mortem*, elasmobranchs, model, muscle, immunohistochemistry
Ethical approval: ☒ Not applicable; ☐ Approved



Scientific Posters

IBERIAN Veterinary Pathology Meeting 2026 JUNE PORTO | PORTUGAL

PO-1

Bridging morphology and omics: a histology-guided approach to chronic cutaneous furunculosis in turbot

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Chronic cutaneous furunculosis, caused by *Aeromonas salmonicida*, is one of the most important infectious diseases in farmed turbot (*Scophthalmus maximus*), yet its underlying molecular mechanisms remain poorly understood. This study applied a histology-guided RNA-seq approach to characterize the cutaneous response associated with chronic lesions under natural outbreak conditions, integrating transcriptomic data with classical morphological techniques.

Histopathology was used as a primary selection tool to define a homogeneous lesional phenotype prior to molecular analysis. Comparative transcriptomics between lesions and paired healthy skin revealed extensive transcriptional reprogramming (>2,000 differentially expressed genes), with coordinated activation of proliferation, immune response, cell migration and extracellular matrix remodeling, alongside reduced structural and metabolic programs.

Histological examination independently demonstrated epidermal hyperplasia, dense dermal inflammatory infiltrates, and marked disruption of dermal architecture, including collagen disorganization and loss of normal tissue structure. These features provide direct morphological evidence of proliferative, inflammatory and tissue remodeling processes consistent with the transcriptomic profile.

Targeted immunohistochemistry, based on upregulated transcripts, enabled in situ validation of key functional axes, including proliferation (PCNA), immune response (IgM), and epithelial adhesion cytoskeletal dynamics (E-cadherin, α-SMA).

These findings highlight the value of integrating morphology and "omic" technologies, positioning histopathology as both a phenotypic anchor and a critical framework for the interpretation and validation of high-throughput molecular data in veterinary pathology.

Keywords: Turbot, Aeromonas salmonicida, furunculosis, RNA-seq, immunohistochemistry

Ethical approval: ☒ Not applicable; ☐ Approved

IBERIAN Veterinary Pathology Meeting 2026
JUNE
PORTO | PORTUGAL

PO-2

Quince extract modulates early HPV-associated lesions in K14HPV16 mice

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Background/Objectives: K14HPV16 mice are a relevant preclinical model of human HPV-related epithelial disease. This study evaluated whether quince leaf extract (QLE) could mitigate early histopathological changes.
Materials and Methods: Thirty-seven mice (18 wild type and 19 K14HPV16) were divided into eight groups receiving water or three concentrations of QLE in drinking water (1.7, 2.6, or 3.6 mg/mL). Groups included WT water (n=4), WT QLE1 (n=4), WT QLE2 (n=5), WT QLE3 (n=5), HPV water (n=4), HPV QLE1 (n=5), HPV QLE2 (n=5), and HPV QLE3 (n=5). After the experimental period, animals were euthanized and samples were collected for histopathological analysis. Lesions were graded using a semi-quantitative rating, ranging from absent/normal to severe. Approval from the Ethics Committee (approval no. 852-e-CITAB-202_A_1-e-122CITAB-2021) and the national competent authority (approval no. 014139) were obtained. Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test (<0.05).
Results: No heart or spleen lesions were detected in any group. In the lungs, the HPV QLE1 group showed the best histopathologic results, with only mild inflammatory infiltrate. In the liver, WT QLE1 and HPV QLE1 groups presented the mildest lesions, limited to hydropic degeneration and mild inflammation. Congestion was observed in kidneys of all HPV-treated groups but was less pronounced at the lowest QLE dose. In ear pavilion skin, HPV water mice exhibited severe deformation, sebaceous hyperplasia, papillomatosis, and grade 2 dysplasia. These lesions were significantly reduced in QLE-treated HPV groups, particularly at low and intermediate doses (QLE1 and QLE2). No papillomas or carcinomas were observed.
Conclusions: Quince leaf extract did not produce deleterious histopathology in wild-type mice and improved epithelial and organ integrity in K14HPV16 mice, especially at lower doses. These findings support quince leaf extract as a non-toxic modulator of early HPV-associated lesions.

Funding: FCT UID/04033/2025 (CITAB), LA/P/0126/2020 (Inov4Agro) and UIDB/0681/2025 (CERNAS).

Keywords: HPV; K14HPV16; quince; dysplasia; histopathology.
Ethical approval: ☐ Not applicable; ☒ Approved

PO-3

Identification of Stable miRNA References for qRT-PCR Normalization in Ovine Fasciolosis

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Introduction: *Fasciola hepatica* establishes chronic infections through complex host-parasite interactions mediated by molecular gene regulation mechanisms. In particular, microRNAs (miRNAs), a class of non-coding RNA, act as post-transcriptional regulators of gene expression modulating the host immune response. They therefore represent key targets for elucidating these interactions, as well as promising biomarkers for diagnosis and potential candidates for novel vaccination strategies. Objective: This study aims to identify reliable reference miRNAs for normalization and internal control in RT-qPCR analyses of plasma samples from sheep infected with *F. hepatica* during early stages of infection. Methods: Thirty Merino sheep were divided into a non-infected control group (n = 6) and an infected group (n = 24), with the latter orally inoculated with 150 metacercariae. Plasma samples from both groups were collected at 1, 3, 9, and 17 days post-infection (dpi). These were processed under controlled haemolysis conditions, and RNA was extracted, quantified, and normalized to 5 ng/µL. Reverse transcription and qPCR were performed in triplicate, including inter-assay controls, using an optimized panel of miRNAs. Validation was carried out using the RefFinder web tool, which integrates multiple algorithmic approaches (NormFinder, the comparative ΔCt method, geNorm, and BestKeeper). Results: Stability ranking of candidate reference genes identified hsa-let-7c-5p, bta-let-7f, and oar-miR-16b as the most suitable miRNAs for use as internal control in RT-qPCR under our experimental conditions. The geometric mean of these three miRNAs was used as a normalization factor, allowing more accurate and reliable quantification of the relative expression levels of three target miRNAs in ovine plasma. This approach revealed time-dependent variation associated with infection progression. Conclusion: This study highlights the value of validated miRNAs as endogenous reference genes to improve sensitivity and specificity in RT-qPCR analyses based on plasma samples in ovine fasciolosis.

Acknowledgments: work funded by PERSEUS (PID2023-152150OB-C22).

Keywords: *F. hepatica*, microRNA (miRNA), RT-qPCR, normalization, reference genes.

Ethical approval: ☐ Not applicable; ☒ Approved

PO-4

Atypic tetraparesis induced by embolic disease associated to aortic valvular bacterial endocarditis in a dog

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Background: A 12-year-old female Labrador Retriever was admitted to the Veterinary Teaching Hospital of the University of Murcia with acute neurological symptoms (apathy and non-ambulatory tetraparesis). On neurological examination, the animal exhibited signs consistent with an intracranial lesion, presumably secondary to a stroke. Clinical investigations revealed leukocytosis (with neutrophilia and monocytosis), and echocardiographic imaging identified an irregular mass located on the left coronary cusp of the aortic valve. Previous medical records indicated that the same mass had been identified five months earlier and was suspected to be valvular neoplasia. The animal's clinical condition deteriorated rapidly, and it died two days after admission. The body was subsequently transferred to the autopsy room of the Veterinary Faculty for examination.

Results: Necropsy revealed macroscopic evidence of encephalomalacia and ecchymoses within the cerebral cortex, signs of myocarditis, and a large mass (6 × 2.5 cm) affecting one of the semilunar valves of the aorta. Additionally, macroscopic evidence of thromboembolism was observed in both the spleen and the kidney. Histopathological analysis demonstrated that the mass identified on the aortic valve exhibited typical features of bacterial endocarditis. Furthermore, the analysis revealed suppurative myocarditis, hemorrhagic infarction, and secondary bacterial meningoencephalitis, along with bacterial thromboemboli in the kidney and spleen, confirming embolic disease with multiorgan septic dissemination.

Conclusion: An infectious etiology should be considered in patients presenting with neurological signs and a prior diagnosis of an aortic valvular mass. Bacterial endocarditis can have a chronic course, leading to the formation of large proliferative lesions in the affected valves. Prompt diagnostic work-up is recommended to enable early initiation of targeted antimicrobial therapy.

Keywords: Dog, tetraparesis, endocarditis, embolic disease

Ethical approval: ☒ Not applicable; ☐ Approved

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PO-5

Periocular Extraskeletal Telangiectatic Osteosarcoma in a Dog

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Extraskeletal osteosarcomas have been reported in dogs and cats affecting a wide variety of visceral and soft tissue locations. These tumors may arise secondary to trauma, injections or chronic inflammatory processes associated with foreign body granulomas. Histologically, they are commonly classified into osteoblastic, fibroblastic, chondroblastic, giant cell, and telangiectatic variants. Periocular presentation is exceptionally rare in the canine species. This report describes the case of a 3.5-year-old male English Setter presented for a painful swelling in the left periocular region with several weeks of progression and incomplete response to medical treatment. Previous trauma in the same area was reported. Cytological evaluation revealed a malignant mesenchymal cell population showing moderate to marked atypia. Computed tomography identified a large retrobulbar and superior palpebral mass extending to the maxillary alveolar process, with extensive mineralization, mild adjacent maxillary osteolysis, and severe compression of the globe. Complete enucleation with excision of the periocular soft tissues was performed. Grossly, a poorly demarcated haemorrhagic periocular mass measuring approximately 3 × 3 cm surrounded and compressed the globe without intraocular involvement. Histologically, the lesion consisted of an infiltrative, unencapsulated mesenchymal neoplasm composed of pleomorphic osteoblast-like cells producing irregular trabeculae of osteoid, frequently mineralized. Multifocally, neoplastic cells formed large blood-filled pseudovascular spaces consistent with a telangiectatic pattern. Immunohistochemically, neoplastic cells were strongly positive for vimentin and alkaline phosphatase, while pseudovascular spaces were negative for factor VIII, ruling out a vascular neoplasm. A diagnosis of periocular extraskeletal telangiectatic osteosarcoma was established. Surgical excision was incomplete because of the anatomical location, and local recurrence developed two months after surgery. This case describes a rare periocular extraskeletal osteosarcoma in a dog with a telangiectatic histological pattern. It highlights the anatomical variability of extraskeletal osteosarcomas and the importance of considering the telangiectatic variant among the differential diagnoses of mineralized periocular masses in dogs.

Keywords: Osteosarcoma, dog, telangiectatic pattern, orbit, periocular neoplasm
Ethical approval: ☒ Not applicable; ☐ Approved

PO-6

Floating in mucin: immunohistochemical decoding of a mucinous mammary carcinoma

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Background/Objectives: Feline Mucinous Mammary Carcinoma (FMMC) is a rare tumour characterised by large amounts of mucinous material and the proliferation of luminal epithelial cells floating within it. The definitive diagnosis is based on mucin identification and the immunohistochemical identification of proliferating cells as luminal epithelial cells. Little is known about the pathological characteristics of FMMC. The aim of this study was to further characterise immunohistochemically this entity.

Methods: a 16-year-old European shorthair female cat was presented with a well define soft nodule with exophytic growth (1.6x1.8 cm), involving the nipple of the third left mammary gland. The mass was thoroughly studied using histochemical staining's as well as an immunohistochemical panel of 17 antibodies, including hormonal-receptors, intermediate filaments, gelatinases, cellular proliferation, and adhesion markers.

Results: histologically, the tumour was pseudolobed, partially capsulated but multifocally infiltrative, characterised by large amounts of extracellular mucin (positive for PAS-diastase, Alcian blue pH 2.5 and mucicarmine) and few to moderate tubule-acini and ribbons of luminal epithelial cells immerse within it. Intratumoral necrosis was moderate. Tumour cells were cuboidal to cylindric, with large, rounded nuclei, stippled chromatin and one prominent nucleolus. Immunohistochemically, tumour cells were positive for oestrogen and progesterone receptors, AE1/AE3+, CK5/6+, CK7+, CK8/18+, CK19+ and CK20+/-, E-cadherin+, MMP2+/-, MMP9+ and vimentin+. No myoepithelial markers were expressed by neoplastic cells, as calponin and actin. Ki67 was highly expressed in the nuclei of neoplastic cells, exhibiting a proliferation index of 55%.

Conclusions: histological and immunohistochemical features of this case provide new knowledge about FMMC. Those are consistent with pure mucinous carcinoma (subtype A) described in breast cancer except for the high proliferation index, which coincides with some types of feline mammary carcinoma.

Keywords: Neoplasia, Feline, Mammary gland, Carcinoma, Mucinous, Immunohistochemistry

Ethical approval: ☒ Not applicable; ☐ Approved

IBERIAN Veterinary Pathology Meeting 2026
JUNE
PORTO | PORTUGAL

PO-7

Spinal Cellular Hemangioblastoma in a Dog: a rare entity

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Background: Hemangioblastoma is a rare vascular neoplasm of the canine central nervous system, typically presenting as an expansile, well-demarcated biphasic proliferation composed of stromal cells and a vascular component. Two histological variants are recognized: reticular, characterized by an abundant capillary network, and cellular, in which the stromal component predominates. The cellular variant is exceptionally rare, with only three canine cases recently reported.

Materials & Methods: A 4-year-old German Shepherd dog was evaluated for a 5-month history of progressive ambulatory to non-ambulatory tetraparesis. Magnetic resonance imaging revealed an approximately 1.5 cm, C7-T1 intradural, indistinctly intraparenchymal, spinal nodular mass, hyperintense on T1- and T2-weighted images and with homogeneous contrast enhancement. The mass was excised though a modified hemilaminectomy. Sections were routinely processed and stained with H&E and Masson's trichrome, followed immunohistochemistry against vimentin, NSE, GFAP, Olig2, neurofilaments, CD31 and Ki-67.

Results: Microscopic examination revealed an intraxial, partially infiltrative, biphasic tumour predominantly formed by neoplastic stromal cells within a capillary network, surrounding a collagen-rich fibrovascular center. Stromal cells were spindle, stellate and polygonal, with variable amounts of cytoplasm, indistinct borders, and oval nuclei with stippled chromatin and visible nucleoli. Anisocytosis and anisokaryosis were moderate, with occasional multinucleation, bizarre nuclei and cytoplasmic nuclear inclusions. Stromal cells were variably positive for vimentin, NSE, GFAP and neurofilaments. The minor vascular component consisted of haphazardly arranged, irregularly shaped small capillaries lined by plump endothelium, highlighted by CD31. The Ki-67 proliferation index was 40%. Surgical excision and physiotherapy resulted in progressive neurological recovery, without recurrence 6 months after surgery.

Conclusion: The findings supported a diagnosis of intramedullary spinal cellular hemangioblastoma. In humans, this rare subtype is associated with increased proliferative activity and a greater risk of recurrence. Further canine cases with extended follow-up are needed to determine whether this variant has similar prognostic significance in dogs.

Keywords: Hemangioglastoma, cellular variant, canine, spinal cord
Ethical approval: ☒ Not applicable; ☐ Approved

IBERIAN Veterinary Pathology Meeting 2026 JUNE PORTO | PORTUGAL

PO-8

***Brucella* spp. in stranded cetaceans of Galicia**

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At least 24 cetacean species occur in Galician waters. The regional stranding network (CEMMA) responds to about 227 strandings per year, involving both live and dead animals. Between January 2020 and October 2024, 230 cetaceans from 11 species were examined through macroscopic and histopathological assessment. Animals were either partially sampled or fully necropsied, with decomposition ranging from very fresh to moderately autolysed. Molecular tests were also performed on 158 individuals from 10 species stranded between January 2021 and August 2024 to detect Brucella spp. using real-time duplex PCR and Cetacean Morbillivirus (CeMV) using real-time PCR. All samples were negative for CeMV. In contrast, *Brucella* spp. DNA was detected in five cetaceans from three species: three Atlantic bottlenose dolphins (*Tursiops truncatus*)—one adult male and two juvenile females—stranded in 2023, and one adult female striped dolphin (*Stenella coeruleoalba*) and one adult male Atlantic common dolphin (*Delphinus delphis*) stranded in 2024. No gross lesions suggestive of brucellosis were observed, meanwhile other findings related with fishery interaction or alive stranding were described. Histopathology study of positive specimens was not complete in most of cases. However, one of the two animals in which the central nervous system was examined, lymphoplasmacytic meningitis in the spinal cord was described. Although the frozen tissues available for molecular testing were not homogeneous among individuals, *Brucella* spp. DNA was detected in the lung, scapulohumeral joint, prescapular and pulmonary lymph nodes, kidney, uterus, testicle, and spinal cord. Given the zoonotic potential of *Brucella* spp., these findings highlight the need to consider this pathogen when handling live or dead cetaceans and emphasize the value of molecular diagnostics for detecting pathogens that may remain undetected through macroscopic or histological examination.

Keywords: *Brucella* sp., Morbillivirus, neurobrucellosis, dolphin, zoonosis
Ethical approval: ☒ Not applicable; ☐ Approved

PO-9

Fibrinous pleuropneumonia in the South African porcupine (*Hystrix africaeaustralis*)

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Pathological conditions in the South African porcupine are poorly described. To date, only a single case of Aspergillus-associated rhinitis has been reported and most of the published studies focused on reproductive aspects. Other respiratory diseases, especially pneumonias, are scarcely documented in porcupines of any species.
We present the case of a 22-year-old adult female South African porcupine with a clinical history of mild to moderate pleural, pericardial effusion and abdominal effusion. The animal experienced a sudden death.
During necropsy, performed at the Pathology Service of the Veterinary Teaching Hospital (CEU Cardenal Herrera University), abundant fibrinous material was observed within the thoracic cavity, predominantly affecting the left hemithorax, along with the presence of clear fluid. The lungs were enlarged and showed multifocal reddish discoloration. On cut section, a whitish-yellow mucous material was also observed in the bronchi. Histologically, the pleura and the lung interstitium were enlarged by the deposition of an abundant eosinophilic fibrillar material (fibrin). A large number of small basophilic coccoid bacteria were identified both within the alveolar lumina and the interstitium, along with abundant cellular debris, neutrophils, macrophages, and, to a lesser extent, lymphocytes and plasma cells. Lung samples were submitted for bacterial culture, from which coagulase-negative Staphylococcus was isolated.
In conclusion, this case report describes the first case of acute fibrinous pleuropneumonia caused by coagulase-negative Staphylococcus in a South African porcupine.
Funding: This study was supported by projects IDOC-25-09 and INDI 25/12 from CEU Cardenal Herrera University.

Keywords: Porcupine, pleuroneumonia, Hystrix, respiratory
Ethical approval: ☒ Not applicable; ☐ Approved

PO-10

Reconstructing Host-Like Conditions *in vitro* to Trigger the L3-L4 Transition in *Anisakis simplex* and Capture Stage-Specific Molecular Signatures

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Anisakis simplex is a zoonotic marine nematode whose infective third-stage larvae (L3) cause anisakiasis and allergic reactions in humans. The L3-L4 transition is a key developmental checkpoint normally triggered only after entry into a warm-blooded host. Access to this stage is limited, constraining molecular studies on development and allergen expression. We aimed to establish a controlled in vitro system that induces the L3-L4 moult and generates high-quality material for stage-specific molecular analyses. L3 larvae isolated from *Trachurus trachurus* were maintained in RPMI-1640 medium and exposed to host-mimicking conditions (37°C; reduced oxygen). Moulting was monitored microscopically. RNA from L3 and in vitro-derived L4 larvae was extracted for cDNA synthesis, PCR, and qRT-PCR of developmentally regulated targets. Approximately 20% of larvae completed the L3-L4 transition within 10 days. Moulting larvae displayed characteristic L4 morphology, including formation of three lips and loss of the boring tooth. qRT-PCR revealed clear stage-dependent transcriptional patterns, including differential expression of allergen-associated genes. Simulating the mammalian gut environment successfully induced the L3–L4 transition in vitro and produced molecularly robust material. This platform provides controlled access to a normally inaccessible developmental stage and supports future transcriptomic and allergen-focused investigations in *A. simplex*.

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Keywords: Fish food safety; parasitic nematode; larval culture; larval moulting; gene expression
Ethical approval: ☒ Not applicable; ☐ Approved

PO-11

Intensity, Morphological and Molecular Identification of *Stephanostomum baccatum* (Trematoda: Acanthocolpidae) Metacercariae in Yellowtail Flounder, *Limanda ferruginea* Commercialized in Portuguese Supermarkets

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Traceability applied to fishery products represents a powerful tool for disease identification and health assessment of wild fish and ecosystems.
The aim of this study was to determine the presence and identification of parasites, their distribution and intensity in steaks of yellowtail flounder (YF), *Limanda ferruginea*, a flatfish sold in Portuguese supermarkets and originated from Northwest Atlantic, FAO zone 21.
A parasitological analysis was performed on YF frozen steaks (*n*= 32). Thawed steaks were weighed and skinned, muscle tissue was sliced and observed directly under a stereomicroscope. The peritoneal cavity and internal organs were also analyzed, when available.
Two species of parasites were recorded. One L3 *Anisakis* larvae was observed at the serosa of a female gonad. Multiple cysts with 1 mm diameter containing the digenetic metacercariae of *Stephanostomum baccatum* were observed in the myotomes on the upper and lower sides of the fish steaks, fin membranes and pterygiophoral muscles.
Cysts were present in 94% of YF steaks, the intensity ranged from 1 to 71 metacercariae/steak, 40% of the YF steaks contained 1 to 5 cysts, and 66% ≥5 cysts.
Ecosystem relationships are discussed. Given the presence of the zoonotic *Anisakis* larvae in the viscera and the high intensity and percentage of metacercariae cysts present in YF steaks, which may negatively affect its quality and market value, our recommendations to the food supply chain stakeholders intended to better discriminate fish parasites are (1) to promote the fully evisceration of the fish and (2) to monitor the fish parasites and the fish origin always before being processed as a frozen fishery product.

Keywords: Fish parasite, digenetic metacercariae, *Stephanostomum baccatum*, yellowtail flounder, food security
Ethical approval: ☒ Not applicable; ☐ Approved

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PO-12

Histopathological characterization of neurobrucellosis in stranded cetaceans in the valencian community (2022–2026)

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Brucellosis is a zoonosis of major importance, endemic in many regions worldwide, caused by different species of the genus *Brucella*. *Brucella ceti* affects mysticetes and odontocetes globally, with both horizontal and vertical transmission. It is associated with neurological, reproductive, osteoarticular, and cardiac lesions, as well as involvement of organs such as the liver and spleen. Infected animals are often found stranded, moribund, or dead, with neurobrucellosis being one of the most relevant manifestations. During the period 2022–2026, necropsies were performed on 42 stranded cetaceans in the Valencian Community. This study describes three cases of striped dolphins (*Stenella coeruleoalba*) infected with *B. ceti* showing lesions in the central nervous system (CNS). Two animals stranded alive and exhibited neurological signs such as impaired buoyancy, tremors, and seizures. All three animals tested positive for *Brucella ceti* by biomolecular techniques (PCR) and/or rapid serological agglutination testing for antibody detection (Rose Bengal test). Histopathological analysis was carried out at the Pathology Service of the Veterinary Teaching Hospital (CEU Cardenal Herrera University). Macroscopically, meningeal congestion was observed, and in one case, a dark red, depressed, multifocal lesion consistent with necrosis. Histologically, the CNS showed liquefactive necrosis, perivascular cuffs and glial nodules. The meninges exhibited lymphoplasmacytic meningitis with congestion and perivascular hemorrhages. To conclude, these results confirm the presence of neurobrucellosis caused by *B. ceti* in stranded dolphins in the Valencian Community, characterized by non-suppurative inflammatory lesions and necrosis in the CNS, with positivity in biomolecular and/or serological techniques.

*This study was funded through a Research + Teaching Project of the CEU Cardenal Herrera University (IDOC–25/09).

Keywords: Brucellosis, Dolphins, central nervous system

Ethical approval: ☒ Not applicable; ☐ Approved

PO-13

Morphologic staging of cutaneous lesions in chronic *Aeromonas salmonicida* infection

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Chronic cutaneous lesions associated with *Aeromonas salmonicida* infection are a frequent but poorly characterized feature of turbot aquaculture, limiting interpretation of disease status under field conditions. This study aimed to define a morphologic framework for these lesions and assess its relevance for understanding disease dynamics. An observational pathological study was conducted during natural outbreaks in a commercial turbot farm in Galicia (Spain). A total of 47 affected fish were examined. Lesions were systematically recorded, digitally mapped, and classified into six morphologic types based on gross appearance. Histopathology and immunohistochemistry were performed to characterize lesion composition and detect A. salmonicida. A total of 916 lesions were analyzed. Six reproducible lesion types were identified, forming a continuous spectrum from early nodular changes to ulcerative and reepithelialized stages. Lesions showed a non-random spatial distribution, with consistent regional clustering. Individual fish typically displayed multiple lesion types simultaneously, although a dominant stage was usually present. Histologically, lesions exhibited a coherent progression from early acute inflammation and intradermal cavity formation to ulceration and subsequent granulomatous remodeling, followed by re-epithelialization. Bacterial antigen was detected in intermediate stages but not in late lesions. These findings indicate that lesion morphology provides a robust proxy for disease stage at both individual and population levels. The coexistence of multiple lesion stages within fish reflects parallel lesion initiation and asynchronous progression, offering a mechanistic explanation for the heterogeneity observed in chronic outbreaks. Importantly, morphologic staging may enable rapid field-based assessment of outbreak phase, supporting more informed management decisions in turbot aquaculture.

Keywords: Turbot, Aeromonas salmonicida, furunculosis, lesion staging, morphometrics
Ethical approval: ☒ Not applicable; ☐ Approved

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PO-14

Osseous metaplasia secondary to sialocele in a dog

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Background: Salivary mucocele is a common disorder affecting the salivary glands. It consists in an accumulation of saliva in a cavity surrounded by granulation tissue. Saliva leakage from the salivary gland parenchyma and/or from associated duct damage causes a chronic inflammatory process which occasionally result in osseous metaplasia. This case reports an unusual case of osseous metaplasia in a mandibular sialocele.

Case Report: A 2-year-old female Chihuahua was referred to the Veterinary Teaching Hospital of the University of Trás-os-Montes and Alto Douro for evaluation of a 3 cm, firm, painless swelling located in the left ventrolateral cervical region. Physical examination and laboratory findings were within normal limits, and there was no clinical evidence of regional lymphadenomegaly. Radiographic examination of the head and neck revealed a large soft tissue opacity with a partially calcified wall in the left intermandibular region. Ultrasonographic evaluation of the cervical area identified a hyperechoic intraductal structure with marked posterior acoustic shadowing, consistent with a sialolith, associated with upstream ductal dilation and mild heterogeneity of the submandibular gland. A ventral surgical approach was performed for mandibular and sublingual sialoadenectomy with duct excision. During dissection of the submandibular gland, a sialocele was identified and completely excised.

Macroscopically fragments of salivar gland and an ovoid, firm, calcified mass measuring 4x2,2x0,5 cm, adjacent to one gland fragment were observed.

Microscopically, subacute adenitis, oedema, and loose, mucinous areas interpreted as saliva leakage were seen. The connective tissue presented plates of mineralized bone, and some areas of bone were colonized by bone marrow. According the macro and microscopic features, a diagnosis of osseous metaplasia secondary to salivar mucocele was made.

Conclusions: The present case ossifying metaplastic cells in the wall of salivary mucocele. The presence of osseous metaplasia in sialoceles is extremely rare, with only few cases described in veterinary medicine.

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Keywords: Osseous metaplasia, dog, salivary gland, sialocele

Ethical approval: ☒ Not applicable; ☐ Approved

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PO-15

Oral neuroma in dog: a rare entity

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Background/Objectives: A neuroma is a circumscribed proliferation of a peripheral nerve; it represents an exaggerated reactive hyperplasia, an attempt to repair a nerve, and is composed of an admixture of fibroblasts, Schwann cells, and axons. Traumatic neuroma is the most frequently recognized neuroma lesion in humans and is well known in the extremities, as well as in the oral cavity. Herein we present a case of oral neuroma, an unusual lesion in the dog.
Case report: A 15-year-old female Pinscher was presented to the Veterinary Hospital of UTAD with periodontal disease. The clinical examination, revealed a lump on the right side of the oral cavity, consisting of a cylindrical mass on the mucosal surface of the lower lip. A dental radiography showed no signs of bone changes. After an inconclusive cytology, the mass was removed surgically.
Macroscopically, it was a well-demarcated, whitish, firm, filiform mass measuring 2.5×0.5×0.3 cm. After fixation, the mass was processed for light microscopy.
Microscopically, we observed a poorly defined mass, consisting of spindle shaped cells forming bands, and coiled structures, mimicking nerves. No mitosis nor Verocay bodies, Antoni type A or Antoni type B patterns were found. A Reticulin stain revealed a delicate network of stromal fibers surrounding individual fusiform cells; these cells were immunopositive to Vimentin and Neuron-Specific Enolase.
Conclusions: The histological and immunohistochemistry features were consistent with a neuroma, an unusual and poorly known entity in the dog. Traumatic neuromas and palisaded encapsulated neuromas consist of proliferation of both Schwann cells and axons. Periodontal disease associated with chronic pain and dental malocclusion could have been the triggering factor for the development of this unusual lesion.

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Keywords: Neuroma, oral cavity, dog, lip.
Ethical approval: ☒ Not applicable; ☐ Approved

IBERIAN Veterinary Pathology Meeting 2026
JUNE
PORTO | PORTUGAL

PO-16

Detection of TNF-α by RNAscope™ in turbot tissues (*Scophthalmus maximus*): contributions to the anatomical-pathological study of the vaccine-induced inflammatory response

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Tumor necrosis factor alpha (TNF-α) is a key pro-inflammatory cytokine in teleosts, involved in cellular activation and in the development of chronic and granulomatous inflammation associated with persistent stimuli such as vaccine adjuvants. Its detection by immunohistochemistry presents limitations, particularly at early stages. In this context, the RNAscope™ technique enables the detection of messenger RNA with high sensitivity and cellular resolution while preserving tissue morphology.

The objective of this study was to optimize and standardize RNAscope™ to evaluate tissue expression of TNF-α in turbot (*Scophthalmus maximus*) and to assess its usefulness as a complementary tool in anatomical pathology. Digestive tract, kidney, and spleen from vaccinated fish were fixed in formalin or Bouin's solution and embedded in paraffin. Samples were examined histopathologically, and chronic inflammatory lesions were identified, mainly well-organized granulomas associated with adjuvants, in which TNF-α expression was evaluated by RNAscope™, applying modifications in heat-mediated and enzymatic retrieval as well as in hybridization conditions.

TNF-α mRNA was detected in all samples, being mainly localized at the periphery of granulomas in mononuclear inflammatory cells, showing a punctate cytoplasmic pattern. This finding allowed the correlation of cytokine expression with areas of greater inflammatory activity. In kidney and spleen, the signal was weak and scarce, limited to isolated cells.

Samples fixed in formalin showed a more intense signal than those fixed in Bouin's solution, indicating better RNA preservation. In addition, the mounting medium DPX was not suitable for preserving the chromogenic signal.

Overall, RNAscope™ is confirmed as a useful tool for in situ detection of TNF-α, providing relevant information for the interpretation of the vaccine-induced inflammatory response in turbot from a morphopathological perspective.

Keywords: RNAscope™, TNF-α, turbot (*Scophthalmus maximus*), granulomatous inflammation, vaccine adjuvants

Ethical approval: ☒ Not applicable; ☐ Approved

PO-17

Use of feather pulp in the diagnosis of Marek's disease: viral replication and immunopathological characterization

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In the last decade, the use of feather pulp (FP) as a gold standard sample for monitoring Marek's disease (MD) vaccination and for the diagnosis of MD has been well established. We had previously demonstrated that FP can be used not only for the diagnosis of chickens with MD but also as an early diagnostic tool to predict the outcome of the disease in apparently healthy chickens. Chickens that are not properly protected against MDV have very high levels of MDV DNA load in the FP at 21 dpi. In the current study, our objective was to elucidate the immunopathological changes that occur in the FP of chickens that were vaccinated with CVI988 vaccine ("healthy"), those that were vaccinated and challenged with a vv+MDV strain 648A ("well protected"), and unvaccinated chickens and also challenged ("no protection") when compared to non-infected naïve chickens. The viral DNA load, viral gene expression, characterization of the lesions by histopathology and immunohistochemistry, and immunophenotypic characterization of the FP infiltrates by flow cytometry was conducted. Our results demonstrate that "non protection" chickens had a significant increase in the percentage of CD3+ T cells, mainly CD4+MHC-II+ cells and CD8+MHC-II+ cells, when compared to all other groups. This group also showed a significantly decreased number of CD8β+ T cells compared to other groups. Interestingly, infection with 648A reduced the percentage of macrophages not only in the unvaccinated but also in the vaccinated and challenged group. All vaccinated groups had higher levels of CD8β+ T cells, suggesting that the vaccine has an enhancing effect on the CTL cells. Our results confirm that the qPCR results obtained in chickens that were inadequately protected against MDV had a correlation with the histological lesions detected in the FP and provide further confirmation that FP is indeed an appropriate sample for the diagnosis of MD.

Keywords: Marek, Feather Pulp, Diagnosis, characterization
Ethical approval: ☒ Not applicable; ☐ Approved

PO-18

A case of Fowl Adenovirus A inclusion body hepatitis in captive Bobwhite quails (*Colinus virginianus*) investigated by the Animal and Plant Health Agency

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A group of captive Bobwhite quails (*Colinus virginianus*) experienced increased mortality, gasping, recumbency and diarrhoea. The birds were of mixed ages (10 weeks to 18 months) and kept in an outdoor aviary which allowed for indirect contact with wild birds. Clinical signs raised suspicion of Highly Pathogenic Avian Influenza (HPAI), which is notifiable in Great Britain (GB). The case was reported to the regulatory body, the Animal and Plant Health Agency (APHA). Statutory investigation ruled out HPAI and carcases were investigated at APHA Bury St Edmunds under the Differential Diagnosis of Negated Report Cases scheme (DDNRC).

Necropsy revealed pale livers with miliary white foci or mottling in three out of five carcases. The other two had urolithiasis and one also had renomegaly. *Escherichia coli* was cultured from liver swabs of all birds. Infectious Bronchitis PCR from lung and trachea of two birds was negative. Formalin-fixed tissues were submitted to the avian pathology centre at APHA Lasswade for histopathology. In the three livers with gross lesions, microscopic examination revealed a moderate acute multifocal fibrino-necrotising hepatitis with basophilic intranuclear inclusion bodies (IBs) in hepatocytes and in the epithelium of major bile ducts. In the Bursa of Fabricius of two birds, necrotising bursitis with similar IBs was detected. No significant lesions were observed in lung sections.

The main findings were suggestive of an adenoviral infection. Whole genome sequencing was performed on samples from the three affected livers and a full genome was recovered. Fowl Adenovirus A (FAdv-A) serotype 1 was detected, with 99% identity and 100% coverage at an e-score of 0. The profile was consistent with Quail Bronchitis Virus (QBV), ruling out the emergence of a new virus. This is the first case of FAdv-A/QBV diagnosed in GB by APHA in a decade and demonstrates the value of the DDNRC scheme.

Keywords: Bobwhite quails, Fowl Adenovirus A, Inclusion bodies, Quail Bronchitis Virus
Ethical approval: ☒ Not applicable; ☐ Approved

PO-19

Coinfections as Modulators of Bovine Tuberculosis Severity in Extensive Cattle Systems in Alentejo, Portugal

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Bovine tuberculosis (bTB), caused by members of the *Mycobacterium tuberculosis* complex, remains a major challenge in extensive cattle systems in southern Portugal, where interactions with wildlife create complex multihost epidemiological scenarios. Although coinfections and micronutrient status, such as vitamin D and calcium, have been shown to modulate tuberculosis severity in other species, their role in shaping bTB pathology in domestic cattle remains poorly understood. This study aimed to evaluate whether host factors, coinfections, and serum micronutrient levels are associated with the presence and lesion pattern of bTB in cattle from the Alentejo region.

A total of 120 intradermal tuberculin test–positive cattle from six extensive farms were sampled at the abattoir. Blood samples were collected directly from the heart, and individual animal data (age, sex, breed) were recorded. A complete post-mortem examination was performed to identify bTB-like lesions and their anatomical distribution. PCR assays were used to detect piroplasms, *Anaplasma* spp., bovine viral diarrhoea virus (BVDV), and infectious bovine rhinotracheitis virus (IBR) in blood samples. Serum vitamin D and calcium concentrations were determined using ELISA-based assays. Associations between host factors, coinfections, micronutrient levels, and the presence and severity of bTB lesions were analysed using non-parametric statistical tests.

None of the evaluated factors was significantly associated with the mere presence of bTB-like lesions. However, when lesion pattern was considered, biologically relevant differences emerged. Animals displaying generalized lesions showed a different distribution of piroplasma DNA load, which was significantly higher in cattle with localized lesions compared with those showing generalized disease (2294 vs. 884 copies/ ng DNA; $p = 0.049$). Additionally, males were more likely to present generalized lesions than females (80% vs. 33.33%), reaching marginal statistical significance ($p = 0.058$).

These findings suggest that modulation of the immune hosts response linked to piroplasm infections and factors associated to males (e.g. level of adrogens) may influence the development of bTB lesions in cattle. However further researches will be necesary to deep into these associations.

Keywords: bovine tuberculosis; coinfections; piroplasmas; lesion pattern; cattle; Alentejo

Ethical approval: ☒ Not applicable; ☐ Approved

PO-20

Early intestinal response to *Fasciola hepatica* juveniles in a perfused *ex vivo* model: a preliminary study

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Increasing anthelmintic resistance in fasciolosis has intensified the search for effective vaccines. Understanding early host-parasite interactions is essential for identifying protective antigens and pathogenic mechanisms. The aim of this study was to characterize the intestinal host response to juvenile forms of *Fasciola hepatica* (NEJs) using an *ex vivo* perfused ovine intestinal model. Metacercariae of *F. hepatica* were excysted in vitro to obtain NEJs. One sheep underwent experimental surgery in which a duodenal loop was inoculated intraluminally with 1400 NEJs, maintained under perfusion with preserved blood supply and immersed in culture medium (infected, INF-group). An adjacent uninoculated duodenal loop served as control (uninfected control, UC-group). Culture medium was analysed to recover NEJs after intestinal migration. Five intestinal samples were processed for histopathology and immunohistochemistry to evaluate apoptosis and T-cell regulatory balance. A low recovery of NEJs was obtained, likely due to the complexity of the model. Histologically, three NEJs were identified within the deep submucosa, close to the muscular layer, without associated inflammatory infiltrates, apoptosis or necrosis. Immunohistochemistry demonstrated significantly increased numbers of $CD3^+$ and $FoxP3^+$ cells in the lamina propria of the INF-group, together with an increased $FoxP3^+/CD3^+$ ratio, suggesting a relative regulatory T-cell component. Multiple variable-sized caspase-3^+ apoptotic foci composed of leukocytes, epithelial cells and apoptotic bodies were exclusively detected in the in lamina propria's INF-group. Numbers of isolated caspase-3^+ leukocytes and epithelial crypt cells were also significantly increased in infected tissue. These findings suggest that intestinal migration of *F. hepatica* NEJs induces epithelial and leukocytic apoptosis together with early immunoregulatory responses, probably associated with parasite excretion–secretion products. This preliminary study constitutes the first perfused *ex vivo* intestinal model reproducing early NEJ migration in the natural host, providing a useful platform for studying early host–parasite interactions in fasciolosis.

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Keywords: *Fasciola hepatica*, *ex vivo* model, ovine, migration, gut.
Ethical approval: ☐ Not applicable; ☒ Approved

IBERIAN Veterinary Pathology Meeting 2026 JUNE PORTO | PORTUGAL

PO-21

Histopathological abomasal lesions in different ruminant species

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Background: *Haemonchus contortus* is a highly pathogenic gastrointestinal nematode of ruminants, yet data on abomasal histopathology particularly for large ruminants remain limited.
Objective: This study investigated abomasal histopathological lesions associated with natural *H. contortus* infection in sheep, goats, cattle and water buffaloes in Greece.
Methods: A total of 2,134 abomasa were examined post-mortem and 200 *H. contortus*-infected abomasa (50 per species), along with non-infected controls, were selected for detailed histopathological evaluation. Adult parasite burden was quantified per litre of abomasal content.
Results: Inflammatory cell infiltration of the lamina propria was the most prevalent lesion across all species and showed a strong association with presence of *H. contortus* and parasite burden. Buffaloes and cattle exhibited significantly higher parasite densities than sheep and goats and developed more pronounced inflammatory infiltration, mucus cell hyperplasia and parietal cell decrease. In contrast, small ruminants displayed marked inflammatory responses despite lower parasite burdens. Epithelial erosion was frequently observed but was not consistently associated with infection, indicating limited diagnostic specificity. Across all species, *H. contortus* infection was associated with a significant reduction in abomasal content volume, reflecting functional impairment of the abomasum.
Conclusions: These findings demonstrate that while the qualitative pattern of abomasal lesions induced by *H. contortus* is broadly conserved among ruminants, lesion severity and diagnostic relevance vary according to host species and parasite load.

Keywords: histopathology, *Haemonchus contortus*, small ruminants, large ruminants, abomasum
Ethical approval: ☐ Not applicable; ☒ Approved

IBERIAN Veterinary Pathology Meeting 2026
JUNE
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PO-22

Synovial lipomatosis: a case report

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Synovial lipomatosis is a proliferative, reactive and pseudotumoral intra-articular lesion, which is very rare, accounting for only 2.1% of synovial biopsies of diarthrodial joints. Although the knee is its classic site of appearance, its presentation in the tarsus is unusual and poses important challenges in the differential diagnosis of malignant neoplastic processes.

We describe the case of a 5-year-old mixed breed dog, whole, who came to the clinic because she had a soft and fluctuating mass of approximately 2.5 cm on the medial aspect of the right tarsus, with more than six months of evolution. Hematology and biochemistry results were normal. During the surgical approach, what was initially suspected as a subcutaneous mass turned out to be an extensive proliferation of adipose tissue within the tarsal joint.

Macroscopically, biopsy fragments exhibited an adipose appearance, multilobed pattern, pale coloration, and soft consistency. The microscopic study revealed an expansive multinodular and papillary proliferation composed of well-differentiated mature adipocytes, without significant anisocytosis or mitotic activity. This proliferation was lined by hypertrophic synoviocytes arranged in up to six layers thick. The fibrovascular support stroma presented areas of myxomatous change, notable neovascularization, fibrosis, and chronic inflammatory infiltrate (lymphocytes, plasma cells, and macrophages with hemosiderin), in addition to foci of ulceration with fibrin aggregates.

Conclusion: The pathological diagnosis confirmed tarsal synovial lipomatosis, a pathology of which only three previous cases described in this joint are recorded. It is imperative to rigorously differentiate this benign reactive hyperplasia from true adipocytic neoplasms (liposarcomas) or synovial sarcomas in order to avoid unnecessary radical surgeries and establish an accurate prognosis.

Keywords: Lipomatosis; dog; synovial
Ethical approval: ☒ Not applicable; ☐ Approved

IBERIAN Veterinary Pathology Meeting 2026 JUNE PORTO | PORTUGAL

PO-23

Canine spinal nephroblastoma

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Dpt. d'Anatomia Patològica, URANOVET

A 3-month-old male Great Dane presented with right-sided monoparesis and a right posterior proprioceptive deficit. Magnetic resonance imaging (MRI) identified an intramedullary nodule at L1-L2. Histological examination of the mass revealed a triphasic neoplastic proliferation composed of blastemal cells, epithelial cells organized into tubular and/or glomeruloid formations, and abundant mesenchymal cells arranged in bundles of variable orientation. Numerous mitotic figures were observed. The histological characteristics along with the positive result for Wilms-1 (WT-1) immunohistochemistry confirm the final diagnosis of nephroblastoma.

Keywords: spinal, nephroblastoma, canine, Wilms-1, immunohistochemistry
Ethical approval: ☒ Not applicable; ☐ Approved

PO-24

***Pteridium aquilinum*–related lesions in K14HPV16 mice: histopathology across organs**

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Background/Objectives: The aim of this study was to evaluate histopathological lesions in wild-type (WT) and K14HPV16 mice exposed to freeze-dried Pteridium aquilinum (PTE) fiddleheads.
Materials and Methods: Thirty mice (10 WT and 20 K14HPV16) were used in this study. A diet containing freeze-dried PTE fiddleheads of 3 different concentrations (PTE1: 12.5%; PTE2: 25%; PTE3: 50%) were administered as a food. Mice were divided into 6 groups (n=5/group): G1 (WT, control), G2 (WT, PTE3), G3 (HPV, control), G4 (HPV, PTE1), G5 (HPV, PTE2), and G6 (HPV, PTE3). After the experimental period, animals were euthanized and samples were collected for histopathological analysis. A semi-quantitative rating, ranging from absent/normal to severe was assigned to each lesion. This study was approved by UTAD's ORBEA and DGAV (014139). Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test (<0.05).
Results: Lung lesions were minimal, with mild inflammatory infiltrate observed only in G3 and G6. No histopathological alterations were detected in heart or spleen tissues. Mild hepatic inflammatory infiltrate was observed in most groups, except G1 and G4. Only a scant number of animals exhibited tumefaction, mild in G4 and G6, and moderate in Group 5. Moderate renal inflammatory infiltrate predominated in K14HPV16 mice, whereas WT animals showed no kidney lesions. In ear pavilion skin, severe deformation was more evident in higher-dose groups (G5 and G6), while dysplasia occurred in all K14HPV16 groups. Mild erosion was observed in G4, whereas ulceration occurred in G3 and G6. Chest skin lesions were dose-dependent, with sebaceous hyperplasia and inflammatory infiltrate present in all K14HPV16 groups. No malignant lesions were identified.

Conclusions: PTE exposure induced dose-dependent epithelial lesions in K14HPV16 mice sparing visceral organs, supporting a synergistic effect between PTE and Human papillomavirus type 16 in epithelial disease progression.
Funding: FCT UID/04033/2025 (CITAB), LA/P/0126/2020 (Inov4Agro) and UIDB/0681/2025 (CERNAS).

Keywords: HPV; K14HPV16; *Pteridium aquilinum*; dysplasia; histopathology

Ethical approval: ☐ Not applicable; ☒ Approved

PO-25

Equine Laryngeal Lymphoma: an atypical presentation of T-cell lymphoma.

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The incidence of lymphoma in horses is 1.3–2.8% of all tumors and has a prevalence of 0.002–0.5% in the equine population. The objective of this study was to describe and characterize an unusual location of lymphoma in a horse.

A 6-year-old Spanish Pure Breed horse with a three-week history of dyspnea, stertor, and exercise intolerance was admitted at the Complutense Veterinary Teaching Hospital. Ultrasonography revealed pleural effusion, and submandibular lymphadenomegaly. Multifocal nodules in the arytenoid cartilage and the epiglottis, occluding 40% of the laryngeal lumen were observed in endoscopy. Computed tomography demonstrated an intramural laryngeal mass reducing the lumen by 80%, with lysis of the cricoid cartilage. The animal was euthanized given poor prognosis.

At necropsy, the laryngeal cartilages contained an irregular, expansile 15 cm mass protruding into the lumen and lymphadenomegaly of regional and submandibular lymph nodes. Additionally, the epiglottis and arytenoid cartilages were hemorrhagic, with multifocal mucosal nodules (1–3 mm). The caudal pulmonary lobe contained a 0.5 cm firm nodule.

Histologically, the larynx contained an infiltrative, densely cellular proliferation composed of sheets of round cells supported by a delicate connective tissue stroma. Cells were moderately pleomorphic and the mitotic index was 13. The mass contained multifocal areas of necrosis and apoptotic cells. Lymph nodes exhibited similar features. The lung presented multifocal parasitic granulomas. Immunohistochemistry demonstrated a CD3+ and CD20− immunophenotype, consistent with T-cell lymphoma.

Laryngeal lymphomas are uncommon and have not previously been described in horses. Mediastinal lymphoma is the most common thoracic neoplasia in horses, usually originating in the thymus or thoracic lymph nodes, and causes similar clinical signs as in this case. This case emphasizes the importance of considering laryngeal lymphoma as a differential diagnosis in horses with severe respiratory distress.

Keywords: lymphoma, laryngeal, horse, T-cell, lymphadenomegaly
Ethical approval: ☒ Not applicable; ☐ Approved

PO-26

Primary Pulmonary Myxoid Liposarcoma in a Dog

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Liposarcomas are malignant mesenchymal neoplasms originating from primitive cells with the capacity for adipocytic differentiation. In veterinary medicine, they are considered uncommon tumors, in contrast to human medicine, where they represent the most common subtype of soft tissue sarcoma. In domestic animals, they are typically located in the subcutaneous tissue, proximal regions of the limbs, abdominal cavity, and retroperitoneal space, while their primary occurrence in internal organs such as the lung is exceptionally rare.

An 11-year-old neutered male Beagle was presented with a six-month history of chronic cough and exercise intolerance. Thoracic radiographs revealed a well-defined, rounded soft tissue opacity superimposed over the cardiac silhouette. Cytological examination showed a proliferation of mesenchymal spindle cells, some resembling mature adipocytes, with cytoplasmic vacuolation and abundant lipid material.

A right intercostal thoracotomy was performed, revealing a nodular mass confined to the right cranial lung lobe, with no evidence of dissemination. A complete lobectomy was carried out without complications.

Grossly, the lesion (7.5 × 7.2 × 5.4 cm) was well demarcated, whitish, soft, and had a fatty appearance, with multifocal cystic areas containing mucoid material. Microscopically, the mass was composed of spindle-shaped, stellate, and polygonal mesenchymal cells arranged in interlacing bundles and surrounding multifocal pools of hypocellular myxoid matrix, which stained positively with PAS-Alcian blue. Immunohistochemically, the neoplastic cells were positive for vimentin and negative for Iba-1, supporting a mesenchymal origin.

The final diagnosis was a well-differentiated myxoid liposarcoma, characterized by low mitotic activity and mild cellular atypia. In the absence of metastatic lesions or similar tumors in other anatomical locations, the mass was considered a primary pulmonary neoplasm.

This case highlights the exceptional location of this tumor and underscores the importance of including liposarcoma in the differential diagnosis of nodular pulmonary masses in dogs.

Keywords: Liposarcoma, myxoid variant, dog, lung, histopathology

Ethical approval: ☒ Not applicable; ☐ Approved

IBERIAN Veterinary Pathology Meeting 2026 JUNE PORTO | PORTUGAL

PO-27

Concurrent leiomyoma and metastatic osteosarcoma in the uterus of a rabbit

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BACKGROUND: an intact female rabbit with a history of reduced appetite, apathy and constipation come to the clinic to examination. Radiologic imaging revealed a large, calcified mass in the lower abdomen and pulmonary nodules. As the presumptive diagnosis was metastatic disease, the animal was euthanized and transferred to the necropsy room for definitive diagnosis. RESULTS: gross examination reveals an enlarged uterus with small homogeneous nodules located mainly in the uterine horns. Additionally, the uterine corpus presented a different gross histopathology, with heterogeneous masses with sporadic hemorrhages. Similar masses were also observed through the mesometrium, reaching the mesentery and renal capsule, liver, spleen and lungs. Histopathology examination revealed that the lesions located in uterine horns consisted of eosinophilic, spindle-shaped cells with minimal pleomorphism, resembling smooth muscle fibers, and positive for α-SMA, features compatible with uterine leiomyoma. The microscopic examination of the masses located on uterine corpus and other locations revealed a small cells subpopulation with basophilic cytoplasm, cuboidal or elongated shape, with marked pleomorphism, positive for vimentin, which were grouped around an acidophilic matrix, which occasionally exhibited areas of heavy mineralization. Additionally, there were signs of vascular invasion. All these features were compatible with a metastatic uterine osteogenic osteosarcoma. CONCLUSION: although leiomyoma is a frequent lesion described in the uterus, the description of a primary uterine osteosarcoma in rabbits is extremely rare. The possible origin of the tumor may be explained by two hypotheses: a) metaplasia arising from the organ's mesenchymal tissue; and b) osteosarcomatous differentiation arising from uterine muscle tumors.

Keywords: Rabbit, uterus, osteosarcoma, leiomyoma
Ethical approval: ☒ Not applicable; ☐ Approved

IBERIAN Veterinary Pathology Meeting 2026
JUNE
PORTO | PORTUGAL

PO-28

Quantification of lymphatic vessels in canine mammary carcinomas and their prognostic value

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Background/Objectives: Mammary tumors are the most common neoplasms in female dogs, and their progression is influenced by lymphatic vessels, which can be identified by LYVE-1 expression. This study aimed to characterize and quantify intra- and peritumoral lymphatic vessels in canine mammary carcinomas and compare them according to histopathological subtype, grade, disease-related mortality, and post-surgical survival.
Materials and Methods: Thirty-six samples of canine mammary carcinomas representing seven histopathological subtypes **(**complex, mixed, tubular, tubulopapillary, intraductal papillary, comedocarcinoma and solid carcinomas) and classified as grade I (48.28%), grade II (41.38%), and grade III (10.34%) were analyzed using immunohistochemistry with an anti-LYVE-1 antibody. Immunolabelling was evaluated in 5 images (total area = 2.75 mm^2) in "hot spots" within the intra- and peritumoral regions. Lymphatic vessels were counted, considering vessels with or without a visible lumen. Luminal areas were measured using the QuPath software. Counts were compared with histological types and grades using unpaired t-tests. A cutoff point was determined using an ROC curve. Survival analysis was performed using the Kaplan-Meier/Mantel-Cox test.
Results: No statistically significant differences were observed in the number of lymphatic vessels or luminal area in both intra- and peritumoral regions when comparing histopathological groups and histological grades. Disease-related mortality was significantly higher in dogs with tumors showing higher intratumoral lymphatic vascularization and luminal area ($p<0.05$). The cutoff of 67 intratumoral lymphatic vessels (AUC=0.6696; p=0.1643) revealed that animals with ≥67 intratumoral vessels in 2.75 mm^2 had significantly shorter survival (p=0.0076; median survival = 790 days) and a 5.33-fold higher risk of tumor-related death compared with dogs with <67 intratumoral vessels.
Conclusions: Although not associated with histopathological subtype or grade, high intratumoral lymphatic vessel counts and larger luminal areas were associated with shorter post-surgical survival and a higher risk of tumor-related death.

Keywords: Carcinoma, dog, mammary gland, immunohistochemistry, lymphatic vessel.
Ethical approval: ☒ Not applicable; ☐ Approved

IBERIAN Veterinary Pathology Meeting 2026
JUNE
PORTO | PORTUGAL

PO-29

Immunohistochemical characterization of a cystic pulmonary adenocarcinoma in an adult cat

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Primary pulmonary tumors in cats are uncommon, accounting for less than 1%. Most are of epithelial origin, and there is an even rarer cystic variant of lung adenocarcinoma. This tumor is characterized by cystic and/or glandular structures lined by atypical proliferative cells. This work describes the histopathological and immunohistochemical findings in a 10-year-old cat. On physical examination, the cat was cachectic, with a six-month history of hyporexia and weight loss. Thoracic radiographs revealed mediastinal changes and a nodular pulmonary pattern. The hemogram showed non-regenerative anemia, neutrophilia, and thrombocytopenia. The cat tested negative for FeLV and FIV and died naturally in the ICU.

At necropsy, no external lesions were observed. The larynx and trachea contained abundant mucinous exudate and showed marked inflammation and hyperemia. The left lung was severely congested and contained a cystic cavity filled with mucopurulent exudate, replacing the normal pulmonary architecture. Microscopically, the cystic structure was lined by a proliferation of cuboidal to columnar epithelial cells and numerous multinucleated cells with neoplastic appearance. In the remaining parenchyma, foci of tumor cells were observed originating from the alveolar cells. The adjacent stroma showed mild lymphoplasmacytic inflammatory infiltrate and areas of fibrosis. Lymphatic vessels contained tumor cell thrombi. This lymphatic invasion was also observed in the right lung, particularly in peripheral vessels.

Immunohistochemistry for TTF-1 and cytokeratin showed positive staining in the neoplastic cells, while vimentin was negative. This immunophenotype (TTF-1+, cytokeratin+, vimentin−) confirms the epithelial origin of the tumor and supports a diagnosis of primary pulmonary carcinoma of alveolar/bronchiolar origin. TTF-1 positivity is consistent with origin from type II pneumocytes or bronchiolar epithelium, while negative vimentin excludes a mesenchymal tumor.

Keywords: Feline, Lung, Neoplasia, Cystic, Adenocarcinoma
Ethical approval: ☒ Not applicable; ☐ Approved

PO-30

Vascular hamartoma in the prepuce of a slaughtered cattle

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Skin tumors in cattle are relatively uncommon, with fibropapillomas and squamous cell carcinomas being the most frequent in prepuces. Fibropapillomas are characterized by the cauliflower-like shape and, microscopically, by acanthosis with epidermal ridges and excessive proliferation of dermal fibroblasts. Hamartomas are excessive growths of normal mature tissue in an area and classified according to the predominant tissue. Vascular hamartomas are those that present an excessive growth of blood vessels. In cattle, vascular hamartomas have been reported in various locations, but never in prepuce. In this work a vascular hamartoma, grossly suspected of being a fibropapilloma, is described in a slaughtered cattle. A rounded mass, approximately 20 cm in diameter and resembling a cauliflower, was observed on the preputial skin of a cattle at slaughterhouse, and was diagnosed as a papilloma. At section, multiple moderately delimited nodules were observed on the surface and supported by a thick oedematous fibrous stalk. Microscopically, no epidermal proliferation was observed and the epithelium was eroded and replaced by severe neutrophilic inflammation that also occupied the superficial dermis. In the dermis, an excessive presence of small, medium, and large vessels was observed, separated by a fibrous stroma of variable cellular density. Small vessels predominated in the superficial epidermis and interstitial areas and were revealed by immunohistochemical staining for von Willebrand factor. Large vessels were observed mainly in the deep dermis and had very irregular shapes with variable proliferation of their walls, as evidenced by desmin immunostaining and Masson trichrome staining. Immunostaining with S100 revealed positivity in the endothelium of some vessels and in some spindle interstitial cells, which approximately corresponded to the capillaries stained with von Willebrand factor. Ki-67 revealed mild cellular proliferation that was mainly observed in the muscular layer of some blood vessels. A mild multifocal lymphoid infiltrate was observed around some blood vessels.

Keywords: Cattle, Hamartoma, Reproductive, Skin, Vascular
Ethical approval: ☒ Not applicable; ☐ Approved

PO-31

Ocular bilateral fibroepithelial hamartoma in a young pig

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Tumors or growth disorders are rare in pigs, with melanomas and lymphomas being the most frequently reported tumors. Hamartomas are excessive growths of normal mature tissue in a specific location. In contrast, choristomas are growths of normal tissue in an abnormal location. In pigs, a cutaneous hamartoma and an ocular choristoma have been reported. This work presents a fibroepithelial hamartoma with immunohistochemical findings in a piglet approximately 6-week-old. No clinical history was available. Lesions were found in both eyes, corresponding to cylindrical masses of approximately 3 x 0,5 cm, protruding from the ventromedial zone of the eyes. Microscopically, the lesions showed abundant connective tissue with numerous small blood vessels and sporadic eccrine glands, surrounded by a stratified squamous epithelium. In some areas, a connexion to the third eyelid was also observed. Pleomorphic inflammatory infiltration, specially subepithelial, was observed in some areas, associated with an ulcerated epithelium. Immunohistochemistry revealed abundant vimentin-positive fibrous tissue surrounded by cytokeratin-positive superficial epithelium. This study represents the first description of a bilateral ocular fibroepithelial hamartoma in pigs.

Keywords: Swine, Hamartoma, ocular
Ethical approval: ☒ Not applicable; ☐ Approved

IBERIAN Veterinary Pathology Meeting 2026
JUNE
PORTO | PORTUGAL

PO-32

Ocular fibrosarcoma in an adult sheep

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Eye tumors are uncommon in sheep and most are palpebral squamous cell carcinomas. Ocular fibrosarcomas are reported less frequently, being more common in other species, such as dogs. Fibrosarcomas have been described sporadically in various locations in sheep; however, there are no reports of ocular fibrosarcomas in sheep, except for one retrobulbar fibrosarcoma. This work describes an ocular fibrosarcoma in sheep. Macroscopically, an exophytic, rounded mass, approximately 5 cm in diameter, was observed in the palpebral area of the right eye, which also affected the cornea and sclera. The surface of the mass was irregular and appeared eroded, with a foul-smelling exudate. Microscopically, irregular and dense aggregates of spindle-shaped and polygonal cells were observed, which presented variable orientations and were surrounded by a marked fibrovascular stroma. The cells showed moderate atypia without well-defined borders, moderate cytoplasm volume, and nuclei of variable size, which had inconspicuous nucleoli and a moderate number of mitoses, some of them atypical. Spindle cells were in close contact with the external layer of the sclera from which appeared to originate. Severe mixed inflammation and occasional foci of necrosis were observed. By immunohistochemistry, the cells were immunostained with the vimentin antibody and were negative for cytokeratin AE1/AE3, desmin, von Willebrand factor and S100. Ki-67 revealed mild cellular proliferation, more intense in the cells near the sclera and less intense in distant cells. This study shows that tumors other than squamous cell carcinoma can appear in the eyes of sheep, and consequently, this case is the first description of an external fibrosarcoma that apparently originated in the outer membrane of the sclera. Also, it shows that immunohistochemistry is important for a correct diagnosis.

Keywords: Ovine, Tumor, Eye, Fibrosarcoma, Immunohistochemistry
Ethical approval: ☒ Not applicable; ☐ Approved

IBERIAN Veterinary Pathology Meeting 2026 JUNE PORTO | PORTUGAL

PO-33

Malignant seminoma with ocular metastasis in a Fulvous whistling duck (*Dendrocygna bicolor*)- A case report

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Seminoma is a primary testicular neoplasm arising from the germinal epithelium of the seminiferous tubules. Although rare in avian species, single-case reports of this tumour exist in Bald Eagles, Psittacines, Guinea fowl, Roosters, Mallard ducks, Trumpeter hornbills, and Columbiformes. Metastasis is rare but has been described in organs such as the liver, lungs, kidneys, heart, brain (optic chiasm), and pancreas.

A 7-year-old male Fulvous whistling duck (*Dendrocygna bicolor*) from a zoo collection was submitted for necropsy following sudden death. On gross examination, the right testis was not observed in its expected anatomical location; replacing the left testis, cranioventral to the left kidney, there was a well-demarcated, multilobulated, moderately firm mass, measuring approximately 6 cm × 5.3 cm × 4 cm and ranging from white to pale pink. Body condition was low and the kidneys were moderately atrophied. No other gross lesions were found.

Histopathology revealed that the mass was composed of polygonal cells with poorly defined cell boundaries, with scant, slightly eosinophilic, and finely vacuolated cytoplasm, organized in cords and sheets, sometimes occupying, sometimes obliterating the seminiferous tubules. Occasionally, giant, binucleated cells were also observed. The findings were consistent with seminoma. Neoplastic cells morphologically identical to those of the mass were also observed within hepatic blood vessels and infiltrating circumferentially the uvea of one eye, in chords and sheets that obliterated the choroid and retina. These findings are evidence of metastatic spread, supporting the final diagnosis of malignant seminoma with systemic dissemination.

The absence of a right testicle may be attributed to severe atrophy, incorporation into the tumour or monorchidism. Histological lesions in the kidneys were unrelated to the tumour and consistent with mild to moderate multifocal lymphoplasmacytic interstitial nephritis.

To the authors' knowledge, this is the first report of malignant seminoma with ocular metastasis in a Fulvous whistling duck.

This study has been funded by CIISA UID/276/2025

Keywords: Neoplasia; malignant seminoma; ocular metastasis; Fulvous whistling duck; wildlife

Ethical approval: ☒ Not applicable; ☐ Approved

PO-34

The impact of human-wildlife conflict on the health and conservation of the Iberian wolf (*Canis lupus signatus*) in Portugal

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The Iberian wolf (*Canis lupus signatus*) is a threatened subspecies. Several factors, both natural and anthropogenic, including illegal hunting, poisoning, and the expansion of human activities, intensify human-wolf conflict and negatively contribute to the health of these populations. Historically widespread across Portugal and Spain, this subspecies suffered a severe range contraction during the 20th century and is now confined to the northwestern Iberian Peninsula.

To support conservation efforts, the Institute for Nature Conservation and Forests (ICNF, I.P.) established the Dead Wolf Monitoring System (DWMS) in 1999. In 2002, a partnership protocol was established to centralize all Iberian wolf necropsies at the currently designated Vairão Veterinary Laboratory – National Institute of Agrarian and Veterinary Investigation (LVV- INIAV, I.P.). This system enables systematic investigation of causes of mortality through forensic necropsy. From 2019 onwards, the notification of these incidents to national authorities has proven advantageous in the context of criminal investigation proceedings by the Public Prosecutor's Office, improving benefits to pathological technicians, namely through access to the police report and the procedures adopted at the crime scene.

This study analyses 145 Iberian wolf specimens collected in Portugal between 1999 and 2025 within the DWMS framework, aiming to characterize mortality patterns and evaluate anthropogenic impacts over time. Anthropogenic causes were identified in 98 cases (67.1%). Among these, 42 deaths resulted from vehicle collisions, while 56 individuals showed evidence of illegal persecution, including gunshot wounds, blunt trauma, steel cable snaring, and poisoning. These results demonstrate a high prevalence of human-induced mortality and highlight the value of long-term forensic monitoring.

Forensic necropsies play a crucial role in understanding the causes of mortality and in providing robust evidence for the investigation of wildlife crimes. These procedures support measures to reduce human–wolf conflict, enforce legal protection, and promote the health and conservation of Iberian wolf populations.

Keywords: wildlife crime, forensic veterinary necropsy, lupine population, biodiversity,

Ethical approval: ☒ Not applicable; ☐ Approved

PO-35

Correlation of splenic immune cells and serum acute phase proteins in wild boars (*Sus scrofa*): a preliminary study

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Immune cell patterns and acute phase proteins are gaining relevance in veterinary medicine, as they are considered an important tool for the detection of infection and inflammatory responses. This preliminary study aimed to evaluate the association between splenic immune cell populations and plasma acute phase proteins in hunted wild boars (*Sus scrofa*) with (n=22) and without (n=36) contact with livestock. A total of 58 wild boars from Salamanca and Asturias (Spain) were sampled. Spleen and blood samples were collected, and immunohistochemistry was performed on spleen samples to detect the populations of the following pathogen exposure related cells: monocytes (C-C motif chemokine receptor 2 (CCR2)), marginal metallophilic macrophages (CD169), marginal zone macrophages (CD209b) and dendritic cells (CD11c). Plasma concentrations of total proteins, albumin, globulins and C reactive protein (CRP) were also determined using a clinical biochemistry analyser. Wild boars from areas without livestock showed a positive correlation between dendritic cells and CRP, marginal zone macrophages and CRP, as well as monocytes and globulins. In contrast, a negative correlation between marginal metallophilic macrophages and total proteins was found in wild boars in contact with livestock. In areas without livestock, the positive correlation between certain splenic cells and CRP and globulins might suggest a coordinated activation of the splenic response and the acute phase response against infectious or inflammatory stimuli. However, the negative correlation observed in animals from areas with livestock might be related to a higher antigen caption by CD169 macrophages and a lower antibody production, which are part of the total proteins; although, a spurious correlation in livestock areas cannot be discarded. Overall, this study suggests an association between splenic and acute phase responses in wild boars, which might also be influenced by the contact with livestock.

Keywords: acute phase proteins, immunohistochemistry, wild boar
Ethical approval: ☒ Not applicable; ☐ Approved

PO-36

First report of an intracranial melanized epidermal cyst in a captive snow leopard (*Panthera uncia*)

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Introduction: Intracranial epidermal cysts are uncommon developmental lesions in veterinary medicine, and their melanized variants are exceptionally rare. This report documents the pathological findings of a unique case in a non-domestic felid.

Case Description: A 12-year-old male snow leopard (*Panthera uncia*) housed in a zoological garden underwent necropsy, revealing a 4 x 2.5 cm extra-axial, space-occupying lesion in the parasellar region of the middle cranial fossa. The mass was firmly adhered to the skull, causing significant compression and deformation of the left temporal lobe. Macroscopically, the encapsulated lesion contained friable, blackish-brown granular material.

Histopathological Findings: Histopathological analysis identified a melanized epidermal cyst lined by stratified squamous epithelium with basal melanin deposition. The cyst lumen was filled with keratin lamellae, cellular debris and polymorphonuclear leukocytes. An extensive granulomatous inflammatory response was observed, characterized by cholesterol clefts, osseous metaplasia (capsular ossification), lymphocytic infiltrates, and multinucleated foreign body–type giant cells, along with aggregates of hemosiderin-laden macrophages and melanophages, consistent with a secondary reaction to cyst wall rupture. Additionally, clusters of melanocytes were noted invading retrobulbar muscle tissue. Microscopic examination of the meninges further revealed multiple transitional meningiomas, characterized by concentric whorls of polygonal cells forming confluent nodules.

Conclusion: To the authors' knowledge, this is the first description of a melanized intracranial epidermal cyst in a snow leopard (*Pantera uncia*). This case emphasizes the importance of including these lesions in the differential diagnosis of central nervous system disorders in zoo species and highlights the occurrence of concurrent primary intracranial neoplasms in geriatric felids.

Keywords: Wildlife pathology, Veterinary necropsy, Central Nervous System, Meningioma

Ethical approval: ☒ Not applicable; ☐ Approved

IBERIAN Veterinary Pathology Meeting 2026 JUNE PORTO | PORTUGAL

PO-37

Osteosarcoma in a California kingsnake: a case report

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Background: In veterinary medicine, neoplasias are frequently found in sauropsids, particularly in their musculoskeletal system, hepatic system, and skin. The highest prevalence of neoplasia is likely in snakes, followed by lizards, testudines, and crocodylians. Herein we report a case of osteosarcoma in a California kingsnake (*Lampropeltis californiae*).
Case Report: An adult snake, age unknown, from the reptile collection at the Maia Zoo presented a dorsal ulcerated neoformation; the clinical exam revealed a subcutaneous, firm mass, adherent to the spinal column, and an ultrasound exam revealed multiple coelomic masses of variable size. The snake was anesthetized, and the dorsal mass was removed and sent for analysis to the UTAD´s Histology and Surgical Pathology Laboratory.
Macroscopically, 3 fragments of a whitish mass measuring 0,6 cm in diameter were trimmed and processed for light microscopy. Microscopic examination revealed fragments of ulcerated skin with superficial bacterial contamination, inflammation, and disorganized proliferation of osteoblasts and bone matrix, admixed with haphazardly arranged spicules of woven bone. According to macro and microscopic features, a diagnosis of osteosarcoma was made.
Conclusions: Neoplasia was once thought to be rare in reptiles, however, in captive snakes, due to a prolonged life span, spontaneous neoplasms are detected more frequently. The prevalence of neoplasia in snakes and other reptiles is not well documented. Reptile collections maintained in zoological parks are important sources of information on spontaneous neoplasia because the animals are usually subjected to fairly constant environmental conditions and are allowed to live out their life span.

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Keywords: Osteosarcoma, snake, neoplasia, sarcoma
Ethical approval: ☒ Not applicable; ☐ Approved

PO-38

Biodiversity of Parasites in the Black Scabbardfish, A*phanopus carbo* from Sesimbra, off the Atlantic Coast of Portugal

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The presence of parasites in fishery products can negatively affect their quality due to their unsightly appearance, as well as consumer safety, especially when dealing with zoonotic parasites. During 2023-2024, four seasonal *Aphanopus carbo* (Ac) samples totaling 123 specimens were obtained from Sesimbra area, on the Portuguese coast (FAO zone 27). This study aimed to characterize the parasitofauna of Ac samples and to evaluate their parasitic infection levels. Each specimen was measured, weighed and examined for parasite detection. For parasitological survey, the integument and visceral cavity were examined by visual inspection, the UV-press method was applied to identify nematodes in the viscera, and gills were observed under a stereomicroscope. Parasite identification was performed by morphological and molecular analyses.

The prevalence and mean intensity of infection were recorded for each parasite and month.

According to our results, eight taxa occurred in the samples: unidentified Monogenea; the Cestoda, *Trypanorhyncha* larvae attributed to the genus *Hepatoxylon* and *Nybelinia* sp,; the acanthocephalan, *Bolbosoma vasculosum*; cysts of Microsporidia and larvae of Nematoda: *Anisakis simplex* (s.s.), *Anisakis pegreffii* and hybrids. A 100% prevalence of nematode *Anisakis* spp. infection was observed all year round and the prevalence in descending order of the other taxa recorded were: 11.4 % of Microsporidia, 5.7% of *Hepatoxylon* pleroceroids, 2.4% of Monogenea, 1.6% of *Nybelinia* sp. and 0.8% of Acanthocephala.

The results show a high prevalence of zoonotic nematode species compared to other taxa, reinforcing the need to improve sanitary control measures and increase consumer awareness of parasite issue in fishery products. Gutting the fish immediately after capture will not only control the migration of *Anisakis* larvae to the muscle, the edible part of the fish, but also physically eliminate Acanthocephala and Cestoda larvae, which, due to their size, impair the quality of the fish and lead to increased food waste.

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Keywords: Black scabbardfish, parasites, biodiversity, fish quality, health risk

Ethical approval: ☒ Not applicable; ☐ Approved

PO-39

Clinicopathological characterization of proliferative cutaneous lesions in a captive population of blue spiny lizards (*Sceloporus serrifer*)

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Background: Neoplasia is an increasingly recognized cause of disease in reptile populations. Neoplasms in lizards (Order Squamata) are reported less frequently than in other reptilian groups. Cutaneous and hepatic carcinomas are among the most frequently diagnosed neoplasms in lizards. The rising incidence of neoplastic processes has been associated with the prolonged lifespan of these animals in captivity. In addition, environmental conditions and viral infections, including papillomaviruses or herpesviruses, may play a key role in the development of neoplastic lesions such as cutaneous squamous cell carcinomas (SCCs).
Objectives: The aim of this study was to characterize the clinicopathological features of cutaneous proliferative lesions in a single captive population of blue spiny lizards (*Sceloporus serrifer*) and to provide insights into their pathogenesis.
Methods: A retrospective study conducted over a 7-year period at Barcelona Zoo identified 14 adult blue spiny lizards of both sexes, housed in different indoor terrariums, presenting with cutaneous proliferative lesions. Clinical findings and environmental conditions were recorded. Skin samples from biopsied and necropsied individuals were collected and processed for histopathological evaluation.
Results: All lizards exhibited exophytic cutaneous lesions on the head, primarily involving the eyelids, nostrils, and lips. These macroscopic changes were detected at different time points, with multiple individuals affected simultaneously. Microscopically, cutaneous lesions were classified as SCCs, keratoacanthomas or hyperplastic dermatitis. UVB exposure and temperature conditions were within normal ranges.
Conclusions: This study describes a spectrum of proliferative cutaneous lesions in captive lizards that may represent a continuum of neoplastic progression. Further studies are required to exclude potential triggering factors, such as viral agents. Understanding the pathogenesis of these lesions may contribute to their prevention and management.

Keywords: Lizards, proliferative lesions, squamous cell carcinoma, keratoacanthoma, hyperplastic dermatitis

Ethical approval: ☒ Not applicable; ☐ Approved

PO-40

Severe parasitic encephalomalacia caused by aberrant trematode migration in a stranded *Delphinus delphis* (Uruguay)

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In late March 2026, a common dolphin (*Delphinus delphis*) was referred to the Diagnostic Pathology Service of the Pathology Academic Unit, Facultad de Veterinaria, Universidad de la República (Udelar, Montevideo, Uruguay). The animal had been found dead on the coast of Punta Colorada, Maldonado, Uruguay.
On arrival, the specimen was identified as an adult female with good body condition and no external lesions. Upon necropsy, the most significant finding was a severe lesion affecting the central nervous system. Gross examination of the brain revealed a focal to regionally extensive area of encephalomalacia involving the left cerebral hemisphere, extending caudally into the cerebellum through a cavitated, fistulous tract filled with necrohemorrhagic material.
For further evaluation, the brain was collected and fixed in 10% neutral buffered formalin. Serial 1-cm-thick coronal sections were performed to characterize the fistulous tract. Representative samples (approximately 0.5 cm thick) from each section were processed routinely, embedded in paraffin, sectioned at 3 µm, and stained with hematoxylin and eosin.
The lesion originated near the emergence of the left VIII cranial nerve and extended from the cerebellum rostrally through the cerebral parenchyma, involving the occipital and medial parietal cortices, and reaching the left frontal cortex.
Histopathological analysis revealed multifocal to coalescing necrohemorrhagic tracts usually associated with abundant parasitic eggs and one adult parasite, which was found on the cerebellar parenchyma. To further characterize the host neuroinflammatory response and neurodegeneration, immunohistochemical analysis using primary antibodies against: Glial Fibrillary Acidic Protein (GFAP), Ionized calcium-Binding Adapter molecule 1 (Iba1), and neurofilaments was carried to define lesions during these aberrant parasitic migrations.
Based on the distribution of the lesions, based on gross pathology and histoplathology, a parasitic migratory origin from the pterygoid sinuses was considered most likely. Trematodes of the genus *Nasitrema* sp. are presumed as the probable etiological agent.

Keywords: *Delphinus delphis*, diagnostic pathology, cetaceans, wildlife, nervous system
Ethical approval: ☒ Not applicable; ☐ Approved

IBERIAN Veterinary Pathology Meeting 2026 JUNE PORTO | PORTUGAL

PO-41

Detection and characterization of lesions caused by canine morbillivirus infection in a grey fox (*Lycalopex gymnocercus*) from Santa Lucía, Canelones, Uruguay

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Canine morbillivirus (CM), a member of the Paramyxoviridae family, causes multisystemic infections with high morbidity and mortality. Although it is primarily associated with domestic dogs, a wide range of susceptible species has been reported worldwide, including both domestic and wild animals, such as carnivores, artiodactyls, rodents, among others.

In Uruguay, little is known about the impact of CM on non-conventional species. In this regard, our research group recently reported a case in a collared peccary (*Pecari tajacu*) from a zoological collection in 2023. Furthermore, we currently have several histopathological studies in progress from animals testing positive by qRT-PCR, including other fox species such as *Cerdocyon thous* and C*hrysocyon brachyurus*, as well as a common marmoset (*Callithrix jacchus*).
The present study aims to contribute diagnostic and pathophysiological knowledge of CM in understudied species. The objective was to detect and characterize CM-associated lesions in a free-ranging grey fox (*Lycalopex gymnocercus*) from Santa Lucía, Canelones, Uruguay. A complete necropsy was performed, and tissue samples were collected. CM infection was confirmed by PCR and anti-CDV immunohistochemistry (IHC), and lesions were characterized in multiple organs, with emphasis on the nervous system, based on criteria recently reported by our group for domestic canines with emphasis on demyelination. Additionally, to understand the responses associated with the infection, markers for astrocytes (GFAP), microglia (Iba1), and neurofilaments were used to evaluate tissue damage and cellular responses.
Finally, the presence of CM associated lesions was confirmed and characterized, demonstrating that the neurological lesions caused by CM in wild canids in Uruguay are similar to previous reports of our group in domestic dogs. These findings provide evidence to better understand CM pathogenesis, support diagnosis, and strengthen sanitary measures in wildlife.

Keywords: canine morbillivirus, diagnostic pathology, *Lycalopex gymnocercus*, wildlife, nervous system
Ethical approval: ☒ Not applicable; ☐ Approved

IBERIAN Veterinary Pathology Meeting 2026
JUNE
PORTO | PORTUGAL

PO-42

Melanophoroma in a bearded dragon (*Pogona vitticeps*)

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Melanophoroma is a neoplasm of reptilian pigment-producing cells, part of a group of neoplasms called chromatophoromas. Chromatophoromas develop from dermal cells carrying pigment and reflecting light (chromatophores) in the skin of reptiles, fish, and amphibians. They include melanophoromas (melanomas), iridophoromas, and xanthophoromas. Melanophoromas or melanomas arise from melanin-producing cells (melanophores) and are considered uncommon in reptiles. Diagnosis is most often confirmed through histological examination.

We describe the histopathological features of a melanophoroma in a 6-year-old bearded dragon (*Pogona vitticeps*). The mass was dark brown to black, measuring 2.9x1.8x0.9 cm, and was located on the ventral aspect of the neck. Histopathological examination revealed a relatively well-circumscribed, non-encapsulated, moderately to highly cellular, multinodular and infiltrative neoplastic proliferation mainly affecting the dermis and extending into the subcutaneous tissue and the muscle layer. The neoplastic cells were organized in sheets and interwoven bundles supported by a fine collagenous stroma. They varied from polygonal to spindle-shaped and had variably defined cell borders, and moderate amounts of eosinophilic cytoplasm containing dark brown to black granular pigment (>60% of neoplastic cells). Their nuclei were irregular to round to ovoid, with moderately to sparsely dense chromatin and up to three nucleoli; frequent multinucleated cells, and occasional binucleate cells, were observed. Anisocytosis and anisokaryosis were moderate to marked, with one mitotic figure in 10 high power fields. These findings supported a diagnosis of melanophoroma.

The prognostic criteria commonly applied for canine cutaneous melanocytic neoplasms does not seem to be applicable for pigmented tumors of bearded dragon. Most cutaneous chromatophoromas in bearded dragons are locally invasive but do not metastasize. Aggressive surgical excision is often curative; however, clinical follow-up is advisable for tumours with increased mitotic count, nuclear atypia, and pleomorphism.

Keywords: Melanophoroma, chromatophoroma, neoplasm, bearded dragon, reptile
Ethical approval: ☒ Not applicable; ☐ Approved

PO-43

Immunohistochemical study of interstitial cells of Cajal and mast cells in an adult dog with acquired idiopathic megaesophagus. Preliminary results

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Megaoesophagus is characterized by diffuse oesophageal dilation and decreased peristalsis. It can be congenital or acquired. The causes of acquired megaesophagus usually remain unknown being classified as idiopathic. Some studies suggest a nervous disorder, although the causes often remain unidentified. This work describes the pathological and immunohistochemical findings of an acquired idiopathic megaesophagus in an 8-year-old neutered Labrador dog. Clinically, the dog was diagnosed with megaesophagus two years earlier. Several clinical conditions associated with megaesophagus were ruled out. These included myasthenia gravis, hypothyroidism, electrolyte disorders, neuromuscular disorders, and toxic problems. Medical management included food with smooth consistence and upright feeding. The dog was also treated with sildenafil and methylprednisolone. However, after two years its condition worsened, and it was euthanized. The necropsy revealed a megaesophagus with no other relevant findings. Microscopically, moderate to severe chronic inflammatory infiltration was mainly observed in the submucosa layer of the medium and caudal oesophagus. No inflammation was observed in the muscular layers. The epithelium was moderately hyperplastic and was infiltrated mostly by neutrophils. The submucosal glands were slightly inactive. Mild aspiration pneumonia was also observed. CD3 staining showed a high number of T lymphocytes in the submucosa, surrounding glands and infiltrating the epithelium. No lymphocytes were observed in the myenteric plexus. Higher number of mast cells were demonstrated by toluidine blue staining and confirmed by c-kit Immunostaining. This marker also showed a decreased number of interstitial cells of Cajal in the muscular layers in comparison with a normal oesophagus. The GFAP and PGP 9.5 markers revealed a decrease in the staining of the myenteric plexus of the caudal oesophagus. This study suggests that interstitial cells of Cajal and mast cells might play a role in some cases of canine acquired megaesophagus, although further studies with a larger number of cases are needed.

Keywords: Canine, Megaesophagus, Idiopathic, Immunohistochemistry, Nervous plexus
Ethical approval: ☐ Not applicable; ☒ Approved

IBERIAN Veterinary Pathology Meeting 2026
JUNE
PORTO | PORTUGAL

PO-44

Biodiversity of Myxozoa Parasites in Two Commercially Important Fish Species, the European Hake, *Merluccius merluccius* (Linnaeus, 1758), and the Pouting, *Trisopterus luscus* (Linnaeus, 1758) in the Eastern Atlantic Waters

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European hake and the pouting are two commercially important fish species in the Portuguese fishing industry. Parasitological analyses were performed on these two fish species samples to evaluate the biodiversity of parasites of the genus Myxozoa in fish landed in Portugal.
Gallbladders, kidneys, and muscles of European hake specimens from the southwest region of Ireland (FAO 27, Region VIIJ) and the Bay of Biscay (FAO 27, VIIIA,C), and pouting specimens from the Portuguese coast (FAO, IX) were analyzed. Tissues were observed in fresh smears under a microscope (x400) for the presence of spores and/or developmental stages of the parasites.
Three species of Myxozoa were found in European hake. In the gallbladder, *Pseudalataspora* sp. were found, with a prevalence of 76.1% (70/92), with no differences between the two sampling regions, nor in relation to the sex of the host. This species coexists with another myxozoan, *Ceratomyxa* sp., with a prevalence of 8.7% (8/92), with a slightly higher prevalence in Southwest Ireland and only infecting females. The kidneys were infected by a species of *Parvicapsula* sp., with a prevalence of 23.5% (51/217), with no variation between the two regions or by host sex. No Myxozoa infection was found in the muscles. In pouting, three species of Myxozoa were found. In the gallbladder, *Sphaeromyxa longa* was found, with a prevalence of 26.1% (6/23). The muscle was infected by *Kudoa* sp. with a prevalence of 5.8% (7/120), frequently in co-infection with *Kudoa thyrsites*. No Myxozoa parasites were detected in the kidneys.
In this study, six species of Myxozoa were identified in European hake and pouting. However, the biodiversity of Myxozoa in these two fish species maybe greater, if a more detailed parasitological analysis was performed, or if fish are captured in other geographic localities.

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Keywords: European hake, pouting, parasite, Myxozoa biodiversity, fish quality
Ethical approval: ☒ Not applicable; ☐ Approved