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Content

SPEAKER ABSTRACTS

WEDNESDAY 16 SEPTEMBER 2026 - RESIDENT DAY

Cytology for anatomic pathologists and histology for clinical pathologists: what about unusual species?

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Eve Ramery[1], Julien Miclard[2]
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Lecture Content:

Abstract

Cytology and histopathology are complementary diagnostic tools in exotic animal medicine, wildlife pathology, and avian/reptile practice. Cytology offers rapid, minimally invasive assessment of lesion type, cellular populations, and the possible presence of infectious agents, whereas histopathology provides architectural context, tissue invasion, lesion depth, and improved etiologic confirmation. This distinction is particularly important in unusual species, where inflammatory responses, tissue composition and architecture, and tumor biology often differ from those of dogs and cats. In birds and reptiles, heterophilic inflammation is a major pattern of acute or recent tissue injury, and granulomatous reactions may develop rapidly in response to fungal, mycobacterial, or foreign body disease. In small mammals, cytology is especially useful because of patient size, anesthesia risk, and the frequent need for minimally invasive sampling. Correlating cytologic and histologic findings across major lesion categories improves diagnostic accuracy, guides ancillary testing, and helps avoid common pitfalls such as misclassifying inflammation as neoplasia or underestimating the extent of disease. Cytology and histopathology should be viewed as a continuum rather than competing methods. In birds, reptiles, small mammals, and wildlife, the best diagnostic strategy often combines both techniques with targeted ancillary tests such as special stains, culture, or molecular diagnostics. A structured approach to cytology-histology correlation improves diagnostic confidence and reduces the risk of misclassification in these challenging species. This presentation reviews the main lesion patterns encountered in birds, reptiles, small mammals, and selected wildlife species.

Keywords
cytology; histopathology; birds; reptiles; small mammals; exotic animals; granulomatous inflammation; neoplasia

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Cytology for anatomic pathologists and histology for clinical pathologists: focus on non-neoplastic changes?|

Edouard Reyes-Gomez (France), Nicolas Soetart (France)

No abstracts has been provided

Notes:

Method Comparison Made Practical

27

A Russell Moore[1]

[1] Colorado State University, Fort Collins, United States

Lecture Content:
Method comparison is a core component of analytical validation in veterinary clinical pathology, yet its interpretation remains a common source of uncertainty. This session focuses on translating statistical outputs into clinically meaningful conclusions, using an example-based format to examine when two measurement methods can be considered interchangeable, when method-specific interpretation is required, and when differences are clinically unacceptable.

The session is framed within the analytical phase of validation, with emphasis on characterizing bias and variability between methods rather than identifying a single "correct" result. Traditional reliance on correlation and linear regression is critically evaluated, highlighting why strong correlation does not equate to agreement and may obscure clinically important differences. Bland–Altman (difference) plot analysis is presented as a primary tool for assessing agreement, with representative datasets used to illustrate constant bias, proportional bias, and random variation, and how these patterns manifest across analytical ranges.

Interpretation focuses on practical questions: the magnitude and direction of average differences between methods, the expected variability for individual samples, and whether observed differences are likely to affect clinical decisions. Statistical elements including mean difference, limits of agreement, and confidence intervals are introduced conceptually, with emphasis placed on pattern recognition, underlying assumptions, and appropriate interpretation rather than formula-based approaches. Common challenges are addressed, including heteroscedastic data, overinterpretation of statistical significance, and limitations of traditional limits of agreement in the presence of proportional bias.

To bridge analytical findings with clinical application, quality goals are incorporated using total allowable error (TEa), sigma metrics, and similar approaches. TEa is used as a benchmark for evaluating whether observed differences are acceptable within a clinical context, while sigma metrics provide a framework for integrating bias and imprecision into overall method performance. Example comparisons are used to demonstrate how these tools inform decisions regarding method interchangeability and clinical impact.

Common pitfalls in method comparison are emphasized, including inadequate sample selection, failure to span clinically relevant ranges, inappropriate use of replicate data, and misinterpretation of outliers. Particular attention is given to the frequent disconnect between statistically detectable differences and clinically meaningful effects.

Case-based scenarios reflecting routine laboratory situations are used to integrate visual interpretation, statistical reasoning, and application of predefined quality goals. The resulting framework supports consistent evaluation of method agreement in a clinically relevant context.

By the end of the session, participants will be equipped to interpret difference plots, recognize key patterns of bias and imprecision, apply TEa and sigma as decision tools, and determine whether methods can be used interchangeably within the context of clinical decision-making.

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Oxidative stress: basics and new concepts

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Jose Ceron

Lecture Content:
This presentation will provide an overview of basic and new concepts related to oxidative stress in veterinary medicine. It will have four main parts:

-Concept of oxidative stress. In this point we will indicate how oxidative stress can be related with the response of the organism to the general stress. In addition the main consequences that a situation of oxidative stress can have to the organism will be discussed.

-Main components of the oxidative stress. We will review the main components of the oxidative reaction and also the main antioxidants that the organism have to counterpart this situation.

-Main causes of the production of oxidative stress in different animal species.

-How to evaluate the oxidative stress by the use of biomarkers of oxidation (1) and antioxidants (2), with emphasis in the importance of a proper analytical validation. In addition the possible use of other sample types different that blood as saliva will be discussed.

References:
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Pitfalls in haematology: preanalytical and analytical errors

31

Catherine Trumel[1]

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Lecture Content:

Title: The hidden hints of in-clinic haematology

In-clinic haematology needs to be defined to highlight and understand the little tricks involved in obtaining reliable results. Most often, haematology at the patient's bedside consists of an analyser giving numerical results and graphs but should also include examination of blood smears. Simple tests such as packed cell volume and slide agglutination tests can also be added.

The first thing is to determine whether the haematological analyser used is reliable for the blood cell population being analysed and therefore to know the methods used by the analyzer. For most good quality feline and canine samples, a properly validated impedance cell counter can provide reliable data for RBCs but cannot quantify reticulocytes. Only two in-clinic analysers use flow cytometry for RBC counting. The LaserCyte was unreliable and no study of the Procyte One has been published. Impedance cell counters are less reliable for WBC counts and differentials. For all platelet quantification methods, platelet aggregation must be absent for the result to be reliable. Impedance methods are unreliable in cats and in the presence of many large platelets. It is therefore important for analysers using impedance variation and cytometry to know which method is used to count platelets.

The second important tip is to check that the machine used is working properly, i.e., that quality checks are regularly carried out and verified.

The third essential point is that the results provided by an analyser are not just numbers, but also graphs, which are just as important as the numbers themselves. By comparing the graphs of sick animals with those of healthy animals, it is possible to detect possible numerical errors and to check the origin of these anomalies on a blood smear.

The fourth trick is to carry out a systematic population-by-population study to produce a list of reliable haematological abnormalities linked to the patient, including: 1/ A critical analysis of results concerning the erythroid population with verification that the MCHC is not above the reference interval, that there are no 'flags', and that the graphs are adequate if available; 2/ a critical analysis of reticulocyte results, checking that the graph is correct and that 'flags' are absent; 3/ a critical analysis of leukocyte results, checking that the graph is correct and 'flags' are absent; and 4/ a critical analysis of platelet results, including a systematic check of the blood smear to exclude the possibility of platelet aggregates in cases of thrombocytopenia.

References:

Scott MA M, Stockham SL. Leukocytes, Erythrocytes, Platelets in Fundamentals of Veterinary Clinical Pathology, Third edition. 2025. John Wiley & Sons, Inc. pp 61-318.

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Medical Device Pathology: Where Innovation Meets Host Response

42

Elie Dagher[1]

[1] NAMSA, Chasse-sur-Rhône, France

Lecture Content:
Medical Device Pathology: Where Innovation Meets Host Response provides a comprehensive and practice-oriented introduction to the unique challenges of evaluating medical devices.

This one-hour session will guide pathology residents through the complete workflow of device-tissue assessment. It begins with study design considerations that influence downstream interpretation, then moves into gross examination, followed by trimming strategies that ensure the most informative device-tissue interfaces are captured for consistent, high-quality evaluation. The presentation will then cover key histological processing methods, ranging from routine paraffin techniques to advanced resin embedding, outlining when and why each method is preferred. Residents will explore how selecting the appropriate embedding technique is guided by factors such as device composition, anatomical location, and the complexity of the tissue-device interface.

To bring these principles to life, the presentation will feature a diverse set of examples, including cardiovascular implants, ocular devices, wound healing devices, hernia repair meshes, etc. These cases will illustrate how device design, material composition shape local and systemic tissue effects, safety and biocompatibility.

By integrating practical methodology with applied case studies, this presentation equips trainees with a foundational understanding of how pathology supports the development, evaluation, and biocompatibility of medical devices.

Common reasons pathology manuscripts are rejected and how to avoid them
9

Mark Dagleish[1]

[1] Journal of Comparative Pathology, London, United Kingdom

Lecture Content:
Many manuscripts are rejected at the desk stage due to avoidable mistakes rendering them unable to be sent for review. Furthermore, other common mistakes within the content of the manuscript leave reviewers no option other than to reject the manuscript. This workshop will highlight and discuss avoidable common mistakes encountered by editors and reviewers specifically handling pathology manuscripts to help authors prior to submission in negotiating the whole process of publishing. The format will be more discussion of specific mistakes to ensure everyone has the chance to benefit from the workshop.

A few classic lesions that may help you with board exams

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Uzal Francisco1

1 California Animal Health and Food Safety Laboratory, San Bernardino, United States

Lecture Content:
During this session we will discuss gross and microscopic lesions of large animals including horses, ruminants, pigs, camelids and poultry. We will provide multiple examples of infectious, metabolic, toxic and neoplastic diseases affecting all system of the body. Most cases will be presented in the form of gross and histology images and multiple choice questions. The session will be highly interactive with cases open for discussion and questions to and from participants. Special emphasis will be put on recently published information in textbooks and scientific journals that may be expected to be included in different anatomic pathology board exams (ECVP and ACVP).

THURSDAY 17 SEPTEMBER 2026

Plenary Session - Liquid Biopsy: From Discovery to Clinical Application – A Revolution in Motion | Catherine Alix-Panabières (France)

No abstracts has been provided

Notes:

Canine and Feline Bone Marrow Histopathology for Anatomic and Clinical Pathologists

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Rose Raskin[1]

[1] Purdue University, West Lafayette, United States

Lecture Content:
The first presentation will involve a review of bone marrow histopathology that covers aspects of cortical bone and its development, trabecular changes, stromal microenvironment, vasculature, and hematopoietic and nonhematopoietic cellular disturbances. Discussion will also include methods to improve collection, processing, and staining of canine and feline core biopsy specimens. A template is provided to assist in composing descriptions of all pertinent aspects necessary to make an interpretation.

Step-By-Step Canine and Feline Bone Marrow Histopathology Using Case Examples

22

Rose Raskin[1]

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Lecture Content:

References:
The second presentation will involve 3-4 cases reviewed in a step-by-step manner following the template form introduced in the first lecture. Hemogram data is added to complement case interpretation. Participants will be able ask questions related to the morphologic changes being discussed. The intent is to make bone marrow core biopsy evaluation less intimidating to the novice as well as provide a structured sequence to follow for experienced pathologists.

Plasma cell neoplasms – An update on entities and diagnosis

28

A Russell Moore[1]

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Lecture Content:
Plasma cell disorders in veterinary medicine comprise a spectrum of malignancies with notable species differences. Multiple myeloma (MM) represents the prototypical plasma cell neoplasm, but myeloma-related disorders also include extramedullary plasmacytomas (cutaneous and non-cutaneous), solitary osseous plasmacytomas (SOPs), and plasma cell leukemia (PCL). Recent advances in understanding tumor biology, biologic behavior and disease progression have warranted updates to these disease entities in human medicine which might be applicable to veterinary disease definitions and diagnostic criteria.

Multiple myeloma is characterized by systemic clonal proliferation of malignant plasma cells and, typically, excessive production of monoclonal proteins (M-proteins), which may occur as intact immunoglobulins or free heavy or light chains. MM accounts for approximately 1% of all canine malignancies and 0.003% to 1% in cats. Clinical consequences include hyperviscosity syndrome, renal insufficiency, and cytopenias due to myelophthisis. Diagnosis has traditionally required at least two of four criteria: bone marrow plasmacytosis, osteolytic lesions, monoclonal gammopathy, and Bence-Jones proteinuria. Biologic behavior varies by species. Canine MM is typically marrow-centric and frequently associated with osteolytic lesions, whereas feline disease often demonstrates visceral involvement (e.g., spleen, liver) with minimal marrow disease.

In human medicine, MM is defined by a plasma cell neoplasm plus ≥1 myeloma-defining event, summarized by the CRAB criteria (hypercalcemia, renal insufficiency, anemia, bone lesions), other imaging-defined mass lesions, and increased clonal immunoglobulin production. Adaptation of some of these criteria to veterinary patients is limited by assay availability but provides a useful conceptual framework. Despite initial responsiveness to chemotherapy, MM is generally fatal, with median survival times of 1.5–2.5 years in dogs and 4–13 months in cats and this tends to hold true when the human diagnostic criteria are used. Pre-malignant states such as monoclonal gammopathy of undetermined significance and smoldering myeloma are recognized in humans and are increasingly suspected in veterinary species.

Extramedullary plasmacytomas (EMPs) are common in dogs, representing approximately 2.5% of all tumors. They most often affect middle-aged to older dogs, with known breed predispositions (e.g., Cocker Spaniels, Golden Retrievers, West Highland White Terriers). Most canine EMPs arise in the skin (~86%) or oral cavity (~9%) and are biologically benign, with surgical excision typically curative. However, non-cutaneous forms (e.g., gastrointestinal, spinal) may be more invasive. A rare multicentric presentation, cutaneous plasmacytosis, involves numerous lesions and often requires systemic therapy.

Feline EMPs differ substantially, often exhibiting more aggressive and systemic behavior and falling within the broader category of myeloma-related disorders with some currently considering these to be the multiple myeloma of cats. Distinct presentations include periarticular plasma cell tumors (commonly involving the tarsus and associated with amyloid deposition) and injection-site plasmacytomas linked to chronic inflammation. Owing to frequent visceral involvement, prognosis is more guarded in cats, and diagnosis often requires immunophenotyping or other testing to distinguish these tumors from other round cell neoplasms.

Solitary osseous plasmacytomas are rare, localized tumors consisting of clonal plasma cells confined to a single bone. They most often occur in vertebrae, pelvis, or long bones of older animals and present with pain, lameness, or neurologic deficits. Diagnosis requires imaging to confirm a solitary lesion, histopathology, and bone marrow evaluation demonstrating <10% plasma cells. Although M-proteins may be present, SOPs lack other myeloma-defining features. Historically considered a precursor to MM, recent studies suggest a more variable course: while ~40% of cases develop additional lesions, only ~15% progress to disseminated MM. Many dogs achieve prolonged survival (median >900 days) with local therapy.

Plasma cell leukemia is an aggressive disorder defined by circulating neoplastic plasma cells in peripheral blood. While older veterinary criteria required >20% circulating plasma cells, updated human guidelines use a threshold of >5%. PCL may occur as a primary disease or as a terminal phase of MM or other plasma cell neoplasms. Regardless of origin, it is associated with rapid systemic progression and poor prognosis.

FIP epizootic

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Alexandra Malbon[1], Charalampos Attipa[1, 2]
[1] The Royal (Dick) School of Veterinary Studies and the Roslin Institute, Edinburgh, United Kingdom
[2] Centre for Inflammation Research/Institute for Regeneration and Repair, Edinburgh, United Kingdom

Lecture Content:
Feline infectious peritonitis (FIP) needs little introduction. Caused by feline coronavirus (FCoV), an alphacoronavirus of the family Coronaviridae, it affects domestic and wild, small and large felid species. Despite the ubiquity of coronaviruses across species, the presentation in felines is unique (excluding a similar disease in ferrets), as is its exceptional mortality rate, at least prior to the recent generation of anti-virals.

Within FCoV there are two biotypes; a widespread largely subclinical enteric form, sometimes referred to as FECV, and a pathogenic form, FIPV which arises almost exclusively as a result of viral mutations occurring within FECV infected individuals, affecting ~5% of cases. Although FECV spreads easily by predominantly faeco-oral transmission, the mutation to a pathogenic form is almost always associated with loss of faecal shedding. This peculiarity has, in the main, prevented the disease causing devastating widespread outbreaks.

There are also two serotypes, FCoV-1 and -2, with seemingly indistinguishable presentations. FCoV-1 is thought to have arisen from a common ancestor to canine coronavirus-1 (CCoV-1) and further recombinations have been identified throughout the virus' history.

At the start of 2023, a wave of cases in Cyprus was detected, from a baseline of a few laboratory-confirmed cases per year to a peak thought to exceed 10,000. This was due to the emergence of a recombinant feline coronavirus named FCoV-23 (a recombinant between an FCoV-1 strain and an S recombination with a pantropic CCoV-2), an epizootic form with efficient horizontal transmission between cats. This recombinant feline–canine coronavirus spread rapidly throughout Cyprus and was subsequently identified in imported cases in the United Kingdom, highlighting the substantial epidemiological risk posed by coronavirus recombination in companion animals.

The clinical symptoms of FCoV-23 FIP are as for classic FIP (e.g. fever, lethargy, anorexia, weight loss, abdominal distension, neurological signs). The most common clinical form of FCoV-23 FIP during the outbreak was effusive (63.72%;), followed by a noticeable neurological presentation (26.05%) with the remainder presenting as non-neurological non-effusive disease. The haematological and biochemical changes are similar to those of classic FIP, with hyperglobulinemia and hyperbilirubinemia being frequently reported. Protein electrophoresis as well as acute phase protein analysis showed a polyclonal gammopathy with increased a2-globulins, whilst serum amyloid A was the most sensitive compared to alpha-1-acid glycoprotein at initial diagnosis of FCoV-23 FIP, specifically in the neurological and dry forms. Multiplex Luminex

cytokine/chemokine analysis showed an acute hyper-inflammatory status in FCoV-23 FIP with a prominent elevation of interleukin-6 compared to non-FIP sick cats. The latter can also explain why in the cytological analysis of effusion due to FCoV-23 there was a higher cell count (typically exudates) with a predominance of neutrophils, rather than a mixed inflammation often reported in traditional FIP. Gross and histological post mortem findings showed no distinguishable features from other FCoV; predominantly multisystemic pyogranulomatous vasculitis and serositis, but fulminant forms with high viral load appear to be common. Thus far still only monocyte/macrophage infection has been detected beyond the intestine.

Early identification of FIP due to FCoV-23 is critical due to the direct transmission potential of the virus. Overall, the clinicopathological findings are similar to classic FIP, and potentially neurological forms, or effusions with high cell counts of neutrophils can raise the suspicion index for this viral variant. However, given the transmission potential versus the classic form, sequencing and ideally a FCoV-23 specific PCR (work in progress) are needed to definitively differentiate infection with FCoV-23. This is also significant as recent studies highlight that similar FCoV recombinations have also occurred in the USA therefore worldwide vigilance by, as well as close communication between, both clinical and anatomical pathologists is of critical importance.

Expanding parasite risks: New and re-emerging parasitic diseases in European animals

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Ard Nijhof[1]

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Lecture Content:
Europe is witnessing changes in the epidemiology of parasitic and vector-borne diseases affecting companion animals, livestock, wildlife, and humans. Climate change, environmental alterations, globalization, and the increasing movement of animals and arthropod vectors are facilitating the emergence and re-emergence of parasites and vector-borne pathogens, including *Babesia canis*, *Leishmania infantum*, *Angiostrongylus vasorum*, and *Dirofilaria* spp., in regions previously considered non-endemic. Exotic arthropod vectors, including ticks such as *Hyalomma marginatum* and *Hyalomma rufipes* as well as invasive mosquito species, are increasingly being detected across parts of Europe. Their introduction and potential establishment raise concerns about the emergence of novel pathogens and the development of new transmission cycles.

For veterinary pathologists, these evolving disease patterns create important new diagnostic and interpretative challenges. These include the recognition of unfamiliar or changing lesion patterns, co-infections, and the appearance of parasitic or vector-borne diseases in unexpected geographic regions and animal species.

This keynote lecture will provide an overview of current trends in emerging parasitic and vector-borne diseases in Europe, with particular emphasis on vector expansion, and the influence of climate and environmental change on parasite ecology.

Intuition and structured knowledge: two sides of a same coin? Understanding reasoning skills to enhance one's practice and supervision skills

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R.Racha Onaisi[1]

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Lecture Content:

In your daily practice, you may find that, as you gain experience, you can solve professional situations on semi-automatic mode. Sometimes, you get this "gut feeling", which is hard to explain but difficult to ignore: you just know that something is not right. At other times, however, you check hypothesis after hypothesis in an intense analytical process.

All of the above are facets of clinical reasoning, which relies on analytical and non-analytical processes that are activated simultaneously, with varying degrees of activation depending on the situation, in order to take the best action or reach the best conclusion in a specific context.

As supervisors, understanding reasoning is beneficial not only for our own practice, as it can enhance metacognition, but also for helping our students develop and strengthen their reasoning skills.

During this session, we will discuss supervision techniques that help students develop and structure the knowledge they use in professional situations.

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Uncertainty: taming it within decision-making processes

47

R.Racha Onaisi[1]

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Lecture Content:

Uncertainty is a constant presence in our practice as professionals. It can have various sources and manifest as various issues that we must learn to deal with when making decisions.

In this era of AI development, it can be tempting to see these tools as our best chance of reducing uncertainty. After all, isn't AI better than us at retaining information? At recognising images? At estimating probabilities?

As tempting as it may seem, a tool is only a tool. Without the proper processes to use and master such tools, there are risks.

Conversely, consolidating decision-making processes and the reasoning skills underlying them is more important than ever in order to deal appropriately with the ubiquitous uncertainty of our professions.

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Emerging Viral Diseases in Livestock in Europe - Drivers, Examples, and Future Risks

7

Angele Breithaupt[1]

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Lecture Content:
Over the past two decades, Europe has experienced the emergence and re-emergence of several viral diseases affecting livestock populations. These developments challenge the traditional perception of Europe as a region with high animal health standards and effective disease control systems.

Emerging diseases, as defined by the World Organisation for Animal Health, include infections arising from pathogen evolution, geographic spread, host shifts, or newly recognized disease events. In the European context, disease emergence is not a random process but is driven by a combination of ecological and anthropogenic factors.

This presentation will highlight three major drivers of viral disease emergence: climate change, globalization of livestock production, and wildlife–livestock interfaces. Vector-borne diseases illustrate the impact of climate-driven expansion of arthropod vectors, while transboundary diseases emphasize the role of animal movement, trade, and biosecurity. Particular attention will be given to the role of wildlife reservoirs, which contribute to the maintenance and spread of viral pathogens and add substantial complexity to disease control efforts at the wildlife–livestock interface. In addition, the presentation will address viral threats that are not yet established in Europe but represent a credible risk for future introduction, reflecting the continent’s increasing vulnerability.

Together, these aspects demonstrate that emerging viral diseases in European livestock are shaped by dynamic interactions between environmental change, human activity, and ecological systems. Given the dynamic nature of this field, the presentation will incorporate the most recent developments — and it may well be that the viruses discussed are not entirely the ones currently anticipated…

Lumpy skin disease: recent advances in our knowledge of this emerging high-consequence poxvirus disease of cattle

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Pip Beard[1]

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Lecture Content:
Lumpy skin disease virus (LSDV) is a poxvirus that causes severe disease in cattle. Over the past ten years this virus has spread from Africa and the Middle East into Europe and across Asia, resulting in substantial loss to cattle industries in these regions (Islam et al., 2026). This seminar will cover the key virological, immunological, epidemiological and pathological features of lumpy skin disease including comparisons with other poxviral diseases. It will also discuss recent progress in our understanding of LSDV and how to control and prevent it.

Lumpy skin disease virus is a member of the capripoxvirus genus, which includes two other species – sheeppox virus and goatpox virus. The three virus species are closely related genetically, and all cause severe disease in ruminants, however their host range is quite different. Sheeppox virus (SPPV) and goatpox virus (GTPV) cause disease in sheep and goats, while lumpy skin disease virus (LSDV) causes disease in cattle, water buffalo and yak. We don't know why such genetically similar viruses have such different host ranges. The gross and histological pathology of all three species is similar, characterised by multifocal dermatitis with necrotising fibrinoid vasculitis and intracytoplasmic inclusion bodies. The very characteristic skin lesions are often accompanied by necrotic lesions in the respiratory and gastrointestinal tract (Embury-Hyatt et al., 2012, Sanz-Bernardo et al., 2020, Gulbahar et al., 2006, Hu et al., 2025). The morbidity and mortality of LSD can vary in different circumstances, usually around 10% of animals develop skin lesions and 1% die. In contrast, the morbidity and mortality in outbreaks of sheeppox and goatpox are usually higher.

The rapid geographic spread of LSDV over the past ten years has been enabled by very efficient vector-borne spread of the virus. LSDV is mechanically transmitted by multiple different vector species including flies and mosquitoes. Recent research into the mechanism of vector-borne transmission of LSDV has provided information about the different roles played by different vector species in the transmission of LSDV.

We have learnt a lot more about LSDV in the past ten years as it has spread into new regions. Research efforts into this previously neglected pathogen have also dramatically increased in response to its expanding impact. The implementation of this new knowledge into effective and proportionate integrated control programmes is a major challenge for veterinary authorities and cattle industries worldwide.

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No TEa? No Problem: Controlling Total Error Anyway

12

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Lecture Content:
Accurate, precise, and clinically reliable laboratory results are fundamental to safe and effective medical decision-making. Even small analytical deviations may therefore affect whether patients are classified as normal or abnormal, stable or progressive, or responsive or non-responsive to treatment.

Total allowable error (TE_a) defines the maximum analytical error that can be tolerated without compromising clinical interpretation. It reflects the combined contribution of random error (imprecision) and systematic error (bias). Together, these components determine the potential difference between a reported laboratory result and the patient's true analyte concentration.

TE_a may originate from different sources. Data on biological variation (BV) can be used to define TE_a for a given analyte; however, these quality goals may be overly stringent for some measurands. In clinical practice, laboratory results are often interpreted at defined decision thresholds. Accordingly, the allowable analytical error should be set with these limits in mind. Alternatively, quality goals can be derived from clinical outcome considerations or from state-of-the-art performance of currently used analyzers to ensure that targets are realistic and achievable. All three concepts are useful when expert-opinion TE_a targets are defined. They have been published for some parameters, but not for all species.

TE_a shall not be confused with the **total observed error (TE_{obs})** of an analyte, which reflects the actual performance of an assay. During method validation studies, TE_{obs} is assessed against TE_a. An assay passes validation if TE_{obs} is $<TE_a$.

TE_a targets are also an important component of quality-control (QC) validation. QC procedures are routinely implemented in clinical laboratories to monitor analytical stability and detect shifts in control results. Formal QC validation—the structured evaluation of whether selected QC rules can reliably detect medically significant deviations—is a final step in the implementation of a new laboratory test. Core components include the TE_a, measures of imprecision and inaccuracy, as well as the probability of error detection (P_{ed}) and false rejection (P_{fr}).

As noted above, in veterinary medicine, TE_a are not universally available and may vary depending on the source. This lack of harmonized TE_a targets complicates QC planning, assay validation, and the assessment of whether analytical quality is sufficient for the intended medical use.

What can we do when no consensus TE_a target exists?

In settings where TE_a is absent, a **reverse approach** can be applied. This strategy starts with observed assay-performance characteristics—specifically, the measured coefficient of variation (CV) and bias—together with the desired P_{ed}, the control rule applied, and the number of control materials run. This approach determines the smallest magnitude of total error that the chosen QC procedure can reliably detect with the desired P_{ed}. This value represents the lowest total

error that can be controlled in routine practice and therefore defines the system's operational analytical capability.

The "grey zone" describes the range of results around a clinical decision threshold in which analytical uncertainty may prevent confident classification of a patient as normal or abnormal. Both imprecision and bias influence this zone, increasing the likelihood of misclassification near cut-offs. Concepts that can help delineate the "grey zone" around laboratory results include **expanded measurement uncertainty (EMU)** and dispersion.

EMU is calculated as $2xCV_A$. It reflects assay imprecision and is therefore expected to be smaller than the lowest total error that can be controlled, as it does not incorporate inaccuracy. EMU can be used to evaluate an unexpected laboratory result: if a sample is repeated and the result falls within the EMU, the results can be considered equivalent. Accurate estimation of CV_A is essential, ideally based on precision studies using patient samples or, as a second choice, calculated from historical QC results obtained during a period of stable performance.

To better characterize the "grey zone" around a clinical decision limit or reference limit, **dispersion** can be calculated. Dispersion requires information on biological variation because it is derived from the within-individual coefficient of variation (CV_I). It also incorporates analytical imprecision in its calculation and is therefore wider than the EMU.

Finally, **bias** is particularly concerning when considering the "grey zone", because it can systematically shift results in one direction, potentially altering disease-prevalence estimates or influencing therapeutic decisions across entire patient populations. Assessing and monitoring bias over time requires robust QC validation of an assay. In addition, preventive measures—such as adhering to QC material stability timelines and evaluating lot-to-lot variation of QC materials—are important to avoid and control bias.

References:

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relationships with total error quality goals and bias in the interpretation of laboratory results. Vet Clin Pathol. 2024;00:1-10.

Making Sense of the 2-Phase Examination System: What is Tested, Why, and How to Approach Each Phase.

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Lecture Content:
The ECVCP Board Examination comprises four parts: General Clinical Pathology (GCP), Haematology, Clinical Biochemistry and Cytology. Traditionally, all sections were tested together following completion of the Residency. In 2024, the Examination was split into two phases: *Phase 1*, run each spring and eligible to any Resident who has completed at least 12 months of their Residency Programme; and *Phase 2,* tested each autumn and eligible to those who have completed their Residency and fulfilled other criteria as outlined in the ECVCP Information Booklet. This talk is open to all: Residents, Supervisors, Educators, and anyone with an interest in postgraduate veterinary medical education. It will be an interactive session designed to:

- explain the ethos behind the two-phase testing
- highlight pertinent information in the available resources
- give examples of how topics are assigned to Phase 1 or Phase 2
- advise how to tackle the core texts in the light of the two phases
- give top-tips for preparing for each section
- advise re current best practice regarding learning and revision techniques
- provide an informal forum for Residents and Supervisors to ask questions.

We hope this session will be of value to all attendees, whether your interest lies in the Examination itself, in supporting Residents through their training, or in postgraduate veterinary education more broadly.

References:
ECVCP Information Brochure, Section 5.3

https://www.ecvcp.org/sites/www.ecvdi.org/files/medias/documents/ECVCP/ECVCP%20Information%20Brochure%20March%202024.pdf

ECVCP 2026 Reading List

https://www.ecvcp.org/sites/www.ecvdi.org/files/medias/documents/ECVCP/2026%20ECVCP%20Board%20Examination%20Reading%20List.pdf

FRIDAY 18 SEPTEMBER 2026

Neglect in swine welfare

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Henrik Elvang Jensen[1]

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Lecture Content:
In forensic veterinary pathology neglect investigations identify failures by caregivers to provide basic needs, e.g. food and health care, for vulnerable animals causing harm or death. These cases are critical issues investigated through post-mortem examinations to establish criminal liability. To carry out investigations of neglect, knowledge of diseases and their progression is essential. Especially, assessing the age of neglect associated lesions is often crucial, as it may be used in judicial proceedings to determine how long an animal has suffered and thereby assign responsibility. Various forms of suffering due to neglect are observed world-wide in swine production systems. In the presentation, wounds, lameness, undernutrition and herniation, which often raise concerns about neglect in swine welfare, will be covered.

Forensic boundaries between infectious disease, trauma and neglect - Challenges in the interpretation of ruminant cases

16

Frauke Seehusen[1]

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Lecture Content:
Neglect in domestic ruminants represents a multifactorial welfare concern situated at the intersection of veterinary pathology, forensic investigation, and herd management. In forensic veterinary medicine, differentiating between naturally occurring disease processes, inadequate husbandry, and deliberate human-inflicted injury remains a considerable diagnostic challenge. This presentation addresses forensic investigations of neglect cases in cattle and small ruminants submitted for pathological examination, with emphasis on disorders of the musculoskeletal system, including inadequate claw care, chronic infectious disease, and deficient management practices. Particular attention is given to cases characterized by cachexia, poor body condition, chronic inflammatory processes, and prolonged suffering associated with delayed or absent veterinary intervention. Some of the documented infectious conditions possessed zoonotic relevance, thereby extending the significance of these cases beyond animal welfare concerns to issues of public health and biosecurity. The forensic evaluation of such cases requires integration of pathological findings with clinical history, husbandry assessment, and laboratory diagnostics to determine whether the disease course was compatible with acceptable standards of animal care or indicative of prolonged neglect. The presented cases illustrate the complexity of forensic assessments in food-producing animals, where pathological findings frequently arise from an interplay of environmental, infectious, nutritional, and management-related factors.

Neglect in compagnion animals

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Leen Van Brantegem[1]

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Lecture Content:

Neglect is a complex and multifactorial issue frequently encountered in companion animal welfare and forensic veterinary pathology. Current challenges in the recognition, assessment, and interpretation of neglect are discussed, with particular attention to the role of the veterinary pathologist. Practical examples illustrate key concepts and highlight the importance of integrating pathological findings with the clinical history and circumstantial evidence.

Comparative aging across species through the lens of DNA methylation and biochemical outcomes

37

Baptiste Sadoughi[1]

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Lecture Content:
Aging is a multi-layered biological process that unfolds from molecular changes within cells to physiological decline in organisms and, ultimately, to the remarkable diversity of aging patterns observed across species. A central challenge in aging biology is to understand how these different levels are connected and how environmental and evolutionary forces shape variation in aging trajectories. This talk will present a comparative perspective on aging that integrates DNA methylation, physiological biomarkers, social environments, and evolutionary comparisons. At the molecular and physiological levels, a fundamental question is how changes captured by DNA methylation relate to functional changes in the organism. While epigenetic biomarkers have transformed the study of biological aging, their interpretation depends on understanding how they align with physiological trajectories. Integrating molecular and biochemical measures provides a more complete picture of biological aging and reveals that different systems within the same individual can age differently. Beyond intrinsic biological processes, aging is also shaped by the environments in which individuals develop and live. A long-term study of free-ranging rhesus macaques provide a unique opportunity to investigate how social experiences, particularly adversity early in life, become biologically embedded and contribute to differences in aging trajectories among individuals. Across these scales lies a common challenge: aging is rarely linear. Instead, biological change may accelerate or decelerate, creating critical windows during which aging trajectories diverge and environmental influences exert their strongest effects. Identifying these non-linear dynamics is therefore essential for understanding both the mechanisms and timing of biological aging. Stepping back from variation among individuals to variation among species raises the broader question of how aging itself evolves. Comparisons of DNA methylation aging across closely related macaque species, which display socio-ecological diversity despite their shared evolutionary history, provide a powerful framework for investigating how ecology and social systems have shaped the evolution of aging. Together, these themes illustrate how integrating molecular, physiological, social, and evolutionary perspectives can provide a more unified understanding of the biology of aging.

Equine endocrine disorders across the lifespan: pathophysiology, age-related evolution, and diagnostic challenges

19

Andrew Durham[1]

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Lecture Content:

Although a broad array of rare endocrine diseases are recognised in horses analogous to those in other species, equine endocrinopathic disease is largely limited to only 2 specific conditions: Pituitary *Pars Intermedia* Dysfunction (PPID) and Equine Metabolic Syndrome (EMS).

Equine Metabolic Syndrome is defined as a collection of risk factors for laminitis associated with insulin dysregulation. Additional common but inconsistent features include typical breeds and types (reflecting genetic risk), obesity, lack of aerobic fitness and dysfunction of adipose tissue reflected by abnormal adipokine concentrations. Laminitis, which is the most concerning consequence of EMS, is unusual before the age of 4 years. Although no studies have investigated time of onset of insulin dysregulation, this is suspected to develop at a similar time after juvenile growth has ceased. There is no upper age limit to development of EMS.

The key effect of insulin dysregulation is excessive post-prandial hyperinsulinaemia which leads to laminar damage via disruption of growth and differentiation via Insulin-like growth factor 1 (IGF-1) receptors on laminar epithelial cells. Diagnosis of EMS requires demonstration of insulin dysregulation. Although several options exist, the most practical test used in practice is measuring insulin response to a standardised oral sugar challenge such as measuring serum insulin between 60-90 minutes following 0.45 mL/kg Karo Lite Corn Syrup. Additionally, hypoadiponectinaemia may be used to support the condition.

Traditional approaches to managing EMS included encouraging weight loss and increased fitness via dietary restriction and exercise although poor compliance was common and success, if achieved, took months to years. Additionally, it was not uncommon in cases where weight loss and increased fitness were achieved, to find that insulin dysregulation was not improved, presumably due to strong genetic factors causing insulin dysregulation.

Instead of long-term strategies targeting obesity and lack of fitness, more logical and successful approaches to managing EMS involve short-term targeting of post-prandial hyperinsulinaemia. Post-prandial testing allows identification of which dietary element(s) is responsible for the excessive hyperinsulinaemia causing laminitis, which can then be changed to have an immediate impact in reducing the insulin response. Where dietary control is impractical then SGLT2 inhibitor drugs may also be used to reduce post-prandial insulin with immediate effects.

Pituitary *Pars Intermedia* Dysfunction arises due to oxidative damage to dopaminergic neurones which normally inhibit the *pars intermedia*. Loss of this inhibitory drive leads to hyperplasia, hypertrophy and micro- and macro-

adenoma formation along with hypersecretion of *pars intermedia* peptides. PPID is uncommon under 15 years of age and rare under 10 years, presumably due to prolonged oxidative insult being required to cause significant neuronal damage. Many clinical signs have been associated with PPID although haircoat changes including delayed or absent summer shedding and hypertrichosis characterised by increased anagen hairs are the only signs firmly associated with PPID. Other signs may include laminitis, excessive sweating, anhidrosis, polydipsia and muscle loss.

Diagnosis generally relies on measurement of basal ACTH concentration in chilled EDTA plasma. Importantly, serum cortisol is normal in PPID cases suggesting that the measured ACTH is not normally bioactive. This might be partially explained by immunoassays cross reacting with peptides other than ACTH (eg. Siemens Immulite 2000XPi ACTH assay also detects 29% of corticotropin-like intermediate lobe peptide (CLIP)) and/or other ACTH inhibitors present in plasma.

Basal ACTH is a highly specific but only moderately sensitive test. Alternatively, ACTH can be measured 10 or 30 minutes after exogenous Thyrotropin Releasing Hormone (a stimulator of *pars intermedia*) which increases test sensitivity. Other *pars intermedia* peptides including alpha-melanocyte stimulating hormone, beta-endorphin and CLIP have been subject to some diagnostic studies although none has yet conclusively outperformed ACTH.

ACTH is very susceptible to degradation post-sampling and must be chilled and analysed promptly to help stabilise the measurable concentration. Several further factors have been shown to affect basal (and post-TRH) ACTH concentrations and must be considered when testing including time of year (peak late September), stress/anxiety, sedation, and breed with more minor effects of geographic location, diet, sex and colour.

Treatment of PPID with dopaminergic agonist drugs (primarily pergolide mesylate) appears effective in the majority of cases in improving clinical signs and decreasing plasma ACTH.

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VCGP as a community project: a collaborative model for standardized veterinary oncology reporting

4

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Lecture Content:
The Veterinary Cancer Guidelines and Protocols (vcgp.org) initiative is a global, community-driven effort to improve cancer care in animals by standardizing tumor evaluation and reporting across institutions and disciplines.

Through expert-reviewed, published literature, continuously updated "living" guidelines and protocols, VCGP aims to establish uniform methods recommended for assessing key diagnostic parameters, such as mitotic count, margin evaluation, lymphovascular invasion, and tumor necrosis, thereby enabling reproducible data collection and robust validation of grading systems. Its interdisciplinary contributors, spanning anatomic and clinical pathology, oncology, surgery, epidemiology, and statistics, have produced influential documents such as guidelines for developing, reporting, and validating histologic tumor grading systems, with a complementary cytologic grading guideline in development.

We will review a selection of VCGP documents, open them for audience feedback, and provide updates on current and upcoming projects (such as standardization of tumor nomenclature and coding). There will be opportunities for colleagues to get involved, so consider this **a call to action**. We particularly encourage trainees in pathology to participate. Contributing to a VCGP document is not only professionally valuable but may also be rewarded with financial support to attend an international conference and present your oncology research.

Protocols are **tumor- and species-specific** tools designed to identify and collect all required data elements for evaluating a particular neoplasm in a given species, while **guidelines** are primarily for methods and serve as expanded "best practices" that provide literature reviews, highlighting methodological weaknesses or conflicting approaches, and outlining future research needs to improve diagnostic methods. Both guidelines and protocols rely on expert review of the literature and aim to enhance **consistency and reproducibility** in tumor evaluation. **Quick Reference Guides (QRGs)** complement these resources by distilling each guideline or protocol into concise checklists that capture key points and essential references for rapid, practical use. There also are ***pathology quick reference guides*** which identify the core (essential) features that have been correlated to outcomes and non-core features that are optional to report. These documents provide the foundation for developing synoptic reports that are tumor and species specific. Examples of each will be presented and discussed.

By providing standardized synoptic reports, image libraries, and tumor-specific guidelines and protocols, VCGP strengthens diagnostic consistency, fosters collaborative research, and supports pathologists through clear definitions of

histological features ultimately enhancing prognostication, comparability of studies, and patient care in veterinary oncology.

References:
https://vcgp.org/

Liver diseases: when cytology is sufficient, when histology is necessary.

25

Carlo Masserdotti[1]

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Lecture Content:
Cytological evaluation of the liver is a valuable diagnostic tool in veterinary medicine, providing a minimally invasive and relatively safe method for assessing hepatic diseases. Techniques such as fine-needle aspiration (FNA) and fine-needle capillary sampling (FNCS) allow collection of cellular material for microscopic examination. FNCS is increasingly favored because it reduces blood contamination by relying on capillary action, an advantage when sampling highly vascular organs such as the liver. Nevertheless, diagnostic accuracy varies depending on lesion type, sampling quality, and integration with clinical data.

Sampling error represents a major limitation. Collected material may not reflect the primary lesion, particularly in focal or heterogeneous diseases. For instance, in biliary disorders, sampling near portal regions may yield hepatocytes with nonspecific reactive changes rather than diagnostic findings. Additionally, cytology does not provide information on tissue architecture, which is essential for diagnosing conditions such as vascular abnormalities, ductal plate malformations, and fibrosis. These require histopathological evaluation.

Another limitation is the overlap of cytological features among different hepatobiliary diseases, which reduces diagnostic specificity. Inflammatory conditions often show nonspecific changes, making classification difficult. Consequently, cytological findings must be interpreted alongside clinical history, physical examination, laboratory data, and imaging studies. In many cases, histology is required for definitive diagnosis.

Cytological findings can be grouped into three categories: definitive diagnoses, suggestive findings, and nonspecific changes.

Cytology alone can be diagnostic in certain conditions, such as septic suppurative hepatitis and cholecystitis, characterized by degenerate neutrophils and intracellular bacteria. Hepatic amyloidosis may also be identified based on extracellular eosinophilic deposits. Cytology is often informative for neoplasia, particularly lymphoma, histiocytic sarcoma, and metastases. However, distinguishing hepatocellular hyperplasia, adenoma, and well-differentiated carcinoma remains difficult due to their similar morphology.

In many cases, **cytology provides useful but limited information**. Cholestasis is readily recognized by bile pigment, but its underlying cause cannot be determined. Fibrosis may be suspected based on spindle cells and collagen, although staging requires histology. Metabolic and degenerative changes are common: glycogen accumulation produces enlarged, pale hepatocytes, while lipid accumulation leads to cytoplasmic vacuolation. These alterations may be associated with metabolic or endocrine disorders or toxic injury. Copper accumulation may occasionally be observed but requires confirmation. Clusters of

cholangiocytes with inflammatory cells may suggest chronic biliary disease, although definitive classification depends on histopathological assessment.

In other cases, **cytologic findings are nonspecific**. Mild or chronic hepatitis may show limited inflammatory infiltrates, often indistinguishable from blood contamination. Acute cholangitis may be missed due to scarce exfoliation of biliary epithelial cells. Vascular abnormalities and developmental disorders cannot be reliably diagnosed cytologically and require imaging and histology.

Overall, liver cytology should be considered part of a multimodal diagnostic approach. It provides rapid and valuable information, helps refine differential diagnoses, and guides further investigation. However, due to its inherent limitations, histopathology remains essential for achieving a definitive diagnosis when required.

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Clostridial diseases of animals: pathogenesis and diagnosis

40

Uzal Francisco[1], Uzal Francisco[2], Horst Posthaus[3]

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[3] University of Bern, Bern, Switzerland

Lecture Content:
In this presentation we will discuss the main features of the most important enteric, histotoxic and neurotoxic clostridial diseases of animals. Enteric diseases will include enterotoxemias and enteritis produced by Clostridium perfringens, Clostridioides difficile, Paraclostridium sordellii, Clostridium piliforme, Clostridium colinum and Thomasclavelia spiroformi. Histotoxic diseases will include black leg (Clostridium chauvoei), gas gangrene (Clostridium septicum, Clostridium chauvoei, Paraclostridium sordellii, Clostridium perfringens and Clostridium novyi) , bacillary hemoglobinuria (Clostridium haemolyticum), infectious necrotic hepatitis (Clostridium novyi type B) and Tyzzer disease (Clostridium piliforme). Neurotoxic diseases will include tetanus (Clostridium tetani) and botulism (Clostridium botulinum). Some of these diseases will be discussed in more detail including etiology, pathogenesis, clinical signs, gross and microscopic lesions and diagnosis, with special emphasis in diagnostic criteria. This will be an interactive session with discussions between the speakers and audience.

Establishment of the ESVCP Sustainability Working Group and Future Perspectives

Nazaré Pinto da Cunha 1,2,3 , Saverio Paltrinieri 4, Katarina Novaković 5

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5 Smartlab Veterinary Laboratory, Belgrade, Serbia

The European Society of Veterinary Clinical Pathology (ESVCP) Sustainability Working Group was established during the ECVCP/ESVCP Congress in Budapest in 2024, in response to the growing need to integrate sustainability principles into veterinary clinical diagnostic laboratories, and with the aim of reducing environmental impact while maintaining high diagnostic standards. Membership is open to all ESVCP members, with recruitment targeting colleagues at all career stages, from residents to experienced practitioners across academic, private, and industry settings, as well as biomedical scientists.

The group's mission is to accelerate the transition toward environmentally responsible veterinary diagnostics by sharing knowledge, inspiring behavioural change, and providing recommendations that support the adoption of sustainable materials, methods, and mindsets. Its vision is to foster laboratories operating with minimal waste, low-toxicity chemistry, and circular resource use, contributing to animal and human health while supporting environmental regeneration.

The initial work focuses on collecting and reviewing relevant literature, existing guidelines, and identifying organizations active in sustainability. Strategic goals include the creation of an open-access knowledge hub, delivery of targeted education and outreach, promotion of safe and circular laboratory practices, and coordinated advocacy toward industry stakeholders for more sustainable products. The group produced a foundational proposal document defining the mission, objectives, and membership structure.

In the next phase, these activities will be translated into consensus-based practical recommendations and dissemination of best practices, raising awareness within the ESVCP community, and driving measurable progress in sustainable veterinary laboratory medicine.

Endocrine flexibility: a new view of glucocorticoids across species. Implications for clinical pathology?

17

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Lecture Content:
Since Seyle introduced the term to physiology, stress has been a major research subject across medicine and veterinary medicine, yet no universally accepted definition exists to this day. Nevertheless, mounting appropriate physiological stress responses when facing stressors is crucial for health, welfare, and fitness. In vertebrates, the hypothalamic-pituitary-adrenal (HPA) axis is the primary system mediating such responses, largely through glucocorticoids — molecules so easily measured that they have become the default metric for quantifying stress from clinical medicine to conservation biology. While differences in glucocorticoid regulation can be associated with differences in health and fitness, how this physiological variation actually causes variation in individual health and fitness remains surprisingly obscure, likely because of the regulatory complexity of the HPA axis. We argue this is because we have long been measuring the wrong thing. Rather than quantifying glucocorticoid concentrations at isolated time points, what matters is the ability of an individual to maintain and recover homeostasis across diverse and unpredictable challenges — what we call endocrine or HPA flexibility, defined as rapid, reversible plasticity in HPA axis function occurring within individuals in response to unpredictable environmental change. The HPA flexibility framework, rather than treating glucocorticoid concentrations as simple proxies for stress, considers glucocorticoids as information-carrying molecules whose functional significance depends on the regulatory range available to an individual — that is, its capacity to mount diverse and contextually appropriate hormonal responses across different stressors. An individual with high HPA flexibility can finely tune its endocrine response to the nature, magnitude, and context of a challenge. An individual with low HPA flexibility is constrained to a narrow repertoire of responses, increasing vulnerability to pathological outcomes under novel or chronic stress. Critically, it is not the magnitude of glucocorticoid elevation that most determines pathological risk, but the failure of negative feedback regulation to resolve it — a distinction with direct relevance to the interpretation of chronic stress lesions in clinical and post-mortem settings. At the molecular level, we suggest that HPA flexibility is substantially regulated by *FKBP5*, the gene encoding FK506 binding protein 51, a co-chaperone in the glucocorticoid receptor (GR) complex . FKBP5 protein exerts potent inhibitory control over GR function via two mechanisms: it reduces GR binding affinity for glucocorticoids and prevents nuclear translocation of the GR complex, together constituting an ultrashort intracellular negative feedback loop. Because *FKBP5* is itself rapidly transcribed in response to GR activation, it acts as a molecular amplifier that calibrates GR sensitivity to prevailing glucocorticoid concentrations. High *FKBP5* expression is therefore associated with GR resistance, impaired negative feedback, and sustained glucocorticoid elevation — the molecular substrate underlying many well-

recognised chronic stress pathologies including lymphoid depletion, adrenocortical hypertrophy, and metabolic dysregulation. Conversely, low *FKBP5* expression is associated with high GR sensitivity, efficient negative feedback, and enhanced stress coping capacity. Importantly, *FKBP5* expression has been validated as an accurate marker of GR signalling and HPA axis regulation in both biomedical and emerging wildlife research contexts. From a practical perspective, quantifying HPA flexibility in clinical or field settings requires multiple repeated hormone measurements across different stressor exposures — logistically demanding in most veterinary contexts. Recent evidence demonstrates that *FKBP5* expression in a single baseline blood sample correlates strongly with both calculated HPA flexibility indices and hypothalamic *FKBP5* expression, raising the prospect of a simple, accessible blood-based biomarker of stress regulatory capacity. Such a marker could transform welfare assessment and pre-mortem diagnostic interpretation by shifting focus from what glucocorticoid concentrations are at a single point in time to what an individual's capacity to regulate those concentrations actually is.

The Journal of Comparative Pathology, a brief history and the benefits of publishing your work here

10

Mark Dagleish[1]

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Lecture Content:
The Journal of Comparative Pathology, established in 1888 by a human doctor who retrained to become a veterinarian, exists to publish papers describing novel pathology and pathogeneses of vertebrate animals, as well as reviews. The Journal is unique in that it is, and always has been, owned and run by veterinary pathologists for veterinary pathology and does not charge to publish under the standard subscription model. This presentation will explain our ethos, ethics and the processes we have in place to ensure your manuscript receives a rigorous, fair and constructive review so you can make an informed decision when deciding which journal to submit your manuscripts to.

Digital Cytopathology Quality Guidelines

29

A Russell Moore[1], Francesco Cian[2]
[1] Colorado State University, Fort Collins, United States
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Lecture Content:
The rapid adoption of digital cytology is transforming veterinary clinical pathology, with significant implications for diagnostic practice, resident training, and the future structure of the profession. While digital platforms offer clear advantages in workflow efficiency, remote accessibility, and case sharing, their integration into routine clinical diagnostics and training programs introduces new challenges that remain incompletely understood and variably addressed across institutions. This session will provide a focused exploration of the current landscape of digital cytology, emphasizing its impact on training, diagnostic quality, and professional practice, while fostering open discussion among participants with diverse experiences in academic and commercial settings.

The session will begin with a brief overview of recently published American Society for Veterinary Clinical Pathology (ASVCP) guidelines, with particular attention to recommendations related to validation, non-inferiority, and residency training. Key considerations include the interpretation of "non-inferiority to glass microscopy" as an acceptable standard for primary diagnosis, and the implications of digital modalities on the development of core diagnostic competencies. Differences between rapid on-site evaluation and comprehensive digital slide review will also be highlighted to contextualize workflow-specific applications of digital cytology.

Perspectives from both academic institutions and high-throughput commercial diagnostic laboratories will be presented to illustrate how digital cytology is currently implemented in practice. These perspectives will address differences in case volume, workflow design, training opportunities, and access to mentorship, all of which influence how residents and pathologists acquire diagnostic skills in a digital environment. Particular attention will be given to the potential benefits of digital systems, including enhanced access to case material and the ability to standardize certain aspects of training, as well as limitations such as reduced exposure to glass microscopy, challenges in teaching search patterns and slide navigation, and variability in scan quality.

A central component of this session will be an interactive, audience-driven discussion designed to capture real-world experiences and concerns. The discussion will examine how digital cytology affects diagnostic accuracy, patient safety, and efficiency. Audience perspectives on quality assurance and accountability in digital workflows, including responsibility for scan-related errors, challenges in standardization, and medicolegal considerations of digital-only diagnoses will be sought. This groundwork will focus on educational impacts, particularly the need for formal training in digital cytology and adaptation of residency programs to preserve core diagnostic skills.

Rather than seeking consensus, this session aims to highlight areas of uncertainty, encourage critical evaluation of current practices, and promote the exchange of observations across practice settings. By integrating guideline review with practical experience and open dialogue, the session will provide attendees with a deeper understanding of both the opportunities and challenges associated with digital cytology in veterinary clinical pathology.

References:

Fish EJ, Hoepp N, Matlow JR, et al. Digital cytopathology quality guidelines. *Vet Clin Pathol*. 2025;54(4):318-337.

ECVP - Train the trainers: Designing mock exams for histology and gross

Giancarlo Avallone

ECVP – Train the trainers: Designing Mock Exams for Histopathology and Gross Pathology

Giancarlo Avallone

The ECVP certifying examination in histopathology and gross pathology tests both pathology knowledge and the ability to describe lesions accurately under considerable time pressure. Effective preparation therefore requires not only knowledge acquisition but also consistent descriptive skills, time management, endurance and familiarity with the examination environment. Mock examinations are an essential training tool because they reproduce these conditions as closely as possible.

Histology mock examinations

The real examination allows approximately 12 minutes per case, with 20 cases completed during one continuous 4.5-hour session. The resulting combination of time pressure, fatigue and stress is fundamentally different from practicing individual cases separately. Mock examinations should therefore reproduce the complete examination environment, including the laptop, microscope, slide sharing, typing and desk arrangement. They should ideally be performed at least annually to assess residents' progress.

Cases should be selected to provide an appropriate balance of species, organs and pathological processes. Each case is worth 20 points: 2 for design, 3 for morphologic diagnosis, 1 for organ identification, 1–2 for an additional question and 12–14 for the description. Scoring should be based on clearly defined keywords and diagnostic hallmarks, with emphasis on features that genuinely support the diagnosis. Different lesion types require specific scoring strategies: neoplasms should address pattern, cellular features and malignancy; inflammatory lesions should emphasize location, recognition and quantification of inflammatory cells; cytology should assess cellularity, quality and background; and TEM should include normal ultrastructural features.

Gross pathology mock examinations

Gross pathology mock examinations should also be performed at least annually and should reproduce the real examination conditions. The examination consists of 60 cases in 2 hours with no opportunity to return to previous cases. Because it follows histopathology, fatigue and concentration are major challenges; therefore, the gross mock exam is ideally scheduled immediately after the histology mock.

Images should be selected primarily for clarity of the lesion and should be reviewed by colleagues before use. Residents should practice all major question types, including morphologic diagnosis, disease name, etiology, etiological diagnosis, pathogenesis, associated lesions, predisposition and histopathological hallmarks. Morphologic diagnosis should be the most common question type, while etiology/etiological diagnosis should be included in approximately half of applicable cases. Each image is worth 3 points, with scoring divided into units of no more than 0.5 points.

Finally, residents should self-score their mock examinations using the official scoring key. This encourages attention to precise keywords usage and careful reading of questions. Supervisors should maintain records of recurrent errors and use this information to refine subsequent mock examinations. Although designing realistic mock exams requires substantial time and effort, the resulting structured approach, descriptive skills and diagnostic discipline provide benefits that extend well beyond success in the ECVP examination

Workshop: Basics for Automated Image Analysis using Machine Learning

45

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Lecture Content:
The rapid advancements in artificial intelligence (AI) are transforming research and diagnostic pathology, offering powerful tools for automated analysis of histological and cytological images. However, many pathologists have limited exposure to AI-driven approaches, creating a need for hands-on training. This workshop provides a gentle introduction to artificial intelligence (AI) for automated analysis of microscopic images. It is intended for pathologists with little prior experience.

This workshop is devided into two parts: Part 1 will provide an introduction into the concepts of machine learning, the relevant pattern recognition tasks for image analysis, and the workflow of algorithm development (including dataset creation, model selection, model training, performance evaluation and model application). The second part will provide a demonstration of model development for two examples (tumor segmentation in histologic images and mitotic figure detection in cytology images) using general purpose pretrained networks and foundation models.

After this workshop, participants will be able to ...
- explain the general concepts of AI
- explain the relevant steps in developing AI models for automated image analysis
- critically judge the performance of an image analysis model

ESVCP Mystery cases in veterinary clinical pathology | Marta Costa (UK), Mariana Serra (UK)

Case 1 - An unexpected guest in the joints of a puppy

An unexpected guest in the joints of a puppy

Contributors

Alexandre Bertin[1], Sébastien Catheland[2], Pierpaolo Romanelli[3], Claudia Scaramella[3], Fanny Granat[1,4], Catherine Trumel[1,5]

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Specimen

EDTA Synovial fluid/ Synovial fluid smear / synovial fluid analysis

Signalment

4-month-old mixed-breed (Griffon x Border Collie) male puppy

History

The puppy was referred for hyperthermia, lameness and joint pain, progressing for the past ten days. Three out of five puppies from the same litter exhibited similar clinical signs. Limited clinical information was available for the other affected puppies, as they were examined by veterinarians at different clinics.

Initial investigations by the referring veterinarian included a complete blood count (CBC), a serum biochemistry profile, and a SNAP 4Dx® test which yielded negative results. The CBC showed mild normocytic normochromic anemia, mild leukopenia due to mild neutropenia, and mild thrombocytopenia. No blood smear was performed to confirm these findings. The biochemistry profile revealed mild hyperglycemia, increased alkaline phosphatase (ALP) activity, mild hypocreatininemia, and elevated C-reactive protein concentration (CRP), compared with reference intervals in adults (Table 1).

The puppy was initially treated with oral doxycyclin (10 mg/kg) and meloxicam (0.1 mg/kg). In the absence of significative clinical improvement, the puppy was then hospitalized for two days, placed on intravenous fluids, and treated with imidocarb. Doxycycline treatment was continued. PCR assays performed on blood samples for *Anaplasma*, *Babesia*, and *Ehrlichia* spp. were all negative. Serological testing for *Anaplasma phagocytophilum* (IgG ELISA), *Borrelia burgdorferi* (ELISA), and *Ehrlichia canis* (ELISA) was also negative. An abdominal ultrasound

examination was performed, with no abnormalities detected. The puppy was then referred for joint taps and further management.

	Result	Reference Interval in adult
Glucose (mmol/L	6.1	4.1 - 7.0
Urea (mmol/L)	4.0	3.2 - 10
Creatinine (µmol/L)	**18.6**	35.4 - 124
Total proteins (g/L)	**77**	50 - 72
ALT (U/L)	18	17 - 78
ALP (U/L)	**121**	15 - 85

Table 1: Results of blood biochemistry (FUJI DRI-CHEM NX500)

Clinical findings

Clinical examination in the referral clinic showed similar abnormalities already described. A computed tomography scan was performed and was suggestive of non-erosive polyarthritis. Synovial fluid examination of knees, shoulders, and elbows was performed (Figure 1,2 and Table 2). Serum capillary zone electrophoresis revealed moderate hypoalbuminemia and hyper-α-globulinemia (Figure 3 and Table 3).

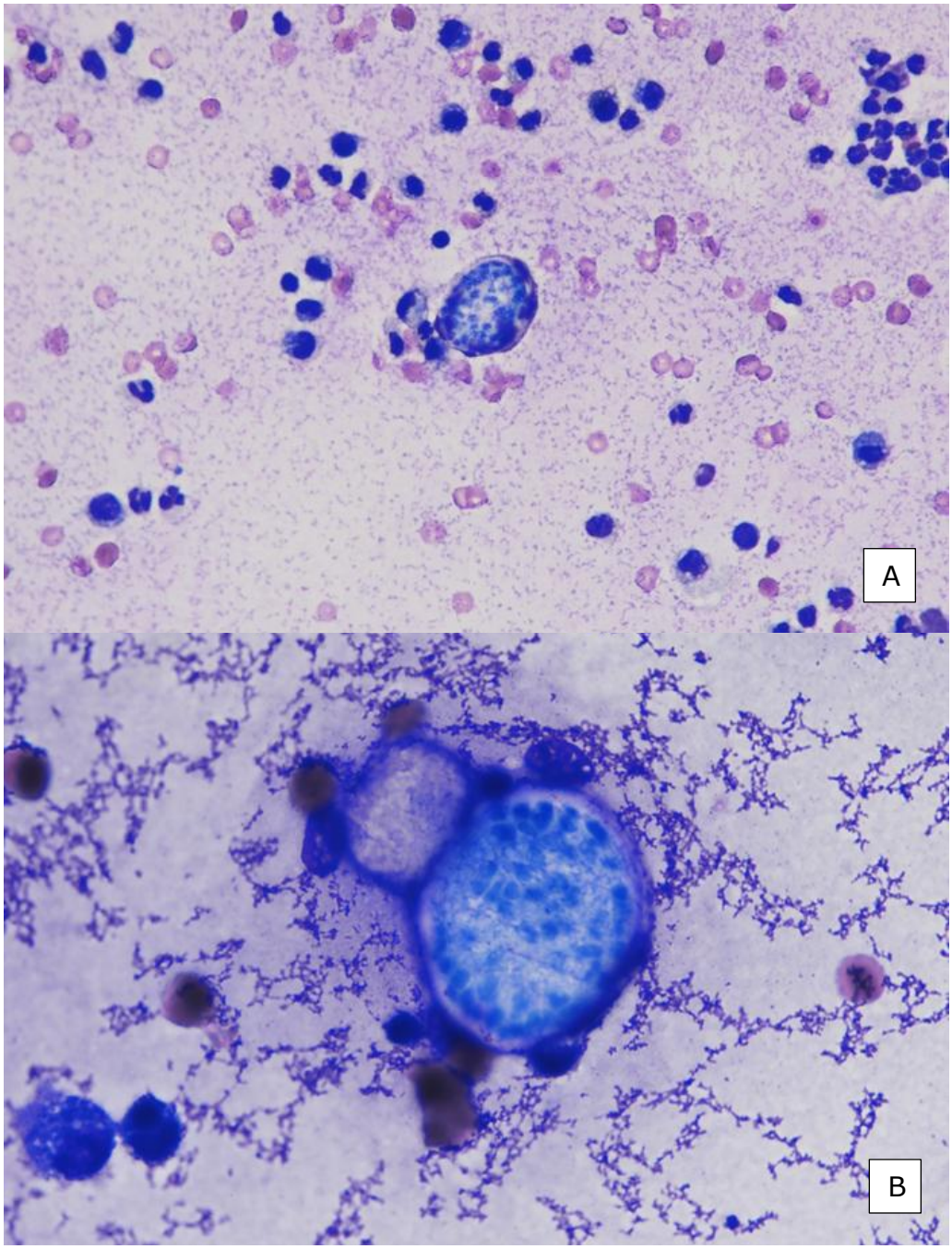

Figure 1 -A, B: Photomicrographs of synovial fluid smears of the puppy, May-Grunwald-Giemsa stain, x500 (A), x1000, oil (B)

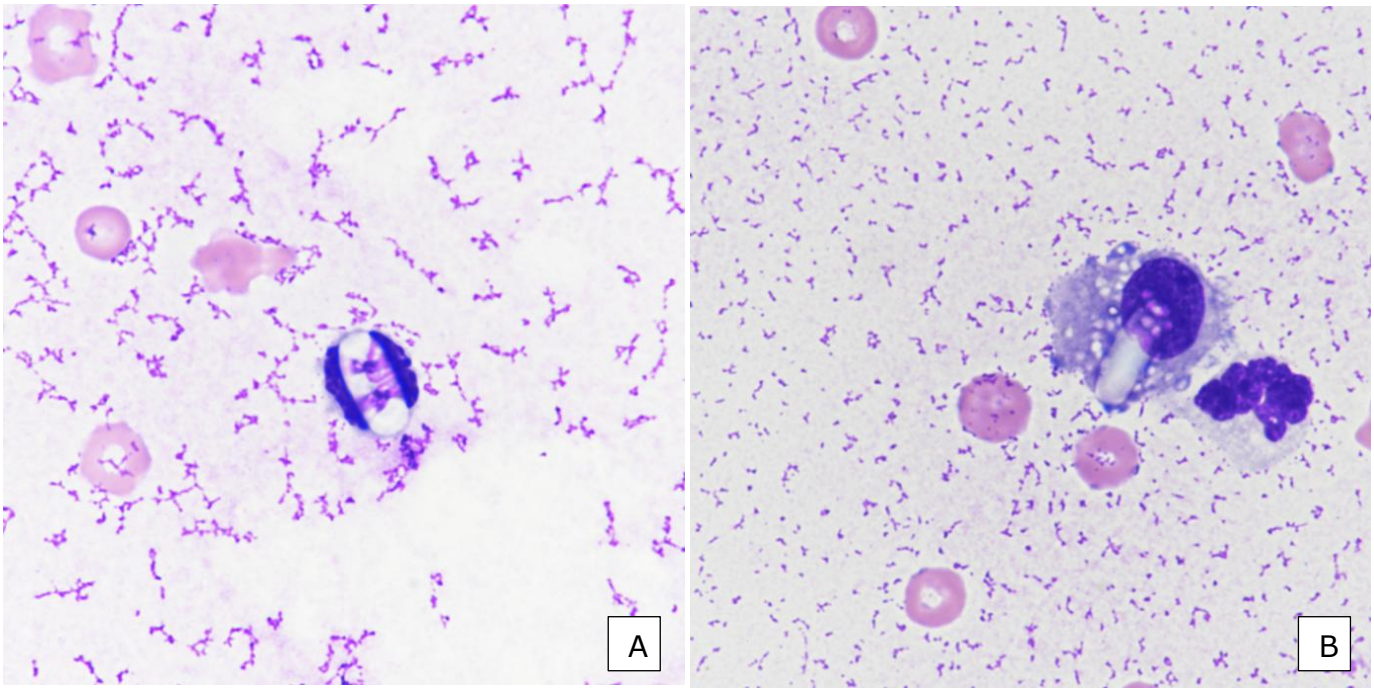

Figure 2 -A, B: Photomicrographs of synovial fluid smears of the puppy, May-Grunwald-Giemsa stain, x1000, oil

	Result	Reference Interval in adult
Proteins concentration (g/L)	**62**	< 30
Nucleated cell count (10^9/L)	**48.30**	< 3.00
Neutrophils (%)	**51**	<5
Macrophages (%)	**49**	>95
Red blood cell count (10^{12}/L)	**0.20**	< 0.01

Table 2: Results of synovial fluid composition. Nucleated cell count was measured using a Sysmex XN-1000V analyzer, and protein concentration was determined by refractometry.

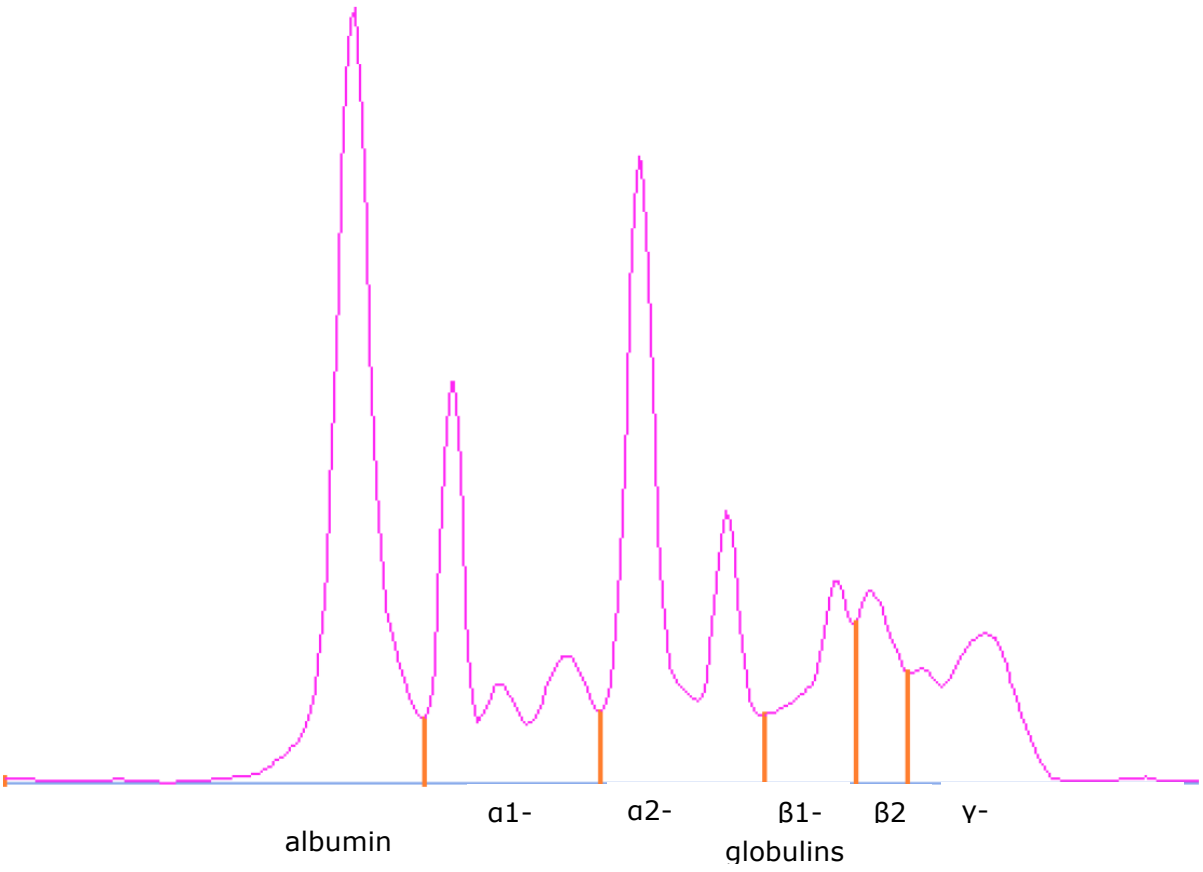

Figure 3: Serum capillary zone electrophoresis profile (Se_ _APILLARYS)

	Result	Reference interval in adult
Albumin (g/dL)	**1.56**	2.86-4.36
α-globulins (g/dL)	**2.34**	0.63-1.21
α1-globulins (g/dL)	**0.92**	0.25-0.47
α2-globulins (g/dL)	**1.42**	0.33-0.83
βglobulins (g/dL)	0.79	0.75-1.52
β1-globulins (g/dL)	0.45	0.31-0.92
β2-globulins (g/dL)	0.34	0.34-0.81
γ-globulins (g/dL)	0.60	0.3-1.2

Table 3: Quantitative composition of serum proteins fractions by serum capillary zone electrophoresis (Sebia CAPILLARYS)

Questions

1) What is your description of the cytological specimens?
2) Based on the clinical and pathological findings, what is your main diagnosis?
3) What additional tests would you use for confirming a diagnosis and further characterize it?

Case 2 – Mass-like lesion in the bone marrow of a dog

Marion Cambrelin[1], Kate Pennick[2], Brittany Lucchetti[3], Emily Rout[1], R. Adam Harris[1]A Russell Moore[1]

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Marion Cambrelin, DVM: Marion.cambrelin@colostate.edu

Specimen

EDTA whole blood, peripheral blood film, bone marrow aspirate smear, bone marrow core

Signalment

Dog, Goldendoodle, 11-year-old, neutered male

History

The dog had a 4-month history of presumptive immune-mediated thrombocytopenia and was treated with prednisone (1.6 mg/kg/day), which was tapered to 0.27 mg/kg/day over this period by the referring veterinarian. The platelet count, however, never exceeded 91 K/µL (RI 120-412 K/µL). He was ultimately referred for evaluation of waxing and waning lethargy, severe progressive thrombocytopenia, and a new-onset moderate non-regenerative anemia.

Clinical Findings

On presentation to the referral hospital, the dog was mildly hyperthermic (39.4°C) with quiet mentation, mild firm swelling of the left tarsus, ventral abdominal ecchymoses, and dorsal erythema and bruising. Complete blood count (Sysmex XN900V-1-L) revealed a moderate non-regenerative anemia (RBC 3.5

M/µL; RI 5.8-8.9 M/µL; HCT 25.8%; RI 41.0-60.0%; hemoglobin 8.5 g/dL; RI 14.6-21.7 g/dL, reticulocytes 18 K/µL; RI 21-140 K/µL), neutropenia (0.2 K/µL; RI 3.0-9.7 K/µL), lymphocytosis (6.1 K/µL; RI 1.0-4.2 K/µL), monocytosis (1.0 K/µL; RI 0.1-0.7 K/µL), thrombocytopenia (22 K/µL; RI 120412 K/µL), and circulating unclassified cells (0.8 K/µL).

Blood film evaluation by a clinical pathologist identified approximately 60% unclassified cells (4,860 cells/µL). These cells were about the size of a medium to large lymphocyte (12-18 micrometers diameter) with round to occasionally indented or lobulated nuclei, finely granular chromatin, variably distinct nucleoli, and scant to mildly increased amounts of light blue cytoplasm (Figure 1). Some cells contained coarse, magenta to purple cytoplasmic granules. These findings raised concern for hematopoietic neoplasia, and bone marrow evaluation was pursued. SNAP™ 4Dx™ Plus Test results (IDEXX Laboratories, Westbrook, Maine, USA) were negative for *Anaplasma spp.*, *Ehrlichia spp.*, *Borrelia burgdorferi*, and *Dirofilaria immitis*. Comprehensive tick-borne PCR testing was also performed and was negative for *Anaplasma spp.*, *Ehrlichia spp.*, *Babesia spp.*, *Bartonella spp.*, *Mycoplasma spp.*, *Rickettsia spp.*, *Borrelia burgdorferi*, and *Dirofilaria immitis*.

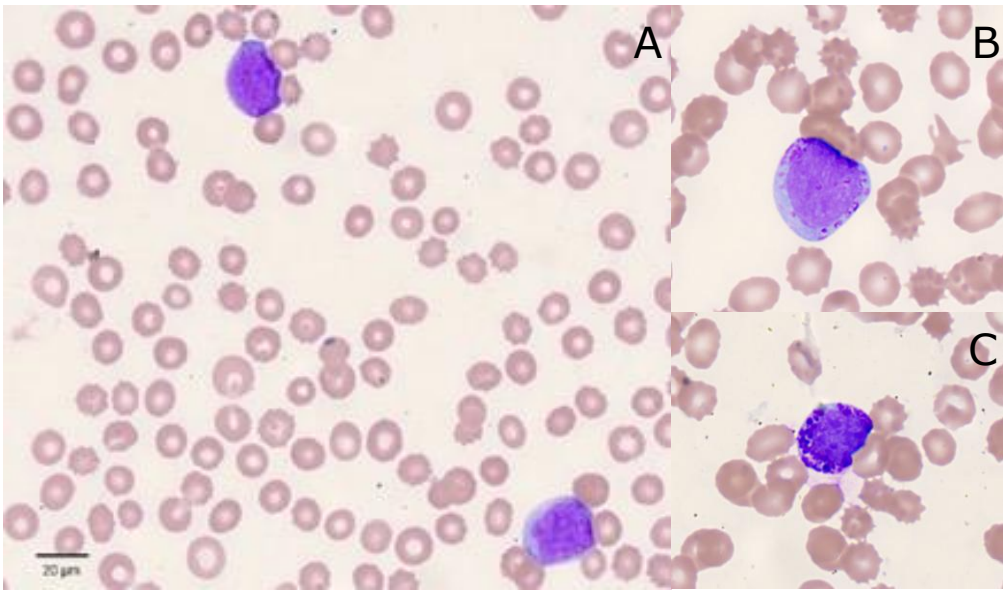


Figure 1. A-C, Blood film demonstrating unclassified cell population. Some of these cells contained coarse magenta to purple cytoplasmic granules (B-C). Wright Giemsa, 100x objective.

Bone marrow aspirate smears (Figure 2) were moderately to highly cellular with numerous particles. Megakaryocytes were mildly decreased (approximately 1–2 per particle) but exhibited appropriate maturation. Hematopoietic elements were nearly effaced by a dominant atypical round cell population consisting primarily of cells that were about the size of a small to intermediate lymphocyte (8-12 micrometers diameter). These cells had a high nuclear to cytoplasmic (N:C) ratio, a scant rim of pale basophilic cytoplasm, coarsely clumped chromatin, and variably prominent nucleoli. An additional smaller subset of atypical cells was present, characterized by a larger size (approximately 12-18 micrometers diameter), a round to ovoid nucleus, coarsely clumped chromatin, and more expanded cytoplasm. Many cells within both populations contained abundant coarse magenta to purple intracytoplasmic granules, and similar granules were also present free in the background. Low numbers of immature erythroid precursors were identified. The background contained moderate blood contribution, platelet clumps, and lysed cellular debris.
Alkaline phosphatase (ALP) reactivity was observed in the majority of the small to intermediate cells (approximately 70-80% showing positive cytoplasmic reactivity) and in approximately 40-50% of the larger cells (Figure 3).

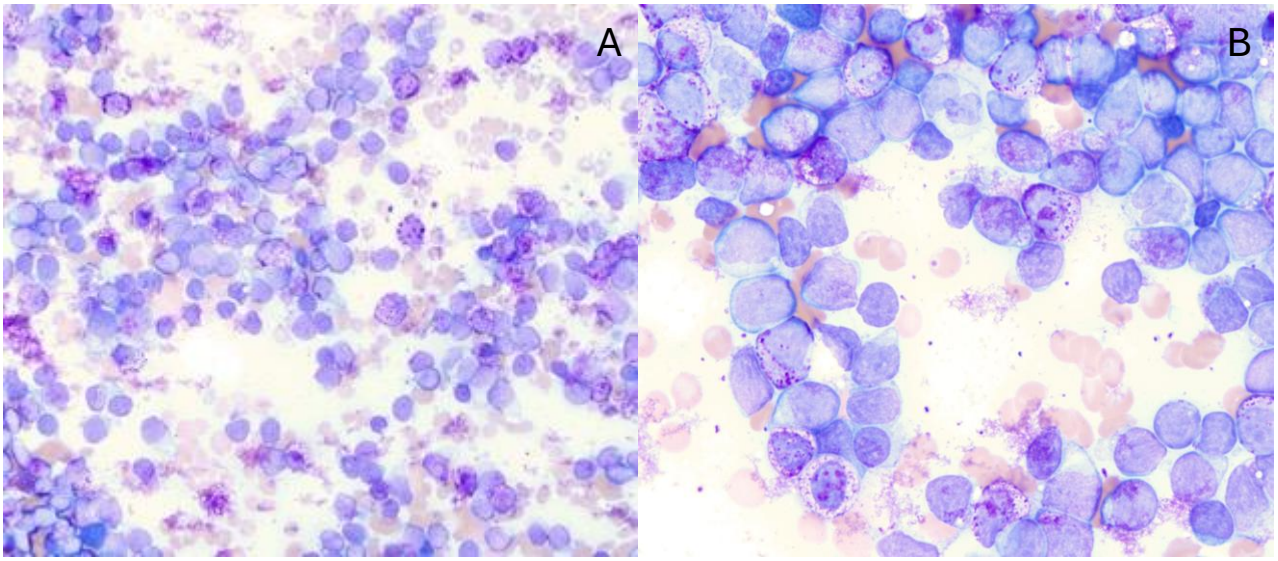


Figure 2. A-B, Bone marrow aspirate smears demonstrating cells with coarse magenta to purple intracytoplasmic granules. Wright-Giemsa stain, 50x objective (A) and 100x (B).

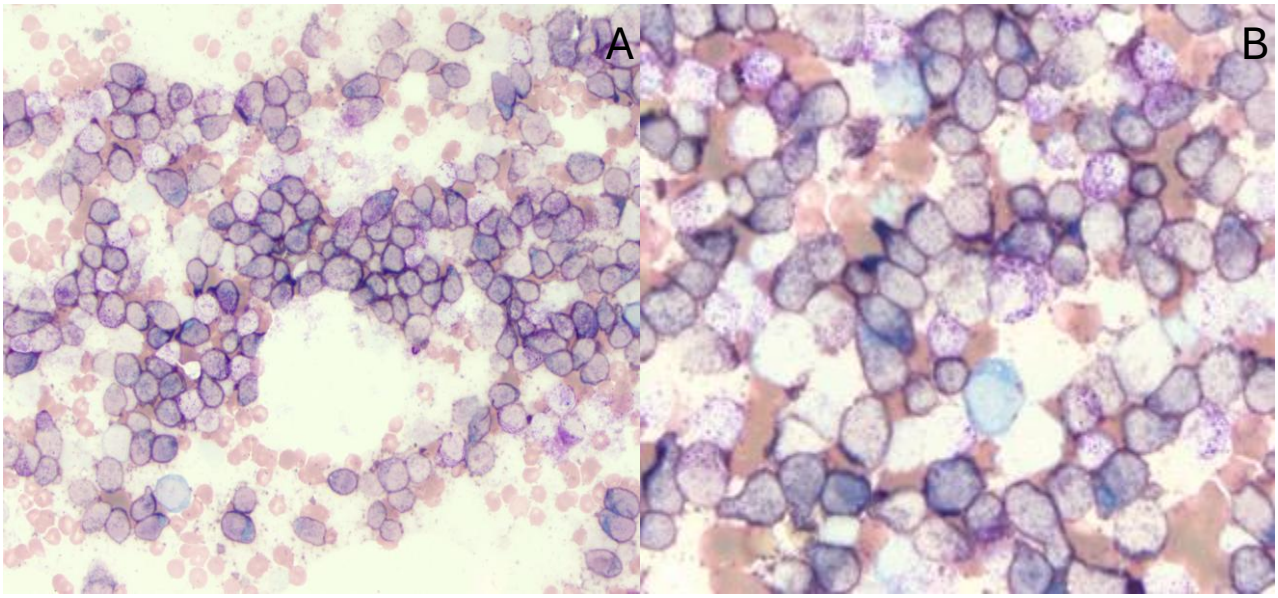


Figure 3. Bone marrow aspirate smear with ALP reactivity. Wright-Giemsa stain, 50x objective (A) and 100x objective (B). Both small to intermediate and the larger cells exhibit ALP reactivity.

The bone marrow core biopsy was markedly hypercellular, with approximately 10-20% adipose tissue within the intertrabecular regions. Consistent with the aspirate findings, normal hematopoietic tissue was effaced by a dominant round cell population exhibiting two morphologies. The subpopulation corresponding to the small to intermediate round cells in the aspirate predominated and was characterized by a high N:C, coarsely clumped chromatin, and 1-3 distinct nucleoli. Interestingly, the subpopulation corresponding to the larger cells in the aspirate formed morphologically distinct aggregates throughout the marrow, which was most apparent at a lower magnification. These cells had more abundant cytoplasm, central to eccentric nuclei, and clumped nuclear chromatin. In contrast to the aspirate, cytoplasmic granules were not evident in the core biopsy sections (Figure 4). The nuclear features of the two populations were still

considered to be similar, which suggested a relationship between them and raised the possibility of maturation within a single neoplastic lineage.

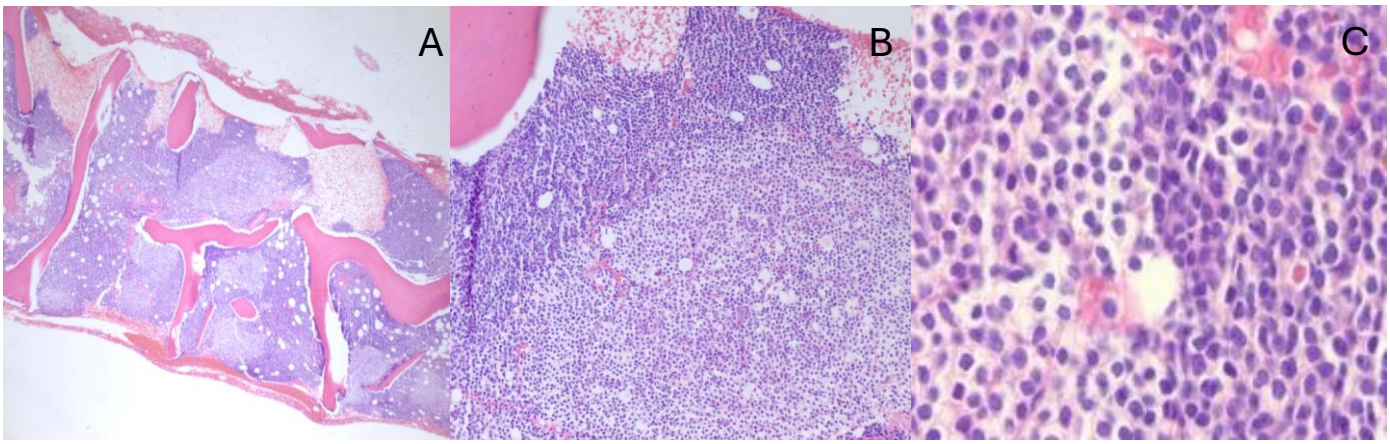
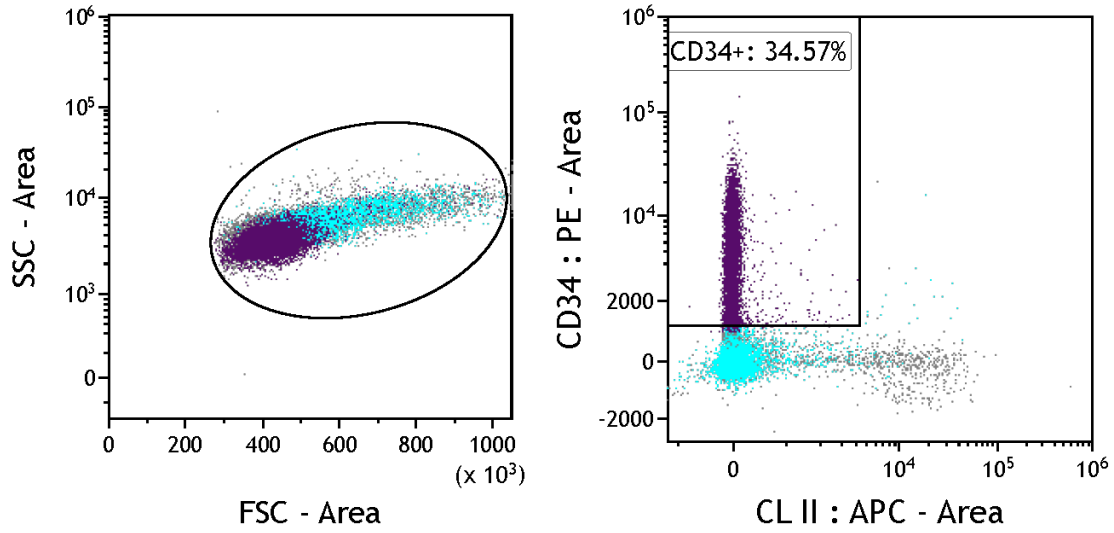


Figure 4. A-C. Bone marrow core, H&E 4x (A), 20x (B), 100x (C).

Routine immunophenotyping by flow cytometry was performed on the bone marrow aspirate (Figure 5). 34.6% of the cells (purple) expressed CD34 and had low forward scatter. A subset of the CD34+ cells co-expressed CD18. A separate population of cells (24.3%; teal) with higher forward scatter expressed CD14, CD18, and low levels of class II.



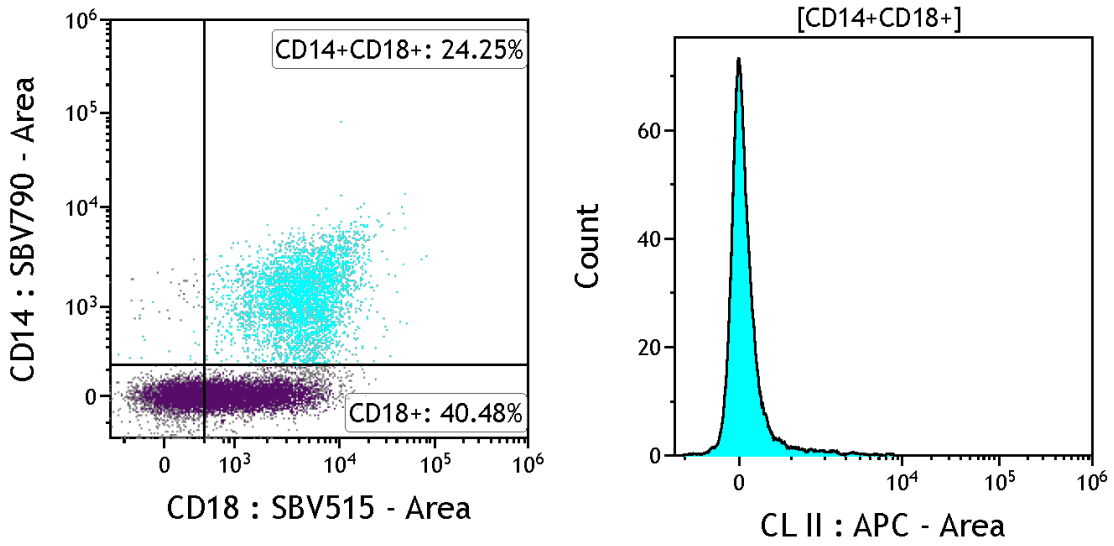


Figure 5. Flow cytometry plots of the bone marrow aspirate. CL II, class II major histocompatibility complex. FSC, forward scatter. SSC, side scatter.

Questions:

1. What are reasonable diagnoses for this neoplasm based on aspirate, core, and flow cytometry data?

2. What additional immunohistochemical or special staining would be most helpful to better characterize the two cell populations described?

3. How would you best explain the discrepancy in granulation between the aspirate and core samples?

Case 3 - Thoracic mass in a dog

Contributors

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Specimen

Fine Needle aspirate biopsy of a thoracic mass

Signalment

12 years old female cross breed dog.

History

The dog was presented on an emergency basis for acute dyspnea following a three-month history of coughing that had shown partial improvement with antibiotic (amoxicillin-clavulanic acid) and corticosteroid therapy. Thoracic radiographs revealed marked pleural effusion, and 1.6 L of fluid were drained by thoracocentesis. The pleural fluid was dark amber in color and moderately turbid. Fluid analysis revealed a total nucleated cell count of 260 cells/µL and a total protein concentration of 4.1 g/dL. No neoplastic cells were identified, and the underlying cause of the effusion could not be determined based on fluid analysis alone. The patient was subsequently referred to the oncology service for further investigation of a suspected neoplastic process.

Clinical findings

Initial clinical examination at the referring hospital revealed mild tachypnea, while the remainder of the physical examination was unremarkable. Hematologic and biochemical analyses showed no significant abnormalities.

Thoracic computed tomography (CT) identified an irregular, heterogeneously contrast-enhancing mass within the ventral mediastinum, surrounding the cardiac apex without evidence of diaphragmatic invasion (figure 1)

Fine needle aspiration of the mass was performed for cytologic evaluation (figures 2-3).

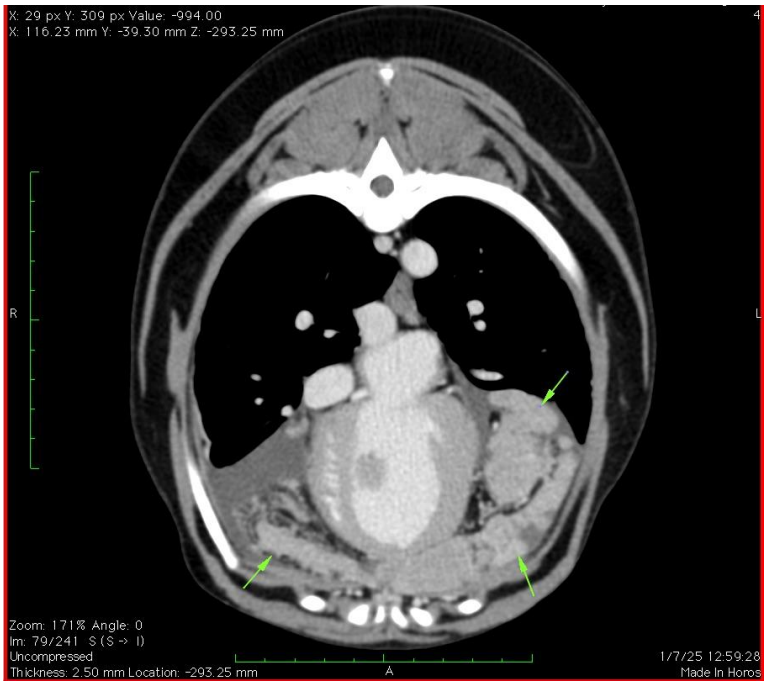

Figure 1. Thoracic CT scan demonstrating a large ventral mediastinal mass (green arrows).

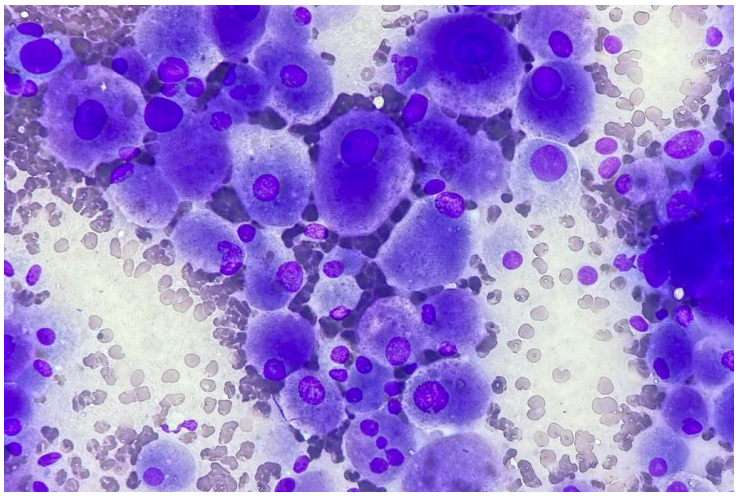

Figure 2. Fine needle aspiration of the thoracic mass (Modified Wright Giemsa) (40x).

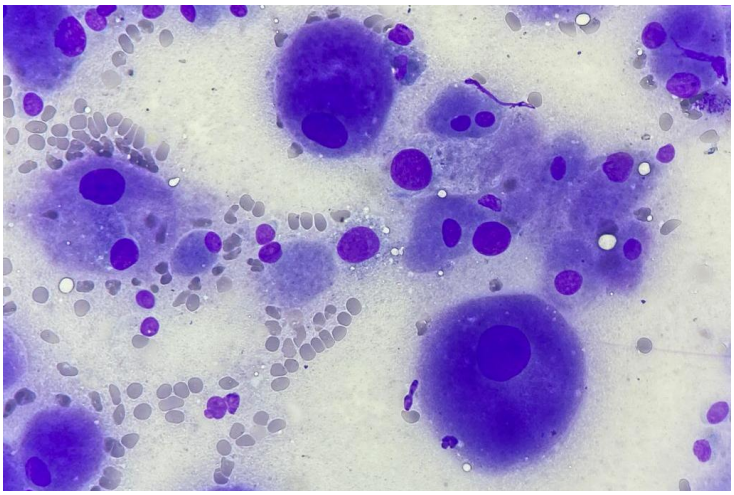

Figure 3. Fine needle aspiration of the thoracic mass (Modified Wright Giemsa) (40x)

Questions

1. What is the cytological description of this lesion?
2. Based on the cytological findings and the location of the mass, what are the differential diagnoses for this lesion?
3. What would be the recommended next steps to further investigate the origin of the mass?

Case 4 - Mystery Case: Thoracic effusion in a horse

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Specimen: Thoracic effusion

Signalment: 14-year-old grey Shagya Arabian horse

History: The horse was presented for signs of colic. Multiple cutaneous nodules were noted on the neck, trunk, and prepuce.

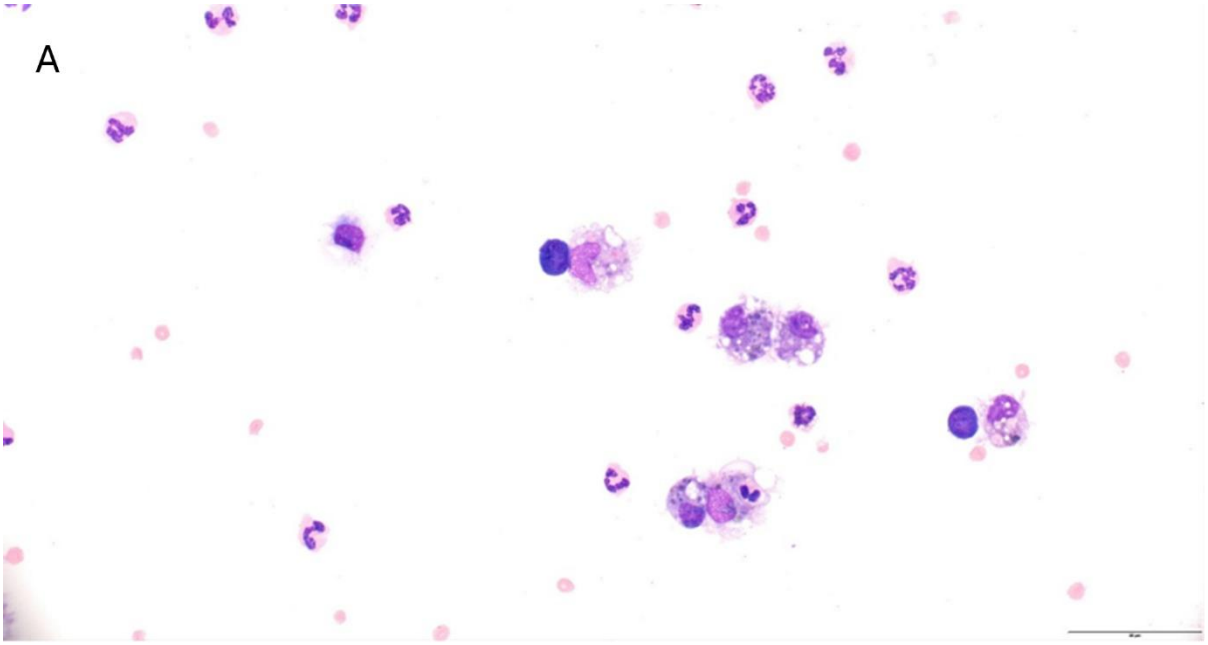
Clinical findings: Hematology, performed using the Sysmex-XN1000V analyzer with a manual differential count, revealed moderate anemia and mild leukocytosis, accompanied by markedly increased serum amyloid A and fibrinogen concentrations. A mild hyperbilirubinemia was also present. (Table 1)

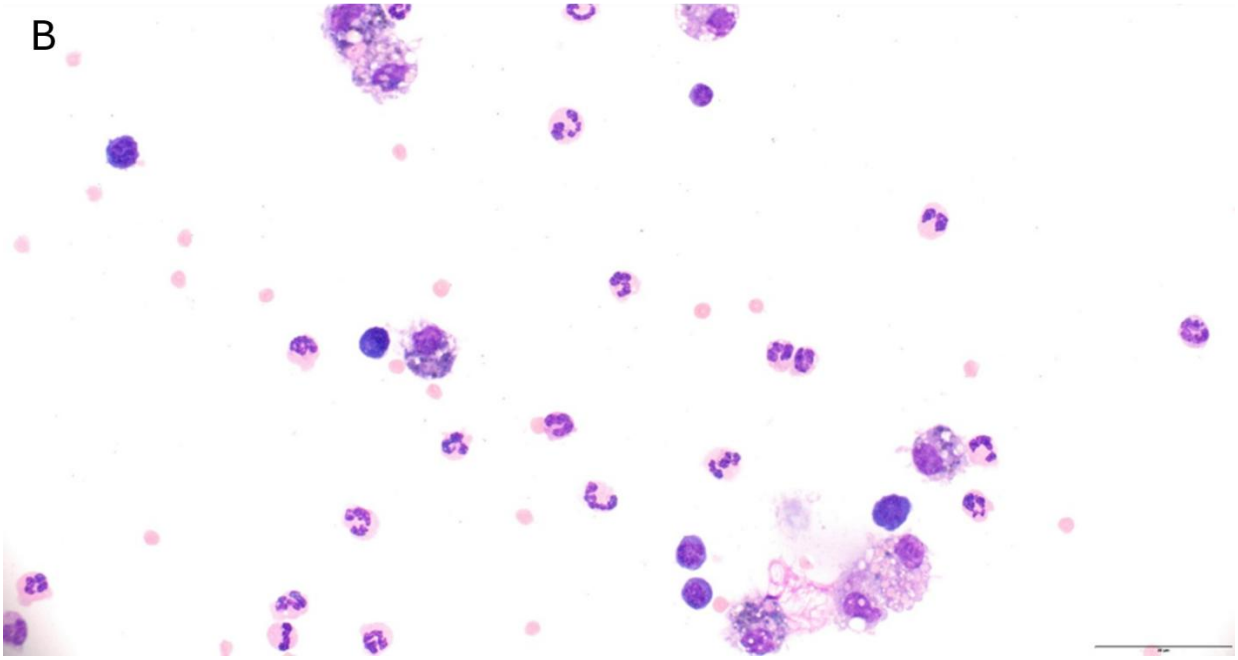
Parameter	**Result**	**Reference interval**
Hematology		
Hematocrit	**25 %**	30 – 42 %
Hemoglobin	**9.3 g/dL**	10.8 – 14.9 g/dL
Erythrocytes	**5.59 × 10^6/µL**	6.2 – 9.0 × 10^6/µL
Thrombocytes	**193 × 10^3/µL**	87 – 168 × 10^3/µL
Leukocytes	**11 × 10^3/µL**	4.7 – 8.2 × 10^3/µL
Band neutrophils	**0.55 × 10^3/µL**	0.00 – 0.08 × 10^3/µL
Neutrophils	**8.66 × 10^3/µL**	3.02 – 5.78 × 10^3/µL
Eosinophils	0.00× 10^3/µL	0.00–0.22 × 10^3/µL
Basophils	**0.11 × 10^3/µL**	0.00 – 0.07× 10^3/µL
Monocytes	**0.27 × 10^3/µL**	0.00 – 0.18× 10^3/µL
Lymphocytes	1.37 × 10^3/µL	1.02–3.47× 10^3/µL
Chemistry		
Total Bilirubin	**54.2 umol/L**	17.4 – 35.2 umol/L
Acute Phase Proteins		
Serum amyloid A	**2'726 mg/L**	0.5–1.2 mg/L

Parameter	Result	Reference interval
Fibrinogen	**7.1 g/L**	1.25–2.85 g/L

Table 1. Relevant hematologic, biochemical, and acute-phase protein parameters in the affected horse.

A large colon impaction was diagnosed and successfully treated. Thoracic ultrasonography demonstrated bilateral pleural effusion, pulmonary consolidation, and a suspected pericardial mass. Radiographs revealed a cranioventral alveolar pattern and/or pleural effusion, as well as a soft tissue mass caudodorsal to the guttural pouch. Endoscopic examination identified dorsal displacement of the soft palate and multifocal lesions within both guttural pouches, most prominent in the left medial compartment. Tracheobronchial secretions were collected for cytological evaluation and revealed a moderate increase in mucus production and moderate neutrophilic inflammation. Thoracocentesis was performed for cytological analysis of the pleural effusion. A total of 2 mL of turbid, orange pleural fluid was collected. Cytological examination revealed a total nucleated cell count of 29'800 cells/µL using the Sysmex XN-1000V, and a protein concentration of 40 g/L measured by refractometry. Manual differential cell count showed a predominance of neutrophils (77%), with macrophages accounting for 13% and lymphocytes for 10%.

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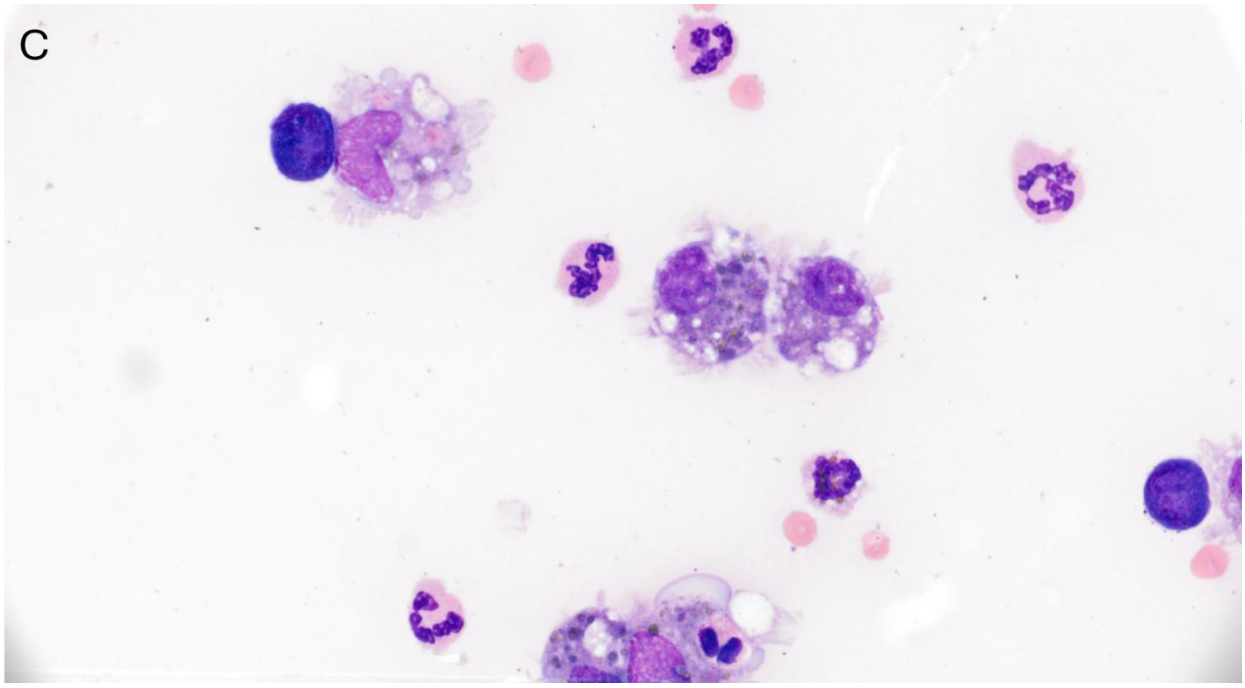
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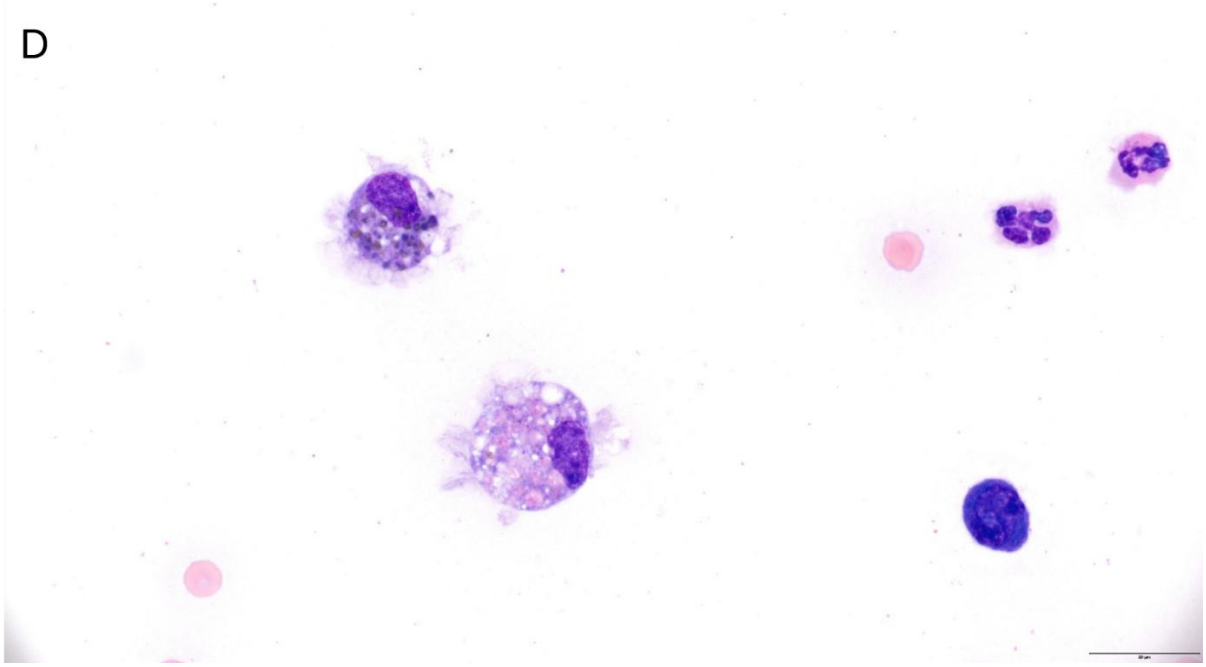

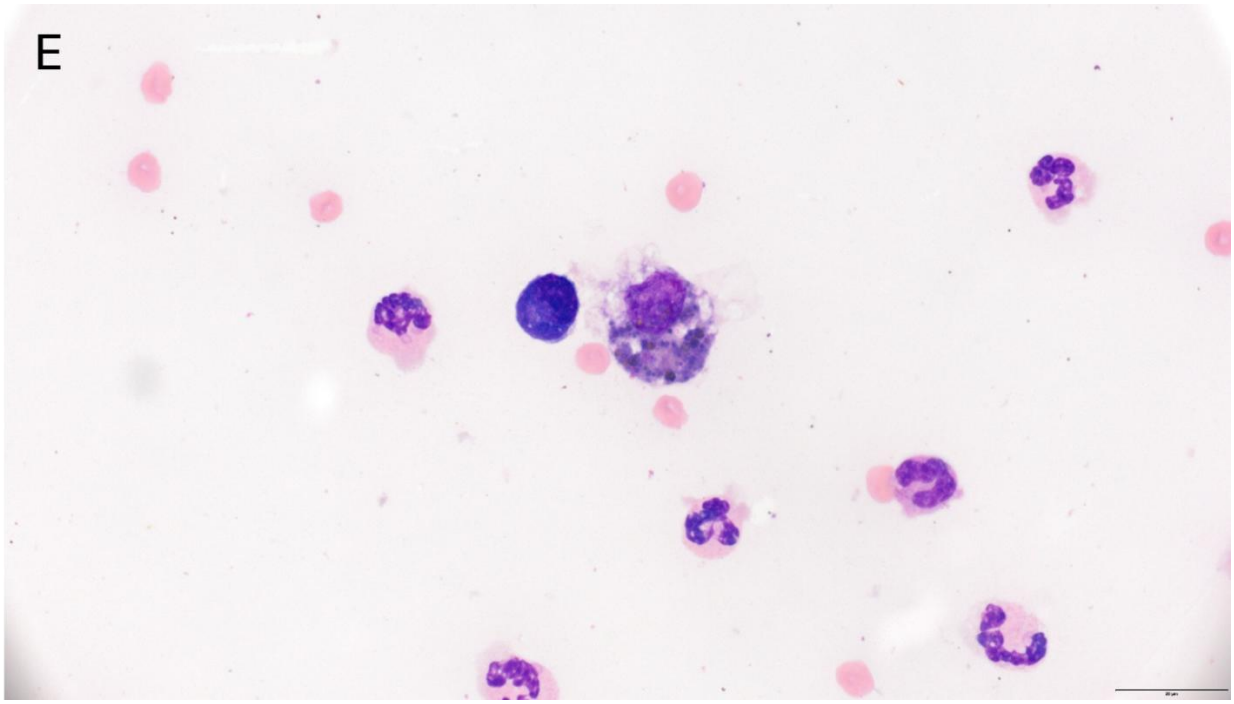

Figure 1. A-B (50x) and C-E (100x). May-Grünwald Giemsa stained direct smear of the thoracic effusion.

Questions:

1. What is the cytological description of the thoracic effusion, and what is your interpretation?
2. What are your main differential diagnoses for the black-greenish pigment seen in the macrophages of the thoracic effusion? What kind of further diagnostic tests would you consider performing?
3. What additional clinical findings and sampling sites should be investigated to clarify the diagnosis?

Case 5 - Intra-abdominal mass in a cat

Contributors

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Specimen

Fine-needle aspiration cytology samples obtained from an intra-abdominal mass of undetermined organ origin.

Signalment

12-year-old spayed female domestic shorthair cat.

History

The cat was presented for excessive licking of the ventral abdomen resulting in alopecia.

Clinical findings

On physical examination, the cat was bright, alert, and responsive. Vital parameters were within normal limits. Symmetrical alopecia was noted on the ventral abdomen and the medial aspects of all limbs.

A complete blood count (XN-V Haematology Analyzer, Sysmex Corporation, Kobe, Japan) was unremarkable, except for a borderline decrease in reticulocyte haemoglobin content (15.2 pg; reference interval [RI]: 15.3 – 22.9 pg). A biochemistry panel (Beckman Coulter AU5800, Brea, CA, USA) revealed a mild increase in lipase activity (49 U/L; RI 0.0 – 45.0 U/L). Serum fructosamine and total thyroxine (T4) levels (Siemens Immulite 2000, Siemens Healthineers, Erlangen, Germany) were within the reference interval.

Abdominal ultrasound revealed an irregular, rounded mass with mildly heterogeneous echotexture located in the mid-cranial abdomen, measuring approximately 30 x 40 mm (**Figure 1**). Based on ultrasonographic findings, no clear association with any abdominal organ could be established. Abdominal lymph node enlargement was suspected, possibly due to neoplasia, particularly lymphoma.

Fine-needle aspiration samples of the intra-abdominal mass were collected and submitted to an external laboratory (IDEXX Laboratories, Kornwestheim, Germany) for cytologic evaluation.

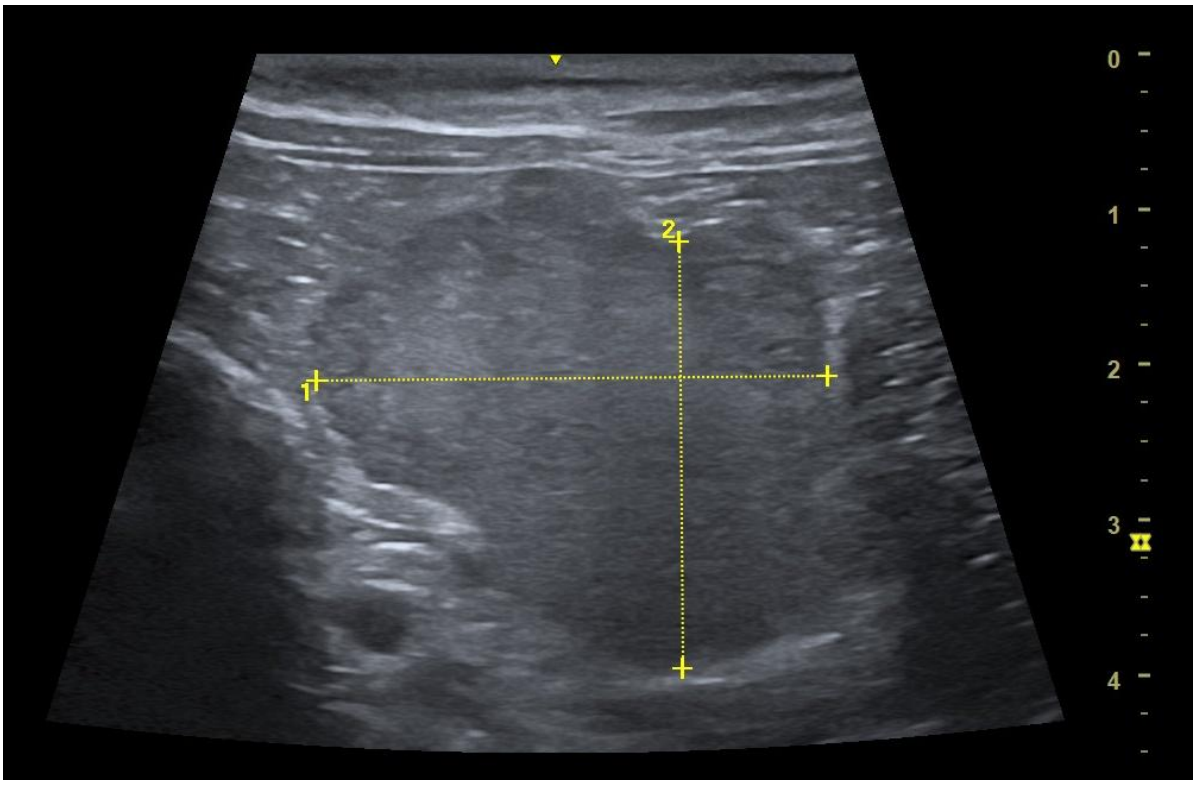

Figure 1. Abdominal ultrasonography showing an approximately 30 x 40 mm mass in the mid-cranial abdomen. Anatomic origin could not be determined.

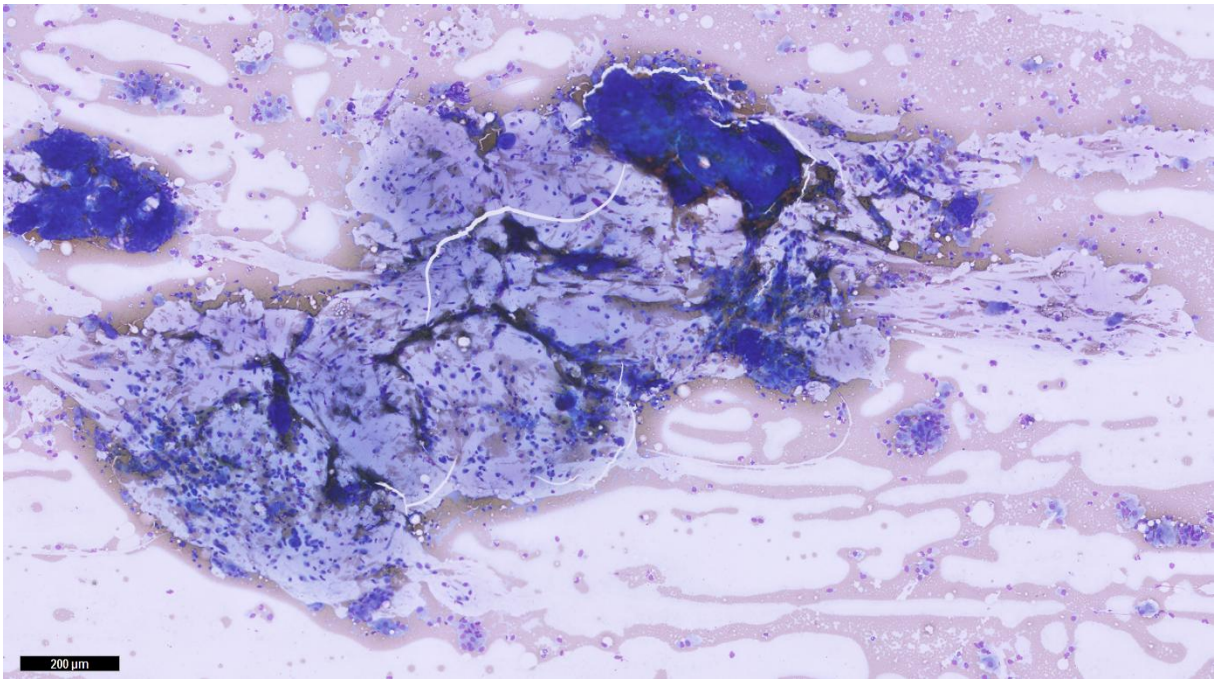

Figure 2. Fine-needle aspiration of an intra-abdominal mass, 50x magnification (May-Grünwald-Giemsa)

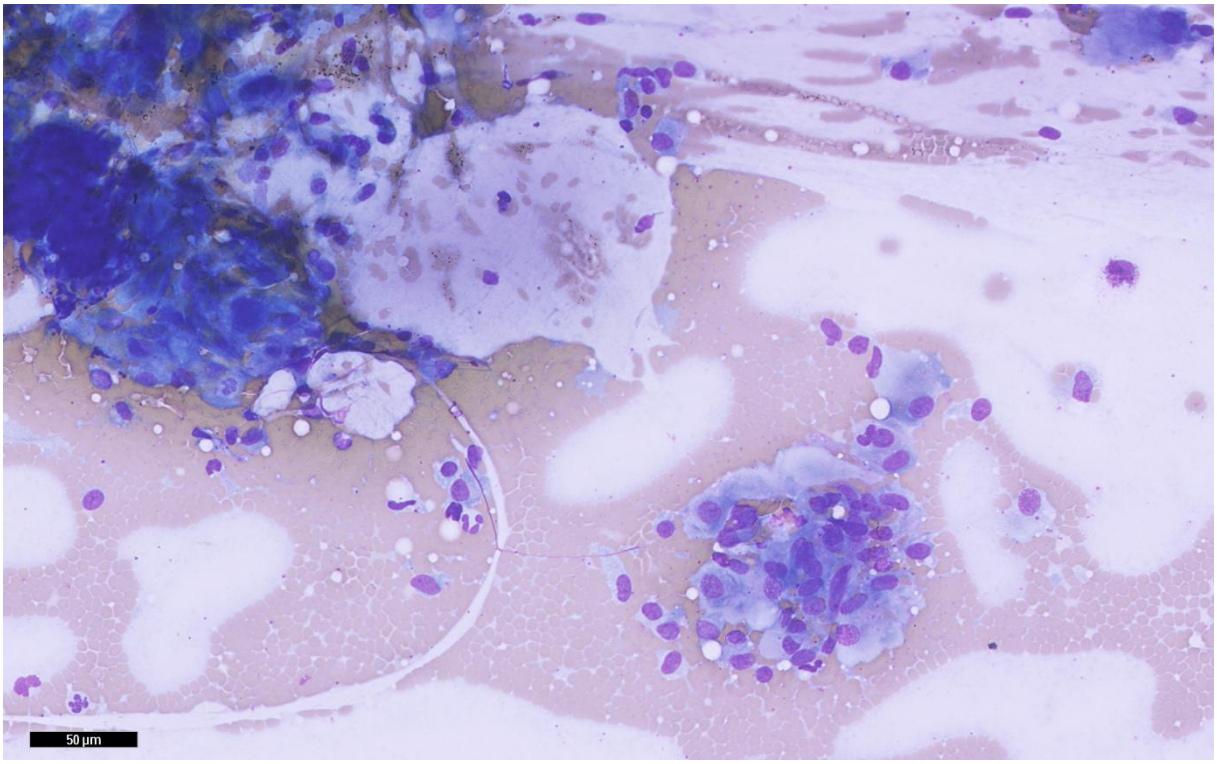

Figure 3. Fine-needle aspiration of an intra-abdominal mass, 200x magnification (May-Grünwald-Giemsa)

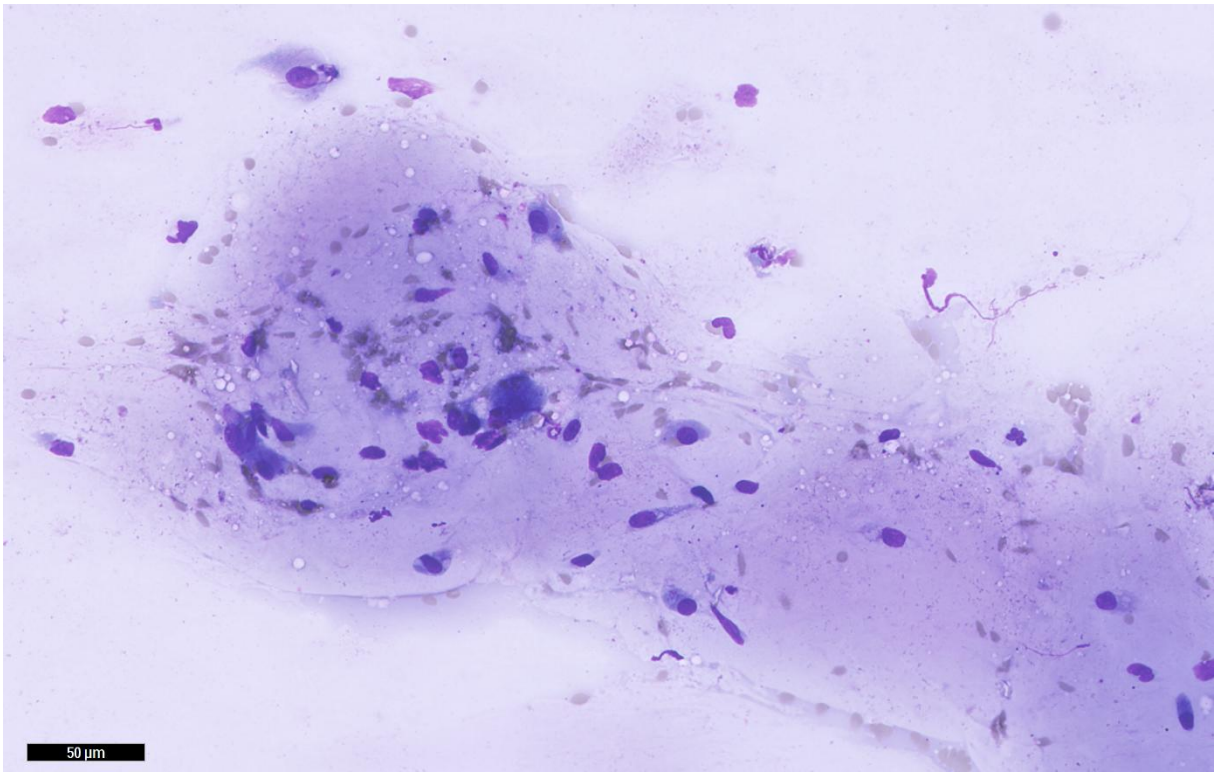

Figure 4. Fine-needle aspiration of an intra-abdominal mass, 200x magnification (May-Grünwald-Giemsa)

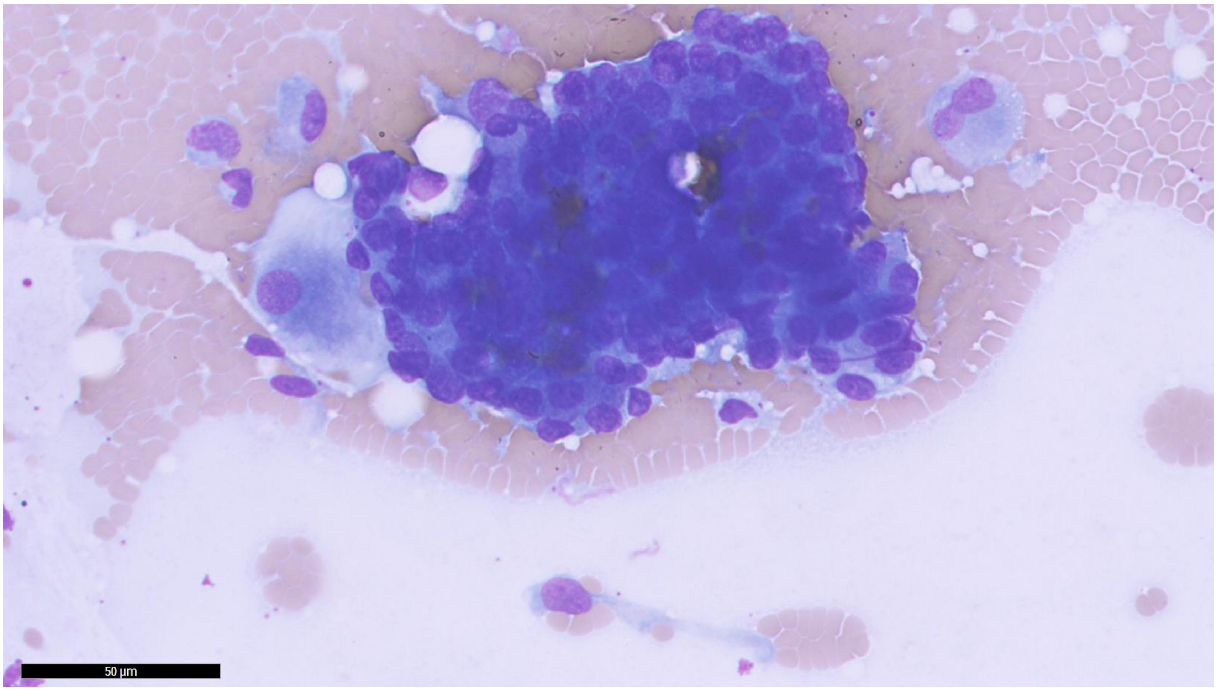

Figure 5. Fine-needle aspiration of an intra-abdominal mass, 400x magnification (May-Grünwald-Giemsa)

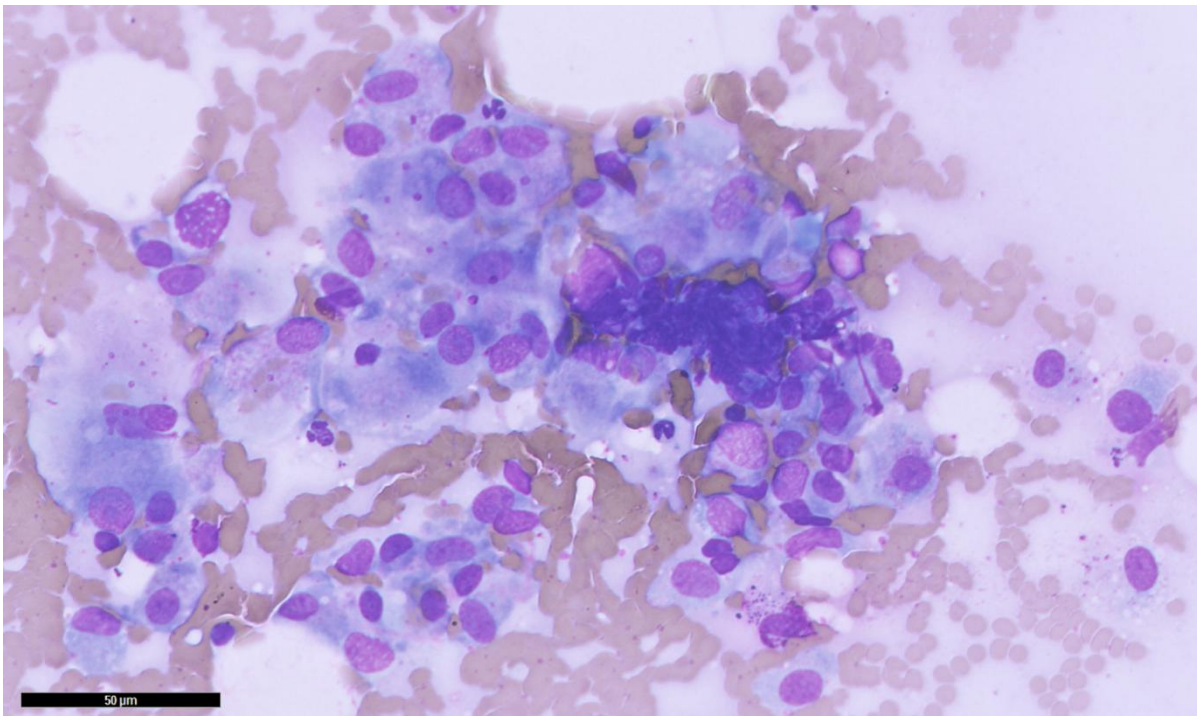

Figure 6. Fine-needle aspiration of an intra-abdominal mass, 400x magnification (May-Grünwald-Giemsa)

Questions

1. How would you describe the origin of the extracellular material observed on the slides?

2. Based on the cytologic picture, what are your top differential diagnoses for the aetiology of the mass and its site of origin?

3. What further diagnostic tests do you think could be helpful in this case?

Case 6 – A Diagnostic Enigma in a Tortoise

Contributors

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Specimen

Fine needle aspirate of three proliferative dermal lesions around the head and neck.

Signalment

21 years and 10 months old male Hermann's tortoise (*Testudo hermanni*).

History

The tortoise was presented at the first opinion veterinary practice with several dermal proliferative lesions around the head and the neck (Figures 1 and 2). The masses were first noticed approximately one year prior to presentation and had gradually increased in size over this period. The tortoise was otherwise clinically well.

Clinical findings

On clinical examination, three proliferative dermal growths were observed. There was a mild left periocular swelling which subsequently resolved. The tortoise was otherwise bright, alert and responsive and no additional abnormalities were seen.

The lesions comprised a prominent nodule on the left mandible, and two nodules (one larger and one smaller) on the right mandible. Fine needle aspirates (FNAs) were obtained and submitted for cytologic examination (Figures 3, 4, 5 and 6). The aspirates were described as consistent with 'solid material', with no evidence of purulent exudate.

Figure 1: Dermal lesions on the right mandibular and neck region.

Figure 2: Dermal lesion on the left mandibular and neck region and mild left periocular swelling.

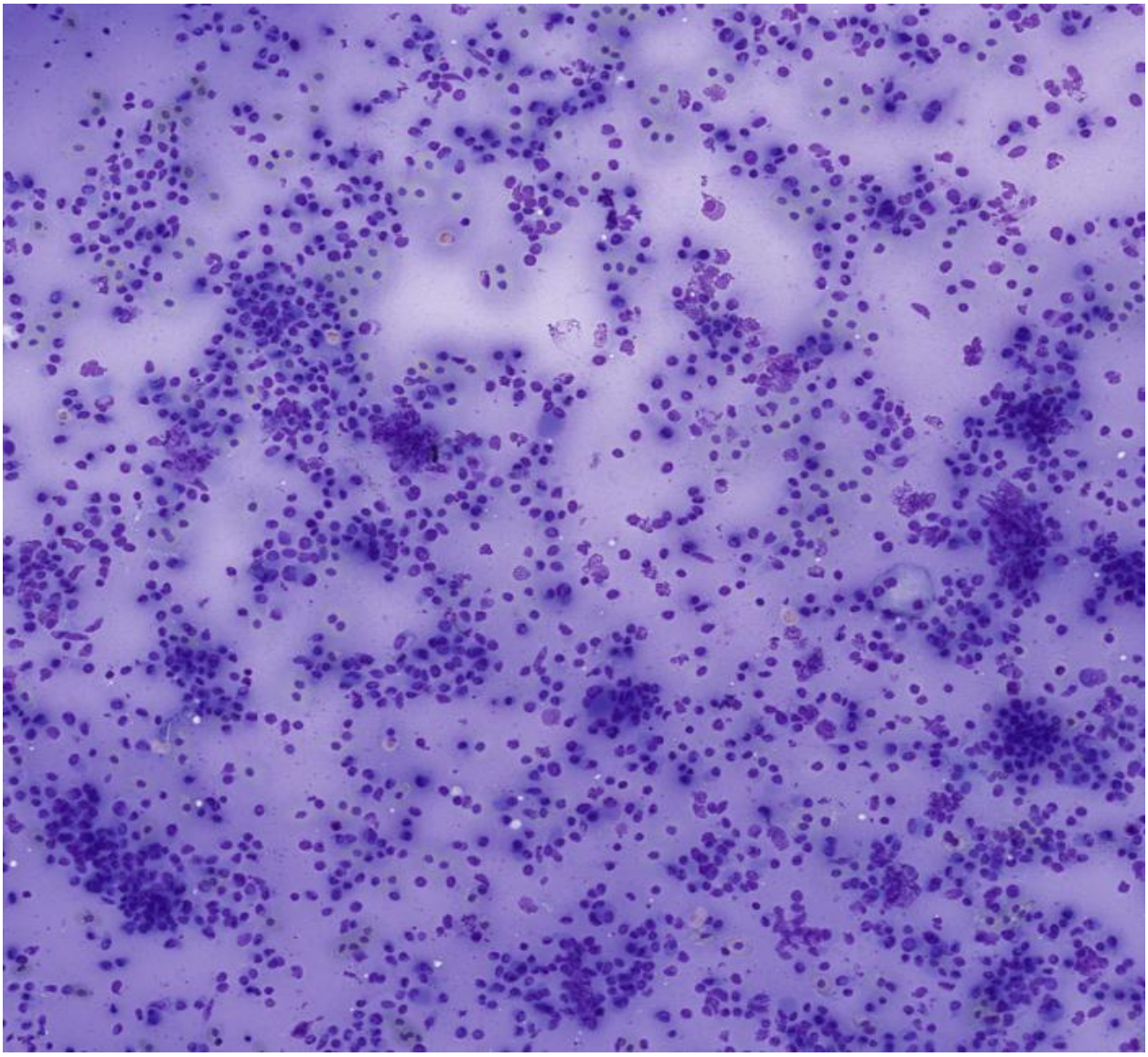

Figure 3: Fine needle aspirate from one of the dermal nodules. Wright-Giemsa stain, 100 x magnification.

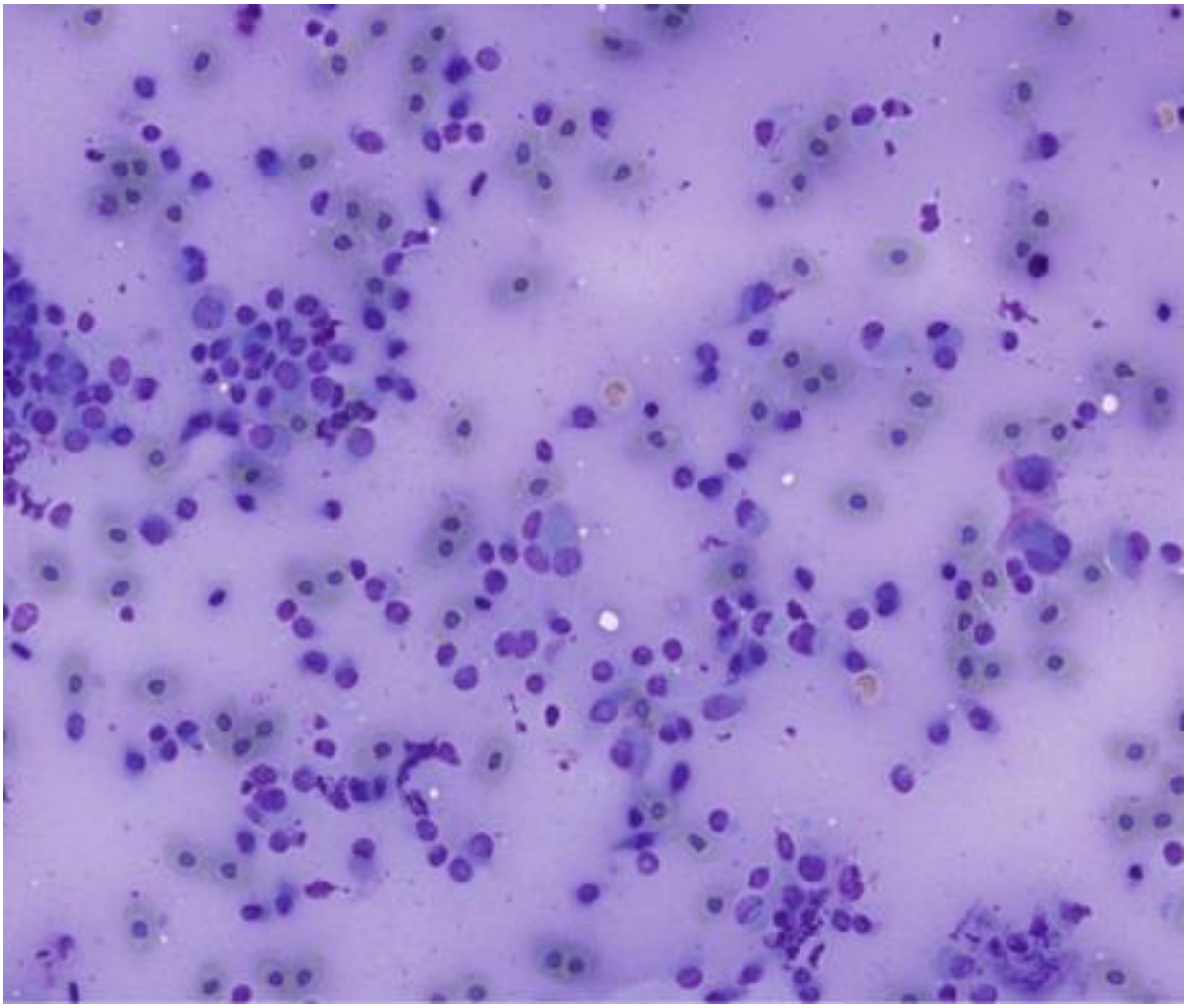

Figure 4: Fine needle aspirate from one of the dermal nodules. Wright-Giemsa stain, 200 x magnification.

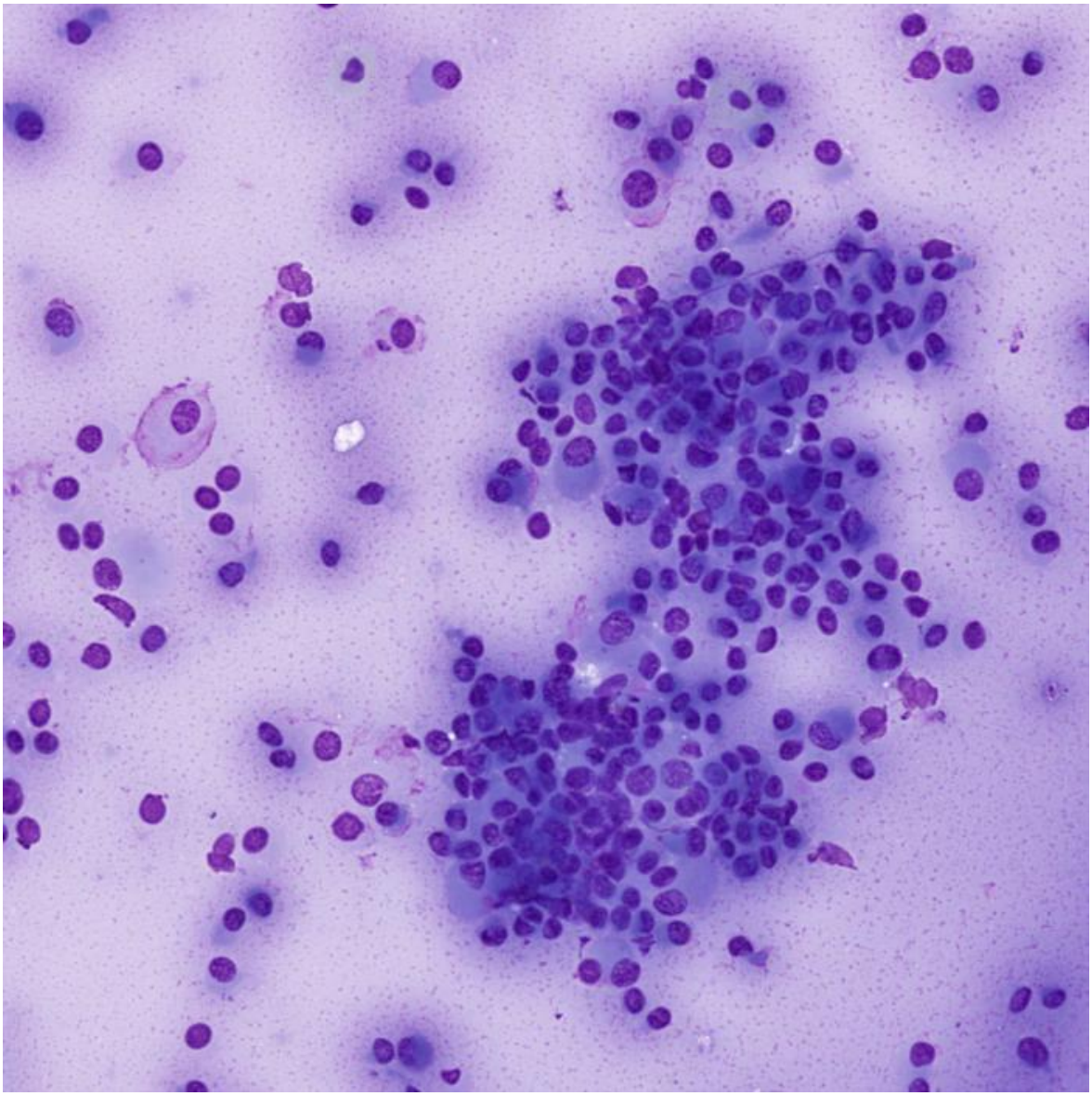

Figure 5: Fine needle aspirate from one of the dermal nodules. Wright-Giemsa stain, 400 x magnification.

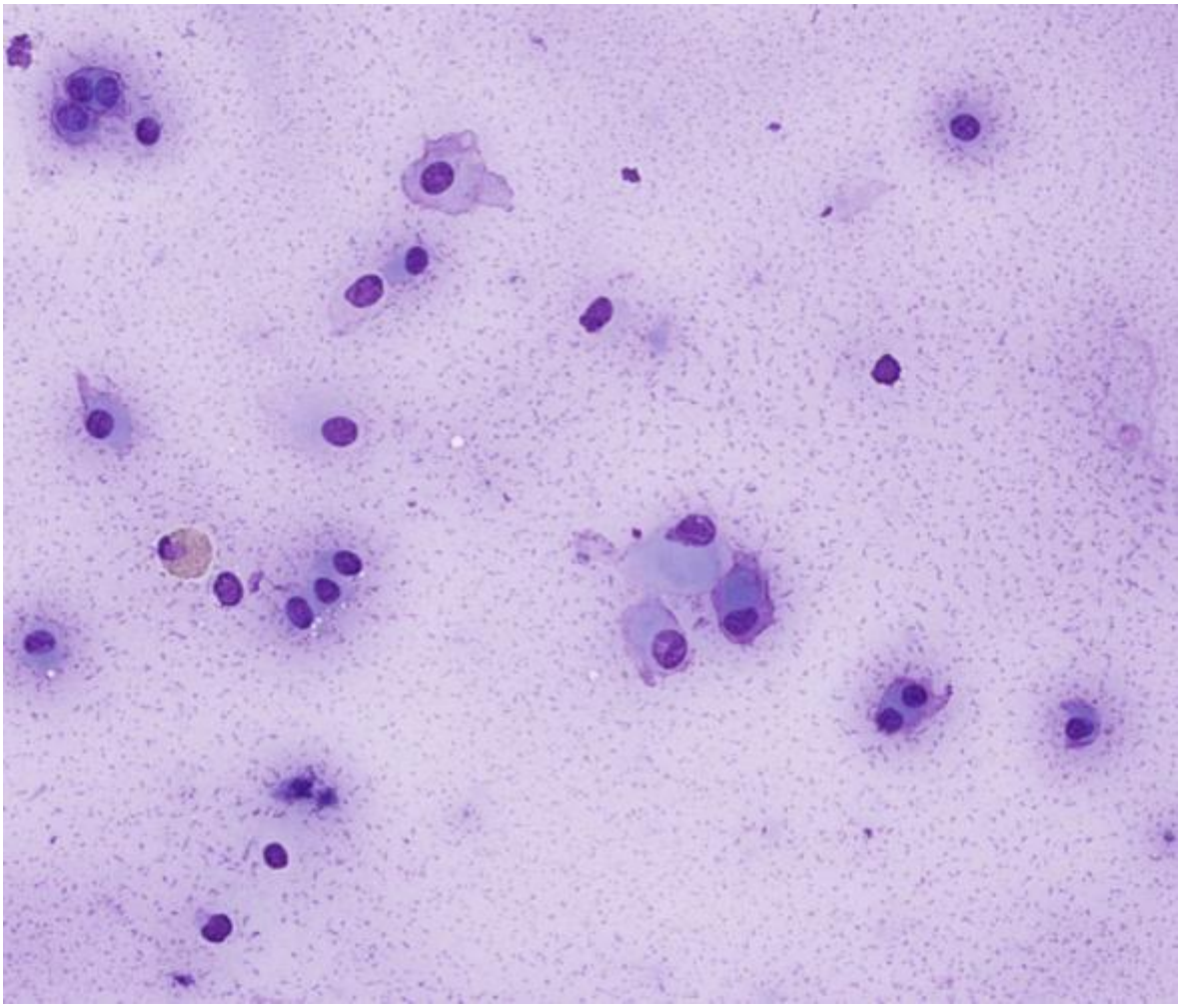

Figure 6: Fine needle aspirate from one of the dermal nodules. Wright-Giemsa stain, 400 x magnification.

Questions

1) What is your diagnosis, based on the cytologic findings and location of the lesions?

2) What are the main differential diagnoses?

3) What further tests would you recommend to clarify the diagnosis?

Case 7 – Abnormal peroxidase scatterplot and marked large unclassified cell counts on the ADVIA 2120i in a mixed-breed dog

Contributors

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Specimen

EDTA whole blood

Signalment

Mixed-breed dog, 13-year-old, castrated male

History

The patient was evaluated after acute smoke exposure from a residential fire. The dog developed acute respiratory distress syndrome (ARDS) secondary to smoke inhalation requiring intensive care; the condition resolved with appropriate treatment. Previous medical history included a mild increase in hepatic transaminase activity managed with nutraceutical therapy. Five months after initial presentation, the dog was re-admitted for recurrence of dyspnea.

Clinical findings

At the initial presentation (Day 1, following smoke exposure), the dog exhibited severe inspiratory dyspnea accompanied by obtunded mentation and marked mucosal cyanosis. Arterial blood gas analysis identified severe carboxyhemoglobinemia (18.7%). Three hours after admission, following oxygen supplementation via nasal cannula (estimated FiO_2 0.5), repeat arterial blood gas analysis showed a reduction in carboxyhemoglobin concentration to 7,6% and a ratio of arterial oxygen partial pressure to fractional inspired oxygen (PaO_2/FiO_2) of 168 mmHg. In conjunction with the acute smoke inhalation injury, diffuse pulmonary infiltrates identified by thoracic imaging and exclusion of cardiogenic pulmonary edema these findings fulfilled the updated ARDS Vet criteria for non intubated mild/moderate ARDS (PaO_2/FiO_2 >100 to ≤300 mmHg) (Balakrishnan et al., 2025).

Point-of-care ultrasound (POCUS) and thoracic radiographs revealed a severe interstitial pulmonary pattern, which progressed during the initial hours of hospitalization. Supplemental oxygen therapy was initially administered via nasal cannula; however, persistent hypoxemia prompted escalation to non-invasive ventilation using continuous positive airway pressure (CPAP) (positive end-expiratory pressure (PEEP) 5–7 cmH_2O, FiO_2 100%). Following initiation of CPAP, the dog demonstrated rapid improvement in carboxyhemoglobin concentration, accompanied by a slower but progressive clinical recovery, paralleled by serial improvement in both POCUS findings and thoracic radiographic abnormalities. The patient was successfully weaned from CPAP after 36 hours and was discharged from the hospital four days later.

A complete blood cell count (CBC) was performed using an ADVIA 2120i (Siemens Healthineers, Germany) on five occasions over a 5-month period (Day

1–3, Week 6, and Month 5). Results from the initial presentation (Day 1, following smoke exposure) are summarized in Table 1; results from subsequent evaluations are summarized in Table 2.

On all five evaluations, the automated WBC differential showed a markedly elevated large unclassified cell (LUC) count (range: 26.1–75.3%). The peroxidase (Perox) scatterplot demonstrated a downward shift of the neutrophil population into the LUC, monocyte and lymphocyte areas, whereas the basophil/lobularity (Baso) channel showed a normal leukocyte distribution (Figure 1).

Manual blood film examination revealed markedly discrepant neutrophil counts: true segmented neutrophil percentages ranged from 61% to 76%, in stark contrast to the 0.1–1.7% reported by the analyzer. Mild to moderate neutrophil toxic changes were present during both hospitalizations (initial admission and the Month-5 readmission) (Figure 2). In addition to the neutrophil toxic changes, blood film review revealed reactive (activated) lymphocytes on several evaluations and occasional activated monocytes at the Day 2 and Month-5 timepoints, graded semiquantitative as detailed in the table footnotes. No dysplastic or atypical features were observed in any leukocyte population.

Table 1. Automated CBC (ADVIA 2120i; Siemens Healthineers, Germany) and manual differential results from Day 1 evaluation.

Parameters	RI	Automated CBC (ADVIA2120i)	Manual differential
WBC (×10³/µL)	*5.0–14.1*	9.34	—
Metamyelocytes (%)	—	—	0
Band neutrophils (%)	—	—	2
Segmented neutrophils (%)	—	**0.8**	**67**
Lymphocytes (%)	—	**67.8**	**15**
Monocytes (%)	—	**1.9**	**15**
Eosinophils (%)	—	3.2	1
Basophils (%)	—	0.2	0
LUC (%)	*0–3.5*	**26.1**	N/A
Metamyelocytes (×10³/µL)	*0*	—	0
Band neutrophils (×10³/µL)	*0–0.3*	—	0.19
Segmented neutrophils (×10³/µL)	*2.9–11.1*	**0.08**	6.26
Lymphocytes (×10³/µL)	*1.0–4.8*	**6.33**	1.40
Monocytes (×10³/µL)	*0.15–1.35*	0.18	**1.40**
Eosinophils (×10³/µL)	*0.1–1.25*	0.30	**0.09**
Basophils (×10³/µL)	*0–0.1*	0.02	0
LUC (×10³/µL)	*0–0.4*	**2.43**	N/A
MPXI	—	22.5	—
RBC (×10⁶/µL)	*5.5–8.5*	8.08	—
HGB (g/dL)	*12.0–18.0*	16.5	—
HCT (%)	*37–55*	52.6	—

MCV (fL)	*62–72*	65.1	—
PLT (×10³/µL)	*170–400*	416	—
RBC morphology	—	—	WNL
WBC morphology	—	—	Act. lymph; neutro tox +/−−
PLT morphology	—	—	Adequate; clumps

Abbreviations: *Act. lymph = activated lymphocytes; HGB, hemoglobin; HCT, hematocrit; LUC, large unclassified cell; MCV, mean cell volume; MPXI = myeloperoxidase index; neutro tox = neutrophil toxic change; N/A = not applicable; PLT, platelet count; RBC, red blood cell count; WBC, white blood cell count; WNL = within normal limits. Morphological findings were graded semiquantitative according to the proportion of affected cells: +/−− (grade 1) = 25–50% of cells affected; ++/− (grade 2) = 50–75%; +++ (grade 3) = >75%. Findings below 25% were recorded as absent/occasional.*

Table 2. Automated CBC (ADVIA 2120i; Siemens Healthineers, Germany) and manual differential results from four subsequent evaluations (Day 2, Day 3, Week 6, and Month 5).

Parameter	**RI**	**Automated CBC (ADVIA2120i)**				**Manual differential**			
		Day 2	**Day 3**	**Week 6**	**Month 5**	**Day 2**	**Day 3**	**Week 6**	**Month 5**
WBC (×10³/µL)	*5.0–14.1*	**18.20**	**17.37**	9.56	13.89	—	—	—	—
Metamyelocytes (%)	—	—	—	—	—	0	0	0	1
Band neutrophils (%)	—	—	—	—	—	17	4	0	3
Segmented neutrophils (%)	—	0.1	0.2	0.6	1.7	63	76	76	76
Lymphocytes (%)	—	27.1	22.9	37.2	28.9	9	8	10	15
Monocytes (%)	—	0.3	0.6	0.2	1.3	10	10	6	2
Eosinophils (%)	—	0.0	0.6	4.7	4.2	1	2	8	3
Basophils (%)	—	0.3	0.4	0.2	0.3	0	0	0	0
LUC (%)	*0–3.5*	**72.2**	**75.3**	**57.1**	**63.6**	N/A	N/A	N/A	N/A
Metamyelocytes (×10³/µL)	*0*	—	—	—		0	0	0	**0.13**
Band neutrophils (×10³/µL)	*0–0.3*	—	—	—	—	**3.09**	**0.69**	0	0.39

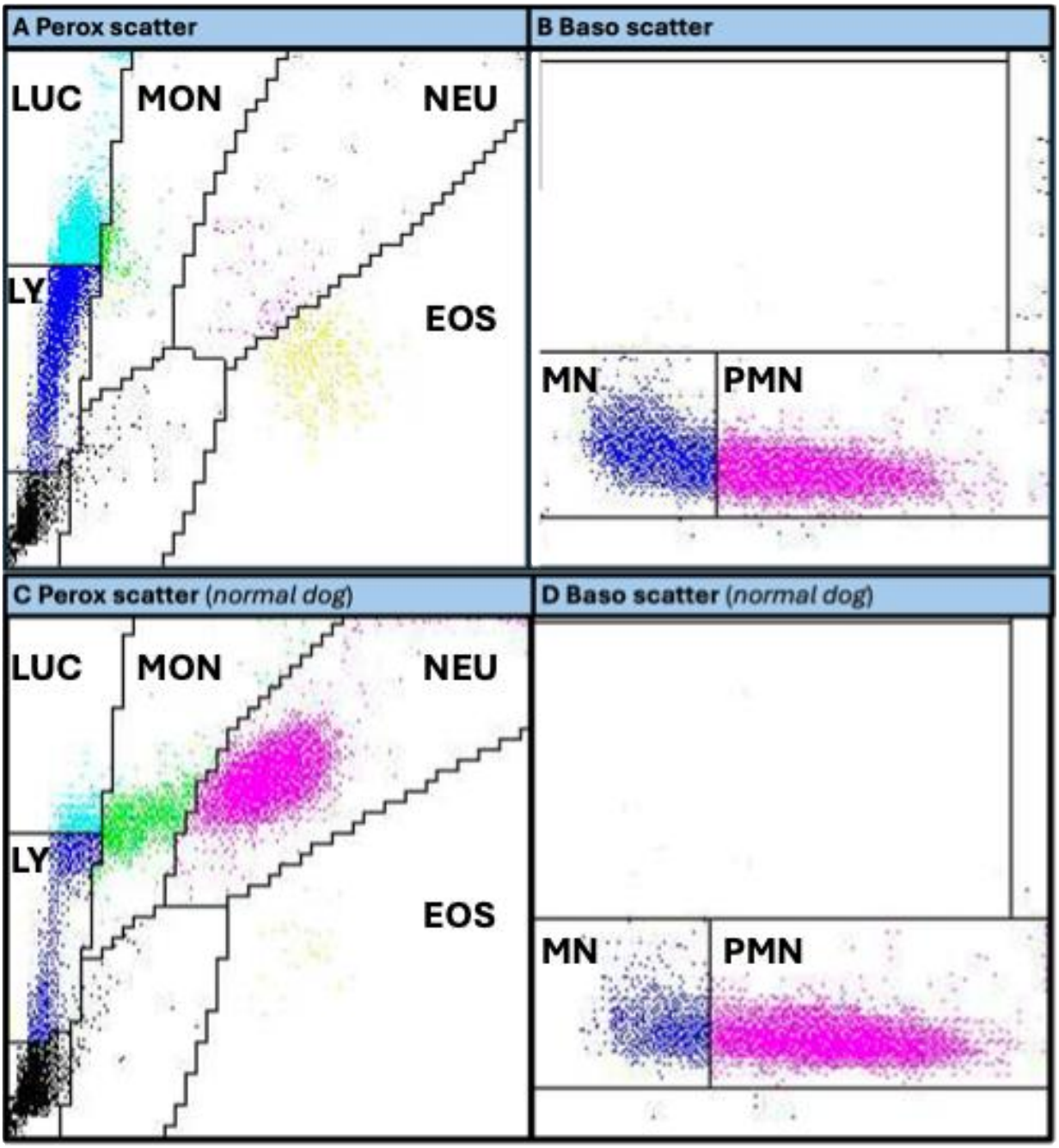
Segmented neutrophils (×10³/µL)	*2.9–11.1*	**0.02**	**0.03**	**0.05**	**0.23**	**11.47**	**13.20**	7.27	10.10
Lymphocytes (×10³/µL)	*1.0–4.8*	**4.93**	3.98	3.56	4.01	1.64	1.40	**0.96**	1.99
Monocytes (×10³/µL)	*0.15–1.35*	**0.05**	**0.10**	**0.02**	0.19	**1.82**	**1.74**	0.57	0.26
Eosinophils (×10³/µL)	*0.1–1.25*	**0.01**	0.10	0.45	0.59	0.18	0.35	0.76	0.39
Basophils (×10³/µL)	*0–0.1*	0.06	0.08	0.01	0.04	0	0	0	0
LUC (×10³/µL)	*0–0.4*	**13.13**	**13.07**	**5.46**	**8.83**	N/A	N/A	N/A	N/A
MPXI	—	**3.2**	**24.0**	**20.8**	**16.8**	—	—	—	—
RBC (×10⁶/µL)	*5.5–8.5*	6.83	6.82	7.23	7.10	—	—	—	—
HGB (g/dL)	*12.0–18.0*	14.4	14.2	15.4	15.3	—	—	—	—
HCT (%)	*37–55*	46.1	45.0	47.2	48.9	—	—	—	—
MCV (fL)	*62–72*	67.4	66.0	65.3	68.8	—	—	—	—
PLT (×10³/µL)	*170–400*	396	395	406	414	—	—	—	—
RBC morphology	—	—	—	—	—	Aniso +/−−	Aniso +/−−	Aniso +/−−	Aniso +/−−
WBC morphology	—	—	—	—	—	Act. lymph; neutro tox ++/−; act. mono +/−−	neutro tox +/−−	WNL	Act. lymph; neutro tox ++/−; act. mono +/−−
PLT morphology	—	—	—	—	—	Adequate; clumps	Adequate; clumps	Adequate	Adequate; clumps

Abbreviations: *Act. lymph = activated lymphocytes; Act. mono = activated monocytes; Aniso = anisocytosis; HGB, hemoglobin; HCT, hematocrit; LUC, large unclassified cell; MCV, mean cell volume; MPXI = myeloperoxidase index; neutro tox = neutrophil toxic change; N/A = not applicable; PLT, platelet count; RBC, red blood cell count; WBC, white blood cell count; WNL = within normal limits. Morphological findings were graded semiquantitative according to the proportion of affected cells: +/−− (grade 1) =*

25–50% of cells affected; ++/− (grade 2) = 50–75%; +++ (grade 3) = >75%. Findings below 25% were recorded as absent/occasional.

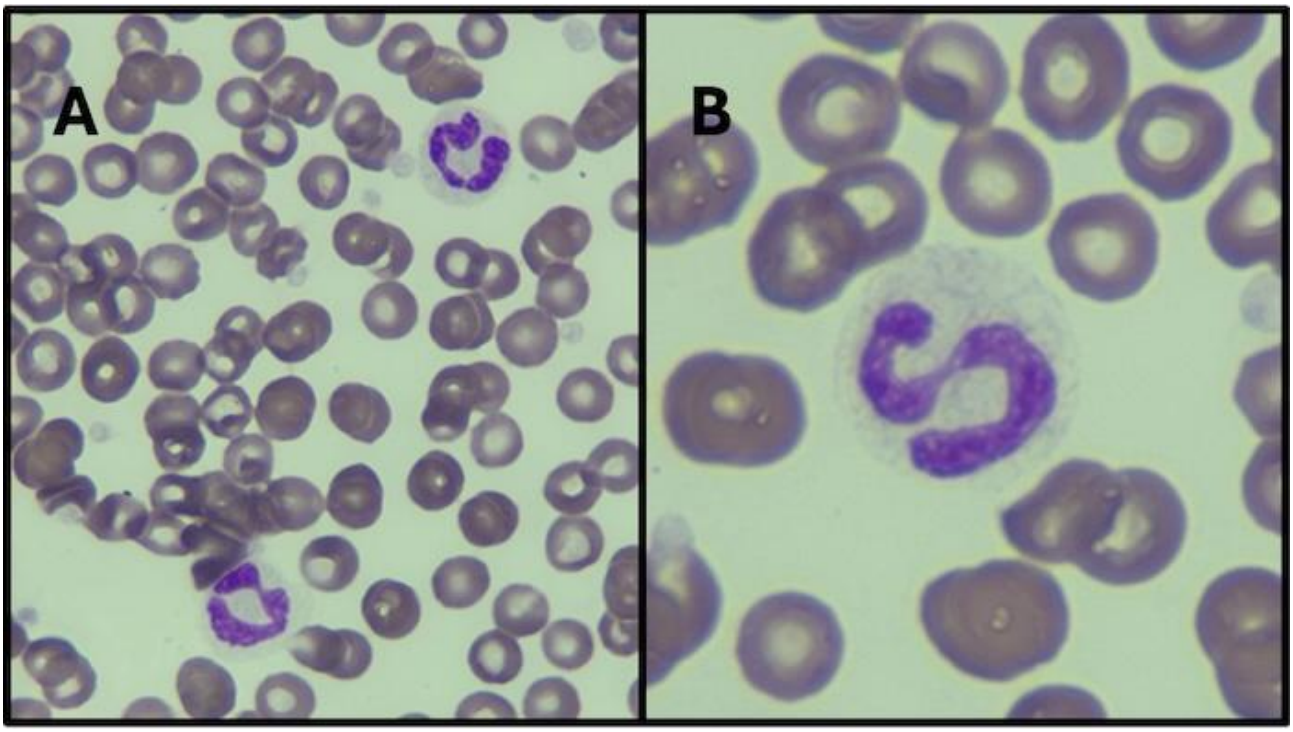
Notes:

Figure 1. Representative ADVIA 2120i scatterplots from the affected dog (Day 1, following smoke exposure) and normal dog.



ADVIA 2120i leukocyte scatterplots from the affected dog (A, B) and a healthy control dog (C, D). In the affected dog, the Perox channel (A) shows loss of the normal neutrophil cluster, with downward displacement into the low-peroxidase region and marked expansion of the LUC area, whereas the Baso channel (B) remains unremarkable. In the control dog, both the Perox (C) and Baso (D) scatterplots show normal leukocyte distribution. Abbreviation: LUC = large unclassified cells, LY = lymphocytes, MON = monocytes, NEU = neutrophils, EOS = eosinophils, MN = mononuclear, PMN = polymorphonuclear.

Figure 2. Segmented neutrophils with mild toxic changes at blood smear (Day 1, following smoke exposure).



Blood film from the affected dog (May-Grünwald Giemsa stain, ×100 oil immersion objective; panel B is a higher-magnification detail). Segmented neutrophils display normal nuclear segmentation with mild cytoplasmic toxicity, evidenced by slight basophilia and vacuolation.

Questions

1 - How do you explain the striking discordance between the automated neutrophil count (0.1–1.7%) and the manual differential (61–76%) across all five evaluations? What analytical or pathological mechanism could account for this finding?

2 - The ADVIA 2120i uses two independent optical channels to classify leukocytes: the Perox channel and the Baso channel. In this case, one channel is grossly abnormal while the other remains normal. What does this dissociation tell you about the underlying mechanism, and what is its diagnostic significance?

3 - Based on the clinical presentation, the CBC findings, and the scatterplot pattern, what is your diagnosis and what confirmatory test would you recommend?

SATURDAY 19 SEPTEMBER 2026

Plenary session: Mystery cytological and histological slides

MYSTERY SLIDE SESSION – ANATOMIC PATHOLOGY AND CYTOLOGY

Chiara Brachelente, Lluís Luján, Carlo Masserdotti

Introduction

Through the examination of digital slides and, where available, direct microscopic evaluation of glass slides, this session aims to encourage participants to exchange opinions on interesting, unusual, challenging or unexpected diagnostic cases in both anatomic pathology and clinical pathology/cytology.

The session

The selected cases will be made available to participants before the congress through the link provided by the Congress Organization. During the main session, the cases will be discussed collectively, and all attendees will be warmly invited to actively participate by answering questions, proposing differential diagnoses, commenting on the findings and discussing the final interpretations.

The session will include both histopathology/anatomic pathology cases and cytology/clinical pathology cases, with the aim of providing a broad and interactive diagnostic discussion across complementary areas of veterinary pathology.

All slides will remain available through the congress platform for the duration of the meeting and, if possible, for a short period after the congress, to allow participants to review the cases again and compare their own interpretation with the final discussion.

Selected cases

Anatomic pathology / histopathology cases

Case 1. Sea snake. Multifocal skeletal lesions and visceral nodules.

Case 2. Horse. Progressive swelling of the fetlock joint.

Case 3. Dog. Intranasal mass.

Case 4. Cat. Pleural effusion with mineralized thoracic lesions.

Case 5. Dog. Ovarian mass.

Cytology / clinical pathology cases

Case 6. Dog. Rarefaction of the vertebral bones, with enlargement of the liver and spleen.

Case 7. Dog. Abdominal mass and multiple nodules in the mediastinum.

Case 8. Cat. Mass in the abdominal skin.

Canine Lymphoma in Focus: Morphology, Immunophenotype, Molecular Insights and Therapeutic Strategies

35

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Lecture Content:
Canine lymphoma comprises biologically diverse lymphoid neoplasms, and primary nodal disease is the setting in which diagnostic standardisation is most relevant. This lecture will provide an integrated, pathology-centred update for veterinary pathologists and residents, moving from routine morphology to immunophenotype, molecular classification and therapy-oriented reporting. The World Health Organization approach remains the diagnostic framework: lymphomas are classified by architecture, cytomorphology, cell size, mitotic activity, maturation stage and immunophenotype. Diffuse large B-cell lymphoma, marginal zone lymphoma, peripheral T-cell lymphoma not otherwise specified, T-zone lymphoma and T-lymphoblastic lymphoma account for most canine nodal cases, but their behaviour differs; therefore, "aggressive" and "indolent" terminology is often more clinically informative than grade alone [1,2]. The lecture will review the practical value and limitations of cytology, histopathology, immunohistochemistry, flow cytometry and clonality testing. Fine-needle aspiration cytology is rapid and useful for detecting many large-cell lymphomas, but it cannot reliably provide complete subtype classification. Histopathology with immunohistochemistry remains required for complete classification, especially for clinical trials, ambiguous cytology, uncommon entities or cases with high clinical suspicion despite a negative aspirate [1,3]. Immunophenotyping is essential: immunohistochemistry preserves tissue architecture, while flow cytometry offers rapid, quantitative assessment from aspirates and is particularly useful for aberrant phenotypes such as T-zone lymphoma. Clonality testing can support difficult diagnoses or minimal residual disease assessment, but it should not replace immunophenotyping because false-positive, false-negative and lineage-discordant results may occur [1]. Recent molecular data are reshaping prognostic thinking. The K9 lymphoma assay has identified distinct B- and T-cell mutational profiles, including TRAF3, TP53, SETD2, SATB1 and FBXW7 alterations with potential biological and prognostic relevance (https://compbiomed.hpc4ai.unito.it/canine-dlbcl/) [4]. Whole-genome sequencing has further expanded the catalogue of coding and non-coding aberrations in canine diffuse large B-cell lymphoma, although routine implementation requires larger validation cohorts [5]. Therapeutically, cyclophosphamide, doxorubicin, vincristine and prednisone-based chemotherapy remains central for aggressive multicentric lymphoma, but relapse is common. Emerging strategies include molecular risk stratification, targeted agents, immunotherapy, anti-CD20 monoclonal antibodies, rabacfosadine and verdinexor, while rescue-protocol evidence remains difficult to compare because subtype and endpoints are inconsistently reported [6-9]. The key message is that modern lymphoma diagnosis should move beyond "B versus T" toward integrated classification that supports prognosis, treatment selection and trial design.

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Recognising, addressing, preventing abuse of power in hierarchical institutions, and the triple failure | Andrea Kübler (Germany)

No abstract has been provided

Notes:

Mistery cases in hematology includig bone marrow pathology

No abstract has been provided

Notes:

POSTER FLASH ABSTRACTS

ESVP/ECVP

Reverse-zoonotic mammalian-derived H5N1 viruses induce fatal multisystemic pathology in chickens and turkeys but predominantly upper respiratory tract lesions in ducks

438
ESVP/ECVP POSTER FLASH

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Background:
Highly pathogenic avian influenza A viruses of clade 2.3.4.4b H5N1 currently drive a panzootic in wild and domestic birds and have caused repeated spillover infections in mammals, including dairy cattle and humans. However, pathological consequences of spillback of mammalian-derived H5N1 viruses into avian hosts remain poorly characterised. This study compared organ tropism and lesion profiles induced by human- and bovine-derived H5N1 viruses in avian species.

Materials & Methods:
Chickens, turkeys and ducks were experimentally infected with human- or bovine-derived clade 2.3.4.4b H5N1 influenza A viruses and subjected to standardised necropsy. Major organs were examined histopathologically. Immunohistochemistry for influenza A virus matrix protein 1 was performed to assess tissue and cellular tropism.

Results:
Distinct species- and virus-dependent lesion patterns were identified. Both viruses induced severe systemic disease in chickens and turkeys, characterised by necrotising pneumonia, pancreatitis, encephalitis, myocarditis and marked lymphoid depletion, accompanied by widespread viral antigen distribution in parenchymal tissues. Endothelial infection was prominent in chickens and less pronounced in turkeys. Ducks showed predominantly upper respiratory tract lesions with limited systemic involvement and sparse viral antigen detection despite efficient viral shedding. The bovine-derived virus showed a trend towards broader parenchymal tropism in ducks, whereas the human-derived virus was associated with more severe encephalitis in chickens.

Conclusion:
Reverse-zoonotic mammalian-derived clade 2.3.4.4b H5N1 viruses remain highly virulent in poultry while inducing distinct host species-specific organ tropism and lesion profiles. Comparative pathological analysis provides important insights into host adaptation and the potential role of birds as amplifying hosts for mammal-adapted influenza viruses.

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

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ESVP/ECVP POSTER FLASH

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Background:
Several mycobacterial species cause diverse clinical syndromes in cats. *Mycobacterium avium* subsp. *hominissuis* (MAH) is an opportunistic 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.

Materials & 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. Complete post-mortem examinations were performed, and FFPE tissue samples were routinely processed for histopathology. Mesenteric lymph nodes were examined by Ziehl-Neelsen (ZN) staining, mycobacterial culture and 16S rRNA gene sequencing.

Results:
Abdominal lymphadenomegaly was the main ultrasonographic finding in both animals. 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 in the liver, spleen, and lungs were found. ZN staining identified an extremely high burden of acid-fast bacilli within the cytoplasm of 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.

Immunohistochemical markers of myocardial damage in horses: a pilot study.

411
ESVP/ECVP POSTER FLASH

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Background:
No immunohistochemical (IHC) markers of cardiac damage have been validated in horses. This pilot study aimed to describe the IHC features of cardiac troponin T (cTnT), desmin and connexin-43 (Cx43) in normal, fresh and autolysed, and damaged equine myocardium.

Materials & Methods:
Twenty-eight post-mortem equine hearts from the University of Liverpool were retrospectively divided into three groups: 8 fresh and 10 autolysed controls without concurrent cardiac pathology and 10 cases with chronic or acute myocardial lesions including fibrosis, haemorrhage, cardiomyocyte degeneration and necrosis. FFPE myocardial sections were immunolabelled for cTnT, desmin and Cx43. Labelling pattern and intensity were correlated with HE features.

Results:
In controls, desmin (7/8) highlighted a moderate granular sarcoplasmic and intercalated-discs (IDs) labelling. cTnT (8/8) showed strong sarcoplasmic fibrillary labelling. Cx43 (8/8) showed predominantly strong IDs' labelling with sparse membranous labelling. In all pathological hearts, IHC abnormalities were topographically associated with the corresponding HE changes: cTnT showed reduced sarcoplasmic and granular labelling. Desmin displayed irregular granular sarcoplasmic and scant IDs labelling. Cx43 showed reduced IDs labelling, with patchy sarcoplasmic and accentuated membranous labelling. All autolytic hearts showed cTnT and desmin diffuse loss of immunoreactivity (cTnT and desmin), while Cx43 showed reduced junctional labelling and variable mild cytoplasmic redistribution. Conduction fibres were better preserved than adjacent myocardium, with prominent membranous Cx43 and granular cTnT/desmin labelling.

Conclusion:
Cx43 IHC revealed a distinct staining pattern unique to pathological myocardium; whereas conduction fibre labelling was preserved in autolysed samples.

Histopathological prognostication and Immune Checkpoint Evaluation in Canine Lung Carcinomas

537
ESVP/ECVP POSTER FLASH

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Background:
Lung cancer is the leading cause of cancer-related mortality in humans but remains uncommon in dogs (15/100,000/year). Although immune checkpoint inhibitors have improved prognosis, further advances require robust preclinical models. This study evaluated pathological parameters, including PD-L1 expression (using a cross-reactive antibody), in canine lung carcinomas to improve prognostication and management, as potential model for human pathology.

Materials & Methods:
Thirty-two dogs with primary lung tumors were retrospectively analysed. Clinical and pathological data, including PD-L1 combined positive score (CPS) and outcomes, were collected. A simplified grading system was defined based on architectural differentiation, nuclear/nucleolar polymorphism, and mitotic count (cut-off: 15/2.37 mm²). Analyses included log-rank and Cox models. Inter-pathologist agreement was assessed using Gwet's AC1.

Results:
Median age was 11.1 years and mean survival time 256 days [84–449]. Histotypes (papillary vs other carcinomas) and simplified histological grade (Grade 1–2 vs 3) were significantly associated with overall survival (OS) (HR=2.49, p=0.0038; HR=3.72, p=0.0041) and disease-free interval (DFI) (HR=2.63, p=0.036; HR=12.07, p=0.00014). All grading features were individually associated with OS and DFI. PD-L1 positivity (CPS >1%) was identified in 84% of cases, similar to humans (72.2%). CPS interobserver agreement was high (AC1=0.76, $p=4.10^{-8}$). As in humans, CPS was not associated with clinical, histological, or survival parameters.

Conclusion:
The simplified histological grading system was strongly prognostic. Although CPS had no prognostic value, its reproducibility supports further investigation as a predictive biomarker for immunotherapy response. The relevance of immunopathological parameters in canine lung carcinomas warrants further investigation.

Microglial acquisition of Listeria monocytogenes from infected neurons drives early bacterial amplification in the CNS

444
ESVP/ECVP POSTER FLASH

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Background:
Neurolisteriosis, caused by *Listeria monocytogenes* (Lm) CNS invasion, affects humans and farmed ruminants and is a significant One Health concern. Resident microglia and infiltrating monocyte-derived macrophages (MDM) are the key phagocyte subsets in the inflamed CNS, but how each interacts with infected neurons remains poorly understood.

Materials & Methods:
Primary bovine microglia and MDM were co-cultured with a bovine neuron-like cell line (FBBC). The impact of cellular interactions on bacterial fate was assessed by confocal microscopy, live-cell imaging, and gentamicin protection assays, and validated against spatiotemporal analysis of microabscesses in bovine neurolisteriosis brain tissue.

Results:
MDM added early after infection reduced bacteria burden by 90% within 24 h without eradicating Lm, which continued to replicate in the neuronal cytosol. In contrast, microglia harbored ~10-fold more bacteria than MDM and increased the overall bacterial load in the culture. Microglia-neuron contacts lasted ~10 times longer than MDM-neuron contacts and were associated with bacterial transfer, while the shorter MDM contacts rarely resulted in bacterial acquisition. Infection with a cell-spread defective mutant and CD11b blockade indicated that MDM were infected predominantly through bacteria-driven spread. In contrast, microglia acquired bacteria not only through bacterial spread but also extracted them from neurons via a CD11b-dependent mechanism: CD11b blockade reduced intramicroglial numbers by ~ 50%. In situ analysis confirmed that microglia were closely associated with infected neurons and heavily infected themselves.

Conclusion:
CNS macrophage heterogeneity and macrophage–neuron crosstalk shape Lm infection dynamics, with microglia and MDM playing contrasting roles that together influence infection outcome.

Canine dementia – are there specific neuropathological findings?

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ESVP/ECVP POSTER FLASH

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Background:
Cerebral beta-amyloid (Aβ) deposition occurs both in normal canine aging and in Canine cognitive dysfunction syndrome (CCDS). Currently, the neuropathological changes specific to CCDS are poorly defined. This study aimed at identifying histopathological findings associated with CCDS, focusing first on Aβ distribution. We hypothesized that vascular Aβ deposition is specifically associated to CCDS.

Materials & Methods:
The cognitive status of 49 aged dogs (8–17 years) was assessed by the Canine Cognitive Dysfunction Rating Scale (CCDR), scores >50 indicating cognitive impairment. We visualized Aβ in post-mortem brain sections using immunohistochemistry (anti-β-amyloid, clone 4G8) and quantified the total Aβ load, leptomeningeal Aβ, capillary Aβ density, and the capillary-to-total Aβ ratio in frontal, occipital, and entorhinal cortex, hippocampus, and thalamus using digital image analysis (QuPath). Results were analyzed using linear regression, with CCDR score as the dependent variable, and adjusted with age.

Results:
Occipital leptomeningeal Aβ load and capillary-to-total Aβ ratio in the occipital cortex were significantly associated with increasing CCDR scores ($p < 0.05$) in age-adjusted analysis. The total Aβ load was not a significant factor in any region.

Conclusion:
We aimed to find Aβ-deposition patterns specifically associated with CCDS, in aging dogs. Our study is based on a substantial canine cohort with known cognitive status and included age as a confounder in the analysis. The results indicate that the occipital cortex may represent a relevant region and vascular-associated Aβ pathology may be a specific histopathological feature of CCDS in dogs.

More Than Just Jelly: Histopathological Progression and Diagnostic Nematocyst Features in Apolemia sp. Exposure in Atlantic Salmon

149
ESVP/ECVP POSTER FLASH

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Background:
Aggregations of gelatinous zooplankton, including siphonophores such as *Apolemia* sp., are an emerging threat to aquaculture. Exposure to venomous cnidocytes can compromise tissue integrity, fish welfare, and production. This study combined controlled in vivo challenges with field case analyses to define histopathological progression following *Apolemia* exposure, with emphasis on lesion development and the diagnostic value of special stains for nematocyst identification.

Materials & Methods:
A total of 150 juvenile salmon (~150 g) were exposed to *Apolemia* and sampled from 2 hours to 14 days post-exposure. Gill and skin tissues were examined histologically using routine and special stains.

Results:
Exposure induced rapid, progressive, gill-dominant pathology with clear nematocyst involvement. Early changes were minimal, although *Apolemia* fragments and nematocysts were present on tissues. Within 2 hours, acute vascular injury developed in the gills (congestion, haemorrhage, oedema, necrosis), with mild epidermal necrosis in skin.

By 2 days, lesions progressed to gill necrosis, thrombosis, and fibrino-suppurative branchitis, often with nematocyst-containing microabscesses. Skin lesions included necrosis, dermatitis, and early melanisation. By 7 days, gills showed epithelial hyperplasia, lamellar fusion, fibrin deposition, and tissue loss, often with secondary bacterial involvement; skin lesions progressed to deep dermatitis. By 14 days, lesions were predominantly chronic.

Conclusion:
Nematocysts appeared as refractile capsules with helical tubules, enhanced by Giemsa, toluidine blue, and Alcian Blue–PAS stains. These findings extend observations presented at EAFP congress 2025* by defining temporal lesion progression and confirming the diagnostic value of specialised staining in *Apolemia*-associated injury.

ESVCP/ECVCP

Comparison between the Siemens Immulite 2000 canine and human total T4 assays in canine and feline serum samples: preliminary results

334
ESVCP/ECVCP POSTER FLASH
Topic: Biochemistry/Endocrinology

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Background:
Although a canine total T4 (CTT4) assay is available for the Siemens Immulite 2000 analyser, some veterinary laboratories use the human total T4 (HTT4) assay for measurement of total T4 (TT4) concentrations in dogs and cats. Our aim was to evaluate the analytical agreement between the CTT4 and HTT4 assays in canine and feline serum samples.

Materials & Methods:
Residual canine and feline serum samples submitted to a diagnostic laboratory were included. TT4 concentrations were measured using both assays on the Siemens Immulite 2000 analyser. Samples with TT4 concentrations outside the analytical range of either assay were excluded.

Results:
Ninety-seven canine and 51 feline serum samples were analysed. In dogs, median (minimum, maximum) TT4 concentrations measured with the CTT4 and HTT4 assays were 2.61 (<0.50, 8.54) µg/dL and 2.06 (<1.00, 7.74) µg/dL, respectively. In cats, median TT4 concentrations were 2.78 (<0.50, 20.0) µg/dL and 2.22 (<1.00, 21.10) µg/dL, respectively. In dogs, Passing-Bablok regression identified significant constant bias (-0.333; 95% CI: -0.583 to -0.025) and proportional bias (0.869; 95% CI: 0.754 to 0.963). In cats, no significant constant (0.067; 95% CI: -0.382 to 0.359) or proportional bias (0.855; 95% CI: 0.745 to 1.010) was detected, although wider inter-assay differences were observed at higher TT4 concentrations in cats. Bland-Altman analysis showed a negative, but not significant mean bias in dogs (-0.682 µg/dL) or cats (-0.353 µg/dL).

Conclusion:
The HTT4 assay underestimated TT4 concentrations in dogs compared with the CTT4 assay. In cats, underestimation at higher TT4 concentrations also appears likely.

Integrating redox biomarkers and machine learning for preoperative grading of canine mast cell tumors

97
ESVCP/ECVCP POSTER FLASH
Topic: Biochemistry/Endocrinology

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Background:
Mast cell tumors (MCTs) are among the most common cutaneous neoplasms in dogs, with highly variable biological behavior. The role of systemic oxidative stress and machine learning in MCT progression remains poorly defined.

Materials & Methods:
Sixty-seven dogs with cytologically and histologically confirmed MCTs were prospectively included. Clinical staging was performed using lymph node, liver and spleen cytology. The tumors were graded according to the Patnaik and Kiupel systems, and Ki-67 index was assessed immunohistochemically. Histopathological evaluation of surgically excised lymph nodes was performed using Weishaar HN classification. Serum oxidative status was evaluated at presentation using reactive oxygen metabolites (d-ROMs), malondialdehyde (MDA), 8-hydroxy-2’-deoxyguanosine (8-OHdG) assays, and antioxidant capacity using the OXY-adsorbent test. Associations between redox biomarkers and clinicopathological variables were analyzed. Statistical analysis and machine learning modelling were performed using Python, and models were developed to predict Kiupel grade preoperatively.

Results:
Malondialdehyde concentrations were significantly higher in dogs with increased proliferative activity (Ki-67>23) ($p = 0.025$) and MDA levels tended to be higher in higher histological grades ($p = 0.051$) and across increasing lymph node HN grades ($p = 0.087$). No significant differences were identified for d-ROMs, 8-OHdG or antioxidant capacity. Machine learning analysis demonstrated good predictive performance, with support vector machines achieving the highest accuracy (0.86). Metastatic status was the most important predictor, while oxidative biomarkers contributed to model performance.

Conclusion:
Systemic oxidative alterations are associated with tumor proliferation in canine MCTs. Redox biomarkers and machine learning may contribute to preoperative risk stratification and prediction of tumor grade.

AGP and SAA in feline infectious peritonitis caused by FCoV-23: cross-sectional and longitudinal evaluation in naturally infected cats from Cyprus

490
ESVCP/ECVCP POSTER FLASH
Topic: Biochemistry/Endocrinology

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Background:
A pathogenic recombinant feline coronavirus emerged in Cyprus, causing an atypical FIP epizootic. This novel strain, termed FCoV-23, has demonstrated efficient direct transmission between cats. This study evaluated the dynamics of AGP and SAA in FCoV-23-associated FIP.

Materials & Methods:
Sixty-three cats (53 FIP cases, 10 controls: non-FIP sick cats) were included. FIP cases were classified as effusive, dry, or neurological. Serum AGP and SAA concentrations were measured using a Beckman AU480 analyser (latex agglutination immunoassay and immunoturbidimetry respectively) at diagnosis for cross-sectional comparison. Samples from a subset of FIP-positive cats that received treatment and had at least two sampling time points available were included for longitudinal analysis.

Results:
At diagnosis, neurological and dry cases exhibited the highest median AGP (2086.6, 1876.85 µg/mL) and SAA concentrations (19.45, 24.75 mg/L). SAA was significantly elevated in neurological and dry forms compared with controls (2.1 mg/L), whereas effusive cases showed high variability (4.75 mg/L). AGP followed a similar pattern but did not reach statistical significance (controls : 1402.1, effusive cases : 723.75 µg/mL). Longitudinally, AGP declined consistently, with the greatest reduction occurring during early treatment followed by stabilisation. In contrast, SAA showed rapid initial decreases but frequent fluctuations.

Conclusion:
In FCoV-23-associated FIP, AGP and SAA concentrations appear dependent on clinical manifestation. SAA appears more discriminatory than AGP at presentation, potentially reflecting the acute, high-virulence phenotype of this recombinant strain. While SAA captures short-term inflammatory dynamics and potential inflammatory flares, AGP may better reflect cumulative inflammatory burden and stabilisation during therapy.

Evaluation of hematological parameters in pre-race trotter horses and their association with racing performance

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ESVCP/ECVCP POSTER FLASH
Topic: Hematology

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Background:
Hematological findings are commonly used to assess health status and racing condition in horses. This study aimed to evaluate pre-race hematological parameters in trotter horses and their association with racing performance.

Materials & Methods:
A total of 92 pre-race hematological evaluations (RBC, HGB, HCT, MCV, MCH, MCHC, RDW, WBC with differential count, PLT, and MPV) were obtained from 17 trotter horses (2–4 years old, mares and geldings). Blood samples were collected on race days in the early morning before feeding. Complete blood counts were performed on Scil Vet abc Plus hematology analyzer (Scil Animal Care Company GmbH, Germany). Racing performance data were retrieved from the publicly available database of the Slovenian Trotting Association. According to race results, evaluations were assigned to three performance groups: P1 (best performance, n = 36), P2 (moderate performance, n = 24), and P3 (poorest performance, n = 32). Statistical analysis was performed using SigmaStat 4.0 and group comparisons were assessed using the Mann–Whitney rank-sum test ($P < 0.05$).

Results:
Horses in the P1 group showed significantly lower RBC, HGB, and HCT values compared to the P3 group ($P < 0.05$). No significant differences were observed among groups for erythrocyte indices, leukocyte counts, or platelet parameters ($P > 0.05$).

Conclusion:
Pre-race erythrocyte parameters differed between performance groups in trotter horses, with lower RBC, HGB, and HCT values observed in the best-performing group. The physiological significance of these findings remains unclear and requires further investigation involving larger populations and repeated measures.

Can the erythrocyte sedimentation rate predict the presence of electrophoretic abnormalities in dogs?

314
ESVCP/ECVCP POSTER FLASH
Topic: Hematology

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Background:
Erythrocyte sedimentation rate (ESR) increases during inflammation and correlates with inflammatory leukogram changes. No information is available about ESR in dogs with inflammatory alterations in serum protein electrophoresis (SPE) nor on its potential ability to predict such changes using ESR.

Materials & Methods:
ESR was measured in samples submitted for routine diagnostics and SPE. The frequency of increased or error-flagged ESR results and ESR values in dogs with normal (negative) versus inflammatory (positive) SPE patterns were statistically compared. A ROC curve was designed to assess the discriminating ability of ESR and determine optimal cut-off values.

Results:
The study included 59 positive and 41 negative samples. Increased/flagged ESR were more frequent in positive dogs using both the published upper reference limit (URL: 10 mm/hr) (73.2% vs 47.5%, P=0.010) and our laboratory URL (14 mm/hr; 68.3% vs 45.8%, P=0.025). Repeated measurements reduced flagged results, particularly in negative dogs, resulting in an even greater significant difference (70.7% vs 35.6%, P=0.000; 65.9% vs 33.9%, P=0.002). ESR was significantly higher (P<0.001) in positive dogs (27 mm/hr; 1-50 mm/hr) than in negative dogs (median ESR: 1 mm/hr, min-max: 1-29 mm/hr). An ESR cut-off of 29 mm/h yielded 100% specificity, while with a cut-off of 11 mm/h the positive likelihood ratio was >10. Sensitivity ranged from 40% to 60%.

Conclusion:
ESR is often increased in dogs with inflammatory SPE patterns. Values exceeding the URL, especially >29 mm/hr, strongly suggest electrophoretic inflammatory alterations, although normal ESR does not exclude them in clinical practice.

Analytical Performance of the Sysmex XN-1000V for Complete Blood Cell Count in Little Owls (Athene Noctua)

378
ESVCP/ECVCP POSTER FLASH
Topic: Hematology

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Background:
Background: Automated complete blood count (CBC) is pivotal for health assessment of wild birds, but is complicated by nucleated erythrocytes and thrombocytes, which interfere with blood cell classification. The Sysmex XN-1000V analyzer, equipped with the PLT-F application, may improve blood cell discrimination in avian species. Aim: To evaluate Sysmex XN-1000V analytical performance in *Athene noctua*, a wild European little owl species, before release from rescue centres.

Materials & Methods:
Materials and Methods: Fifty-five heparinised blood samples were analysed with the Sysmex XN-1000V using the WNR and PLT-F channels with specific gating strategies and results were compared with RBC and thrombocyte+leukocyte (T+WBC) counts obtained with a Neubauer hemocytometer and blood smear examination. Paired statistics and method comparison tests were performed.

Results:
Results: RBC in native samples exceeded the detection limit for nucleated cells count with WNR channel, requiring a 1:3 dilution for analyses, whereas PLT-F channel allowed direct analysis. Instrumental RBC counts were highly correlated (r=0.822), with Passing–Bablok (intercept −0.09; slope 1.04) and Bland–Altman showing no bias or proportional error. Manual RBC counts were higher than instrumental ($p<0.0001$) and variable. For T+WBC, no significant differences were found between manual and instrumental counts ($p= 0.372$ vs PLT-F and $p= 0.466$ vs 1:3 WNR), but correlation and agreement were poor ($r<0.3$; mean difference= 2.6 vs PLT-F; 4.2 vs 1:3 WNR).

Conclusion:
Conclusion: Sysmex XN-1000V appears suitable for CBC in little owls. PLT-F and WNR channels were interchangeable for RBC counts, although PLT-F avoided sample pre-dilution. T+WBC measurement requires specific gating strategies and blood smear validation.

Relationship between Immature Platelet Fraction, C-Reactive Protein and iron status in thrombocytopenic dogs: a preliminary study

392
ESVCP/ECVCP POSTER FLASH
Topic: Hematology

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Background:
Thrombocytopenia is a common haematological abnormality in dogs and may arise from heterogeneous aetiologies, including immune-mediated diseases, infectious agents, neoplasia and bone marrow disorders. The immature platelet fraction (IPF) reflects bone marrow platelet production, whereas C-reactive protein (CRP) and serum iron are indicators of systemic inflammation and iron metabolism. However, the relationship between platelet regenerative response, inflammatory status and iron homeostasis in canine thrombocytopenia remains unclear.

This study aimed to investigate the association between IPF, CRP, and serum iron in thrombocytopenic dogs and evaluate whether IPF stratification using a cut-off is associated with different inflammatory and iron profiles.

Materials & Methods:
Eighty-five thrombocytopenic dogs, confirmed by peripheral blood smear examination, were included. IPF, CRP and serum iron values were recorded. Dogs were further stratified into two groups based on IPF values (≤6.9% and >6.9%) and statistical analyses were conducted.

Results:
No significant correlation was identified between IPF and CRP or serum iron in the overall population or within stratified groups. A significant inverse correlation was observed between CRP and serum iron in the overall cohort and in both IPF subgroups.

Conclusion:
In thrombocytopenic dogs, IPF does not appear to be associated with systemic inflammatory status or iron levels, suggesting platelet regenerative activity is probably independent of these pathways. Conversely, the inverse relationship between CRP and serum iron confirms the expected link between inflammation and iron sequestration. Further studies are warranted to determine whether these associations may differ when thrombocytopenic dogs are categorized according to the underlying aetiology.

Effect of plasma interferences on MCHC and optically derived MCHC (MCHC-O) in canine samples using the Sysmex XN-1000V

440
ESVCP/ECVCP POSTER FLASH
Topic: Hematology

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Background:
Increased mean corpuscular hemoglobin concentration (MCHC) in canine samples is commonly interpreted as an analytical artefact, often limiting erythrocyte evaluation. Interferences such as hemolysis, lipemia and icterus are known to falsely increase MCHC, particularly when hemoglobin is measured using spectrophotometric methods. However, their impact on alternative hemoglobin-derived parameters remains incompletely characterized.

Materials & Methods:
Canine complete blood count results obtained with a Sysmex XN-1000V, from 2021 to 2025, were retrospectively reviewed. After data cleaning, 3531 samples were included as a non-interfered group and 611 with analytical interferences (hemolysis, lipemia, and icterus). Samples were classified according to the presence or absence of interferences based on visual plasma assessment. MCHC and MCHC-O, derived from spectrophotometric and RET-channel hemoglobin measurements, respectively, were compared using non-parametric tests. The proportion of results exceeding the upper reference limit (URL; 37 g/dL) was evaluated.

Results:
Compared with non-interfered samples, MCHC was significantly increased in hemolyzed, lipemic, and hemolyzed-lipemic samples (all $p < 0.0001$), whereas MCHC-O was significantly affected only by hemolysis ($p = 0.0194$). Icterus did not significantly affect either parameter. The proportion of results exceeding the URL was significantly higher for MCHC in interference groups, increasing from 2.8% in non-interfered samples to 31.8%, particularly in lipemic samples. No significant increase was observed for MCHC-O.

Conclusion:
Lipemia and hemolysis increased the proportion of MCHC results exceeding the URL, whereas MCHC-O remained largely unaffected. MCHC-O may represent a more reliable parameter when analyzing samples with hemolysis or lipemia.

Analytical Validation of an Automated Immunoturbidimetric Assay for Feline Serum Alpha-1-Acid Glycoprotein

243
ESVCP/ECVCP POSTER FLASH
Topic: Lab Quality Management

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Background:
Several analytical methods for measuring serum α1-acid glycoprotein (AGP) have been reported. Recently, a feline-specific latex agglutination immunoassay (Shima Lab) has been released. The study aims to validate this method in feline serum using the AU480 Beckman chemistry analyser.

Materials & Methods:
Imprecision, linearity, limits of blank, detection and quantification, interference, stability and spike recovery were assessed. Frozen patient sample pools (FPSP) were validated as quality control material (QCM), and TEa was calculated using a "reverse engineering" approach. Overlap performance was preliminarily assessed in 57 cats with confirmed FIP, inflammatory and non-inflammatory diseases.

Results:
The assay demonstrated good intra- and inter-assay imprecision (1.5%-1.6% and 2.7%-3.7%, respectively) and acceptable bias based on spike recovery experiments (7.8%). Linearity was confirmed across the range indicated by the manufacturer. Storage at +4°C and -20°C did not significantly affect AGP for the tested periods (five and 90 days, respectively). Haemolysis and lipaemia did not significantly interfere, while marked hyperbilirubinaemia affected AGP determination. FPSP QCM was fully validated with an established TEa of 16% using a $1_{2.5s}$ control rule. The preliminary overlap performance results showed significantly different AGP concentrations in the three groups ($p<0.05$) with a pattern similar to that reported in previous literature.

Conclusion:
Shima Lab assay demonstrated acceptable performance for serum fAGP determination. FPSP can be reliably used as QCM following appropriate validation. The assay can differentiate between cats with FIP, inflammatory diseases, and non-inflammatory diseases. However, establishment of kit-specific medical decision limits and assessment of their sensitivity and specificity are warranted.

ORAL ABSTRACTS ESVP-ECVP

How much do you know about pre-neoplastic, noninvasive and dysplastic lesions in the urinary bladder of dogs and cats?

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Topic: Small Animals

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Background:
Preneoplastic, non-invasive and dysplastic bladder lesions in domestic animals are very common in diagnostic settings but lack a standardised classification. Consequently, dysplastic lesions and urothelial carcinoma in situ may be under-recognised in routine practice, while some hyperplastic lesions may represent biologically distinct entities. This study aims to characterise the morphological features of these lesions in dogs and cats and propose a classification system based on human criteria.

Materials & Methods:
Forty-one canine and feline bladder biopsies and surgical full-thickness samples (MyLav, Italy; UQ, Australia) were re-assessed based on WHO 2022 criteria. Cases were evaluated in HE-stained sections using standardized histological features: growth pattern, maturation, polarity, stratification, stroma-epithelial interface, cytological atypia, mitotic activity, umbrella cells, denudation, inflammation and microvascular proliferation. The classification system included flat and papillary hyperplasia, dysplasia, reactive atypia, carcinoma in situ (CIS), papilloma, and papillary urothelial neoplasms of low malignant potential (PUNLMP), von Brunn's nests, florid von Brunn's nests, cystitis cystica, cystitis glandularis and inverted papilloma.

Results:
Von Brunn's nests was the most common diagnosis (N=12), followed by reactive atypia (N=10), flat hyperplasia (N=8), CIS (N=7), papillary hyperplasia and dysplasia (N=6). Florid von Brunn's nests and cystitis cystica occurred in 4 and 3 cases, and inverted papilloma and papilloma, 2 each. PUNLMP and cystitis glandularis accounted for 1 case each. Three lesions were borderline CIS/dysplasia.

Conclusion:
This study shows that a human-based classification system can be applied to bladder lesions in dogs and cats, helping define the morphological spectrum from reactive changes to dysplastic lesions and CIS.

Rethinking canine portal vein hypoperfusion: evidence for a porto-sinusoidal vascular remodeling spectrum

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Topic: Small Animals

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Background:
Porto-sinusoidal vascular disease (PSVD) in humans comprises non-cirrhotic vascular liver disorders. In dogs, reduced portal venous profiles (RPVP) are associated with portal vein hypoperfusion (PVH) secondary to portosystemic shunting or portal vascular abnormalities. Because primary portal vein hypoplasia is considered a diagnosis of exclusion, this study investigated PSVD-like clinicopathological features in dogs without shunting and/or portal vein thrombosis.

Materials & Methods:
Dogs with histological evidence of RPVP and clinical suspicion of portal hypertension and/or increased liver enzymes were retrospectively included. Histological assessment included portal vein reduction or stenosis, portal fibrosis, and nodular regeneration. Immunohistochemistry with CD31, factor VIII-related antigen, and α-smooth muscle actin (α-SMA) was performed to assess vascular remodelling.

Results:
Fifteen liver biopsies from 14 dogs, including one follow-up biopsy, were evaluated. Seven dogs had congenital portosystemic shunts (PSS), two acquired PSS, and five had no shunting. Two dogs showed portal hypertension, and all dogs had elevated liver enzymes. Vascular histological changes were non-specific across groups and included portal vein reduction or stenosis and increased arteriolar profiles. Portal fibrosis without cirrhosis was identified in acquired shunting. CD31 highlighted sinusoidal capillarization more consistently than factor VIII in all biopsies, whereas α-SMA confirmed portal stromal remodeling in 2 cases. Follow-up in four non-shunting dogs showed stable clinical conditions under medical management, with partial improvement or stabilization of liver enzymes in three cases.

Conclusion:
RPVP in non-shunting dogs may reflect a spectrum ranging from primary portal vein hypoplasia to adaptive porto-sinusoidal vascular remodeling, overlapping with PSVD-like conditions.

Diagnostic utility of bacterial 16S rRNA qPCR and fluorescence in situ hybridization in canine endocarditis

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Topic: Small Animals

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Background:
Identifying the causative agent of endocarditis is frequently challenging. The aim of this study was to assess whether bacterial 16S rRNA qPCR and fluorescence in situ hybridization (FISH) were of diagnostic benefit in determining the cause of endocarditis in dogs.

Materials & Methods:
This retrospective study included 43 cases of endocarditis in dogs submitted from 2004 through 2019 at North Carolina State University. The presence and location of bacteria within the lesion were assessed using H&E sections and FISH with the eubacterial probe EUB-338. One punch biopsy per block, either at the site of bacteria (if visualised on H&E) or within an area of inflammation, was used for DNA extraction. Quantitative PCR was performed targeting four bacterial 16S rRNA gene regions. For FISH positive qPCR negative cases, DNA extraction and qPCR were performed on a second biopsy at the site of bacteria visualised by FISH.

Results:
Bacteria were visible on H&E sections in 25 cases (58%), and by FISH in 20 cases (47%). All the FISH positive cases were H&E positive. After comparison of the serial sections, 3 FISH negative cases were lacking H&E bacteria clusters. Among the 16 qPCR positive cases (37%), including 3 cases identified only following FISH, bacteria were appreciated on H&E sections in 15 cases. The most frequently identified bacteria were *Streptococcus* (*n* = 8) and *Pasteurella* (*n* = 3, including 1 dog co-infected with *Bartonella henselae*).

Conclusion:
Bacterial 16S rRNA qPCR and FISH were of diagnostic utility in cases in which bacteria were visible on H&E sections.

Morphologic, Histochemical, and Immunohistochemical Features of Feline Nasal Hamartomas

437
Topic: Small Animals

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Background:
Feline nasal hamartomas are rare non-neoplastic lesions poorly characterized in veterinary pathology. This study aimed to describe their morphological, histochemical, and immunohistochemical features and evaluate proliferative activity and hormone receptor expression.

Materials & Methods:
Ten feline nasal hamartomas (1 seromucinous epithelial, 1 vascular, 4 COREAHs, and 4 MNHs) were retrospectively evaluated. Cats were predominantly Domestic Shorthairs (mean age, 7.43 years; 2 intact females, 4 spayed females, and 4 neutered males). Computed tomography identified space-occupying nasal masses in all cases. Immunohistochemistry included pancytokeratin, CK19, α-SMA, ER, and PR, with factor VIII-related antigen applied to the vascular hamartoma. PAS, PAS-diastase, Alcian blue pH 2.5, and Masson's trichrome were performed on the seromucinous hamartoma. Ki-67 index was quantified using QuPath in the most immunoreactive 1 mm^2 area

Results:
Morphological classification was straightforward in adequately represented samples, whereas small endoscopic biopsies limited diagnostic interpretation. All epithelial components showed positivity for pancytokeratin and CK19. α-SMA-positive cells were identified in all lesions except the seromucinous hamartoma. Endothelial cells in the vascular hamartoma were positive for factor VIII-related antigen. Histochemical analysis of the seromucinous hamartoma demonstrated neutral and acidic mucins within an edematous stroma. ER expression was detected in all cases, whereas PR expression was variable. The Ki-67 index was low (median, 2.6%; range, 1.6–6.5%).

Conclusion:
Adequate sampling allowed morphologic classification, whereas small biopsies remained diagnostically challenging. Feline nasal hamartomas are heterogeneous lesions characterized by low proliferative activity and variable epithelial and mesenchymal components. Hormonal receptor expression may suggest a role in their pathogenesis.

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

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Topic: Large Animals

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Background:
Catalonia has been officially bovine tuberculosis (TB)-free since 2023. However, domestic and wildlife reservoirs continue to threaten this status. Within eradication programs, slaughterhouse surveillance remains a key component for early outbreak detection alongside testing-and-culling strategies. The Catalan slaughterhouse support network (SESC; SESC.cat) coordinates the analysis of lesions compatible with TB (i.e. granulomas). Nevertheless, submission rates of suspect lesions remain suboptimal and largely depend on the ability of meat inspectors and assistants to recognize compatible gross lesions. To address this limitation, we developed an AI-based tool to classify lesions as TB-risk (requiring laboratory confirmation) or Non-TB.

Materials & Methods:
A dataset of 1,000 annotated images with associated diagnostic metadata was retrieved from the SESC archives. Due to the scarcity of well-annotated images, particularly for less frequent granuloma categories, a hybrid training strategy combining synthetic and real data was implemented. Synthetic images were generated using a 3D blending pipeline embedding annotated lesions into tissue backgrounds, followed by augmentation to enhance variability and realism. The model was subsequently fine-tuned using approximately 1,000 real-world images representative of routine slaughterhouse inspection conditions.

Results:
On an independent test set of 104 images, the model achieved 90.7% sensitivity and 60.6% specificity for discriminating TB-risk granulomas from Non-TB lesions and normal tissues, corresponding to an overall accuracy of approximately 81%.

Conclusion:
Combining limited real-world datasets with synthetic image generation enabled robust model performance. This approach provides a strong basis for AI-assisted support tools in slaughterhouse surveillance, improving granuloma detection towards performance levels comparable to trained pathologists.

Lumpy skin disease outbreak in Catalonia

367
Topic: Large Animals

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Background:
Lumpy skin disease (LSD), caused by lumpy skin disease virus (LSDV; *Capripoxvirus lumpyskinpox*), affects cattle and water buffalo and is transmitted mainly by hematophagous arthropods. The disease is characterised by fever, lymphadenopathy and nodular dermatitis progressing to necrosis and ulceration. In the European Union, LSD is classified as a category A disease requiring immediate eradication. In October 2025, LSD was diagnosed in Spain for the first time, following outbreaks in North Africa and Southern Europe. The index outbreak was detected through passive surveillance in northeastern Catalonia (Alt Empordà), followed by 19 additional outbreaks in Catalonia and Aragón, mostly within compulsory vaccination zones.

Materials & Methods:
Skin samples from all Catalan outbreaks and tissues from three complete necropsies were collected to characterise lesion distribution and viral tropism. Viral presence was confirmed by qPCR, lesion chronicity was assessed histologically, and viral replication was evaluated using in situ hybridisation. Vaccination response was additionally assessed in two vaccinated farms.

Results:
Histopathology revealed the characteristic proliferative and nodular dermatitis associated with LSD, with viral RNA detected in epidermis, follicular epithelium, adnexal glands, piloerector muscle, blood vessels and dermal cells. Viral RNA detection decreased in chronic lesions. In addition, lesions not commonly described in LSD, including interstitial emphysematous pneumonia and proliferative pleuritis, showed high levels of viral replication. Anatomopathological staging suggested viral circulation in Catalonia since at least August 2025.

Conclusion:
These findings expand the pathological characterisation of LSD during its first emergence in Spain and support the value of pathology-based surveillance for recognition and investigation of this emerging transboundary disease.

Novel finding of Rustrela virus in Swedish horses with encephalitis

166
Topic: ESVP/ECVP Abstract

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Background:

Rustrela virus (RusV; species *Rubivirus strelense*; family *Matonaviridae*) has been described in cases of encephalitis in a wide range of animal species, including a donkey, but so far not in horses. Recent research indicates RusV as the cause of feline Staggering disease, a serious neurological disease seen endemically in cats within the lake Mälaren region in Sweden. In this study we explored RusV as a potential etiology of encephalitis in Swedish horses.
Materials & Methods:

Formalin-fixed, paraffin-embedded brain and/or spinal cord tissues from 11 horses, comprising 6 cases with a diagnosis of non-suppurative encephalitis, encephalomyelitis or meningoencephalitis and 5 without, were evaluated. All horses were examined postmortem at the Swedish University of Agricultural Sciences, Uppsala, Sweden, between 2007-2021. From each horse, a minimum of two locations in the central nervous system were investigated for presence of RusV capsid protein using immunohistochemistry, and for RusV RNA using RT-qPCR. Additionally, RT-qPCR for Borna disease virus-1 (BoDV-1) was performed in encephalitic horses.
Results:

RusV capsid protein was detected in three encephalitic horses. Immunostaining was variably detected intracytoplasmically in brain and spinal cord neurons, and in occasional glial cell. RusV RNA was detected by RT-qPCR in all three cases. All encephalitic horses were negative for BoDV-1. Horses without encephalitis were negative for RusV antigen and RusV RNA.
Conclusion:

The results indicate RusV as a cause of encephalitis in Swedish horses. Further investigations into the pathogenesis, including transmission routes, are warranted.

Exploring the etiology of intestinal dilatation syndrome in commercial brown layers

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Topic: Large Animals

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Background:
Intestinal Dilatation Syndrome (IDS) is an emerging disease of free-range breeder and laying hens characterized by severe heterophilic enteritis and marked jejunal dilatation. Although an infectious origin has been proposed, the etiology remains unresolved. This study aimed to demonstrate the IDS clinicopathological progression, transmissibility and etiology.

Materials & Methods:
Eight healthy, 67-week-old hens (Group A) were co-housed with eight IDS-affected, 75-week-old hens (Group B) for 80 days to evaluate natural transmission. Clinicopathological progression was monitored using periodic hematology and serum protein electrophoresis. Four hens from each group were euthanized 50 days after the beginning of the experiment, and the remaining hens were euthanized after 80 days. Postmortem examination, histopathology, and intestinal metatranscriptomic analysis by RNA sequencing were performed.

Results:
Hematological analysis revealed heterophilia in Group A and lymphocytosis and monocytosis in Group B at the moment of euthanasia; both supported by increased α-2, β-1, and γ-globulin fractions. From Group A, 75% of hens developed IDS-compatible lesions, characterized by heterophilic/lymphoplasmacytic jejunitis with crypt hyperplasia and ganglioneuritis. RNA sequencing revealed intestinal dysbiosis in IDS-affected hens, with enrichment of *Campylobacter jejuni*, *Enterococcus cecorum*, *Clostridium colinum*, *Escherichia coli* and *Fusobacterium mortiferum*. Viral sequences, including galliviruses and gammacoronaviruses, were predominantly detected in IDS-affected birds.

Conclusion:
This study suggests that IDS is a naturally transmissible disease. Likely, intestinal dysbiosis plays a fundamental role in the development of this syndrome. However, the limited number of animals and the molecular limitations of the study do not yet offer a clear infectious candidate for this syndrome.

Comparative pathology and viral tropism of HPAIV H5N1 clade 2.3.4.4b in wild birds from Germany (2025-2026)

146
Topic: Exotic/wildlife animals

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Background:
Highly pathogenic avian influenza virus (HPAIV) H5N1 clade 2.3.4.4b causes significant mortality in wild birds and poultry worldwide. Comparative inter-species analyses of lesion patterns and viral antigen distribution are limited. This study investigates lesions and viral antigen distribution in different wild bird species during a major outbreak in Germany (2025-2026), aiming to identify distinct host-dependent patterns.

Materials & Methods:
A total of 26 wild birds, including 5 Eurasian cranes, 15 geese, 1 teal and 5 raptors (3 common buzzards, 2 peregrine falcons), were examined postmortem. HPAIV infection was confirmed in all animals by RT-qPCR. Multiple organs were sampled for histopathology (HE) and immunohistochemistry (IHC) targeting influenza A matrix protein. Lesions and antigen were scored semi-quantitatively based on severity and distribution. Additionally, viral genome sequencing was conducted in a subset of birds to investigate viral phylogeny.

Results:
Across all species, widespread multisystemic disease was observed. Lesions were mainly lymphohistiocytic to granulomatous and necrotizing inflammation, depending on the species. In cranes, antigen-associated lesions were evenly distributed, whereas other species showed lesions more confined to individual organs, especially in the liver, pancreas, and spleen. Immunohistochemistry revealed especially high numbers of antigen-positive cells in the central nervous system and pancreas across species. Sequencing identified closely related viruses of the EA-2024-DI.2.1 genotype; cranes formed a monophyletic cluster, while other species showed greater variability.

Conclusion:
These findings demonstrate systemic HPAIV infection with indication of species-specific lesion patterns. Further studies are needed to clarify underlying mechanisms and epidemiological implications of the observed variations in organ involvement.

Between the devil and the deep blue sea: avian cholera can cause mass mortality amidst H5N1 HPAI outbreaks in Antarctica

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Topic: Exotic/wildlife animals

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Background:

Antarctica's relative paucity of people and high wildlife densities cause wildlife mortality to be easily missed, leading to lack of knowledge of such events. Avian cholera, similar to high pathogenicity avian influenza virus infection, can cause peracute death and mass mortality in a variety of bird species. Our aim is to describe how diagnoses was made based on samples taken for detection of avian influenza.
Materials & Methods:

March 2024, an unusual mortality of seabirds at the Danger Islands in Antarctica was detected, including thousands of Adélie penguins (*Pygoscelis adeliae*). Carcasses were mainly adults in good body condition. Samples were collected for molecular detection of infectious agents (33 penguins, 11 skuas, 3 sheathbills, 1 giant petrel) and histology (8 penguins, 1 sheathbill).
Results:

Pathological findings revealed that although influenza virus was present at low levels in some carcasses, the most likely cause of the mortality for the adult Adélie penguins was avian cholera. Diagnosis was based on the combination of *P. multocida* DNA by quantitative PCR, detection of micro-abscesses by histology in liver, and co-localization of the *P. multocida* bacteria with micro-abscesses, demonstrated via a newly developed *in situ* hybridization assay. Sequencing and phylogenetics on *P. multocida* bacteria from this outbreak as well as historical ones showed the same strain has been circulating for 25 years in the Antarctic Peninsula.
Conclusion:

This study confirms avian cholera as a relevant cause of mortality in Antarctica. Based on our results differentiation from avian influenza related mortality can be achieved through using molecular techniques of bird carcass tissues.

Systemic Apolipoprotein A-II Amyloidosis in Toco Toucans (Ramphastos toco)

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Topic: Exotic/wildlife animals

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Background:
In avian species, amyloidosis has so far been classified into systemic AA amyloidosis and cerebral Aβ amyloidosis. Here, we describe systemic apolipoprotein A-II (ApoA-II) amyloidosis in two toco toucans (*Ramphastos toco*), representing a novel form of avian amyloidosis.

Materials & Methods:
Two captive toco toucans, aged 3 and 7 years, that died of bacterial necrotising hepatitis were examined histopathologically. To characterise the amyloid precursor protein, immunohistochemistry, mass spectrometry-based proteomic analysis, and genetic analysis were performed.

Results:
In both cases, amyloid deposition was observed predominantly in the villi of the gastrointestinal tract and in the adjacent adipose tissue. In the villous lamina propria, amyloid was diffusely deposited and replaced the normal architecture, resulting in marked thickening of the villus. In the adipose tissue, numerous concentric amyloid deposits were observed. In contrast to typical distribution in avian AA amyloidosis, deposition was minimal or absent in the liver, spleen, and kidneys. Immunohistochemically, amyloid deposits were positive for ApoA-II, whereas only some deposits in the lamina propria were weakly positive for serum amyloid A (SAA). Mass spectrometry identified ApoA-II as a major component of amyloid deposits and SAA as a minor component. Genetic analysis of *APOA2* and *SAA* revealed no pathogenic mutations.

Conclusion:
These findings suggest systemic ApoA-II amyloidosis with concurrent AA amyloid deposition in toco toucans. To our knowledge, this is the first report of ApoA-II amyloidosis in avian species.

Pathology-led investigation of multifactorial zinc toxicosis in mixed-species aviary birds

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Topic: Exotic/wildlife animals

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Background:
An episode of recurrent mortality in a mixed-species aviary prompted pathology-led investigation after multiple birds showed a consistent lesion pattern of upper gastrointestinal irritation, koilin damage and, in selected cases, pancreatic necrosis.

Materials & Methods:
Post-mortem findings were reviewed alongside hepatic mineral analysis for 53 birds. Koilin pathology was graded and correlated with hepatic zinc, copper and iron concentrations. Water, soil, plants, fresh produce, dried foods, pellets, dust, ropes and paint chips were tested to identify plausible exposure sources.

Results:
Twenty-five of 53 birds had hepatic zinc above reference values; 30/53 had increased iron and 12/53 increased copper. Koilin pathology was present in 25 birds, predominantly in Coraciiformes, Musophagiformes and Piciformes, and in birds housed in the main aviary. Hepatic zinc showed a moderate correlation with koilin damage across the full dataset and a strong correlation in main-aviary cases; copper and iron correlations were weak. Environmental testing identified theming rope, dust and paint chips as the highest zinc sources, with dust also prominent for copper and iron and paint chips for iron. Dried insects and pellets represented additional dietary mineral sources. Following dust removal, rope replacement and soil replenishment, subsequent liver testing showed marked reductions in average zinc, iron and copper concentrations, although gastrointestinal lesions persisted in some birds.

Conclusion:
This outbreak illustrates zinc toxicosis as a pathology-driven, multifactorial diagnosis rather than a single-source event. Lesion pattern recognition, targeted mineral testing and environmental source tracing were central to risk reduction in a complex managed-care aviary.

Histopathological and immunohistochemical evaluation of vital reactions for estimating trauma-to-death interval in cats

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Topic: Forensic

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Background:
Determining the interval between trauma and death is a critical issue in veterinary forensic pathology. This study aimed to assess the usefulness of histological, histochemical, and immunohistochemical markers in estimating the trauma-to-death interval in cats.

Materials & Methods:
An observational study was conducted on 25 cats with traumatic injuries (road traffic accidents, animal attacks, and abuse) for which the timing of trauma was reliably documented. The interval between trauma and death ranged from immediate death to 15 days. All animals underwent complete post-mortem examination. Tissue samples from injury sites were collected and processed for histological, histochemical, and immunohistochemical analyses. HE-stained sections were evaluated for the presence of haemorrhage, inflammatory infiltrate, and erythrophagocytosis. Perls' Prussian blue, Mallory's phosphotungstic haematoxylin, Toluidine blue, and Masson's trichrome stains were used to detect hemosiderin, fibrin, mast cells, and collagen, respectively. Immunohistochemistry was performed to assess endothelial activation and coagulation markers (P-selectin, E-selectin, Factor VIII and CD31), pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) and to characterize inflammatory cells (CD3, CD18, CD20, tryptase and IBA-1).

Results:
The results demonstrated that histopathological and immunohistochemical findings correlate with the known trauma-to-death interval. In particular, a temporal progression was observed, starting from the expression of endothelial activation and coagulation markers, the evolution of the inflammatory response up to fibrosis, erythrophagocytosis and haemosiderin formation.

Conclusion:
These findings support the usefulness of combined histopathological and immunohistochemical approaches in assessing lesion vitality and improving estimation of the timing of trauma in feline forensic cases.

Skull and rib fractures in dogs and cats: can location and distribution help differentiate between accidental and non-accidental injury?

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Topic: Forensic

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Background:
Diagnosis of non-accidental injury (NAI) requires multifactorial consideration of history, presenting injuries and diagnostic tests to differentiate it from accidental injury (AI). As skull and rib fractures are common in NAI, we compared location and distribution of these in canine and feline NAI and AI cases to identify patterns which may support more robust diagnoses.

Materials & Methods:
NAI cases were identified from the Royal Veterinary College forensic pathology database 2017-2025 (22 dogs, 18 cats), and AI cases from the VetCompass database 2020-2023 (65 dogs, 45 cats). Inclusion criteria involved only dogs and cats with skull and/or rib fractures and a history of NAI or AI, respectively. Fractures were characterised according to computed tomography, radiographic and postmortem reports.

Results:
NAI skull fractures occurred most in the cranial vault (frontal (62%), parietal (55%), temporal (62%) and occipital (34%) bones). Fractures of the tympanic bullae occurred in cats only (NAI: 33%, AI: 3%). NAI rib fractures were more often of mixed ages (NAI: 44%, AI: 0%), affecting non-consecutive ribs (NAI: 64%, AI: 21%), bilateral (NAI: 60%, AI: 18%) and affected a higher mean number of ribs (NAI: 6.2, AI: 3.6).

Conclusion:
In both dogs and cats, cranial vault skull fractures and bilateral, numerous, non-consecutive rib fractures of mixed ages within a wider suspicious context are suggestive of NAI. These findings may assist in better recognising NAI in pets, enabling detection of wider abuse within the household putting others at risk: a known link exists between child, animal and intimate partner abuse.

Sublethal Shootings in a German Eurasian Lynx (Lynx lynx) Population: Linking CT-Guided Pathology Findings to European Union (EU) Legal Frameworks

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Topic: Forensic

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Background:
The Eurasian lynx species is strictly protected across most of its EU range under the Habitats and Environmental Crime Directives. Unlawful lethal shootings directly contravene this legislation. Sublethal projectile injuries raise additional, important individual animal welfare concerns.

Materials & Methods:
24 Eurasian lynx found dead or euthanized with overt trauma underwent whole-body CT. Only one had a suspected shooting history. All animals with CT-detected metal-dense particles underwent systematic post mortem examination. ChatGPT-5.5 assisted in identifying and translating relevant EU and national legal texts.

Results:
Metal-dense fragments were identified in 4 out of 24 lynx (17 %) by CT scans. 3 cases were consistent with fragmenting bullets and one with shotgun pellets, as determined by necropsy. 3 animals had sublethal shootings and were emaciated. Specific lesions included: an organized comminuted elbow fracture; retrobulbar shotgun pellets lacking detectable entry wounds or wound tracts; and an elbow fracture with loss of the distal limb and marked shoulder muscle atrophy. Legal mapping identified three relevant EU-level legal frameworks: Article 13 TFEU, the Habitats Directive, and the Environmental Crime Directive. Sublethal shooting of wildlife seemed not, on their own, to act as a distinct EU legal offence of animal-cruelty. Individual animal welfare harms seem to remain primarily subject to non-harmonized national animal-welfare laws.

Conclusion:
Sublethal projectile injuries in transboundary wildlife populations such as lynx, bare an EU-level harmonization gap: species protection and environmental-crime obligations are EU-framed, whereas prevention and sanctioning of prolonged individual animal suffering of wildlife may depend largely on non-harmonized national animal-welfare laws.

Brain Gas Bubbles in Cetaceans: A Histopathological Approach to Distinguishing Lesions from Putrefaction

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Topic: Forensic

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Background:
Gas bubble formation in cetaceans has been associated with several pathological conditions, as well as peri- and postmortem off-gassing from supersaturated tissues. In stranded cetaceans, putrefactive bacterial proliferation also contributes substantially to gas accumulation, both macroscopically and microscopically. However, distinguishing true intravascular or intratissue gas bubbles formed in vivo from postmortem artifacts remains particularly challenging in the central nervous system.

Materials & Methods:
Ten stranded odontocetes of different species and decomposition states presenting cerebral gas bubbles were evaluated. Histochemical analyses using Oil Red O and Luxol Fast Blue, combined with chromic acid pretreatment to improve lipid and myelin preservation, were performed to characterize intraparenchymal gas bubbles.

Results:
In well-preserved brains (n = 6), gas bubbles were consistently associated with hemorrhage, congestion, edema, and compression of the neuropil, supporting an antemortem origin. Histochemical analyses frequently revealed concentric laminated ring-like structures compatible with intramyelinic separation previously described ultrastructurally in experimental decompression sickness. These bubbles were generally small to medium sized and showed regular, well-defined borders. In contrast, bubbles attributed to putrefaction (n = 4) displayed marked morphological heterogeneity, variable size, irregular contours, and predominant localization within white matter. Their vascular association was inconsistent, and some contained bacterial colonies with amorphous degenerative material.

Conclusion:
Findings suggest that gas may disrupt myelin integrity and potentially provide nucleation sites for bubble formation. The combination of hemorrhage, congestion, edema, and demonstrable myelin separation may represent useful histological criteria for identifying antemortem cerebral gas bubble formation in cetaceans.

Too Cold to Fight: Immune Profiling and Hippo Pathway Dysregulation in Equine Sarcoids — A Prospective Case Series

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Topic: Cancer

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Background:
Equine sarcoid accounts for up to 90% of all cutaneous tumours in horses, with Bovine papillomavirus type 1 (BPV1) considered the major etiological agent and no universally effective treatment available. Given the emerging role of Hippo signalling in PV-oncogenesis and tumour immune microenvironment (TIME) regulation, this study aimed to characterize the TIME of equine sarcoids and to investigate the expression of selected Hippo pathway-associated genes.

Materials & Methods:
Tissues from twelve equine sarcoids were collected. All cases were tested for BPV1, BPV2, and BPV13 by qPCR. Relative expression of selected Hippo pathway-associated and immune-related genes was assessed by RT-qPCR in total RNA of whole-tissue sarcoid samples compared with healthy skin controls, using B2M as the reference gene for normalization. IHC was performed for CD3, CD20, IBA1, CD204, CD163, and MUM1.

Results:
All sarcoids were positive for BPV1. Gene expression analysis revealed different expression of selected Hippo pathway-associated genes in sarcoids compared with healthy skin, with higher mRNA copy numbers of WNT5A and lower mRNA copy numbers of APC2, CTNNA3, and FZD3, where detected. Likewise, higher mRNA copy numbers of IL1B and IL2 and lower mRNA copy numbers of IL5, IL18 and IL23 were observed where detected. Sarcoids showed abundant macrophage infiltration, mainly represented by CD204+, CD163+, and IBA1+ cells.

Conclusion:
BPV1+ equine sarcoids seem characterized by a macrophage-rich and immunologically "cold" TIME, supported by scarce TILs infiltration, predominance of M2-like macrophages, and modulation of selected Hippo pathway-associated genes together with altered cytokine expression, suggesting an ineffective antitumour immune response.

Stress shapes the tumor immune microenvironment and worsens outcome in feline mammary carcinoma

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Topic: Cancer

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Background:
Evidence from murine models suggests that chronic stress may promote tumor dissemination by inducing an immunosuppressive microenvironment enriched in neutrophils. The objective of this study was to investigate the interaction between stress and immunity in the outcome of feline mammary carcinomas (FMC).

Materials & Methods:
This retrospective study included two independent cohorts of queens with FMC. The first cohort (N=28) was used to evaluate stress levels using a questionnaire completed by owners, assessing behavioral indicators stratifying cats in high stress (N=18) vs low stress (N=10). A second cohort (N=107), with exhaustive histopathological characteristics, was used to investigate the prognostic significance of immune microenvironment, assessed by immunohistochemistry in both cohorts through quantification of myeloperoxidase+ neutrophils and Foxp3+ regulatory T cells (Tregs). Prognostic analyses evaluated specific survival (SS) and disease-free interval (DFI).

Results:
High stress levels were associated with poorer SS (HR=4.58; p=0.0014) and shorter DFI (HR=7.45; p=0.0004) compared with low-stress cats. Stressed cats also showed a higher intratumoral Neutrophils/Tregs ratio (82% of tumors with ratio >1.2 vs 33%; p=0.038). In the second cohort, a high Neutrophils/Tregs ratio (n=59) was associated with decreased SS (HR=1.85; p=0.0076) and DFI (HR=2.04; p=0.0078). By multivariable analysis, the Neutrophils/Tregs ratio remained a prognostic factor for SS (HR=1.95; p=0.0072), independent of nodal status (HR=2.58 for tumors with vs without nodal metastasis; p=0.0003) and tumor COX2 expression (HR=1.93 for high COX2 expression versus low; p=0.0065).

Conclusion:
This study shows that stress significantly influences FMC progression through tumor immune microenvironment modulation, supporting development of stress-targeted or immunomodulatory therapies.

Exploring the spatiotemporal changes in residual disease using BRCA1 p53-deficient mammary tumours and a derived 3D tumoroid model

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Topic: Cancer

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Background:
In the oncology field, therapy resistance remains the major cause of death of patients with disseminated tumours. Typical examples are triple-negative breast cancer patients, which are frequently defective in the repair of DNA double-strand breaks, e.g. due to loss of BRCA1 function. While these patients initially respond well to DNA damage-inducing therapies, the tumours are usually not eradicated and resistant tumour cells are eventually selected. Understanding the drug tolerance mechanisms of residual tumours is essential to establish better therapeutic strategies.

Materials & Methods:
The *K14cre;Brca1$^{F/F}$;p53$^{F/F}$* (KB1P) mouse model for hereditary breast cancer provides a unique tool to study those mechanisms in an immunocompetent model. Using single-cell RNA sequencing and spatial transcriptomics, we explore the intratumoral heterogeneity and microenvironment of residual disease at the tissue and molecular level. Additionally, a 3D tumoroid model was derived from KB1P tumours for *in vitro* studies.

Results:
We identified specific subpopulations of tumour cells with survival benefits upon treatment. Those cells display mesenchymal features and are supported by a highly reactive stroma and pro-tumoral immune cells. In relapsing tumours, both morphological and transcriptional changes reversed, emphasizing the plasticity of drug tolerance mechanisms. While the heterogeneous tumour subpopulations are preserved in the tumoroid model, their proportions following treatment differ from the *in vivo* data, underlining limitations of *in vitro* models.

Conclusion:
With this project, we demonstrated the significant changes that tumours undergo to survive treatment in a spatial context and we hope to help develop better therapeutic approaches to target drug-tolerant residual cells.

Ovine tumours detected through passive surveillance in ireland

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Topic: Cancer

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Background:
Ovine tumours detected through passive surveillance in Ireland are described.

Materials & Methods:
Ovine carcasses and part-carcasses submitted for post-mortem examination at DAFM (January 2010–April 2026) were reviewed. Congenital tumours in foetal submissions were not revised.

Results:
Of a total of 22,103 submissions received, 244 tumours were diagnosed (228 carcasses; 16 part-carcasses) from 195 farms. Body condition was poor (60/228), moderate (49/228), good (68/228), or unrecorded (49/228); age ranged from 4-months-old to 10-years-old; 205 were females, 25 males and 14 were unrecorded.

Tumours consisted of pulmonary adenocarcinoma -OPA- (166), intestinal adenocarcinoma -IAC- (22), lymphoma (15), hemangiosarcoma (5), unspecified carcinoma/adenocarcinoma -C/A- (8), hepatocellular carcinoma (4), cholangiocarcinoma (3), unspecified sarcoma (2), chondrosarcoma (1), granulosa cell tumour (1), hemangiopericytoma (1), melanoma (1), mesothelioma (1), oligodendroglioma (1), squamous cell carcinoma (1), mammary carcinoma (1), renal adenocarcinoma (1). Ten tumours were not further detailed. OPA was grossly masked by or accompanied by bacterial/verminous pneumonia in 77/166 cases; the youngest OPA-affected animal was four months old, and 5/166 cases showed uncommon extra-thoracic metastases (diaphragm, mesentery, and/or liver). IAC involved the small (14/22) and the large (3/22) intestine, or had generalised or not recorded intestinal localisation (5/22), and often (10/22) severely compressed/occluded the lumen. Hemangiosarcoma was frequently associated with extensive haemorrhage (3/5). C/A had a high percentage of metastases (mainly at the regional lymph-nodes, diaphragm, lung and liver). Lymphoma was multicentric/metastatic in 13/15 cases.

Conclusion:
To the authors' knowledge, this is the first tumour case-series detected in ovine submissions through passive surveillance in Ireland.

Analyses of influenza A virus infection in raccoons (Procyon lotor) using a respiratory ex vivo culture model

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Topic: Experimental

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Background:
Influenza A viruses (IAV) are globally distributed, have a high propensity to cross species barriers and are frequently responsible for fatal disease outbreaks in humans as well as domestic animals and wildlife. Due to their opportunistic and peridomestic behavior raccoons act as potential intermediate hosts for a variety of infectious diseases. Precision-cut lung slices (PCLS), as a three-dimensional *ex vivo* model, represent a useful approach to simulate the natural lung environment and to investigate respiratory viral infections.

Materials & Methods:
Lungs from six raccoons were collected and filled with low melting agarose. Using an automated slicer, tissue cylinders were cut into PCLS and infected with seasonal IAV (H1N1 A/Victoria/2570/2019). Virus infected PCLS were fixed in 4% paraformaldehyde at 1-, 24-, 48-, and 72-hours post-infection. Subsequently, PCLS were processed for histology, immunohistochemistry, immunofluorescence, electron microscopy, and molecular analyses.

Results:
IAV infection led to cytopathic effects in PCLS with attenuation of bronchial epithelial cells visible histologically and by electron microscopy. Virus infected cells, expression of interferon-regulated proteins and markers of cellular apoptosis were detected in the bronchial epithelium via immunostaining of fixed PCLS.

Conclusion:
The bronchial epithelium of raccoons is highly susceptible to IAV infection and shows irreversible degenerative changes. PCLS derived from wildlife species thus provide an authentic respiratory model in which to investigate virus-host interactions and assess susceptibility to cross-species virus infection. Such models will help to reduce animal testing in accordance with the 3R-principle (refinement, reduction, replacement).

Host defence mechanisms in precision-cut lung slices reveal strain-dependent infectivity to canine distemper virus infection in an ex vivo model

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Topic: Experimental

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Background:
Canine Distemper Virus (CDV) is a highly contagious morbillivirus causing severe respiratory and systemic disease in carnivores. Innate immune defenses—such as mucin secretion, ciliary clearance, and interferon responses—are critical in limiting viral spread, yet their roles in CDV pathogenesis remain poorly understood in physiologically relevant models. Therefore, we established a precision-cut lung slice (PCLS) model to evaluate cell tropism and host defense mechanisms across different CDV strains.

Materials & Methods:
Four CDV variants were used: CDV-R252, CDV-5804 All-See GFP, SLAM-blind GFP, and Nectin-4-blind GFP. PCLS were infected and harvested at 1, 2, and 3 days post-infection (dpi). Viral replication was assessed by immunohistochemistry (IHC) using monoclonal antibody D110 and $TCID_{50}$ titration. Mucin secretion was quantified via Alcian Blue staining, and ciliary beating frequency was monitored by live imaging.

Results:
CDV R252 and CDV 5804 All-see induced significantly higher numbers of infected cells compared to SLAM-blind and Nectin-blind variants. Viral titers were detectable only in cultures infected with CDV R252 and CDV 5804 All-see. Mucin secretion was decreased in infected PCLS versus controls. No significant changes in ciliary beating frequency were observed post-infection. Submucosal glands appear to be a target tissue based on HE and CDV IHC staining.

Conclusion:
CDV R252 and CDV 5804 have higher infectivity in *ex vivo* models. Submucosal glands appear to be an important viral target in CDV infections, leading to increased mucus production. The PCLS model provides a valuable *ex vivo* platform for studying CDV pulmonary pathogenesis and host-virus interactions.

Integrated quantification and immunohistochemical characterization of drug-induced Polyploid Giant Cancer Cells in a preclinical model of High-Grade Serous Ovarian Cancer

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Topic: Experimental

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Background:
High-Grade Serous Ovarian Cancer (HGSOC) is the most aggressive epithelial ovarian cancer subtype, characterized by high recurrence, metastasis, and poor prognosis. Chemotherapy represents the standard treatment; however, increasing evidence indicates therapy may induce the emergence of Polyploid Giant Cancer Cells (PGCCs), a subpopulation associated with tumor progression and drug resistance. This study developed an integrated workflow to quantify and characterize PGCCs in a preclinical HGSOC mouse model.

Materials & Methods:
C57BL/6 female mice (n=46) were intraperitoneally injected with ID8 $p53^{-/-}$/$PTEN^{-/-}$ cells and treated with vehicle or carboplatin. FFPE intra-abdominal tumor samples were analyzed. PGCCs quantification was performed using manual counting, Digital Image Analysis (DIA), and an AI-based ploidy assessment tool (PloiViT). IHC staining was performed using markers associated with key PGCCs features: yH2AX (DNA damage), N-cadherin (EMT), Vimentin (EMT), CD44 (stemness), p21 (cell cycle), Cyclin D1 (cell cycle), and LC3B (autophagy).

Results:
Carboplatin significantly reduced tumor burden while increasing PGCCs and tumor ploidy compared with controls across all quantification methods. Treated tumors had higher expression of yH2AX, N-cadherin, CD44, p21, and LC3B, whereas no significant differences were observed for Vimentin and Cyclin D1. All tested antibodies identified PGCCs; however, none were PGCCs-specific.

Conclusion:
This integrated strategy highlights chemotherapy-induced polyploidization as a potential adaptive mechanism underlying treatment resistance in HGSOC and provides a reproducible framework for PGCCs quantification. Immunohistochemical characterization suggests the involvement of DNA damage, epithelial–mesenchymal transition, stemness, cell cycle regulation, and autophagy, while underscoring the need for PGCCs-specific markers.

Photogrammetry as an accessible tool for veterinary pathology education and field practice

358
Topic: Experimental

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Background:
Photogrammetry holds great promise across veterinary education, including veterinary pathology. Photogrammetry generates precise, high-fidelity three-dimensional (3D) models from a series of overlapping photographs. Crucially, a smartphone and an application with minimal training are sufficient to produce accurate results.

Materials & Methods:
We reviewed photogrammetry-based animations generated over a two-year period at two veterinary schools and one commercial abattoir. The animations were created from routine diagnostic cases and abattoir rejections. The image acquisition was performed by members of the teaching and technical teams or by students. Various smartphones and tablets were used for image acquisition using the Polycam® application.

Results:
A total of 90 animations of samples from various domestic species (ruminants, poultry, equine, feline, and canine) were reviewed and evaluated. Lesions encompassed bronchopneumonia, malformations and neoplasia, affecting single organ or whole carcass. 87 animations were considered adequate in terms of immersivity and accuracy, regardless of the operator, device or acquisition setting.

Conclusion:
Photogrammetry has the potential to enable practitioners to generate high-quality 3D animations directly in the field. This may be valuable for unusual cases or suspected notifiable diseases, where rapid and accurate documentation is essential. The ability to capture and share precise visual data could support remote consultations, and more effective communication; three-dimensional animations offer a more immersive representation than conventional two-dimensional photography. Beyond its educational and research applications, photogrammetry therefore represents an inclusive and widely accessible tool that can extend advanced diagnostic documentation capabilities to veterinary colleagues outside academia.

Clostridium perfringens, new genomic perspectives from the pathologist's view

473
Topic: ESVP/ECVP Abstract

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Background:

Clostridium perfringens is an enteric pathogen of animals but also a common intestinal commensal. Current classification schemes often fail to distinguish pathogenic from commensal isolates, complicating the interpretation of bacteriological findings in suspected enteric clostridioses. Recent advances in whole-genome sequencing (WGS) provide unprecedented opportunities to identify disease-associated *C. perfringens* traits.
Materials & Methods:

216 animal derived disease and non-disease associated *C. perfringens* isolates with comprehensive pathological and clinical metadata were projected to WGS. 383 publicly available genomes were included for contextualization purposes and to calculate a comprehensive *C. perfringens* pangenome. Assemblies and annotations were analysed using bioinformatic tools.
Results:

Isolates derived from some diseases, including porcine necrotic enteritis, small-ruminant enterotoxemia, and canine acute hemorrhagic diarrhoea syndrome, displayed conserved gene repertoires. In contrast, isolates associated with bovine and lorikeet necrohemorrhagic enteritis were genetically diverse and lacked conserved virulence-associated genes, suggesting that disease development may depend more strongly on host or environmental factors. Non-disease-associated isolates showed comparable genomic diversity and were distributed throughout the phylogeny without clear clade association. Moreover, disease-associated isolates carried higher numbers of conjugative plasmids than non-disease-associated isolates. Beyond canonical plasmid-encoded virulence factors, these accessory genes may facilitate rapid adaptation and represent a reservoir of previously uncharacterized virulence-associated genes.
Conclusion:

WGS based investigations enable comprehensive analysis of *C. perfringens* as a pathogen and may allow discovery of disease associated characteristics of clinical isolates.

Enterotoxemia in Akkaraman Lambs: Pathological and Molecular Findings

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Topic: ESVP/ECVP Abstract

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Background:

Enterotoxemia caused by *Clostridium perfringens* is a major cause of peracute mortality in small ruminants, although definitive diagnosis remains challenging because the organism is part of the normal intestinal flora. This study characterized the clinical, pathological, and molecular features of enterotoxemia in Akkaraman lambs with sudden death.
Materials & Methods:

Forty Akkaraman lambs (20 days–3 months old) from multiple flocks were examined postmortem following peracute death. Clinical history, gross lesions, and histopathological findings were evaluated. Multiplex PCR was performed on intestinal and fecal samples to detect *C. perfringens* toxin genes.
Results:

Clinical signs included abdominal distension, lethargy, and diarrhea. Gross findings consisted of generalized pallor, hemorrhagic abomasitis, intestinal serosal hemorrhages, and pulmonary edema. Histopathology revealed intestinal mononuclear infiltration and vascular hyperemia, pulmonary edema, hemorrhage and emphysema, renal tubular degeneration with vascular hyperemia, hepatic congestion, and prominent lymph node reticular framework. Multiplex PCR detected *cpa* (alpha toxin) and *etx* (epsilon toxin) genes in 33/40 lambs, supporting type D enterotoxemia. The remaining seven lambs showed similar pathological findings but were negative for both toxin genes.
Conclusion:

Enterotoxemia should be regarded as a systemic toxemic disease characterized by widespread vascular injury rather than a purely enteric disorder. Seven lambs initially suspected of enterotoxemia based on pathological findings were PCR-negative and therefore were not considered confirmed cases. Their diagnostic relevance lies in demonstrating that pathological findings alone may be insufficient for definitive diagnosis and that molecular confirmation is essential in suspected cases of enterotoxemia.

ORAL ABSTRACTS ESVCP-ECVCP

TOPIC: BIOCHEMISTRY/ENDOCRINOLOGY

Validation of an Ultrasensitive ELISA for Measuring Feline Insulin

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Topic: Biochemistry/Endocrinology

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Background:
Oral hypoglycaemic drugs for treating feline diabetes mellitus require preserved endogenous insulin production. However, validated methods for routine measurement of feline insulin remain limited. This study aimed to validate an ultrasensitive ELISA assay for insulin manufactured by Mercodia AB (Uppsala, Sweden).

Materials & Methods:
Linearity under dilution was evaluated across the measurement range. Precision was assessed at three concentration levels against quality goals derived from published biological variation data. Expected biological response was assessed using a previously established ex vivo model of isolated feline pancreatic islets, which enabled assessment of glucose- and depolarization-induced insulin secretion. Sample stability was evaluated at 20°C and at 4°C for seven days, as well as the effect of four freeze–thaw cycles.

Results:
The assay demonstrated linearity across the measurement range with recovery of 92-117%. Within-run CVs ranged from 3.5-8.9% and between-run CVs from 4.2-9.5%, meeting predefined quality goals with the highest variability observed at low concentrations. The assay detected physiologically appropriate increases in insulin secretion from isolated islets in response to glucose and potassium stimulation. The sample was stable at 4˚C for 7 days, but only for 24 hours at 20°C. Four freeze-thaw cycles had no effect on the results and were within the precision of the assay.

Conclusion:
The assay demonstrated robustness and good analytical performance, and the observed biological response to beta-cell stimulation supported analytical validity. These findings support its potential for clinical use; however, establishment of a reference interval is required and is the subject of ongoing research.

Stability of iodothyronines in canine and feline serum under varying temperatures and influence of multiple thawing-cycles when measured with LC-MS/MS

294
Topic: Biochemistry/Endocrinology

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Background:
Proper pre-analytical management of samples is an important factor in achieving reliable hormone measurement results, and mass spectrometry is generally more sensitive to pre-analytical errors than traditional immunoassays. Currently, there are no studies on the effect of storage or multiple freeze-thaw cycles on the concentrations of feline and canine iodothyronines measured with liquid chromatography-tandem mass spectrometry (LC-MS/MS).

The objectives of this study were to investigate the stability of total thyroxine (TT4), triiodothyronine (TT3) and reverse triiodothyronine (TrT3) in canine and feline serum samples stored for up to two weeks at various temperatures and to investigate the influence of multiple freeze-thaw cycles.

Materials & Methods:
Leftover serum samples with medium and high iodothyronine concentrations were aliquoted and stored at 23, 4 and -21 °C for two weeks. Measurements, using a validated LC-MS/MS-method were performed in five replicates on day 0, 1, 3, 7 and 14. Four freeze-thaw cycles were examined.

Results:
On day 7 the recovery rates for all hormones ranged from 96 to 114%, irrespective of the storage temperature applied. On day 14, low recovery rates ranging from 62% to 67% were observed for TT3 and for feline TT4 at high concentrations. Except for dogs with high iodothyronine concentrations, reduced recovery rates of 33% to79% were observed after the fourth freeze-thaw cycle.

Conclusion:
TT4, TT3 and TrT3 in canine and feline serum are stable for at least 7 days when stored at room temperature, cooled or frozen. Up to three freeze-thaw cycles have no effect on iodothyronine measurements.

Analytical and clinical agreement between the Siemens Immulite 2000 veterinary cortisol and standard cortisol assays in canine serum

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Topic: Biochemistry/Endocrinology

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Background:
In 2020, a permanent change in the antibody used in the Siemens Immulite 2000 cortisol assay resulted in a negative bias of 23%. In response, the manufacturer introduced a veterinary-specific cortisol assay. The objective of our study was to compare the analytical and diagnostic agreement between the Siemens Immulite 2000 standard cortisol (SC) and veterinary cortisol (VC) assays.

Materials & Methods:
Residual canine serum samples submitted to a veterinary diagnostic laboratory were used. Cortisol concentrations were measured using both SC and VC kits on the Siemens Immulite 2000 analyser.

Results:
A total of 105 dogs contributed 154 cortisol measurements. Median (minimum, maximum) cortisol concentrations for VC and SC assays were 4.19 (<1.00, >50.00) µg/dL and 4.67 (<1.00, >50.00) µg/dL, respectively. Further analysis included only one measurement per dog. Cortisol concentrations measured by the two assays were highly correlated (Spearman's rho = 0.981, P < 0.001). Passing-Bablok regression demonstrated no significant constant bias (intercept -0.035; 95% CI: -0.184 to 0.061) or proportional bias (slope 1.036; 95% CI: 0.997 to 1.098). Bland-Altman analysis showed no significant mean bias between assays (-0.494 µg/dL; 95% CI: -3.524 µg/dL to 4.513 µg/dL), with limits of agreement of -4.353 µg/dL to 5.342 µg/dL. Diagnostic agreement was excellent (Cohen's κ = 0.961); 3/105 cases were classified differently, with discordant results occurring near diagnostic decision thresholds.

Conclusion:
The SC and VC assays demonstrate strong analytical and diagnostic agreement in canine serum. Use of either assay is unlikely to alter clinical interpretation in most cases, and both assays appear to share similar analytical characteristics.

Systemic Immune Inflammation Index (SII) in dogs: comparison with C-reactive protein (CRP) in a clinical hospital population

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Topic: Biochemistry/Endocrinology

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Background:
Systemic Immune Inflammation Index (SII) integrates neutrophil, platelet and lymphocyte counts, reflecting both innate and adaptive immune responses. In dogs, C-reactive protein (CRP) is a validated biomarker of systemic inflammation. This study aimed to evaluate the relationship between SII and CRP and assess how SII as a composite marker of inflammation-related hematological responses, classify dogs as inflammatory or having a low CRP concentration.

Materials & Methods:
Samples from 432 dogs (August 2024–October 2025) were analyzed, including CRP (Gentian, Norway) and hematology (Advia 2120i, Siemens Healthineers) including manual blood-smear evaluation; samples with platelet aggregates were excluded. SII was calculated as (neutrophils×platelets)/lymphocytes. Inflammation was defined as CRP ≥10 mg/L. Differences in SII between groups were assessed using the Wilcoxon rank sum test. Youden's index evaluated SII's classification of inflammatory status. Spearman correlation between CRP and SII was calculated for all samples.

Results:
181 (41.9%) dogs were inflammatory with CRP (median; range) 29.3 (10.0–297.6) mg/L and SII 1284 (256–6866)×10^9/L. 251 (58.1%) dogs had CRP within range (median; range) 4.1 (0–9.9) mg/L and SII 768 (253–3325)×10^9/L. SII differed significantly between groups ($p<0.001$). Diagnostic performance of SII was weak, with a Youden's index of 0.29 at a cut-off of 1034. A weak positive correlation between CRP and SII was observed ($\rho=0.242$;$p<0.0001$).

Conclusion:
SII differed significantly between CRP-defined groups but showed limited concordance for individual classification. This indicates that SII cannot substitute CRP for identifying inflammatory dogs in a heterogenous clinical population, likely reflecting broader immune alterations beyond acute-phase responses.

Use of the Spike-and-Recovery Method for Validation of Biochemical Analyses in Canine Serum

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Topic: Biochemistry/Endocrinology

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Background:
Validation of canine biochemical analyses using human reagents remains challenging due to limited species-specific validation data. Differences in sample matrix may affect the performance of assays intended for use with human samples when applied to canine specimens. Therefore, this study assessed spike-and-recovery testing as a preliminary validation approach in canine serum.

Materials & Methods:
Human n = 2 and canine n = 6 serum samples were analyzed for albumin, urea, creatinine, total protein, cholesterol, ALT, ALP, CK, calcium, sodium, potassium, and chloride using human commercial reagents on an automated biochemical analyzer.

Precision and preliminary applicability were evaluated by triplicate measurements, 9:1 serum-water dilution, and 9:1 spike-and-recovery testing using five-fold concentrated human quality control serum. Spike and dilution recovery (%) were calculated as measured-to-expected concentration ratios ×100. Mean, SD, CV%, and recovery were calculated using jamovi (v2.7.27).

Results:
Recovery in canine serum ranged from 92.87% to 103.11% (ALP and creatinine, respectively), while recovery in human serum ranged from 81.57% to 104.57% (urea and CK, respectively). Dilution recovery ranged from 81.57% to 104.57% (ALT and CK, respectively) in canine serum and from 72.58% to 111.33% (ALT and CK, respectively) in human serum. Precision was generally acceptable (CV ≤ 2.5%), with a maximum CV of 8.55%.

Conclusion:
The favorable recovery values indicate good agreement between expected and measured concentrations in canine serum under the tested conditions and support the use of spike-and-recovery testing as a preliminary validation tool in veterinary laboratory diagnostics.

IGF1 AS Genotype Correlates with Plasma IGF 1 Levels and Morphometric Diversity in Healthy Dogs

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Topic: Biochemistry/Endocrinology

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Background:
Canine morphological diversity is attributed to a small number of genetic loci, notably *Insulin-like Growth Factor type 1*(IGF-1) gene. A regulatory variant in the antisense long non-coding RNA IGF1-AS, defined by the presence of either a cytosine(C) or thymine(T) base, has been identified as a major determinant of body size, yielding three genotypes: CC (small dogs), CT (medium), and TT (large). This study aimed to assess the correlation between IGF1-AS genotype, IGF-1 concentrations, and morphometric traits in healthy dogs.

Materials & Methods:
Seventy-three adult healthy dogs were prospectively recruited based on normal physical examination and hematobiochemical profile, and divided into three size categories (n=20<10 kg, n=31[10–25 kg], n=22>25 kg). Body weight(BW), length(BL), height at withers, plasma IGF-1 (Radioimmunoanalysis Mediagnost) and *IGF1-AS* genotyping (Sanger sequencing) were assessed. Non-parametric statistical evaluated correlations among IGF-1concentration, genotype, and phenotype.

Results:
Plasma IGF-1 strongly correlated ($p<10^{-4}$) with BW (r=0.87), height (r=0.84) and lenght (r=0.80). TT dogs had significantly higher IGF-1 than CT (339 vs. 164 ng/mL; p=0.042) and CC (137 ng/mL; p<0.001), with a further CC vs. CT difference (p=0.031). TT dogs also showed greater BW compared to CT (32.8 kg, p=0.038) and CC (8.3 kg, p<0.001), height (CT: 62 cm, p=0.033; CC: 35 cm, p<0.001), and BL (CT: 103.5 cm, p=0.029; CC: 146.8 cm, p<0.001), with significant CC vs. CT differences across all traits (p≤0.01).

Conclusion:
This study demonstrates a direct relationship between IGF1-AS genotype, plasma IGF-1, and canine diversity, strengthening the role of IGF-1 in somatotropic regulation and supporting predictive genomic applications in veterinary medicine.

Summary of state of current doable performance in veterinary endocrinology: implication in the elaboration of future Total Allowable Error consensus

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Topic: Biochemistry/Endocrinology

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Background:
Total Allowable Error (TEa) consensus was established in veterinary hematology and biochemistry but is lacking in endocrinology. Common quality goals are highly desirable for harmonization in laboratory quality control and in result interpretation.

Materials & Methods:
Cumulative internal quality control data from multiple laboratories for a 2-month period and reported analytical performance from a literature review was collated for: cortisol, ACTH, tT4, fT4, TSH, insulin, testosterone, and progesterone. For each, coefficient of variations (CVs) and bias were plotted as a function of concentration. Performance was characterized via 2 approaches. Approach-1: Max-CV or Max-bias were elected as the upper limit of the CI95% at the clinical concentration(s) of interest; we calculated Max-TEa =|Max-bias|+ 4.5xMax-CV; 4.5 was elected as the minimum sigma for which QC monitoring is doable (sufficiently high probability of error detection) at 2 or 3 QCM levels. Approach-2: Sigma was determined as a function of increasing TEa, and Doable-TEa assigned as the lowest TEa for which median sigma >4.5.

Results:
Max-TEa (higher TEa) and Doable-TEa (lower TEa) were obtained for each endocrine analyte, providing a current summary of state of analytical performance at clinically relevant concentrations. Max-TEa and Doable-TEa determined based on current performance only were consistently slightly lower than when including the performance data reported in the literature.

Conclusion:
We suggest that consensus clinical TEa should be elected somewhere between Doable-TEa and Max-TEa. If consensus clinical TEa is chosen as <Doable-TEa, many laboratories currently would risk not being able to achieve this analytical quality goal.

Long-term biological variation biochemistry results in a cohort of Labrador Retrievers, Beagles and Norfolk Terriers

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Topic: Biochemistry/Endocrinology

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Background:
Biological Variation (BV) data assesses usefulness of population-based reference intervals. Influence of different canine breeds is relatively unknown. The aim of this study was to evaluate long-term BV data in three breeds.

Materials & Methods:
Retrospectively, results of 16 biochemistry parameters from routine health checks of 146 dogs living in the same housing conditions sampled from 01/2024-10/2025 were analyzed. Samples were excluded if medical records suggested that dogs were unwell. Data was screened and filtered for outliers and analytes with a significant temporal trend were adjusted to steady state. Within and between-subject coefficients of variation (CV_I, CV_G) were evaluated by restricted maximum likelihood estimation using linear mixed-effects models with patient as a random effect. Analytical variation (CV_A) was assessed by evaluation of pooled patient samples. Index of Individuality (II) was calculated. Linear mixed-effects models with breed as a fixed effect evaluated the influence of breeds.

Results:
N=38 Beagles, n=28 Norfolk Terriers, and n=80 Labrador Retrievers with 10-18 measurement timepoints were included in the study. An II of <0.7 was observed for Na, Cl, and K. Remaining parameters showed intermediate individuality. II interpretation did not change when breed effect was considered. Breed means values were significantly different (p<0.05) in all parameters except for UREA, total protein, and globulin.

Conclusion:
Low II for Na, Cl, and K indicates that population-based reference intervals can be safely used. Breed mean values were significantly different in most parameters, but clinical significance of the differences is unlikely for Norfolk Terriers, Labradors and Beagles.

TOPIC: HEMATOLOGY

Neutrophil, monocyte and lymphocyte identification in samples from dogs with band neutrophils, with Sysmex XN-1000v, ProCyte Dx and ElementHT5+

431
Topic: Hematology

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Background:
White blood cell classification has been proven challenging for several hematology analyzers in samples with band neutrophils present. The aim of this study was to evaluate the performance of ElementHT5+ (HT5+) in this population.

Materials & Methods:
Surplus EDTA-blood from dogs analyzed with Sysmex XN-1000v (Sysmex) where band neutrophils had been identified in manual blood smear interpretation by a blinded 200-cell manual differential (m-diff) count, was additionally analyzed with ProCyte Dx (ProCyte) and HT5+ within 24 hours. Pearson correlation, Passing Bablock and Bland-Altman plots were analyzed between instruments and m-diff.

Results:
45 blood samples were included. Correlation for neutrophils between all instruments was excellent (r=0.93-0.99, intercept 0.07-0.60, slope 0.96-1.0) while poor for all instruments compared to m-diff (r=0.31-0.47, intercept -4.0 - -4.4, slope 1.1-1.3). Bias for neutrophils was mild between instruments; no bias was seen for neutrophils and m-diff between HT5+ and Sysmex, and a mild negative bias between m-diff and ProCyte. Correlation between monocytes and lymphocytes was poor for all comparisons, except excellent for monocytes between Sysmex and ProCyte. A negative proportional bias was seen between HT5+ and ProCyte and Sysmex for monocytes, and between HT5+ and ProCyte for lymphocytes. A positive proportional bias was seen between Sysmex and ProCyte and m-diff for monocytes.

Conclusion:
In samples from dogs with band neutrophils present, correlation for neutrophils between instruments and m-diff was poor in all instruments. Sysmex and ProCyte counted more monocytes than HT5+ and m-diff, and ProCyte counted more lymphocytes than HT5+.

Neutrophil, monocyte and lymphocyte identification in samples from cats with band neutrophils with Sysmex XN-1000v, ProCyte Dx and ElementHT5+

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Topic: Hematology

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Background:
White blood cell classification has been proven challenging for several hematology analyzers in samples with band neutrophils present. The aim of this study was to evaluate the performance of Element HT5+ (HT5+) in this population.

Materials & Methods:
Surplus EDTA-blood from cats analyzed with Sysmex XN-1000v (Sysmex) where band neutrophils had been identified in manual blood smear interpretation by a blinded 200-cell manual differential (m-diff) count, was additionally analyzed with ProCyte Dx (ProCyte) and HT5+ within 24 hours. Pearson correlation, Passing Bablock and Bland-Altman plots were analyzed between instruments and m-diff.

Results:
20 samples were included. Correlation for neutrophils between all instruments was poor (r=0.34-0.67, intercept 1.20-2.52, slope 0.92-1.45). Correlation for neutrophils was excellent between m-diff and HT5+ (r=0.99, intercept=0.24, slope 1.0), fair for ProCyte and poor for Sysmex. Bias for neutrophils was moderate positive between HT5+ and both Sysmex and ProCyte and moderate negative between Sysmex and ProCyte and m-diff. Correlation was poor for all comparisons for monocytes and lymphocytes, except excellent for monocytes between Sysmex and ProCyte and good for lymphocytes between HT5+ and m-diff. For lymphocytes a negative proportional bias was seen between HT5+ and ProCyte and Sysmex, a positive proportional bias for Sysmex and ProCyte compared to m-diff.

Conclusion:
Sysmex and ProCyte counted more lymphocytes than HT5+ and m-diff in samples from cats with bands present. HT5+ had excellent correlation for neutrophils to m-diff.

Low Optical Platelet Counts in Dogs and Cats: A Multiregional European Study

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Topic: Hematology

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Background:
A decreased optical platelet count (PLT) can be characterised using platelet indices (PI), particularly mean platelet volume (MPV) and plateletcrit (PCT). Increased MPV in fresh samples may indicate macroplatelets or platelet clumping, whereas decreased PCT mainly suggests a reduced platelet mass. Concurrent cytopaenias may reflect ineffective haematopoiesis. This study aimed to compare associations between decreased PLT, PI and other haematologic abnormalities in cats and dogs.

Materials & Methods:
This retrospective study analysed in-house haemograms processed using the ProCyte Dx™ Haematology Analyser in 34 European countries between January 2024 and December 2025. Cases were grouped into northern, central, western, and southern regions using a simplified Köppen–Geiger classification. PLT, PI, haemoglobin, and neutrophils were classified according to analyser reference intervals. Decreased PLT was graded as mild, moderate, or marked using species-specific thresholds.

Results:
Decreased PLT was more common in cats (23.8%) than dogs (13.8%) and was observed more frequently in southern and western regions in both species ($p < 0.05$). In cats, decreased PLT was mainly associated with non-increased MPV and low PCT in southern and western regions, while adequate PCT predominated in northern and central areas. Cats in southern regions more often showed concurrent anaemia and neutrophil abnormalities. In dogs, decreased PLT was typically associated with increased MPV, particularly in northern regions, where concurrent cytopaenias were less frequent ($p < 0.05$).

Conclusion:
In summary, regional differences in both the prevalence and severity of decreased PLT in dogs and cats suggest that climatic factors may contribute to its pathogenesis.

Clinicopathological abnormalities and outcome of acute Babesia canis infections in 23 dogs treated with imidocarb dipropionate

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Topic: Hematology

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Background:
Babesia (*B.*) *canis* infections are considered an emerging tick-borne disease with most often unspecific clinical signs. Two injections of 6.6 mg/kg bodyweight (BW) imidocarb dipropionate (ID) in a 14-days-period are recommended for treatment. *Babesia* antibody levels are protective against severe and life-threatening disease.

Materials & Methods:
The prospective study aimed to describe clinicopathological abnormalities and *Babesia* antibody levels in 23 dogs with acute *B. canis* infections, defined by positive PCR with sequencing results revealing *B. canis* on EDTA-blood, and to follow these dogs from T0 (diagnosis, first ID) to T1 (laboratory control, second ID), and T2 (laboratory control). Complete blood counts, biochemistry, coagulation profiles, Coomb`s testing, and *Babesia* spp. antibody levels were determined.

Results:
Thrombocytopenia, anemia, hyperbilirubinemia, and acute phase-responses were the most detected. ID dosage ranged from 1.7-7.0 mg/kg BW (median: 4.2 mg/kg BW). At T1 (median 15 days after T0), all PCR-results were negative, and laboratory abnormalities significantly improved. At T2 (median 32 days after T0) laboratory abnormalities did not significantly improve further compared to T1. Initially serologically negative dogs built up *Babesia* antibody levels by T1, which mostly dropped by T2. All 23 dogs were PCR negative at T1 besides the wide range in ID dosage.

Conclusion:
Laboratory abnormalities resolved quickly with an uncomplicated course of disease. A second PCR-test is recommended 14 days after the first ID injection. If the PCR-result is negative and clinicopathological abnormalities have significantly improved, a second injection should be avoided to not decrease protective antibody levels.

Flow cytometric evaluation of leukocyte subpopulations in dairy cows with subacute ruminal acidosis (SARA) under continuous reticulorumen pH monitoring

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Topic: Hematology

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Background:
Subacute Ruminal Acidosis (SARA) is common in periparturient dairy cows. While alterations in innate immunity have been described, the impact on adaptive immunity remains poorly characterised. This study aims to evaluate peripheral leukocyte subsets in cows classified based on acidosis events.

Materials & Methods:
Fifty-four periparturient dairy cows were equipped with indwelling reticulorumen pH sensors for continuous monitoring. K3EDTA-anticoagulated blood samples were collected at four time points (T0 −5 days (d), T1 +5 d, T2 +30 d, T3 +60 d relative to parturition). Peripheral leukocyte subsets were analysed by flow cytometry. Cows were classified as controls (no acidosis events), SARA6, SARA5.8, or SARA5.5 (pH <6.0, <5.8 and <5.5 for ≥300 min, respectively); controls and SARA6 were considered healthy, and SARA5.8–5.5 were categorised as diseased. Data were analysed using a linear mixed-effects model with time, acidosis category (healthy vs. diseased), and their interaction included as fixed effects; post-hoc comparisons used estimated marginal means ($p<0.05$). P-values were adjusted using the Benjamini–Hochberg False Discovery Rate method.

Results:
A significant time effect was observed for some leukocyte subsets ($P<0.001$): γδ T-lymphocytes ($WC1^{+}CD4^{-}$) increased between T2 and T3 ($p<0.001$), T-helper lymphocytes decreased between T0 and T1 ($p=0.041$) and increased between T2 and T3 ($p<0.001$). Monocytes decreased between T0 and T1 ($p=0.015$). No effects of acidosis category or time × category interaction were observed.

Conclusion:
Changes in peripheral leukocyte subsets were associated with time but not with SARA classification, suggesting that periparturient physiological changes rather than SARA may drive the observed immune variations.

TOPIC: CYTOLOGY

Formalin Fumes: The End of an Era? - Validation of a Formalin Artifact Reversal Protocol in Cytopathology

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Topic: Cytology

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Background:
Formalin fume contamination of unstained cytological smears is a well-known cause of non-diagnostic samples. Romanowsky staining, the veterinary cytology standard, is highly vulnerable to formalin exposure, which disrupts tinctorial affinity. Consequently, this artifact remains a significant global challenge, especially for laboratories receiving courier-sent cases. This study aimed to validate a protocol to reverse formalin fume artifacts on unstained smears, enabling diagnostic May-Grünwald Giemsa (MGG) staining without compromising cellular morphology.

Materials & Methods:
Nineteen cases (6 inflammatory, 13 neoplastic) were evaluated. For each, three air-dried smears were prepared: one MGG-stained morphological control, and two experimentally exposed to 10% neutral buffered formalin fumes for 24 and 48 hours. The reversal protocol comprised three consecutive five-minute heat cycles in an EDTA-based buffer, followed by standard MGG staining. Cytological features and diagnoses were compared.

Results:
The protocol effectively restored staining properties and cytomorphological detail, enabling a definitive diagnosis in 17/19 cases exposed for 24 hours. Under these conditions, staining intensity was slightly lower than in unexposed samples, preventing diagnosis in 2 cases (one neoplastic, one infectious), while cell detachment was minimal. Notably, bacteria were visible, but *Leishmania* organisms were inconspicuous. At 48 hours, diagnostic success decreased slightly (15/19) due to faint staining (2/19) and cell detachment (2/19) across three neoplastic cases and one infectious case.

Conclusion:
Despite minor limitations with prolonged exposure, this simple, rapid, and cost-effective protocol efficiently recovers most formalin-compromised smears, circumventing resampling and minimizing diagnostic delays. Further validation in larger cohorts is planned.

Agreement and Reliability of Cell Differential Counts in Body Cavity Fluids: Manual Assessment Versus an Automated Analyser

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Topic: Cytology

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Background:
Automated haematology analysers are routinely employed for total nucleated cell counts in body cavity fluids; however, their role in differential cell counts (DCC) is poorly characterized. This study evaluated agreement between automated and manual DCC in canine and feline effusions and assessed their analytical reliability.

Materials & Methods:
Body cavitary fluids from dogs and cats submitted for routine diagnostics were included, encompassing a wide range of cellularities and fluid types. Automated total and differential cell counts were obtained using the Sysmex XN-1000V™ and compared with manual DCC, used as reference method. Manual DCC (200 cells per sample) was independently performed by three board-certified clinical pathologists following standardized guidelines. Interobserver agreement was assessed by intraclass correlation coefficient (ICC); manual-to-automated agreement was evaluated using Passing-Bablok regression, Pearson correlation, and Bland-Altman analysis. Fluid classification followed published criteria.

Results:
A total of 60 effusions were included in the study. Interobserver agreement was moderate for neutrophils (ICC 0.73), lymphocytes (ICC 0.71), and macrophages/monocytes (ICC 0.66). Despite this variability, fluid classification showed almost perfect concordance among pathologists (Cohen's Kappa 0.89%). Correlation between manual and automated DCC was moderate for neutrophils and lymphocytes and poor for other cell populations. The analyzer failed to generate DCC in low-cellularity transudates and reported eosinophil and basophil fractions not supported by cytologic evaluation. However, WDF scattergrams showed recognizable patterns for lymphoid neoplasia, lymphorrhagic effusions, and exudates.

Conclusion:
Although manual cytologic differentials showed moderate interobserver agreement, fluid classification was highly concordant. Automated DCC exhibited substantial discordance compared with cytology, though WDF scattergram patterns may aid effusion categorization.

Flotillin 1 Expression in Serum and Effusions of Dogs with Neoplastic and Septic Diseases

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Topic: Cytology

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Background:
Effusion analysis is important for differentiating neoplastic from septic conditions in body cavities. Extracellular vesicles (EVs), particularly exosomes, play an important role in intercellular communication through transport of bioactive molecules such as flotillins. Flotillins are lipid raft-associated proteins involved in signalling and endocytosis and are considered promising biomarkers in neoplastic and inflammatory processes. This pilot study evaluated flotillin 1expression in sera and effusions from dogs with neoplastic and septic diseases.

Materials & Methods:
Samples included sera and effusions from 5 dogs with neoplastic diseases (3 adenocarcinomas, 2 lymphomas) and 5 dogs with septic peritonitis, together with sera from 5 clinically healthy dogs. EVs were isolated by size-exclusion chromatography (qEV, IZON Oxford). Flotillin 1 expression was assessed semiquantitatively by Western blot using the VWR Mini Vertical PAGE System and Mini Electroblotting System. Statistical analyses were performed in R (v3.6.1) using least squares models, analysis of variance and *emmeans* for adjusted means and post hoc comparisons.

Results:
Flotillin 1 expression differed significantly ($p < 0.05$) between neoplastic and septic sera, with higher expression detected in sera from dogs with neoplastic diseases. The highest flotillin 1 expression was observed in septic effusions compared with neoplastic effusions and sera.

Conclusion:
These preliminary findings demonstrate different flotillin 1 expression in sera and effusions from dogs with neoplastic and septic diseases. Increased flotillin 1 expression in septic effusions may reflect immune system activation, while higher serum expression in neoplastic diseases supports further investigation of flotillin 1 as a potential adjunct biomarker. These findings warrant larger studies with further EV characterization.

Copy numbers of the S-adenosylmethionine synthetase gene in 32 dogs with active Leishmania infantum infection

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Topic: ESVP/ECVP Abstract

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Background:
Relapses of canine leishmaniasis under allopurinol treatment are common. *S*-adenosylmethionine synthetase (*METK*) gene copy numbers (CNs) <3.0 were demonstrated in allopurinol resistant *Leishmania infantum* strains *in vitro*. Hypothesis include that *METK* gene CNs >4.0 (*L. infantum* wildtype) occur in dogs with efficient allopurinol therapy, in relapses linked to immunosuppression, and in dogs not receiving allopurinol yet. *METK* gene CNs <3.0 might occur in dogs under allopurinol therapy and in recent cases not receiving allopurinol yet.

Materials & Methods:
METK gene CNs were quantified by ddPCR in this prospective multicenter study.

Results:
METK gene CNs ranged from 0.7-4.96 in 32 dogs with active *L. infantum* infection (positive *L. infantum* PCR with corresponding clinicopathological abnormalities; CNs <3.0: 19 (59%), CNs 3.1-3.9: 8 (25%), CNs >4.0: 5 (16%)). *METK* gene CNs <4.0 occurred in dogs with recurring clinicopathological abnormalities of active leishmaniasis under allopurinol and in dogs with recent diagnosis not receiving allopurinol. *METK* gene CNs >4.0 were exclusively observed in dogs without allopurinol (n=4) or with immunosuppressive treatment (n=1). In 13/19 dogs (68%) with *METK* gene CNs <3.0 and allopurinol treatment, recurring dermatological signs (10/13, 77%), anemia (9/13, 69%) and decreased albumin/globulin ratios (11/13, 85%) were noted.

Conclusion:
Identification of resistance is crucial for further management and should be considered in dogs with *METK* gene CNs <4.0 *in vivo*. Long-term management with allopurinol may cause *METK* gene CNs <4.0 in *L. infantum*. CNs <4.0 in dogs with active leishmaniasis prior to treatment make transmission of resistant strains by sand flies likely.

TOPIC: LAB QUALITY MANAGEMENT

Verifying reference intervals: validation of a novel and practical tool for the veterinary diagnostic laboratories.

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Topic: Lab Quality Management

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Background:
The aim of this study was to evaluate a novel indirect model for RI verification (VeRUS – Verification of Reference Intervals based on Uncertainty of Sampling) and to compare its performance with the traditional binomial verification method.

Materials & Methods:
A healthy population of canine blood donors was used to establish RIs for 10 biochemical and hematologic measurands. Three direct RIs were established per measurand: a correct RI (95% of the population), an intentionally widened RI (99.9% of the population), and an intentionally narrowed RI (80% of the population). The VeRUS model compared each of these with an indirect RI derived from the laboratory patient population, assessing the difference between the two as acceptable or unacceptable based on sampling uncertainty; the area of statistical uncertainty that would be present if both RIs had been estimated using the standard nonparametric approach with 120 reference individuals. Verification outcomes obtained with VeRUS were compared with those from the traditional binomial verification method, randomly selecting 20 dogs from the healthy population.

Results:
The binomial method did not reject any of the excessively wide RIs (0/10), whereas VeRUS rejected 6 out of 10. For excessively narrow RIs, the binomial method rejected 7 out of 10 intervals, whereas VeRUS rejected 10 out of 10. Both methods correctly accepted all appropriate RIs (10/10).

Conclusion:
The VeRUS method showed superior performance compared with the traditional binomial approach, consistently rejecting excessively narrow RIs and identifying a substantial proportion of excessively wide RIs, which are generally accepted by binomial verification.

POSTER ABSTRACTS ESVP-ECVP

Forensic animal welfare cases in veterinary pathology in Finland, 2013–2023

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Topic: Small animals w/o cancer

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Background:
Forensic veterinary science is a developing, multidisciplinary field. In Finland, forensic veterinary pathology examinations are conducted by the Finnish Food Authority (FFA). We describe the animal welfare-related forensic cases of selected diagnosis groups examined at the FFA during 2013–2023 and investigate the associations between case numbers and geographical or population-related factors.

Materials & Methods:
Forensic autopsy cases submitted to the FFA between 2013 and 2023 were retrieved from electronic records. Cases representing the most common animal welfare-related diagnostic groups (starvation, trauma, dental disorders) were included. Species, breed, diagnosis group, and individual diagnoses were collected. Municipal population data were obtained from Statistic Finland. Chi-square tests and Spearman correlations were applied ($p<0.05$). Case numbers were visualized relative to municipal population size.

Results:
Of 995 forensic cases, 353 involved diagnoses related to starvation, trauma, or dental disorders. Dogs and cats accounted for 263 cases, while 90 cases involved other companion or production animals. Among dogs and cats, no significant differences were observed between diagnosis groups. Compared with dogs and cats, other species had significantly fewer cases with dental disorders. Case numbers correlated positively with municipal population size and the proportion of rental and urban housing. No correlation was observed with the proportion of residents of foreign nationality.

Conclusion:
This is the first description of Finnish forensic animal welfare case autopsies. Differences in examination protocol between species may have influenced the detection of dental disorders. Greater case numbers in densely populated areas may reflect increased recognition and reporting of animal welfare concerns.

Canine Ovarian Lesions: Diagnostic Challenges in a Retrospective Study

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Topic: Small animals w/o cancer

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Background:
Canine ovarian (CO) lesions are commonly encountered in veterinary pathology, often assessed in conjunction with uterine changes. This study aims to characterize these lesions and their association with uterine pathology, highlining rare CO findings not currently represented in existing classification.

Materials & Methods:
A total of 257 CO cases were retrospectively reviewed from a database of 27,763 diagnostic submissions (2017-2026). CO cases were categorized as cysts, neoplasms, normal, or developmental abnormalities. CO neoplasms were classified according to World Health Organization (WHO, 1999) criteria. Uterine findings associated with 123 CO cases were categorized as endometrial hyperplasia, inflammation, neoplasm, or normal.

Results:
CO cysts were most frequent (43%), followed by neoplasms (36%), normal ovaries (18%), and developmental abnormalities (3%). Among neoplasms, sex cord-stromal tumors predominated (56%), followed by epithelial (26%), germ cell (14%), undifferentiated (4%), and mesenchymal tumors (2%). Bilateral neoplasms were rare (3%). Three neoplasms showed features not included in current veterinary classification and were interpreted, based on comparative literature, as Müllerian-type adenosarcoma, adenoma with squamous differentiation, and bilateral monophasic teratoma. A subset of CO cases (neoplasms, cysts, and normal) showed multifocal, extracortical, sex cord-like islands and tubules negative for synaptophysin, chromogranin A, Grimelius, and positive for pan-cytokeratin. Endometrial cystic hyperplasia was identified in 75% of the 123 associated uterine cases, with no association with CO neoplasms ($p=0.66$) and a significant inverse association with CO cysts ($p=0.039$).

Conclusion:
CO lesions are heterogeneous and include rare patterns not fully encompassed by current classification. Further studies may help clarify their biological and diagnostic significance.

Large-scale retrospective evaluation of canine adrenal lesions with identification of adrenohepatic fusion mimicking neoplastic invasion

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Topic: Small animals w/o cancer

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Background:
Adrenal lesions in dogs are heterogeneous and include non-neoplastic changes as well as cortical and medullary neoplasms. Retrospective studies in canine diagnostic databases are scarce but may identify lesion frequencies and diagnostic pitfalls. Among these, adrenohepatic fusion (AHF), defined by direct adrenal-hepatic continuity due to absence of the intervening capsule, is recognised in humans but has not been described in dogs. This study evaluated canine adrenal lesions, identified AHF cases, and assessed their diagnostic implications.

Materials & Methods:
A retrospective analysis was conducted on 202 canine adrenal lesions (2017–2026). All cases were morphologically evaluated using haematoxylin and eosin staining, with Grimelius staining applied where necessary. Cases showing direct adrenal–hepatic continuity were identified as AHF and further evaluated using clinical, imaging, histological and immunohistochemical data (pan-cytokeratin, synaptophysin, chromogranin A, and vimentin).

Results:
Common diagnoses were pheochromocytomas (34.5%), adrenocortical carcinomas (30%) and adenomas (24%), while less defined lesions were classified as cortical neoplasia (4.5%) or unspecified adrenal neoplasia (2.5%). Mixed-breed dogs predominated, and the mean age was 10.4 years. Among the most diagnostically challenging lesions were five AHF cases (2.5%). These were mainly interpreted as adenomas or cortical hyperplasia, although some had previously been diagnosed as carcinomas because AHF histologically mimicked infiltrative neoplasia, making immunohistochemistry and clinical findings crucial for diagnosis.

Conclusion:
This study provides a large-scale survey of canine adrenal lesions, characterises AHF in dogs, and highlights features useful for diagnostic recognition to avoid misdiagnosis and misclassification.

Regional myocardial remodelling in dogs with myxomatous mitral valve disease: histopathological and immunohistochemical characterization of the left ventricle

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Topic: Small animals w/o cancer

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Background:
Myxomatous Mitral Valve Disease (MMVD) in dogs causes progressive volume overload and left ventricular remodelling. Although global cardiac changes are well recognized, the regional heterogeneity of myocardial remodelling remains poorly understood. This study aimed to characterize region-specific myocardial changes within the left ventricle, focusing on the mechanical gradient between the apical and basal portions of the papillary muscles.

Materials & Methods:
Myocardial tissue samples were collected at necropsy from 10 dogs with naturally occurring MMVD at different Whitney grades and ACVIM stages and from 5 controls. Specimens included the left ventricular free wall, interventricular septum, and left papillary muscles, further subdivided into apical and basal portions. Histochemical stains were performed to assess fibrosis (Masson's trichrome) and early ischemic damage (Phosphotungstic Acid Haematoxylin). Immunohistochemistry for Connexin 43 (Cx43) and Matrix Metalloproteinase-2 (MMP-2) was used to investigate whether mechanical stress correlates with localized gap junction redistribution and extracellular matrix turnover.

Results:
The degree of fibrosis and myofibrillary integrity correlate with MMVD severity and varied across different myocardial regions. Papillary muscles showed marked Cx43 lateralization and increased MMP2 expression, particularly in the apical portions compared with the basal portion of the papillary muscle and the left ventricular free wall, suggesting a topographical gradient of remodelling.

Conclusion:
These findings contribute to clarifying the topographical distribution of myocardial damage in canine MMVD. Characterization of the regional expression patterns of Cx43 and MMP-2 may help elucidate the structural basis of electrical instability and contractile dysfunction during the disease progression.

More Than Meets the Eye: Forensic Evaluation of Canine Firearm Fatalities – A Case Series

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Topic: Small animals w/o cancer

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Background:
Firearm-related injuries in animals represent a significant but underreported component of veterinary forensic pathology. Animal abuse is associated with broader social violence. Accurate interpretation of ballistic trauma requires integration of necropsy findings with imaging and forensic principles. This study aimed to characterise the distribution patterns and morphological features of injuries in canine firearm-related fatalities and to evaluate their forensic significance.

Materials & Methods:
Three canine forensic firearm fatalities were evaluated using gross necropsy, radiographic imaging, and histopathology when applicable. Wound morphology, projectile distribution, organ damage, and trajectory characteristics were assessed. Forensic context, postmortem changes, and entomological findings were also considered.

Results:
All cases showed severe multisystem trauma with distinct ballistic characteristics. A single-projectile case demonstrated a clear entry–exit trajectory causing cardiac, pulmonary, and hepatic perforations with hemothorax and hemopericardium. Shotgun-related cases showed widespread pellet distribution with numerous metallic foreign bodies detected radiographically and recovered at necropsy. Extensive soft tissue and organ damage, including jugular vein disruption and cardiac perforation, resulted in hypovolemic shock and, in one case, cardiac tamponade. In one case, advanced postmortem changes and entomological evidence contributed to postmortem interval estimation.

Conclusion:
These findings highlight the diversity and complexity of firearm-related injuries in dogs and emphasise the importance of integrating necropsy with imaging in forensic evaluations. Recognition of injury distribution patterns and morphological features improves diagnostic accuracy and supports medico-legal investigations. Considering the established association between animal abuse and broader societal violence, accurate forensic evaluation of such cases is of significant forensic and societal importance.

Hereditary feline hepatic amyloidosis in a european shorthair cat

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Topic: Small animals w/o cancer

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Background:
Feline hepatic amyloidosis is a genetically predisposed disease in Abyssinian and Siamese breeds, although it may result from infectious, autoimmune, or neoplastic causes. Hepatic amyloid accumulation causes nonspecific clinical signs, including weight loss, vomiting, jaundice and hepatomegaly. In severe cases, hepatic rupture and fatal hemorrhage may occur.

Materials & Methods:
A necropsy was performed on a 1-year-old neutered male European Shorthair cat up-to-date on vaccinations and deworming. The cat had been hospitalized for shock, hypothermia, hypotension, and bradycardia. Blood analysis showed increased ALT and haptoglobin without serum amyloid A elevation. Prior ultrasound revealed changes compatible with acute hepatitis, moderate abdominal effusion, and reactive lymphadenopathy.

Results:
Macroscopically, pale mucous membranes and severe hemoperitoneum were observed. The liver was diffusely brownish, with multiple linear parenchymal discontinuities on the parietal surface. Parenchymal ruptures were present in all lobes on the visceral surface. Irregular, dark red, raised, well-defined subcapsular areas extended into the parenchyma as hemorrhagic tracts. Microscopically, the liver presented subcapsular and parenchymal hemorrhages. Between hepatocyte cords, amorphous, homogeneous acidophilic material was present in the spaces of Disse. This material stained positive for Congo red, confirming amyloid deposition. Polarized light microscopy and immunohistochemistry for amyloid A were positive. In the spleen, red pulp depletion and amyloid deposits in arterial walls were detected.

Conclusion:
The age and absence of chronic infections suggest hereditary hepatic amyloidosis, a rarely reported condition in this breed. Therefore, it should be considered in the differential diagnosis of chronic hepatitis in young cats, even in non-predisposed breeds.

Oral Ectopic Sebaceous Glands (Fordyce Granules) in a Cat

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Topic: Small animals w/o cancer

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Background:
Ectopic sebaceous glands of the oral cavity, also known as Fordyce granules, are clusters of sebaceous glands not associated with hair follicles. They are well recognized in humans and have been reported in laboratory rats, whereas veterinary descriptions in domestic animals remain extremely limited. In the oral mucosa, they are usually considered incidental findings; however, their recognition is important because they may mimic other oral lesions clinically.

Materials & Methods:
A 14-year-old male European shorthair cat was presented with a round, approximately 1 cm plaque on the palate, associated with discoloration and mild swelling of the right faucial isthmus. A clinical suspicion of eosinophilic plaque was raised. Three small oral mucosal biopsies were collected.

Results:
Histologically, the samples consisted of oral mucosa containing salivary glands and ectopic sebaceous glands. A severe inflammatory infiltrate involved the surrounding connective tissue, sebaceous ducts, and salivary ducts. The final diagnosis was severe lymphoplasmacytic stomatitis with multifocal erosions, associated with ectopic sebaceous glands, consistent with Fordyce granules.

Conclusion:
This case documents Fordyce granules in the oral mucosa of a cat. Fordyce granules should be considered among the possible histological findings in oral mucosal biopsies, even in species in which they are rarely reported. Their presence may complicate the interpretation of palatal plaques or erosive oral lesions. This case expands the comparative pathology of oral Fordyce granules and highlights the diagnostic value of histopathology in feline oral lesions.

Diagnostic utility of molecular techniques and bacterial visualisation techniques in canine endocarditis caused by Bartonella

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Topic: Small animals w/o cancer

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Background:
Bartonella spp. are facultative intracellular Gram-negative bacilli with a worldwide distribution, affecting both humans and animals. In dogs, an important clinical manifestation is endocarditis/myocarditis often caused by *Bartonella vinsonii* subsp. *berkhoffii* (*Bvb*). This subspecies is fastidious and slow-growing, and is not visible on H&E sections, potentially contributing to misdiagnosis. The goal of this study was to assess the diagnostic utility of different molecular and bacterial visualisation techniques in three *Bvb* infected dogs.

Materials & Methods:
We obtained the heart from three dogs with echocardiographic evidence of endocarditis and high anti-Bartonella antibody titers by indirect fluorescent antibody assays. Results from histology, immunofluorescence using an in-house polyclonal anti-Bartonella antibody, RNAscope™ In Situ Hybridization (ISH) using a *Bartonella* 23S rRNA probe and molecular testing (*Bartonella* genus qPCR, digital PCR) targeting the 16S-23S rRNA internal transcribed spacer region were compared.

Results:
Histology findings included aortic endocarditis with calcification (3/3), mitral valve endocarditis (1/3) and myocarditis (1/3). Bacteria were not visualised on H&E sections. Amplicon sequence analysis documented infection with *Bvb* genotype II in the aortic lesion (3/3) and in other cardiac regions (1/3). Despite numerous copies of *Bartonella* DNA by dPCR in all 3 cases, *Bvb* antigens and RNA were visualised within the lesional aorta in only 1 dog in which immunofluorescence detected more bacteria compared to ISH, suggesting a mixed population of dead and live bacteria. While the majority of organisms were extracellular, bacteria were visible within macrophages.

Conclusion:
Molecular techniques should be used in conjunction with bacterial visualisation techniques in dogs with culture-negative endocarditis or suspected bartonellosis.

Ultrastructural Heterogeneity of Storage Material in Selected Canine and Feline Lysosomal Storage Diseases: A 30-Year Experience

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Topic: Small animals w/o cancer

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Background:
Lysosomal storage diseases (LSDs) are inherited metabolic disorders caused by impaired lysosomal degradation and intracellular accumulation of undegraded substrates, frequently associated with neurodegeneration. Transmission electron microscopy (TEM) enables characterization of storage material, revealing lamellar, fibrillar, curvilinear, granular, and electron-dense inclusions. Although some ultrastructural patterns have traditionally been linked to specific disease categories, morphological overlap may occur among different LSDs. Therefore, TEM remains valuable in selected veterinary cases, especially when biochemical or genetic confirmation is incomplete or unavailable, or when storage material shows atypical features.

Materials & Methods:
We retrospectively reviewed ultrastructural findings from 11 veterinary LSD cases archived and examined by the authors over 30 years, including 3 dogs and 8 cats. Canine cases included adult-onset LSDs in one Azawakh and one Schipperke dog, and neuronal ceroid lipofuscinosis (NCL) in an Australian Blue Heeler dog. Feline cases included GM1- and GM2-gangliosidosis, mucopolysaccharidosis types I and VI, sphingomyelin lipidosis, alpha-mannosidosis, and NCL-11.

Results:
TEM revealed variable combinations of lamellated membranes, fibrillar material, curvilinear profiles, empty vacuoles, granular osmiophilic deposits, and electron-dense inclusions, with marked variability among diseases and cell types.

Conclusion:
Our findings highlight ultrastructural heterogeneity across species, diseases, and cell types, suggesting that storage patterns are not strictly disease-specific. Although the underlying gene defect remains a major determinant, the fine morphology of lysosomal storage material may also reflect substrate composition, affected cell populations, and disease progression. These observations support the continued relevance of TEM as a complementary and, in selected cases, unique diagnostic and investigative tool in veterinary neuropathology.

Identification of a proteomic signature in cavitary effusions of cats with feline infectious peritonitis

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Topic: Small animals w/o cancer

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Background:
Recent advents in antiviral medication offer promise for clinical interventions in the management of feline infectious peritonitis (FIP), an otherwise almost inevitably fatal coronavirus-induced disease of cats. However, these therapeutics come at significant cost, and definitive diagnosis still largely depends on invasive sampling and immunohistology. We hypothesised that the cavitary fluids in the effusive form of the disease would be characterised by protein biomarkers differentiating these cases from other effusive conditions.

Materials & Methods:
Using ultra-high sensitivity quadrupole time-of-flight mass spectrometry, we analysed the proteomes of cavitary fluids from cats with confirmed FIP (n=23) and cats with other diseases (n=23). The peptide data from these analyses were compared to identify differentially expressed proteins (DEP) using Bioconductor in R. To further elucidate the functions of DEP and potential pathways in which they were involved, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed.

Results:
Mass spectrometry identified a panel of DEP between the two groups, of which the majority pertain to proteins upregulated in FIP. Antiviral effectors, such as interferon-induced ISG15 stand out amongst proteins with the most significant and greatest enhancement in FIP effusions. Enrichment analysis of GO terms and KEGG pathways indicated the DEPs are mainly implicated in immune regulation and inflammatory signalling pathways associated with both innate and adaptive responses.

Conclusion:
FIP effusions are characterised by a proteomic fingerprint that reflects host immune activation, including upregulation of antiviral effectors, and offers potential for development of specific diagnostic tests.

Liver lobe strangulation by a thrombosed vessel in a dog

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Topic: Small animals w/o cancer

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Background:
Hemorrhagic infarction of the liver is rare in dogs, with hepatic torsion being the most common cause. While most cases of hepatic torsion are idiopathic, a few cases of quadrate lobe torsion have been associated with entrapment by the hepatogastric ligament. We report a case of hepatic hemorrhagic infarction associated with strangulation by a mesenteric vessel.

Materials & Methods:
An adult, female castrated Golden Retriever was submitted for student necropsy training; clinical history was unavailable.

Results:
The left lateral liver lobe exhibited an abrupt constriction at the middle of the lobe, narrowing from approximately 8 to 2 cm, with hemorrhagic infarction of the ventral portion. The constricted segment was encircled by a 4-mm-thick structure and a small fragment of fatty tissue (suspected mesenteric origin). The structure was contiguous with a 1mm diameter mesenteric vessel. Histologically, the constricted hepatic segment showed severe fibrosis (confirmed by Masson's trichrome staining). The circular structure was consistent with a thrombosed vessel with a muscular wall (confirmed immunohistochemically) and a lumen occluded by fibrosis, within which were numerous small endothelial-lined channels.

Conclusion:
Vascular strangulation of a liver lobe should be considered a differential diagnosis for liver lobe torsion and may be grossly distinguished by the location of hemorrhagic infarction (i.e., hilar region vs. middle of lobe). In the present case, the suspected pathogenesis is a mesenteric tear with herniation of the liver lobe and entrapment by a vessel. Subsequent thrombosis and progressive constriction of the vessel likely resulted in strangulation of the liver lobe.

Associations between signalment and clinicopathological variables with histological features in 3,464 dogs with chronic inflammatory enteropathy

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Topic: Small animals w/o cancer

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Background:
Chronic inflammatory enteropathy (CIE) is common in dogs. Histopathological features likely influence treatment response and outcome; however, associations with signalment and clinicopathological variables are lacking.

Materials & Methods:
Small intestinal endoscopic biopsy reports consistent with CIE were identified from the IDEXX-UK database (2019–2024) using keyword searches (e.g. "enteritis", "enteropathy"). Histological inflammatory severity and phenotype were graded according to World Small Animal Veterinary Association guidelines; morphological features, breed, and clinicopathological data (within the preceding 90 days) were recorded. Associations were examined using univariable (chi-squared, Kruskal–Wallis) and multivariable regression models in RStudio.

Results:
3,464 dogs were included. Phenotypes included lymphoplasmacytic (n=1,721, 49.7%), eosinophilic (n=1,265, 36.5%), neutrophilic (n=241, 6.9%), mixed (n=195, 5.6%) and granulomatous (n=42, 1.2%). Eosinophilic inflammation was overrepresented in Rottweilers, boxers and Siberian huskies, and granulomatous inflammation in French bulldogs and miniature schnauzers. Inflammatory phenotype was moderately associated with inflammatory severity ($p < 0.001$; Cramér's V = 0.22), with neutrophilic, granulomatous, and mixed phenotypes overrepresented in cases with marked inflammation. Epithelial loss/necrosis had the strongest association with inflammatory phenotype and severity ($p < 0.001$; Cramér's V = 0.265 and 0.304), with neutrophilic (OR 5.87), mixed (OR 5.33), and granulomatous (OR 3.93) phenotypes independently predicting epithelial injury. Granulomatous inflammation (OR 3.78, 95% CI 1.88–7.37) and decreased cobalamin (OR 2.46, 95% CI 1.25–4.85) were independently associated with lymphangiectasia.

Conclusion:
Inflammatory phenotype is associated with histological inflammatory severity and epithelial injury. Neutrophilic, mixed, and granulomatous phenotypes are associated with greater epithelial injury, suggesting a potential mechanism for their clinical presentation.

Differential mRNA levels of adipocyte-associated mediators in the bone marrow of dogs with Visceral Leishmaniasis

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Topic: Small animals w/o cancer

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Background:
Bone marrow adipocytes play an active role in regulating haematopoiesis through the secretion of adipokines and inflammatory mediators. In canine visceral leishmaniasis (CanL), alterations in bone marrow microenvironment may contribute to haematological dysfunction; however, the contribution of marrow adipose tissue-derived mediators to bone marrow dysfunction in CanL remains poorly understood. This study aimed to evaluate the expression of adipocyte-related genes in the bone marrow of infected dogs.

Materials & Methods:
Bone marrow samples were obtained from dogs naturally infected with *Leishmania infantum* (n=18) and healthy controls (n=5). Total RNA was extracted and reverse-transcribed for quantitative real-time polymerase chain reaction analysis. Relative expression of adiponectin, leptin, prostaglandin E2 synthase (PGE2), and interleukin-6 (IL-6) was assessed. Relative gene expression between groups was compared using the Mann–Whitney U test, considering $p<0.05$ as statistically significant.

Results:
Infected dogs exhibited significantly lower adiponectin ($p=0.0303$) and leptin ($p=0.0049$) mRNA levels than controls, whereas PGE2 mRNA levels were significantly higher ($p=0.0303$). No significant difference was observed in IL-6 levels ($p=0.1296$). These findings indicate differences in the bone marrow mRNA profile of adipocyte-associated mediators between infected and control dogs.

Conclusion:
Differences in bone marrow mRNA levels of adipocyte-associated mediators were observed in dogs with CanL, indicating alterations in the molecular profile of the bone marrow microenvironment during chronic infection. These findings provide new insights into bone marrow alterations associated with CanL and may contribute to a better understanding of mechanisms involved in haematopoietic disturbances during infection.

Biliary hamartomas in cats and dogs: retrospective study of 83 cases

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Topic: Small animals w/o cancer

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Background:
Biliary hamartoma (BH), also known as von Meyenburg complex, is a congenital malformation belonging to the group of ductal plate malformations (DPMs), and is thought to be rare in animals. BH is a small cystic structure composed of irregular bile duct profiles, often located subcapsular or within the hepatic parenchyma. The aim of this study was to retrospectively evaluate a large number of BHs in dogs and cats, analyzing their epidemiological characteristics and hepatic comorbidities.

Materials & Methods:
83 liver samples from cats and dogs, submitted for histopathological examination during 2021–2026, were included. All were routinely processed with HE staining and were diagnosed as BHs.

Results:
BHs were identified in 66 cats and in 17 dogs. Sex distribution was similar in both species. The mean age at diagnosis was 11 years in cats and 12 years in dogs. European Shorthair cats and mixed-breed dogs predominated. Commonly observed concurrent hepatic findings included ductular hyperplasia, hepatocellular swelling, vacuolation, inflammation, and cyst destruction. In cats, two BHs were accompanied by diffuse adenocarcinoma (probably metastatic), and in one cat by cholangiocellular carcinoma, closely associated with BH. In dogs, one BH was closely associated with hepatocellular carcinoma. Only one coexisting renal cyst was observed in a Persian cat.

Conclusion:
BHs are more common in cats, are typically diagnosed in older animals without breed or sex predispositions. Ductular hyperplasia was the most common concurrent hepatic finding. BHs are extremely rarely linked to tumorigenesis, as we observed only two closely associated tumors.

Concurrent exocrine pancreatic lesions in cats with inflammatory bowel disease and intestinal T-cell lymphoma

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Topic: Small animals w/o cancer

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Background:
Feline pancreatitis is frequently associated with concurrent gastrointestinal, hepatobiliary and
metabolic disorders.

Materials & Methods:
32 cats diagnosed with inflammatory bowel disease (n=19) or intestinal lymphoma (n=13),
presenting chronic vomiting, diarrhea, anorexia and weight loss were included. Intestinal and pancreatic tissue samples, collected during enterectomy (n=22) or exploratory laparotomy (n=10) were concurrently evaluated by gross and histopathological examination following routine fixation in 10% neutral-buffered formalin process and hematoxylin–eosin staining. Serum cobalamin, feline pancreatic lipase immunoreactivity (fPLI), and trypsin-like immunoreactivity (TLI) concentrations were measured in all cats and compared with the histopathological findings to assess concurrent pancreatic abnormalities.

Results:
Gross pancreatic abnormalities were observed in 28/32 cats and consisted of mild pancreatic enlargement, focal discoloration, and altered lobular appearance. Histopathological examination revealed multifocal to coalescing lymphoplasmacytic infiltrates within the pancreatic interstitium and periacinar regions. Chronic lesions consisted of variable interstitial fibrosis and acinar atrophy, whereas lymphocytes and eosinophils were commonly observed within inflammatory infiltrates. Pancreatic inflammatory changes were detected in 18/19 cats with inflammatory bowel disease and in 6/13 cats with intestinal lymphoma. Increased feline pancreatic lipase concentrations and reduced trypsin-like immunoreactivity values were frequently associated with histopathological findings.

Conclusion:
Concurrent pancreatic lesions were commonly observed in cats with inflammatory bowel disease and intestinal lymphoma being frequently associated with alterations in pancreatic biomarkers. The compared histopathological and biochemical findings support the frequent coexistence of pancreatic and intestinal abnormalities in affected cats and emphasize the value of pancreatic evaluation in feline chronic enteropathies and intestinal lymphoma.

Intestinal toxoplasmosis mimicking neoplasia in a cat

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Topic: Small animals w/o cancer

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Background:
Cats are the definitive hosts for *Toxoplasma gondii* (*Tg*). In this species, infection is usually subclinical, with clinical disease typically indicating systemic dissemination. Apart from the parasite's intraepithelial sexual reproduction, gastrointestinal involvement is uncommon. Tumour-like presentations have been described in intermediate hosts, but appear to be rare in cats.

Materials & Methods:
A nine-year-old neutered male domestic shorthair cat was presented with a ten-day history of depression, loss of appetite, and gastrointestinal signs, in a context of treated hyperthyroidism and suspected primary hyperaldosteronism. Abdominal ultrasonography revealed masses in the intestines, as well as hepatomegaly and jejunal lymphadenopathy. Fine-needle aspirates of lymph nodes and intestines yielded non-specific cytological findings. Due to clinical deterioration, euthanasia was chosen and a limited necropsy was performed by the clinicians.

Results:
Histologically, the intestines showed severe asymmetric circumferential thickening with transmural infiltration of neutrophils, macrophages, lymphocytes, and plasma cells. This was associated with foci of necrosis, numerous protozoal cysts, and free zoites. Protozoans were also identified in the liver and spleen. Giemsa staining highlighted the organisms, which were identified as *Tg* by immunohistochemistry and by qPCR targeting the REP-529 sequence. Retrospective examination of the cytological slides showed that rare zoites were present.

Conclusion:
This report describes an atypical presentation of feline toxoplasmosis characterised by segmental intestinal thickening mimicking neoplasia. To the authors' knowledge, such a presentation has not been documented previously in cats. Pathologists should be aware of this presentation, particularly when dealing with cytological specimens, as parasites can be rare and difficult to identify.

Lung Lobe Torsion Associated with Bronchial Cartilage Hypoplasia in Two Adult Spitz Dogs: Clinicopathological Findings

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Topic: Small animals w/o cancer

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Background:
Lung lobe torsion is an uncommon and potentially life-threatening condition in dogs. Although many cases are considered idiopathic, congenital bronchial cartilage dysplasia or hypoplasia has been proposed as a predisposing factor; however, histopathologic confirmation remains rare, particularly in puppies. This report describes pulmonary lobar torsion associated with abnormal bronchial cartilage in two adult small-breed dogs.

Materials & Methods:
A 2-year-old intact male Volpino Italiano and a 7-year-old female Pomeranian were presented for evaluation of acute respiratory distress. Lung lobe torsion was suspected on thoracic radiographs. Computed tomography confirmed torsion of the left cranial lung lobe in one dog and of the right middle lung lobe in the other case. Following surgical lobectomy, histopathologic, histochemical, and immunohistochemical examinations were performed.

Results:
In both dogs, histopathology revealed severe diffuse infarction of lung parenchyma characterised by extensive haemorrhage, coagulative necrosis, oedema, fibrin thrombi, and fibrinous pleuritis with reactive subpleural fibroplasia, consistent with the consequences of the pulmonary lobar torsion. Multifocally, medium- to large-calibre bronchi showed marked reduction to complete absence of cartilage rings. Residual cartilage was histologically unremarkable. In perivascular and peribronchial areas, marked disorganized smooth muscle proliferation was also observed. Both dogs recovered uneventfully following surgery.

Conclusion:
These cases describe an unusual association between lung lobe torsion and hypoplastic bronchial cartilage in two adult dogs. These findings suggest that bronchial cartilage abnormalities may occur beyond juvenile cases and could represent an underrecognised histopathologic feature of pulmonary lobe torsion in adult dogs, including cases currently classified as idiopathic.

Histopathological and Immunoglobulin Light Chain V Gene Analysis of Canine Localised AL Amyloidosis

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Topic: Small animals w/o cancer

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Background:
In canine localised AL amyloidosis, variable regions of immunoglobulin light chains (IgLs) constitute amyloid. In this study, we compared the histological features, IgL V gene usage, and mRNA expression of amyloid-positive and -negative canine extramedullary cutaneous plasmacytomas (EMPs).

Materials & Methods:
FFPE samples from 10 amyloid-positive and 30 amyloid-negative cases of canine EMP were used. Tumour subtypes and amyloid deposition were histologically examined. RT-PCR and real-time PCR were performed using total RNA extracted from FFPE samples to determine the IgL V gene sequences and assess IgL mRNA expression. The obtained sequences were aligned with the IMGT database to identify V gene usage.

Results:
Regarding tumour subtypes, amyloid-positive cases consisted of monomorphoblastic and mature types, whereas amyloid-negative cases tended to exhibit pleomorphism. Real-time PCR showed IgL mRNA expression levels were higher in amyloid-negative cases than in amyloid-positive cases. High-frequency usage of certain V genes was observed in both positive and negative cases, but no V gene was specifically associated with amyloid deposition.

Conclusion:
These findings indicate that neither IgL V gene usage nor IgL mRNA expression is sufficient to explain amyloid formation, suggesting that protein secretion capacity and local microenvironment may contribute to amyloid deposition. This study provides the first systematic comparison of IgL V gene usage and histopathological features in animal AL amyloidosis.

Systemic Vasculitis and Neuroangiostrongyliasis in a Puppy

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Topic: Small animals w/o cancer

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Background:
Angiostrongylus cantonensis, commonly known as the rat lungworm, is a significant cause of zoonotic eosinophilic meningitis. While primarily recognized for causing central nervous system (CNS) lesions, this report characterizes a case in a 5-month-old puppy that exhibited extensive systemic organ involvement and fibrinoid vasculitis.

Materials & Methods:
A 5-month-old male Maltese dog presented with acute mentation change, non-ambulatory paraparesis, and seizures. Following a poor response to high-dose prednisolone, the animal was euthanized due to clinical deterioration. A comprehensive necropsy and histopathological assessment were performed. Definitive pathogen identification was achieved through Polymerase Chain Reaction (PCR) and sequence analysis of fresh CNS tissue.

Results:
Gross examination revealed pale mucosae, thoracic and pericardial effusions, hepatomegaly, and multifocal white pinpoint lesions on the spleen. Histopathology confirmed multifocal, subacute to chronic, pyogranulomatous and eosinophilic encephalomyelitis with intralesional nematode larvae. Notably, multifocal subacute fibrinoid vasculitis was identified in the heart, liver, thymus, kidney, and intestinal tract. Splenic findings included focal granulomas and extramedullary haematopoiesis. PCR sequences demonstrated 95–98% identity to *A. cantonensis*.

Conclusion:
The final diagnosis was neuroangiostrongyliasis with systemic dissemination. These findings are consistent with larval migration via the vascular circulation, resulting in systemic vasculitis and granulomatous inflammation in multiple extra-neural organs. In endemic regions, *A. cantonensis* should be a key differential diagnosis for canine patients presenting with acute neurological deficits and multi-organ dysfunction.

Animal Welfare Perspectives of Giantism in Dogs: Health Consequences Versus Requirements in Traditional Breed Standards

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Topic: Small animals w/o cancer

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Background:
Selection for extreme body size in certain dog breeds has been associated with adverse effects on various bodily functions. However, it is unclear how these health issues relate to requirements specified in certain breed standards. The aim of this study was to provide an evidence-based compilation of all health risks reported in the scientific literature, followed by an assessment of traditional breed standards with regard to requirements for giantism.

Materials & Methods:
A systematic review of international peer-reviewed scientific literature was conducted using a complex search syntax. Furthermore, all 364 breed standards currently recognised by the Fédération Cynologique Internationale (FCI) were screened for requirements meeting the most commonly used definition for giantism in the literature.

Results:
Giantism was most commonly defined as a body weight threshold of > 45 kg. Various established health consequences include increased risks of osteosarcoma, several orthopaedic conditions and potentially fatal gastric-dilatation-volvulus. Increased incidence of other malignant tumours and reduced life expectancy were also repeatedly reported. Giantism is required by at least 20 FCI breed standards and tolerated by an additional five. Affected breeds include, but are not limited to, Great Danes, Newfoundlands, Rottweilers, Saint Bernards, Irish Wolfhounds and Dogues de Bordeaux.

Conclusion:
Breeding for giantism in dogs is clearly associated with increased risk of various forms of pain, suffering or harm which is prohibited by European and several national animal welfare laws. This raises the question of whether certain breed standards may require health-oriented revisions.

Gallbladder agenesis associated with congenital hepatobiliary developmental abnormalities in two Maltese dogs from the same breeder

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Topic: Small animals w/o cancer

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Background:
Gallbladder agenesis (GBA) is a rare congenital anomaly in dogs, previously reported in 26 canine cases, including three Maltese dogs. Concurrent histopathological abnormalities have been identified in the majority of reported cases, most commonly ductal plate malformation (DPM) and primary hypoplasia of the portal vein (PHPV). This report describes the clinicopathological, imaging and histopathological findings in two Maltese dogs from the same breeder diagnosed with GBA.

Materials & Methods:
Two Maltese dogs, aged 1.5 and 2.5 years, were investigated for persistent increases in liver enzyme activities, especially alanine aminotransferase (ALT). Diagnostic investigations included haematology, serum biochemistry, abdominal ultrasonography, computed tomography, exploratory laparoscopy, liver histopathology and culture.

Results:
One dog was clinically asymptomatic, whereas the other showed signs consistent with hepatic encephalopathy. The symptomatic dog had hyperammonaemia, microcytosis, hypoalbuminaemia, portal hypertension and acquired portosystemic shunts. Gallbladder absence was confirmed by imaging techniques and exploratory laparoscopy. Liver histopathology revealed congenital hepatobiliary abnormalities in both cases, including DPM in the dog with hepatic encephalopathy and PHPV in the asymptomatic dog. Both dogs received medical management including hepatoprotective treatment and dietary modification. Both dogs remain clinically stable 3 and 5 years after diagnosis, respectively.

Conclusion:
These findings suggest that GBA in dogs may represent part of a congenital hepatobiliary developmental disorder rather than an isolated biliary anomaly. The occurrence of this rare condition in two Maltese dogs from the same breeder, together with the apparent overrepresentation of Maltese dogs in the veterinary literature, raises suspicion of a possible hereditary predisposition.

Strain-dependent infection dynamics and immune modulation in spleen tissue and splenocyte cultures during canine distemper virus infection

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Topic: Small animals w/o cancer

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Background:
Canine distemper virus (CDV) causes devastating disease in domestic and wildlife carnivores, affecting lymphoid tissues and host immune responses. The spleen plays a pivotal role in antiviral defense; however, the mechanisms underlying the CDV-associated immune modulation remain incompletely understood. This study aimed to investigate CDV infection in spleen tissue and splenocytes cultures, focusing on cytopathic effects and myeloid cell polarity.

Materials & Methods:
Archived spleen tissue samples from naturally infected dogs were characterized by histology and leukocyte-specific immunohistochemistry. Splenocyte cultures were infected *in vitro* with different CDV strains and analyzed at different time points. In addition to evaluation of infection dynamics and cytopathic effects, myeloid cell polarity was investigated by immunostaining using M1- and M2 specific markers.

Results:
Natural CDV infection was associated with transient splenic depletion with a loss of CD3+ T cells, CD79a+ B cells, and MHC class II+ antigen-presenting cells. Splenocyte cultures infected with the CDV R252 strain showed a time-dependent increase in infection accompanied by reduced cell viability and increased cell death. Immunofluorescence analyses demonstrated TNF-α expression in infected splenocyte cultures, indicative of an M1-associated immune response.

Conclusion:
CDV infection induces cytopathic effects and modulates immune cell responses in the spleen. Preliminary immunofluorescence findings suggest altered expression of polarization-associated markers in CDV-infected splenocyte cultures, supporting further investigation of the cellular immune responses associated with CDV infection.

Spontaneous ectopic ossification affecting multiple visceral organs in a domestic rabbit

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Topic: Small animals w/o cancer

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Background:
Ectopic ossification is characterized by the formation of mature bone in non-osseous tissues. It may occur secondary to trauma, surgery, genetic factors, metabolic disturbances, or experimental induction, but can also arise idiopathically. Although rabbits exhibit a unique calcium metabolism and disturbances of calcium-phosphorus homeostasis have been associated with ectopic mineralization, the pathogenesis of spontaneous ectopic ossification remains poorly understood. This report describes spontaneous multifocal ectopic ossification affecting multiple visceral organs in a domestic rabbit.

Materials & Methods:
An 8-year-old neutered female dwarf rabbit was euthanized due to acute neurological signs and submitted for necropsy. A complete postmortem examination was performed, and tissue samples from major organs were routinely processed for histology.

Results:
Macroscopically, the lungs and liver contained multiple well-demarcated, slightly raised, white nodules measuring 0.1–0.2 cm in diameter. Histologically, the nodules consisted of anastomosing mature bone trabeculae lined by osteoblasts and occasional osteoclasts and supported by loosely arranged connective tissue. While most nodules were well demarcated, some exhibited a reactive and partially invasive phenotype accompanied by inflammation.

Conclusion:
This case describes extensive, multifocal ectopic ossification involving multiple visceral organs in a rabbit. The lesions were considered incidental and represent a rare finding. The underlying pathogenesis could not be determined. Although rabbits exhibit a unique calcium metabolism and disturbances of calcium-phosphorus homeostasis have been associated with ectopic mineralization and, occasionally, ossification, the pathogenesis of spontaneous ectopic ossification remains poorly understood. Awareness of this lesion may facilitate recognition of similar cases in the future.

When morphology is not enough: three cases of Mycobacterium smegmatis group infection in Italian cats

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Topic: Small animals w/o cancer

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Background:
Members of the *Mycobacterium smegmatis* group (MSG) are opportunistic pathogens capable of causing dermatitis and panniculitis in cats. As with other mycobacterial infections, diagnosis may be challenging and often requires a multimodal approach to improve diagnostic accuracy. This study describes three cases of MSG infection in Italian cats.

Materials & Methods:
Samples from three cats (two neutered females and one male) were submitted to a private diagnostic laboratory to investigate nodular (cases 1 and 3) or fistulous (case 2) cutaneous and subcutaneous lesions, associated with lymphadenomegaly in case 1. Histopathological examination was performed in cases 1 and 3, while case 2 underwent cytological examination. Fite–Faraco staining was used in all cases to detect acid-fast bacteria. Samples were also submitted for microbiological culture, followed by MALDI-TOF MS evaluation considering scores ≥ 2.0 significant for identification.

Results:
Cases 1 and 3 showed granulomatous to pyogranulomatous dermatitis and panniculitis, associated with pyogranulomatous lymphadenitis in case 1. Fite–Faraco staining was negative in both cases. Case 2 showed neutrophilic and macrophagic inflammation with abundant extracellular bacilli that unexpectedly stained intensely positive with Fite–Faraco. In all cases, microbiological investigations identified infection by members of MSG as confirmed by MALDI-TOF MS. All cats responded successfully to targeted antimicrobial therapy.

Conclusion:
MSG infection should be considered whenever granulomatous or pyogranulomatous inflammation is observed, even when histological or cytological findings are inconclusive or misleading as observed in this work. Indeed, our cases highlight the importance of a multimodal diagnostic approach including microbiological investigations.

Comparative Evaluation of Anti-CD1a Antibody and Serum from a Naturally Infected Dog for Leishmania Detection in FFPE Tissues

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Topic: Small animals w/o cancer

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Background:
In endemic countries, immunohistochemical confirmation of intralesional *Leishmania* in canine FFPE samples may be limited by the lack of commercial antibodies. Recent studies in humans and dogs have demonstrated that selected anti-CD1a clones cross-react with *Leishmania* amastigotes, allowing their identification in FFPE samples.

Materials & Methods:
To compare the diagnostic performance and organism detection rate of a commercial anti-CD1a antibody with serum from a naturally infected, Leishmania-positive dog, twenty FFPE samples with intralesional Leishmania amastigotes were retrospectively selected. Serial sections were immunolabeled with either canine serum or anti-CD1a and each sample was classified as positive or negative. Immunolabeled amastigotes were manually counted in five paired hotspot images per sample, selected from corresponding areas on serial sections.

Results:
Both methods detected *Leishmania* amastigotes in 19/20 cases, corresponding to a sensitivity of 95%. However, canine serum labeled significantly more amastigotes than anti-CD1a in paired hotspot counts ($p<0.05$). Conversely, CD1a provided a cleaner background, facilitating recognition of labeled protozoa.

No cross-reactivity towards *Neospora*, *Toxoplasma*, *Histoplasma* and *Malassezia* was noted.

Conclusion:
Although based on a limited number of cases, our data show that anti-CD1a has the same sensitivity as canine serum, despite labeling fewer amastigotes. Moreover, sera from different dogs may vary in titer and specificity, affecting reproducibility. Dog-on-dog methodology may increase background staining due to endogenous canine immunoglobulins. Therefore, commercial anti-CD1a can provide better standardization for detecting Leishmania amastigotes in FFPE samples. Further validation on PCR-confirmed cases with low parasite burden is needed to assess its sensitivity in weakly positive tissues.

Atypical Interstitial Pneumonia in Rabbits (Oryctolagus cuniculus): Pathologic Findings from a Longitudinal Study

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Topic: Small animals w/o cancer

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Background:
Recently, increased post-weaning mortality has been reported in Italian commercial rabbiteries, reaching up to 20–30% per production cycle. Affected rabbits develop hyperacute respiratory disease at 52–60 days of age, with sudden death despite limited clinical signs and only transient response to antimicrobial therapy. This study investigated the pathologic features and temporal development of pulmonary lesions associated with this syndrome.

Materials & Methods:
Initial investigations included necropsy, histopathology, RK13 cell culture, transmission electron microscopy (TEM), and next-generation sequencing (NGS) on symptomatic or deceased rabbits from affected farms. Subsequently, a longitudinal study was performed on 90 rabbits from three rabbiteries (two affected, one control). Three rabbits per farm were euthanised weekly for ten weeks and underwent necropsy, histopathologic, and immunohistochemical (IHC) analyses.

Results:
Investigations revealed severe fibrino-suppurative and necrotizing pleuropneumonia frequently associated with *Pasteurella* spp., together with histiocytic and lymphoplasmacytic interstitial pneumonia, type II pneumocyte hyperplasia, and multinucleated interstitial cells. Cell cultures developed cytopathic effects, while TEM identified intracytoplasmic electron-dense structures potentially compatible with viral particles, both in RK13 and putative pulmonary epithelial cells. However, NGS did not identify specific viral pathogens. In the longitudinal study, rabbits from affected farms developed severe histiocytic and lymphoplasmacytic bronchointerstitial pneumonia from 5 weeks of age, preceding suppurative lesions. Multinucleated cells were pan-cytokeratin negative and positive for CD204 and MAC387 by IHC, supporting a histiocytic lineage

Conclusion:
These findings depict an early atypical severe interstitial pneumonia preceding secondary bacterial respiratory disease in affected rabbits and suggest the role of a potential underlying infectious agent to be investigated further.

A blastomycosis-like dermatitis caused by alternaria or curvularia: a diagnostic pitfall

526
Topic: Small animals w/o cancer

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Background:
*Alternaria*spp. and *Curvularia* spp. are dematiaceous fungi occasionally reported as causes of fungal dermatitis in immunocompromised animals, and they may histologically mimic blastomycosis. This report describes two cases of granulomatous dermatitis in a horse and a dog from the Valencian Community, Spain, with fungal structures morphologically resembling *Blastomyces* spp. yeasts but later identified as *Alternaria* spp. and *Curvularia* spp.

Materials & Methods:
In December 2025-January 2026, two skin biopsies were submitted for histopathology. The first was from an 8-year-old mixed-breed mare with multiple cutaneous nodules along the alba line. The second was from a 7-year-old male Ibizan Hound with immune-mediated polyarthritis, multiple depigmented papules on the nose, and a cutaneous nodule on the nape.

Results:
Histopathology was similar in both cases, showing mild to moderate superficial and deep granulomatous dermatitis with macrophages, neutrophils, lymphocytes, and plasma cells. Numerous extracellular and intrahistiocytic yeast-like structures, 7-20 µm in diameter, with an approximately 2 µm thick, refractile, non-pigmented, double contour wall, a basophilic nucleus, and occasional structures resembling broad-based budding were observed. PAS and Grocott stains highlighted fungal walls and short septate, narrow hyphae (4-5 µm in diameter). Panfungal PCR and sequencing showed sequences matching *Alternaria* spp. (2/2) and *Curvularia* spp. (1/2; horse).

Conclusion:
Based on the histopathology, a granulomatous dermatitis associated with *Blastomyces* spp. was initially diagnosed. Molecular analyses identified *Alternaria* spp. and *Curvularia* spp. as the causative agents, highlighting them as important differential diagnoses in suspected blastomycosis. Special stains should be performed to better reveal short fungal hyphae.

First report of feline straelensiosis in Italy

539
Topic: Small animals w/o cancer

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Background:
Straelensiosis is a trombidiasis caused by *Straelensia cynotis* larvae that affect wild mammals but has also been reported in dogs (southern European countries and Israel), and infrequently in cats (France and Israel). Both species share similar clinical presentation, with a generally non-pruritic papulo-nodular dermatitis with possible crusts.

Materials & Methods:
A 11-year-old spayed female domestic shorthair cat developed a pruritic papulo-nodular dermatitis with crusts, localized on the dorsal trunk, abdomen, limbs, pinnae, and tail. Histopathological examination of biopsy specimens from dorsal trunk and abdomen, and molecular PCR identification of the parasite on FFPE blocks were performed.

Results:
Both specimens were characterized by dilated infundibula containing a variably degenerated arthropod larva surrounded by an eosinophilic, variably calcified stylosome (consistent with *S. cynotis*.), follicular hyperplasia with pseudoepitheliomatous features, and perifollicular mucinosis. A mild (abdomen) to moderate (dorsal trunk), perifollicular and interstitial mixed inflammatory infiltrate was present. The dorsal trunk specimen was also characterized by a severe pustular dermatitis with ulceration, serocellular crusts, epidermal hyperplasia and hyperkeratosis, and neutrophilic luminal folliculitis (suggestive of secondary bacterial infection). *S. cynotis* infection was confirmed by isolation and analysis of the parasite's molecular sequences.

Conclusion:
To the authors' knowledge, this is the first description of feline straelensiosis in Italy, with macro- and microscopic findings and molecular identification of the parasite. The presence of the parasite can elicit negligible inflammation, or may be associated with severe pustular dermatitis with ulceration and pruritus, likely due to secondary bacterial infection.

Histopathological Characterization of Ocular Lesions in Guinea Pigs (Cavia porcellus): A Retrospective Case Series

560

Topic: Small animals w/o cancer

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Background:
Ocular diseases are increasingly recognized in guinea pigs; however, reports describing their associated histopathological findings remain limited. This study aimed to characterize the histologic spectrum of ocular lesions in enucleated guinea pig eyes submitted for diagnostic evaluation.

Materials & Methods:
Guinea pig eyes submitted to LABOKLIN GmbH & Co. KG for histopathological examination between 2017 and 2025 were retrospectively reviewed. All animals presented with a clinically suspected ocular disorder. Available data, including age, sex, and clinical history, were recorded. A total of 15 globes from 14 animals were included in the study.

Results:
Seven of 15 globes (46%) were diagnosed with intraocular osseous choristomas (IOC), characterized by mature bone arising from the ciliary body and extending into the anterior chamber. Inflammatory lesions were identified in 6/15 globes (40%), including suppurative (2/15), lymphoplasmacytic (2/15), and pyogranulomatous inflammation (2/15). One globe (7%) contained an osteosarcoma in an animal with contralateral IOC, while one eye (7%) was histologically unremarkable. The lesions most frequently present alongside IOC were chronic follicular conjunctivitis (7/7) and keratitis (5/7), either lymphoplasmacytic or ulcerative-suppurative in nature. Significant intraocular inflammation was uncommon and primarily involved the ciliary body.

Conclusion:
Although IOC was previously considered rare and likely incidental, it accounted for nearly half of all ocular submissions in this series. These findings suggest IOC may be more prevalent than currently recognized or may disproportionately result in enucleation because of associated gross changes, despite their described, apparent, benign nature.

Histopathological and clinicopathological findings in 227 dogs with chronic neutrophilic inflammatory enteropathy

574
Topic: Small animals w/o cancer

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Background:
Neutrophilic inflammatory enteropathy (NIE) is poorly characterised in dogs, with no large studies describing signalment, clinicopathological, or histological characteristics.

Materials & Methods:
Retrospective study examining canine small intestinal biopsy submissions from the UK IDEXX laboratories database (2019–2024), with cases identified using keyword-based coding of histopathology reports. The keywords that were used in this study included "enteritis", "enteropathy" and "neutrophilic". The severity of neutrophilic inflammation was categorised as mild, moderate or marked, as per World Small Animal Veterinary Association guidelines. Univariable and multivariable analyses were performed using RStudio.

Results:
Neutrophilic inflammation was identified in 227 dogs: mild in 142 (62.5%), moderate in 49 (21.6%), and marked in 36 (15.9%). No significant associations were identified between neutrophilic severity and sex ($p=0.81$), age ($p=0.18$; $\eta^2=0.006$), or breed ($p=0.266$). Clinicopathological data were available for 65/227 (28.6%) cases, with median (IQR) values available for haematocrit (45.4 (36.5–53.6) L/L; n=18), neutrophil count (8.65 (5.48–15.74) $x10^9$/L; n=18), albumin (26.4 (22.7–31.3) g/L; n=14), globulin (29.4 (23.6–34.4) g/L; n=14), cholesterol (5.73 (4.02–7.18) mmol/L; n=12), folate (12.4 (7.2–15.8) ng/mL; n=36) and cobalamin (424.5 (237.5–556.2) ng/L; n=38). No associations were identified between these variables and neutrophilic severity (all $p>0.05$). Mucosal epithelial loss/necrosis was strongly associated with neutrophilic inflammatory severity ($p<0.001$, Cramer's $V=0.47$), with higher epithelial loss scores across inflammatory categories (9% mild, 12% moderate, 58% marked).

Conclusion:
NIE is strongly associated with mucosal epithelial injury, suggesting neutrophilic inflammation contributes to or is the result of mucosal damage, potentially contributing to clinical presentation.

Postmortem detection and occurrence of feline enteric viruses

578
Topic: Small animals w/o cancer

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Background:
Feline viral gastroenteritis is a significant cause of morbidity and mortality in cats. Despite the known role of feline panleukopenia virus (FPV) and feline coronavirus (FCoV) many cases remain a diagnostic challenge, suggesting involvement of less characterized enteric viruses. This study aimed to detect FPV, FCoV, feline bocavirus (FBoV) and feline astrovirus (FeAstV) in cats with (gastro)enteritis.

Materials & Methods:
Cats (n=20) aged 4 months to 16 years (median 1.3 years) were diagnosed with (gastro)enteritis based on postmortem examination (necropsy and histopathology), and then underwent PCR analysis with Sanger sequencing. Genetic material was isolated from intestinal and mesenteric lymph node samples.

Results:
Infection with at least one virus was detected in 80% (16/20) of cats. FPV was most common (14/20), followed by FCoV (11/20) and FBoV (9/20). FeAstV was rare (1/20). FPV was the only virus detected as a mono-infection (in 4 cats). Co-infection FPV+FBoV+FCoV was predominant (5/20). Lesions consistent with panleukopenia were found in only 3/14 FPV-positive cats. Lesions consistent with feline infectious peritonitis (FIP) occurred in 3/11 FCoV-positive cats. On the other hand, panleukopenia-consistent lesions were present in 1 FPV-negative cat and FIP-consistent lesions in 2 FCoV-negative cats.

Conclusion:
Multiple viral infections were common in cats with (gastro)enteritis, with FPV most frequently detected. The presence of novel enteric viruses, FeAstV and FBoV, implies their role in feline enteric infections. Postmortem diagnosis of panleukopenia and FIP was not always consistent with molecular results. Our results indicate that combining postmortem examination and molecular testing may increase accuracy of diagnosis.

TOPIC: CANCER

Mitotic Figures with Atypical Morphology in Non-Neoplastic Tissue:Time to Revise Current Morphological Criteria?

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Topic: Cancer

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Background:
Atypical mitotic figures (AMFs) are prognostically relevant in various animal and human neoplasms, yet identification remains challenging due to overlap with normal morphologies. This study evaluated whether AMF morphologies, as described in current guidelines, are specific for malignant tumours.

Materials & Methods:
From HE-stained whole-slide images, 300 mitotic figures (MFs) from non-neoplastic tissue (canine gingiva, feline skin, canine intestine) and 300 MFs from neoplastic tissue (canine oral squamous cell carcinoma, feline cutaneous squamous cell carcinoma, canine intestinal carcinoma) were annotated, cropped, and uniformly resized. MFs with borderline morphology between normal and atypical were overrepresented. The MFs were randomised and sent to five pathologists for binary classification. Non-neoplastic MFs labelled 'atypical' by at least one pathologist were classified by a single person based on their most likely morphology (Bridging, Lagging, Tri-/Multipolar, Dispersed, C/V/U-shape, Bipolar asymmetry, Other).

Results:
Inter-rater agreement for normal versus atypical was fair (Fleiss' $\kappa = 0.39$). Of 300 non-neoplastic MFs, 214 were labelled 'atypical' at least once. Among non-neoplastic tissues, skin had the most 'atypical' labels (83/100), followed by gingiva (68/100) and intestine (63/100). The vast majority of presumed AMFs met 'Lagging' criteria (93/214), followed by 'Bipolar asymmetry' (23/214).

Conclusion:
The high number of suspected AMFs in non-neoplastic tissue raises concerns that current definitions may lack specificity, potentially leading to overestimation of AMFs. Existing AMF guidelines, particularly for 'Lagging' and 'Bipolar asymmetry', should be evaluated and revised in future studies.

Pd1 in serum small extracellular vesicles as a prognostic biomarker in canine mammary carcinomas

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Topic: Cancer

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Background:
Small extracellular vesicles (SEVs) are cell-derived particles smaller than 200 nm in diameter, enclosed by a lipid bilayer and incapable of self-replication. The programmed cell death protein 1 (PD1) is an immune checkpoint receptor in the PD1/PDL1 pathway. SEVs can carry PD1 on their surface and exert immunosuppressive effects at sites distant from the tumour. The aim of this study was to quantify PD1+ SEVs in the serum of female dogs with mammary carcinomas and to assess the prognostic value of this biomarker.

Materials & Methods:
Serum samples from 28 female dogs with mammary carcinomas and 12 healthy controls were analysed. SEVs were isolated via ultracentrifugation. Nano-flow cytometry was used for PD1 quantification using an anti-human PD1 antibody (4F12, Novus Biologicals), following recommendations for bead size (100-240 nm) and fluorescence selection. The percentage of PD1+ SEVs was assessed for each case.

Results:
Dogs with mammary carcinomas showed higher percentages of PD1+ SEVs ($p=0.002$) compared with the control group, specifically in dogs with higher histological grades (II, $p=0.024$; III, $p=0.011$), and those that died due to the disease ($p=0.010$). Based on a ROC curve analysis, a cutoff of 14.7% was established. Dogs with ≥14.7% of serum PD1+ SEVs exhibited a significantly shorter survival ($p=0.022$; hazard ratio = 5.675; 95%CI = 0.917 to 35.12).

Conclusion:
The percentage of PD1+ SEVs in the serum of dogs with CMCs is a potential prognostic marker for post-surgical survival.

Odontogenic malignant tumour in a young cat probably derived from a feline inductive odontogenic tumour.

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Topic: Cancer

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Background:
Odontogenic tumours are rare in cats, as in other domestic species. The aim of this study is to describe a case of odontogenic malignant tumour, with a sarcomatous component, probably arising from a feline inductive odontogenic tumour (FIOT).

Materials & Methods:
A 3-year-old neutered male Scottish Fold cat was presented with an intraoral soft tissue swelling around tooth 204, which had been present for one year. Dental radiographs revealed a mixed pattern of radiopaque and radiolucent areas. The mass was excised, and samples were submitted for histological examination and immunohistochemical investigation using cytokeratin, Osterix, and SOX10 markers.

Results:
Histological examination revealed proliferating neoplastic odontogenic epithelial and mesenchymal cells, the latter displaying malignant features. The mesenchymal cells were associated with an eosinophilic mineralizing extracellular matrix (osteoid). In a small area of the samples, mesenchymal induction around epithelial islands resembling the cup stage of odontogenesis was observed. Immunohistochemical analysis demonstrated positive cytoplasmic immunoreactivity for cytokeratin in the epithelial component. Nuclear-positive Osterix immunostaining was present in both epithelial and mesenchymal cells, while SOX10 was negative.

Conclusion:
This is the first report of an odontogenic osteosarcoma probably resulting from loss of proliferation control of mesenchymal neural crest–derived cells of a FIOT. Osterix and SOX10 are both involved in the process of odontogenesis. Osterix expression in the mesenchymal cells, together with the presence of osteoid, indicates malignant transformation of FIOT odontoblasts towards osteoblasts. In contrast, the lack of SOX10 expression suggests its down-regulation following differentiation of neural crest–derived ectomesenchymal cells towards osteoblasts.

Expression of candidate therapeutic targets in canine splenic non-angiomatous non-lymphomatous sarcomas (NANLS): an immunohistochemical study

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Topic: Cancer

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Background:
Canine splenic non-angiomatous, non-lymphomatous sarcomas (NANLS) are rare, typically aggressive primary neoplasms arising from heterogeneous stromal cells. Given their poor prognosis and the limited efficacy of conventional chemotherapy, this study aimed to investigate the expression of potential therapeutic targets.

Materials & Methods:
14 NANLS retrospectively collected between 2011 and 2023 were included. FFPE sections were immunostained for vimentin, Iba-1, CD31, α-smooth muscle actin (αSMA), nerve growth factor receptor (NGFR), and glial fibrillary acidic protein (GFAP) for tumor diagnosis. Antibodies validated in dogs were used to assess CD117 (KIT), vascular endothelial growth factor receptor 2 (VEGFR2), fibroblast growth factor receptor 1 (FGFR1), platelet-derived growth factor receptor β (PDGFRβ), fibroblast activation protein (FAP), and oxytocin receptor (OXTR) expression. Samples of non-neoplastic canine spleens were included as controls. Target expression was semi-quantitatively scored based on percentage of labeled cells (0–10%, 11–50%, 51–80%, 81–100%) and staining intensity (low, moderate, high). A target was considered promising if >80% of neoplastic cells were positive.

Results:
Based on morphology and immunophenotype, NANLS included 9 stromal sarcomas (vimentin only), 2 leiomyosarcomas, 2 nerve sheath tumors, and 1 liposarcoma. In NANLS, CD117, VEGFR2, and FGFR1 were always negative. PDGFRβ, FAP, and OXTR labelled 2/14, 5/14, and 13/14 cases respectively, with low to moderate intensity. None of the therapeutic targets were expressed in non-neoplastic splenic stroma.

Conclusion:
Based on the immunohistochemical analysis performed and the limited sample size, FAP and OXTR may represent promising theranostic and/or therapeutic targets and warrant further investigation.

Primary leptomeningeal gliomatosis with astrocytic differentiation in a dog

175
Topic: Cancer

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Background:
Primary leptomeningeal gliomatosis (PLG) is characterized by the dissemination of neoplastic glial cells within the subarachnoid space in the absence of a parenchymal glioma. Most reported canine cases are leptomeningeal oligodendrogliomas, with only one documented astrocytomas. Here we report a canine case of PLG with histologic and immunohistochemical features of high-grade astrocytoma.

Materials & Methods:
A 3-year-old castrated male American Bully was evaluated because of a 5-week history of progressive vestibular signs and visual impairment. Magnetic resonance imaging of the brain revealed multifocal, predominantly left-sided parenchymal and meningeal lesions in the cerebrum and thalamus. Clinical signs progressed, and euthanasia was elected.

Results:
Autopsy revealed bilateral gelatinous leptomeningeal thickening extending from the ventral frontal lobes to the hypothalamus and mesencephalon. The left basal nuclei and piriform lobe were mildly swollen. Histologically, the leptomeninges of the ventral frontal cortex, hypothalamus, mesencephalon, and pons were expanded by bundles of elongate neoplastic cells with moderate pleomorphism and marked anisokaryosis, abundant eosinophilic cytoplasm, and round to elongate nuclei with coarse chromatin and 1-2 nucleoli. There were 4 mitoses in 2.37 mm^2 (10 FN22/40X fields). Neoplastic cells extended into the perivascular spaces of the underlying gray and white matter. Approximately 50% of neoplastic cells had moderate nuclear immunolabeling for OLIG2. There was widespread, robust cytoplasmic immunolabeling for GFAP, with no immunolabeling for E-cadherin.

Conclusion:
Our findings support that rare canine leptomeningeal astrocytomas share histologic and immunophenotypic features with parenchymal astrocytomas, similar to PLG with oligodendroglial differentiation.

Presumed mass-forming mural follicular lymphoid hyperplasia associated with intestinal T-cell lymphoma in two cats: A diagnostic challenge

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Topic: Cancer

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Background:
Lymphocytic infiltrations of the intestine in cats are common and range from lymphocytic enteritis to intestinal lymphomas. We present two unusual cases with intestinal small T-cell lymphoma and a concurrent mass with prominent lymphoid follicles extending from the submucosa to the mesentery in the jejunum.

Materials & Methods:
The histomorphology of the two concurrent jejunal lesions was characterized in both cats using HE and IHC for CD3, CD20, CD79a and Pax5. Clonality testing (PCR for antigen receptor rearrangements (PARR)) was performed on FFPE sections from both lesions separately.

Results:
In both cases, the mucosal lesions consisted of small T-cell infiltrates (CD3 positive; B-cell markers negative) in epithelium and lamina propria with low mitotic activity and T-cell receptor clonality, compatible with small T-cell lymphoma. The concurrent mural masses were composed of numerous B-cell-exclusive lymphoid follicles, frequently with germinal centers (CD20, CD79a and Pax5 positive, CD3 negative), surrounded by numerous small T-cells, mimicking mucosa associated lymphoid tissue (MALT). This lesion also showed T-cell receptor clonality with PARR.

Conclusion:
A similar case has recently been published by Sakai et al. interpreted as T-cell lymphoma with prominent lymphoid follicle formation. As there is evidence that clonal T-cell expansion may also occur in reactive lesions and as the architecture of the mural lesion resembles MALT, we question that the mass lesion corresponds with lymphoma and propose a diagnosis of atypical reactive hyperplasia of MALT, as described in humans. These controversial cases warrant further investigation including clinical follow-up to determine pathogenesis and prognosis.

From indolent to aggressive: Immunohistochemical and clonal evidence for intestinal lymphoma transformation in cats

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Topic: Cancer

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Background:
Lymphoma frequently affects the feline alimentary tract, with small T-cell lymphoma (enteropathy-associated T-cell lymphoma type 2, EATL-2) being the predominant form, typically following an indolent clinical course. Concurrence with aggressive large-cell lymphoma is rarely described, and common clonal origin has been described in only one previously reported cat.

Materials & Methods:
Eleven cats with concurrent intestinal small-cell and large-cell lymphoma based on histopathology were included. All cases underwent immunohistochemistry (CD3, CD20, CD79a, Pax5). PCR for antigen receptor gene rearrangement (PARR) was performed separately for each lymphoma in all animals, targeting immunoglobulin heavy, light chains and kappa deleting element (IGH-VDJ, IGH-DJ; IGL; Kde) and T-cell receptor gamma chain (TRG1, in all cases; TRG2, in 8/12). Bidirectional Sanger sequencing was performed in two selected cases.

Results:
All small-cell lymphomas showed T-cell phenotype. Large-cell lymphomas comprised 6/11 T-cell, 2/11 B-cell and 3/11 lymphomas positive for CD3 and CD79a. PARR was non-diagnostic in 2/11 cases. In the remaining, all small-cell and 7/9 large-cell lymphomas were clonal by tested TRG, while one large-cell tumor was clonal by IGH. PCR products from two cats were sequenced, confirming identical TRG clones in both lymphomas in both animals.

Conclusion:
These findings provide evidence that feline intestinal small T-cell lymphoma (EATL-2) can, in individual cases, undergo clonal transformation into large T-cell lymphoma. Larger studies with comprehensive molecular characterization are needed to further elucidate this relationship.

Adopting evidence gap maps (EGMs) to guide the identification of research priorities in canine prostate oncology

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Topic: Cancer

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Background:
Evidence-based medicine guides clinical decision-making; however, rare conditions such as canine prostatic carcinoma (PC) remain poorly represented in the literature, resulting in fragmented knowledge and predominance of low-level evidence. Although studies on canine PC exist, their overall volume and quality have not been systematically evaluated. Evidence Gap Maps (EGMs) provide a structured, visual method to assess the distribution and strength of research.

Materials & Methods:
A structured search was conducted in PubMed and Web of Science using predefined criteria. Retrieved records were screened according to established criteria. Eligible studies underwent full-text review, and data were extracted into a standardised form. Each study was classified based on the adapted Oxford Centre for Evidence-Based Medicine levels of evidence. As a final step, all records will be imported into EPPI Reviewer® software, adapted for the purposes of this project.

Results:
In total, 238 studies were selected, and all the available evidence for canine PC was organised into a structured framework where tumour subtypes and descriptors represent rows and columns, respectively. Pockets of low evidence and lack of high-evidence levels were flagged in multiple fields assessed by this study. These findings will be displayed in a visual and interactive map.

Conclusion:
This study provides a structured overview of the available literature on spontaneous canine PC. The resulting map offers a visual summary of existing evidence, supporting clinicians and researchers in decision-making and helping to define future research priorities. This represents the first application of EGM methodology in veterinary medicine.

Comparison of clinical and pathological approaches to tumour size assessment in canine mammary carcinomas and their association with molecular subtype

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Topic: Cancer

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Background:
Tumour size and behaviour are key factors for surgical treatment and prognosis in canine mammary carcinomas (CMCs). CMC size is essential for staging at diagnosis, while molecular subtype may reflect biological behaviour but remains less characterised than in humans. We analysed association between various tumour dimensions and histopathological and molecular characteristics.

Materials & Methods:
62 CMCs from 51 female dogs were included. Clinical tumour size (cT) was estimated by surgeon, while pathological (gross pT) and (histo)pathological (HP pT) tumour dimensions (length and width) were measured by a pathologist. Molecular subtypes were examined by immunohistochemistry for expression of estrogen receptor (ER), progesterone receptor (PR), HER2, Ki67, basal (CK5/6, CK14, p63), and luminal markers (CK19).

Results:
All tumour dimensions were significantly positively correlated (r=0.7 to 0.8) but gross pT length was significantly shorter than cT ($p<0.001$) and HP pT dimensions were significantly shorter than gross pT dimensions ($p<0.001$). Larger gross pT length was significantly associated with complex carcinoma ($p=0.022$), invasive growth ($p=0.018$), lymphovascular invasion ($p=0.015$), and higher histological grade ($p=0.001$). PR-negative CMCs showed significantly greater HP pT ($p=0.002$) and gross pT lengths ($p=0.017$). In contrast, neither molecular subtype nor ER, HER2, Ki67 expression were significantly associated with CMC dimensions. Among basal/luminal markers, p63-positive CMCs had significantly shorter gross pT length ($p=0.008$).

Conclusion:
Clinical, gross and HP tumour dimensions are correlated but they should not be considered interchangeable. Gross pT length appears to be most diagnostically informative. Our results suggest that CMC size does not reflect underlying molecular tumour biology.

Characterisation of a Novel Tumour Type in the Cervical Spinal Cord of a 3-Year-Old Lagotto Romagnolo Dog

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Topic: Cancer

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Background:
Primary intramedullary spinal tumours are uncommon in dogs and typically aggressive. These lesions cause progressive neurological deficits, and prognosis is generally poor. Ependymomas, astrocytomas, and oligodendrogliomas are gliomas that arise within the spinal cord. Here, we report a novel type of canine spinal tumour.

Materials & Methods:
A 3-year-old female Lagotto Romagnolo dog with progressive, treatment-resistant paresis was examined clinically, by MRI, pathologically and immunohistochemically (vimentin, GFAP, periaxin, S100, p75, neurofilament, α-SMA, desmin, Olig2, melan-A, c-Kit), as well as tumour samples undergoing whole exome sequencing (WES) and for proteomic analysis (MALDI-MSI).

Results:
A mass measuring 1.7 x 1.1 x 1.0 cm was identified in the cervical spinal cord (C2-C3) via MRI and gross pathology. Histologically, the tumour did not fit current canine or human glioma classifications. The lesion showed intra-axial and subarachnoid infiltration, a predominantly angiocentric pattern, abundant production of extracellular matrix, and complex differentiation, including some glial-like cells. With exception of α-SMA, desmin, and melan-A, all markers examined were expressed with high intercellular variability. WES analyses identified three protein-altering somatic mutations: an in-frame insertion in *EGFR* (p.N705_H707dup; in the tyrosine kinase domain), a nonsense mutation in *NDK1* (p.C447*; stop-gained), and a frameshift deletion in *SH3D19* (p.R392Dfs*70). First MALDI-MSI analyses failed to yield a proteomic profile indicative of its histogenesis or possible classification.

Conclusion:
The tumour could not be classified under any established entity in veterinary or human medicine and likely represents a novel type of glioma. Further characterisation of this tumour may allow identification of additional cases.

Proteomic profiling of drug-induced Polyploid Giant Cancer Cells in a preclinical model of High-Grade Serous Ovarian Cancer

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Topic: Cancer

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Background:
High-Grade Serous Ovarian Cancer (HGSOC) is the most aggressive ovarian cancer subtype, characterized by late diagnosis and chemotherapy resistance. Polyploid Giant Cancer Cells (PGCCs) are a chemotherapy-induced subpopulation associated with tumor progression, drug resistance, and relapse. However, their molecular characterization remains incomplete. This study aimed to define the proteomic profile of PGCCs in a preclinical HGSOC mouse model.

Materials & Methods:
C57BL/6 female mice (n=10) were intraperitoneally injected with ID8 $p53^{-/-}$/$PTEN^{-/-}$ cells and treated with vehicle (n=5) or carboplatin (n=5). Primary tumors were collected and Laser Capture Microdissection (LCM) was employed to isolate PGCCs and conventional neoplastic cells. Samples were processed for liquid chromatography tandem mass spectrometry. 19 samples across groups were profiled: PGCCs-vehicle (n=4), PGCCs-carboplatin (n=5), conventional neoplastic cells-vehicle (n=5), and conventional neoplastic cells-carboplatin (n=5).

Results:
Proteomic analysis revealed a clear separation between PGCCs and conventional neoplastic cells under vehicle conditions, partially reshaped by carboplatin, suggesting activation of shared stress-response programs. Overall, PGCCs displayed increased expression of proteins involved in mitotic stress, spindle assembly, cell-cycle regulation, and DNA damage response. Pathway analysis revealed enrichment of cell-cycle, DNA repair, cytokinesis, and chromatin remodeling pathways, supporting mechanisms of polyploidization and genomic instability. Unique proteins of PGCCs (Pkp3, Aldh1b1, Cps1, Eif2ak3, Spc24, Usp34, and Scel) linked to stress adaptation, metabolic remodeling, and cellular plasticity were also identified.

Conclusion:
PGCCs exhibited a distinct proteomic landscape associated with stress adaptation and cellular plasticity, supporting their potential role in HGSOC progression and therapy adaptation while providing insights for biomarker discovery and targeted therapeutic

strategies.

Assessment of Molecular Subtypes in Highly Malignant Canine Mammary Carcinomas

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Topic: Cancer

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Background:
Research on molecular subtypes of canine mammary cancer (MC) remains limited, although their characterisation is important for developing canine-specific therapies and validating canine MC as a comparative model for human, particularly triple-negative, breast cancer. This study aimed to characterise the molecular subtypes of highly malignant canine MCs.

Materials & Methods:
Thirty-nine grade III tumours were retrospectively selected. After histopathological review of the H&E-stained slides, immunohistochemistry for ER, PR, HER2, Ki-67, CK14, and AR was performed on paraffin-embedded sections to determine the molecular subtype: luminal A (ER/PR+, HER2−, Ki-67 low), luminal B-HER2 negative (ER+, PR− or low, HER2−, Ki-67 high), luminal B-HER2 positive (ER+, PR− or low, HER2+, Ki-67 high), HER2-overexpressing (ER/PR−, HER2+, Ki-67 high), triple-negative (TN) (ER−, PR−, HER2−), and the TN subtypes luminal androgen receptor (LAR) (ER−, PR−, HER2−, AR+) and basal-like (ER−, PR−, HER2−, CK14+). The statistical study included epidemiological, clinical, and pathological variables.

Results:
Luminal A was the most frequent molecular subtype (48.7%), followed by triple-negative (30.8%) and luminal B-HER2(−) tumours (17.9%). Only one case was HER2-overexpressing, and no TN-LAR tumours were identified. Molecular subtype differed significantly in tumours with an inflammatory phenotype (n=15) (p=0.029), which showed a predominance of triple-negative tumours (66.7%), whereas most luminal A tumours and all luminal B-HER2(−) tumours occurred in non-inflammatory cases (n=24). Molecular subtype was also associated with clinical stage (p=0.02).

Conclusion:
Although there are similarities, important differences exist between the molecular subtypes of canine and human breast cancer, and these should be considered in comparative studies.

Plexiform uterine mesenchymal tumour in a dog resembling human uterine plexiform lesions: a diagnostic challenge

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Topic: Cancer

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Background:
Plexiform growth patterns in uterine mesenchymal tumours are uncommon and represent a recognised diagnostic challenge in human pathology, where they may occur in smooth muscle tumours, endometrial stromal tumours, and rare polyphenotypic entities such as uterine tumours resembling ovarian sex cord tumours (UTROSCT). In dogs, plexiform patterns have rarely been described in leiomyosarcomas, and not with the architectural extent and histomorphology observed here.

Materials & Methods:
A uterine horn mass from a dog was evaluated histologically using H&E and by immunohistochemistry, including CD10, desmin, α-SMA, muscle-specific actin (MSA), calponin, vimentin, AE1/AE3, inhibin and Ki-67. Normal uterine horn tissue from two dogs was used as controls.

Results:
The tumour was centred in and effaced the myometrium while sparing the endometrium. Histologically, it consisted of monomorphic epithelioid cells arranged in thin anastomosing cords and ribbons forming a plexiform pattern, associated with small arterioles and hyalinised stromal collagen. Atypia, mitotic count and Ki-67 index (<1%) were low. Neoplastic cells were strongly positive for CD10, desmin and vimentin, while α-SMA, MSA, calponin, AE1/AE3 and inhibin were negative.

Conclusion:
This case describes an unusual uterine mesenchymal tumour in a dog with extensive plexiform architecture and lack of convincing smooth muscle differentiation, not previously described with these features in veterinary pathology. Morphology and immunophenotype show similarities to plexiform uterine lesions described in human pathology, including stromal tumours and UTROSCT-like entities. This case highlights the diagnostic difficulty of classifying such tumours and underscores the phenotypic complexity of uterine mesenchymal tumours in veterinary pathology.

Diagnostic Accuracy of Deep Learning Foundation Models for Differentiating Canine Oral Amelanotic Melanoma from Fibrosarcoma on H&E-Stained Whole Slide Images

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Topic: Cancer

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Background:
Canine oral amelanotic melanoma is frequently misdiagnosed as fibrosarcoma due to overlapping morphological features, yet these tumours carry markedly different prognoses. This study evaluated the diagnostic accuracy of deep learning foundation models for differentiating these tumour types on haematoxylin and eosin-stained whole slide images (WSIs).

Materials & Methods:
Seventy WSIs (38 melanoma, 32 sarcoma) were digitised from immunohistochemically confirmed canine archival cases at the University of Liverpool. Tiles of 512 x 512 pixels were extracted, yielding 82,678 labelled tiles. Eleven publicly available foundation models (nine pretrained on human pathology, two general-purpose baselines) were benchmarked using 5-fold cross-validation, and a 2-layer multilayer perceptron classifier was trained on frozen model features with slide-level splitting.

Results:
The top-performing models, CONCH v1.5 and Virchow2, both achieved 78.7% balanced accuracy, with area under the receiver operating characteristic curve values of 0.882 and 0.884 respectively. Pathology-specific models outperformed general-purpose baselines by 5.4 percentage points. CONCH v1.5 showed the most balanced per-class recall (melanoma 80.7%, sarcoma 76.7%). Two-thirds of tiles (54,851/82,661; 66.4%) were classified consistently by at least 10 of 11 models, while 12,535/82,661 tiles (15.2%) were classified at near-random accuracy. Using conformal prediction at the 99% coverage level, CONCH v1.5 produced definitive single-class predictions for 30.1% of tiles with 96.7% accuracy.

Conclusion:
Pathology foundation models, despite human-only pretraining, differentiate canine oral amelanotic melanoma from fibrosarcoma on routine haematoxylin and eosin sections with moderate accuracy. Conformal prediction reliably flags morphologically ambiguous tiles where immunohistochemistry remains essential.

Tumor Immune Microenvironment and Immune Phenotypes of Canine Squamous Cell Carcinomas Assessed by Multiplex Immunohistochemistry

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Topic: Cancer

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Background:
Prognostic and predictive immune biomarkers remain poorly characterized in canine squamous cell carcinomas (SCCs). This study investigated the tumor immune microenvironment (TIME) of canine SCCs through characterization of immune cell populations and definition of specific immune phenotypes.

Materials & Methods:
Thirty-four canine SCCs were selected (20 cutaneous, 14 mucosal). Multiplex immunohistochemistry was performed using an automated staining platform (Discovery Ultra, Roche Diagnostics) with anti-CD3, -CD20, -IBA1, -CD40, and -CD206 antibodies. According to the spatial distribution of immune infiltrates, tumors were classified as immune-inflamed (intratumoral lymphocytic infiltration), immune-excluded (lymphocytes restricted to the invasive margin), immune-desert (minimal or absent immune infiltration). Immune cell populations were quantified on digitized whole-slide images using QuPath software.

Results:
Based on CD3, CD20, and IBA1 expression, 27/34 SCCs (79%) were classified as immune-inflamed, 5/34 (15%) as immune-excluded, and 2/34 (6%) as immune-desert. Immune-inflamed tumors showed predominance of CD3+ T lymphocytes and IBA1+ macrophages, with similar proportions in cutaneous SCCs and higher T-cell infiltration in mucosal SCCs. In contrast, immune-excluded and immune-desert tumors were characterized mainly by IBA1+ cells. Macrophage profiling revealed a predominance of CD206+ (M2-like) macrophages in both cutaneous and mucosal SCCs. Conversely, CD40+ (M1-like) macrophages were less represented and mainly localized within pyogranulomatous inflammatory foci surrounding keratin deposits.

Conclusion:
Most canine SCCs exhibited a T-cell-rich inflamed phenotype, potentially associated with responsiveness to immunotherapeutic strategies. The predominance of M2-polarized macrophages suggested an immunosuppressive pro-tumoral microenvironment. Multiplex immunohistochemistry combined with digital image analysis represented an effective and accessible approach for TIME characterization in canine SCCs.

Clinicopathological Analysis of Feline Cervical Nodal Lymphomas

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Topic: Cancer

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Background:
Feline cervical nodal lymphomas, primarily T-cell-rich B-cell lymphoma (TCRBCL) and Hodgkin-like lymphoma (HLL), often present with overlapping morphological features despite having distinct clinical prognoses. Precise differentiation is therefore essential for clinical management. The identification of Reed-Sternberg-like (RS-like) cells, typically characterised by CD20 and CD30 immunoreactivity, is pivotal for diagnosing HLL. This study aims to evaluate the histomorphological characteristics, immunophenotypes, and clinical presentations of these neoplasms within a Taiwanese cohort.

Materials & Methods:
A retrospective cohort of 31 cases was evaluated. Histomorphological features were assessed via hematoxylin and eosin (H&E) staining, and immunophenotypic profiles were established using CD3, CD20, PAX5, and, where indicated, supplementary CD30. Clinical data were integrated and analyzed to identify key features for differentiating these nodal neoplasms.

Results:
The mean age of affected cats was 10.9 years (range 0.5–18.5), with 16 females. Domestic Shorthairs represented 87% of the cohort. All tumors were localized to the submandibular and retropharyngeal lymph nodes. Diagnoses comprised 16 TCRBCL (51.6%), 3 diffuse large B-cell lymphomas (DLBCL), 1 HLL, and 11 other nodal conditions.

Conclusion:
TCRBCL emerged as the most prevalent subtype, underscoring its significance in feline cervical lymphadenopathy in Taiwan. Given the consistent anatomical predilection and morphological overlap between subtypes, a targeted immunohistochemical panel (CD3, CD20, PAX5, and CD30) is indispensable for definitive classification. These findings highlight the necessity of precise pathological diagnosis to inform clinical management and prognostic assessments.

GATA3 and Uroplakin II Expression in Canine Bladder and Prostatic Urothelial Carcinoma

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Topic: Cancer

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Background:
Canie urothelial carcinoma (UC) arises from the bladder urothelium or the prostate. In the bladder, it is the most common malignant tumor, accounting for 2% of all canine cancer. In the prostate, its occurrence is low (0,2-0,6%), but it is highly aggressive with high metastatic potential. The aim of this study was to characterize two urothelium markers in bladder (BUC) and prostatic UC (PUC) in dogs.

Materials & Methods:
Eighteen samples of BUC and 15 PUC samples were evaluated. Immunohistochemistry was performed using a polymer-based detection system with primary antibodies against GATA3 and uroplakin II (UPKII).

Results:
GATA3 immunolabelling was found in 94% (17/18) of BUC and UPKII in 89% (16/18). In PUC, GATA3 showed 100% positivity and UPKII 87% (13/15). In BUC, GATA3 expression was predominantly high, with 71% of cases scoring 4 (>76%), while the remaining cases showed low to moderate distribution among the neoplastic cells. UPKII in BUC was more heterogeneous, most frequently scores 2 (37%) and 3 (25%). In PUC, GATA3 also showed predominantly high expression, with 67% scoring 4 and 20% scoring 3. In contrast, UPKII in PUC was more variable, with most cases scoring 2 (47%), followed by scores 3 (23%) and 4 (20%).

Conclusion:
GATA3 and UPKII are consistently expressed across both bladder and prostatic urothelial carcinomas, reinforcing their robustness as markers of urothelial differentiation.

Integrated assessment of WHO histopathological classification, immunohistochemistry, and clinicopathological biomarkers as predictors of survival in 118 cats with intestinal lymphoma

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Topic: Cancer

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Background:
Intestinal lymphoma is the most common neoplasm in cats and represents a significant diagnostic and therapeutic challenge.

Materials & Methods:
This retrospective study included 118 feline intestinal samples, which were routinely processed and stained with H&E, CD3 (50/118) and PAX5 (40/118) antibodies. The cohort consisted of cats, aged from 8-13 years, presenting with chronic diarrhea, vomiting, anorexia, and weight loss. Serum cobalamin, folates, and feline leukemia virus were tested. Histological classification was performed according to the WHO criteria, tumor site, degree of epitheliotropism, and pattern of transmural lymphocytic infiltration, to identify the median survival time.

Results:
Lymphomas were classified in mucosal and transmural lymphoma. Mucosal lymphoma was the predominant subtype (79/118), corresponding to enteropathy-associated T-cell lymphoma type II, and was associated with prolonged median survival (3 years). Histologically, these lesions were characterized by epitheliotropic infiltration of small-to-intermediate CD3-positive lymphocytes involving the villous, crypt epithelium, and lamina propria. Transmural T-cell lymphoma (39/118), consistent with enteropathy-associated T-cell lymphoma type I, exhibited aggressive transmural invasion by large lymphocytes, associated with markedly shorter survival (2.5 months). Intestinal T-cell lymphomas were characterized by distinctive mucosal architecture, CD3 positive expression (50/50), and negative PAX5 results (40/40). Cases occurred predominantly in female cats, the jejunum being the most frequently affected site, followed by the ileum and duodenum. Increased serum cobalamin and folate concentrations were detected in 89/188 patients. 69/188 were positive for feline leukemia virus.

Conclusion:
This study supports that WHO histopathological classification associated with immunohistochemistry and clinicopathological data is key to predicting survival outcomes in cats with intestinal lymphoma.

Soft Tissue Oncology Research in Mammals (STORM): a pilot database study on mesenchymal tumors in pets

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Topic: Cancer

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Background:
Demographic and anatomical distribution data for mesenchymal tumors in pets are outdated and based on superseded classifications. The STORM project aims to create a canine and feline mesenchymal lesions database, updating epidemiological data.

Materials & Methods:
Neoplastic and non-neoplastic mesenchymal lesions diagnosed in dogs and cats at a single institution between 2015 and 2024 were retrieved. Breed, sex, age, and sites were recorded, diagnoses updated to the recent classification, equivocal cases reviewed and appropriate IHC stains performed to confirm the diagnosis when needed. Statistical analyses were performed to investigate associations among histotype, site, age, and sex.

Results:
Overall, 2548 cases were collected (2144 canine and 404 feline): 1833 were soft tissue lesions, 535 visceral, and 180 skeletal. Preliminary analyses focused on canine soft tissue and visceral tumors. Mean age was 9.9 ± 3.0 years. The most frequent sarcomas were perivascular wall tumors (PWT), hemangiosarcoma, soft tissue sarcoma NOS, and fibrosarcoma. Males were younger than females ($p=0.003$), and visceral tumors occurred in older animals ($p<0.001$). Histotype was associated with site and age ($p<0.05$). PWT occurred mainly on limbs, cutaneous/subcutaneous hemangiosarcoma on the trunk, nerve sheath tumors (NST) and fibrosarcoma on head, neck and trunk. Correspondence analyses identified four clusters: head and neck (fibrosarcoma, NST, PWT), trunk (vascular and adipocytic tumors), limbs (PWT, smooth muscle tumors, NST), and visceral (hemangiosarcoma and smooth muscle tumors).

Conclusion:
This study represents the first step toward a systematic collection of mesenchymal lesion data in pets. Future developments include Vet-ICD-O coding and collaborations with additional institutions.

Lymphoma in snakes: attempts at classification

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Topic: Cancer

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Background:
Reports on neoplasias in snakes are generally rare. Nonetheless, their association with boid inclusion body disease (BIBD) has frequently been claimed for boid snakes. Lymphomas are among the most frequently diagnosed neoplasias in snakes. Their classification was so far hampered by the lack of reliable immunohistochemical markers. Since a reliable B cell marker is now available, we have attempted their systematic characterisation and classification in the present study.

Materials & Methods:
Twenty lymphoma cases in snakes (7 *Colubridae*, 6 *Pythonidae*, 6 *Boidae*, 1 *Viperidae*) were re-examined using consecutive sections stained with haematoxylin-eosin and by immunohistology for T and B cells, using commercially available cross-reactive antibodies against CD3 and PAX5. A multiplex PCR for reptarenaviruses was performed with RNA extracted from the paraffin-embedded tumour material.

Results:
In most cases (82%), multicentric involvement was observed. Overall, the gastrointestinal tract was most frequently affected (88%), followed by the urinary tract (52%). In half of the cases, intravascular lymphoid neoplastic cells were detected, indicating leukaemia. The tumours were classified as T cell (n = 8), B cell (n = 2), double-immunolabelling (n = 1), and non-T/non-B cell (n = 6) lymphoma. There was no evidence of BIBD and reptarenavirus infection, and no significant association between lymphoma category, affected organs, morphological features, and snake family.

Conclusion:
Lymphomas in snakes are most frequently multicentric, involve the gastrointestinal tract and often show evidence of leukaemia. No association with reptarenavirus infection was identified across the snake families examined.

Oral rhabdomyosarcoma in a young sheep

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Topic: Cancer

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Background:
Rhabdomyosarcomas are rare in sheep, with only a few reports in the muscles or the lungs.

Materials & Methods:
One year after neutering, a 1.5-year-old male sheep was presented with an oral mass that had been growing for two months. The mass was approximately 6 cm in diameter, firm, exophytic, ill-defined, necrotic and had infiltrated the right palate and superior gingiva. Biopsies were submitted. In addition to haematoxylin, eosin and saffron stain, immunochemistry was performed for vimentin, desmin and myogenin.

Results:
Histologically, the lesion infiltrated the lamina propria and was heterogeneous with dense sheets of "small round blue cells" resembling lymphoma admixed with streams of pleomorphic spindle to polygonal cells in a variably abundant collagenous stroma. Some of the latter population, were multinucleated cells with aligned nuclei, a hypereosinophilic cytoplasm and occasional striations (myotubes). The immunohistochemical profile revealed strong cytoplasmic vimentin expression in all cells, strong cytoplasmic desmin expression in approximately 40% of cells and most of the multinucleated cells, and strong nuclear myogenin expression in half of the cells. A diagnosis of embryonal rhabdomyosarcoma was made. The animal died at its owner's place, and a necropsy could not be performed to assess the presence of metastasis.

Conclusion:
This is the first reported case of an oral rhabdomyosarcoma in a sheep, arising from the palate and gingiva. Interestingly, oral rhabdomyosarcomas have also rarely been described in young cattle but seem to predominantly affect the tongue in this species.

Feline Oral and Nasal Squamous Cell Carcinoma: A Comparative and Translational Microenvironment Study

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Topic: Cancer

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Background:
Squamous cell carcinoma (SCC) is a common neoplasia in cats. The tumour microenvironment is composed of tumour, stromal and immune cells, which support the survival and proliferation of neoplastic cells.

The objectives of this study were to (i) characterize the immune cells in the SCC microenvironment, (ii) assess cell proliferation, (iii) compare the microenvironment of oral and nasal SCC between themselves and with human SCC, and (iv) to investigate associations between cell count/proliferation and clinical and histopathological variables.

Materials & Methods:
17 oral and 17 nasal samples from cats diagnosed with SCC were subjected to an immunohistochemistry protocol for the quantitative evaluation of the positive cells to Iba-1 (total macrophages), CD163 (M2 macrophages), CD3 (T-cells), PAX-5 (B-cells), and Ki-67 (cell proliferation).

Results:
This study found no differences between cell populations and anatomical locations, histological subtypes, nor clinical data, or correlations between CD163 and CD3, CD163 and PAX-5, or any cell marker with Ki-67 index. Correlations observed: higher Ki-67 index with poorly differentiated SCC (p= 0.0067); Iba-1 with CD163 (p= 0.0372); Iba-1 with CD3 (p= 0.0006); Iba-1 with PAX-5 (p= 6.523×10^{-6}); CD3 with PAX-5 (p= 0.0001). Among the immune cells studied, T-cells predominated, followed by total macrophages, and lastly B-cells and M2 macrophages.

Conclusion:
These results suggest that feline oral and nasal SCC share a mixed and complex tumour microenvironment similar to human head and neck SCC, supporting their classification as a single biological entity and as a relevant comparative model for prognostic and immunotherapeutic research.

Dna polymerase theta as prognostic marker in canine cutaneous mast cell tumours

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Topic: Cancer

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Background:
Mast cell tumour (MCT) is a prevalent canine neoplasm, accounting for a significant proportion of skin tumours in this species. DNA Polymerase Theta (POLQ) is the main protein involved in Theta-Mediated end-joining (TMEJ), one of the pathways responsible for DNA repair. TMEJ is an error-prone repair mechanism frequently altered in human cancers, leading to genomic instability, as well as to chemo- and radioresistance. This study aimed to characterise the prognostic value of POLQ expression in canine cutaneous mast cell tumours.

Materials & Methods:
Twenty-nine cases of cutaneous MCTs were subjected to immunohistochemistry using a polyclonal anti-POLQ antibody (Invitrogen). Protein expression was quantified in five high-power field images from hot spot areas (total area = 0.4 mm²). All neoplastic mast cells were counted (immunolabelled or not) to calculate the percentage of positive cells. The results were evaluated in relation to disease-specific mortality and overall survival time following surgery.

Results:
Dogs that died due to the disease (n=12) showed lower POLQ expression compared with those that were censored in survival analysis (n=17) (p=0.0257). Using ROC curve analysis (AUC=0.7549; p=0.0213), we observed that dogs with tumours showing less than 75% of POLQ immunolabelled cells had shorter post-surgical survival times (p=0.0151; hazard ratio = 8.256; median survival for POLQ<75% = 164 days).

Conclusion:
Our findings indicate that lower DNA polymerase Theta expression in canine cutaneous mast cell tumours is a potential indicator of shorter post-surgical survival. The causes and consequences of POLQ expression in canine cutaneous MCTs remain to be further elucidated.

Immunosupressive landscape in canine mammary carcinomas: an analysis of treg tumour-infiltrating lymphocytes and pdl1+ vasculature

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Topic: Cancer

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Background:
Programmed death-ligand 1 (PDL1) is expressed in immune, endothelial, and tumour epithelial cells within the cancer microenvironment and may stimulate immune tolerance. In addition, PDL1 expression promotes regulatory T-cell (Treg) expansion and immunosuppressive activity. The aim of this study was to investigate the association between endothelial PDL1 expression, the Ki67 index, and Treg infiltration with the traditional TNM staging and histopathological grading in canine mammary carcinomas (CMCs).

Materials & Methods:
Thirty-five CMCs were subjected to immunohistochemical analysis using anti-FoxP3, anti-PDL1 and anti-Ki67 antibodies. The Ki67 index was calculated in a minimum of 1,000 cells. The percentage of Treg tumour-infiltrating lymphocytes (TILs) and the number of intratumoral PD-L1+ vessels were assessed in 5 photomicrographs from hotspot areas (total area = 0.4 mm^2).

Results:
The Ki67 index and the percentage of Tregs were significantly higher in grade III CMCs (30.0±11.0% and 7.7±6.3%, respectively) than in grade I CMCs (11.3±9.8% and 2.0±3.0%, respectively; p=0.002 and p=0.019, respectively). The percentage of Tregs and the number of PDL1+ vessels were significantly higher in stage IV–V cases (8.6±7.0% and 20.7±17.9%, respectively) than in stage I–III cases (2.9±4.0% and 9.9±10.2, respectively; p=0.010 and p=0.046).

Conclusion:
An immunosuppressive tumour microenvironment, characterised by the presence of Tregs and PDL1+ vasculature, is associated with more aggressive CMC phenotypes.

Intra and interobserver agreement on histologic subtype classification of canine meningiomas between expert and generalist veterinary pathologists

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Topic: Cancer

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Background:
Some histologic subtypes are prognostic in human meningiomas; however, the reproducibility of subtype classification in canine meningiomas remains uncertain. This study aimed to evaluate intra- and interobserver agreement in the histologic subtype classification of canine meningiomas, as a prerequisite for assessing its prognostic value.

Materials & Methods:
Digitized hematoxylin and eosin-stained slides from 186 canine meningiomas were independently and blindly reviewed twice by 9 pathologists, including 5 generalist veterinary pathologists and 4 veterinary neuropathology experts. Each tumor was assigned a predominant histologic subtype, without consideration of tumor grade. Intra- and interobserver agreement were assessed using Cohen's kappa coefficient (κ).

Results:
Intraobserver agreement ranged from good to very good overall, and was higher among neuropathology experts (κ = 0.787-0.978) than among generalist pathologists (κ = 0.602-0.803). Overall interobserver agreement was fair in both groups (κ = 0.394 for experts; κ = 0.332 for generalists). Specifically, moderate agreement (κ = 0.4-0.6) was achieved among experts for anaplastic, meningothelial, and transitional subtypes, and in both groups of pathologists for fibrous and microcystic subtypes. The psammomatous subtype showed very good agreement (κ > 0.8) among generalist pathologists. For the remaining subtypes, agreement was below 0.4, including for chordoid, rhabdoid, and papillary meningiomas.

Conclusion:
Histologic subtype classification shows limited reproducibility, even for subtypes with prognostic value in human medicine, suggesting that it may not represent a robust

prognostic parameter in canine meningiomas.

Pathological and immunohistochemical characterization of an adrenocortical carcinoma in a drill (Mandrillus leucophaeus) with Multiple Endocrine Neoplasia-like syndrome.

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Topic: Cancer

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Background:
Endocrine disorders, including neoplasia, have been reported in several primate species. Described cases were diagnosed during necropsy, with clinical signs varying from asymptomatic individuals to changes in morphology, skin coloration, body condition, external sexual characteristics, and behavior.

Materials & Methods:
A 22-years old female drill (*Mandrillus leucophaeus*) living at a multispecies exhibit was presented with masculinization, weight loss, and alopecia associated with neoplasia-related hormonal imbalances.

Results:
An adrenal mass with malignant characteristics was diagnosed ante-mortem, using abdominal ultrasound and computed tomography. During postmortem examination, a heterogeneous mass measuring 5 cm in diameter was identified in the medial aspect of the left adrenal gland. The mass did not invade the adjacent renal or adrenal blood vessels. Histologically, the mass corresponded to an unencapsulated, poorly demarcated, multilobular, densely cellular neoplastic growth that infiltrated the adrenal cortex and capsule and compressed the medulla. The mass was composed of polygonal cells arranged in cords, nests, and packets with mild to moderate anisocytosis and anisokaryosis and 10 mitotic figures in 2.37 mm². Neoplastic cells were positive for vimentin and androgen receptor, faintly positive to melan-A, but negative for synaptophysin D, chromogranin, and S100. The morphological and immunohistochemical results were consistent with adrenal gland adenocarcinoma. In addition, this animal presented a small adenoma in the *pars intermedia* of the pituitary gland, and bilateral thyroid hyperplasia.

Conclusion:
A drill was presented with concurrent multiple endocrine disorders, making this case a case of Multiple Endocrine Neoplasia-like syndrome. Ante mortem diagnosis was possible thanks to imaging techniques.

Argyrophilic nucleolar organizer regions count in feline injection site fibrosarcoma: a pilot study.

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Topic: Cancer

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Background:
Feline injection-site fibrosarcoma is an aggressive malignant tumour with variable biological behaviour. Reliable proliferation markers may improve the assessment of tumour aggressiveness. The aim of the study was to evaluate argyrophilic nucleolar organizer regions (AgNORs) staining in feline injection-site fibrosarcomas and its association with histological malignancy indicators.

Materials & Methods:
Fifteen cases of feline injection-site fibrosarcomas were included. The diagnosis was established on HE-stained sections. Tumours were classified into three histological grades (grade I, II, III; n=5 per group) based on mitotic count, degree of inflammatory infiltrate, and percentage of tumour necrosis area. AgNORs staining was performed on paraffin-embedded tissue sections using a commercial kit (Bio Optica, Italy). The number of AgNORs per nucleus was assessed in 100 tumour cell nuclei with FN22/100×, corresponding to 0.038 mm². Data distribution was assessed using the Shapiro-Wilk test. Correlations with histological parameters were analysed using Spearman's rank correlation coefficient ($p \leq 0.05$).

Results:
AgNORs counts increased with tumour grade. A positive correlation was observed between AgNORs count and tumour grade ($r=0.83$; $p=0.000$), mitotic count ($r=0.87$; $p=0.000$), and tumour necrosis score ($r=0.64$; $p=0.009$). No significant correlation was observed with the degree of inflammatory infiltrate.

Conclusion:
AgNOR staining reflects proliferative activity in feline injection-site fibrosarcomas and shows a strong association with established histological indicators of tumour aggressiveness, particularly mitotic count and tumour grade. These findings suggest that AgNORs may serve as a useful supplementary marker in the evaluation of feline injection-site fibrosarcoma; however, further studies on larger cohorts, including clinical outcome data, are needed.

Topographic characterization of cell proliferation in canine meningioma

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Topic: Cancer

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Background:
The mitotic count (MC) is a key histological parameter for grading canine meningioma, though it is generally assessed without standardized topographic guidance. This study aimed to characterize the spatial distribution and localization of the highest proliferative areas in canine meningiomas, to identify the most appropriate area for MC assessment.

Materials & Methods:
Ki-67 immunohistochemistry was performed on 95 paraffin-embedded canine meningiomas. For each tumor, a Ki-67+ density map was generated using QuPath software, and classified by distribution (multifocal, focal, or diffuse) and main localization (peripheral, central, or scattered). The hotspot was identified and classified as inside or outside the main tumor mass. "Inside" hotspots were further categorized by localization (peripheral, central); peripheral hotspots were classified as deep or superficial, based on proximity to nervous tissue or dura mater, respectively.

Results:
Cell proliferation was predominantly multifocal in 51/95 (59%), followed by focal in 32/95 (33.7%), and diffuse in 7/95 (7.3%) tumors. Cell proliferation was mainly localized at the periphery of the tumor in 51/95 (53.7%), with scattered (36.8%) and central (9.5%) distributions less frequent. Hotspots were located within the main tumor mass in 68/91 cases (74.7%), of which 54/68 (79.4%) were peripheral. Among peripheral hotspots, 20/28 (71.4%) were closer to nervous tissue than to dura mater.

Conclusion:
These findings indicate that the most proliferative regions in canine meningiomas are predominantly located at the tumor periphery, specifically in areas adjacent to the nervous tissue. Therefore, mitotic count assessment should be preferably performed in these areas to ensure accurate tumor grading.

Comparison of TLR2 and TLR4 mRNA expression in feline oral squamous cell carcinoma, pyogenic granuloma, and healthy gingiva.

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Topic: Cancer

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Background:
Feline oral squamous cell carcinoma (FOSCC) is a common malignant tumour of cats, and its cause(s) are poorly understood. Human oral squamous cell carcinoma (HOSCC) displays similar biologic behaviour, and periodontal disease (PD) is implicated as a risk factor. Expression of Toll-like receptors (TLRs) in response to oral bacteria has been investigated in this context in HOSCC, but similar research has not been conducted in FOSCC, despite the high prevalence of PD in cats.

Materials & Methods:
Formalin-fixed, paraffin-embedded samples of healthy gingival tissue, inflamed oral tissue (pyogenic granuloma) and neoplastic oral tissue (FOSCC) from cats (n=22 of each) were subjected to RNA in-situ hybridisation (RNA-ISH) for *TLR2* and *TLR4*. Pyogenic granuloma and FOSCC samples were examined histologically and features recorded. RNA-ISH signals in the epithelial compartments were quantified using image analysis software. Levels of TLR expression were compared between tissues and with histologic features.

Results:
All samples expressed *TLR2* and *TLR4* mRNA, with higher expression in FOSCC than healthy tissue (*TLR2*: p=0.0029; *TLR4*: p=0.0296). *TLR4* correlated with inflammation in FOSCC (ρ_s=0.49, p=0.0216), while both *TLR2* and *TLR4* correlated with inflammation in pyogenic granulomas (ρ_s=0.52 and 0.47; p=0.0137 and 0.0262, respectively).

Conclusion:
Increased TLR2 and TLR4 mRNA expression was observed in FOSCC. Whilst the cause is uncertain, the oral microbiome may influence TLR expression in epithelial cells. The association between TLR expression and inflammation may support a role for oral inflammation in the pathogenesis of FOSCC and highlights the complex interplay between cancer, the immune system and the microbiome.

Establishment and Histopathological and Molecular Characterization of Patient-Derived Xenograft (PDX) Models from Feline Mammary Carcinomas

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Topic: Cancer

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Background:
Patient-derived xenograft (PDX) models are generated by implanting surgically resected tumors into immunodeficient mice. In human medicine, PDX models faithfully retain the histopathological and molecular features of the original tumors, contributing to anticancer drug development. Although feline mammary tumor PDX models have been reported, their fidelity to the original tumors and characteristics remain insufficiently evaluated.

Materials & Methods:
PDX models were established from six feline mammary carcinomas (FMCs), including five primary lesions and one nodal metastatic lesion. Tumor samples were cut into 1–2 mm^3 pieces, transplanted into the mammary fat pad of severe combined immunodeficient (SCID) mice, and serially passaged after reaching 500 mm^3. Histopathological, immunohistochemical, and RNA-seq analyses were compared with those of the corresponding original tumors.

Results:
PDX models were established up to P5 (after four serial passages) from three primary lesions and one metastatic lesion. All PDX models retained the histopathological growth patterns and immunophenotypes for estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 of the original tumors. RNA-seq revealed high correlations in gene expression profiles between each PDX model and its corresponding original tumor. Analysis of species-separated RNA-seq reads detected mouse-derived transcripts in all PDX tumors, including vascular and stromal genes. Consistently, vascular structures in the xenografts showed immunoreactivity with a mouse-reactive CD31 antibody, suggesting partial replacement of the original tumor stroma by host-derived stromal and vasculature components.

Conclusion:
FMC-derived PDX models preserved the histopathological and molecular characteristics of the original tumors, although stromal components were partially replaced by mouse-derived tissues.

Preclinical assessment of the Nupr1 inhibitor ZZW-115 in Canine Mammary Tumors: A comparative study in 2D and 3D culture models

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Topic: Cancer

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Background:
Canine mammary tumor (CMT) remains a significant challenge in veterinary oncology, because only few CMTs are aggressive. Nupr1 has been widely described as a pro-oncogenic factor in different human tumors. Nupr1 inhibition in CMTs remains uninvestigated. This study aims to evaluate the efficacy of ZZW-115 (Nupr1 inhibitor), as a standalone treatment and in combination with doxorubicin in CMT cells.

Materials & Methods:
Comparative cytotoxicity was assessed in 2D and 3D CMT-U27 cell model. The 3D spheroids, generated using an innovative silicone mold system technique, were processed as histological specimens following treatment with ZZW-115 and doxorubicin. This approach enabled the immunohistochemical evaluation of Nupr1, Ki67, and Caspase-3 expression.

Results:
3D spheroid models successfully replicated the tumor microenvironment, including spatial cell-cell interactions and metabolic gradients, which were absent in traditional 2D cultures. The CMT-U27 cells were confirmed to express Nupr1 messenger RNA (mRNA). ZZW-115 demonstrated high cytotoxic activity in 2D and 3D models. Morphologically, the combination of ZZW-115 and doxorubicin triggered rapid spheroid disaggregation and widespread apoptosis. IHC analysis confirmed a downregulation of Nupr1 and Ki67, correlating with the observed antiproliferative effects. The combination treatment with ZZW-115 and doxorubicin resulted in a greater reduction in cell viability than treatment with doxorubicin alone in the CMT-U27 cells.

Conclusion:
The 3D spheroid data showed ZZW-115 functions as a superior inhibitory agent compared to doxorubicin in the CMT-U27 cells, acting through the direct downregulation of oncogenic targets and cellular proliferation. These 3D models provide a robust platform for validating new therapeutic targets.

Diagnostic Challenges in Canine and Feline Cutaneous Tumors with Incomplete Clinical Histories

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Topic: Cancer

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Background:
Cutaneous tumors in companion animals comprise a heterogeneous group of neoplasms that may present diagnostic difficulties because of overlapping histomorphological features, complex differentiation patterns, clinicopathological discrepancies, and limited clinical information. In such cases, selecting appropriate immunohistochemical markers for further characterization may itself be challenging. This study aimed to evaluate diagnostic difficulties encountered in canine and feline cutaneous tumors and identify factors contributing to diagnostic uncertainty.

Materials & Methods:
This study presents cases from an ongoing project evaluating canine and feline cutaneous tumors submitted to the Department of Pathology. Twenty-eight neoplastic cases in which diagnostic interpretation was complicated by limited clinical information, unknown treatment history, overlapping histomorphological features, or difficulties in determining cellular lineage and differentiation were evaluated. Immunohistochemical markers were selected according to histomorphological findings and differential diagnostic considerations. Diagnoses were established through integration of histopathological and immunophenotypic findings.

Results:
Diagnostic challenges were most evident in squamous cell carcinoma, basosquamous carcinoma, fibrosarcoma, myofibroblastic sarcoma, fibroadenocarcinoma, and pleomorphic adenoma. In several cases, inflammatory, hyperplastic, and neoplastic components coexisted within the same lesion, creating marked histological heterogeneity and complicating interpretation. Morphological overlap hindered differentiation between fibrosarcoma and myofibroblastic sarcoma, whereas determination of cellular lineage and differentiation represented a major challenge in basosquamous carcinomas, fibroadenocarcinomas, and pleomorphic adenomas.

Conclusion:
Diagnostic difficulties arose from histological heterogeneity, overlapping morphology, and limited clinical information. In several cases, overlapping histomorphological features complicated not only diagnosis but also selection of appropriate immunohistochemical markers. These findings emphasize the importance of integrating clinical, histopathological, and immunophenotypic data when evaluating cutaneous neoplasms with complex morphological features.

ATP-sensitive potassium channel subunits expression in Canine Mammary Gland and Canine Mammary Gland Tumours: an immunohistochemical study

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Topic: Cancer

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Background:
ATP-sensitive potassium channels (K_{ATP} channels), composed of Kir6.x pore-forming subunits and SUR regulatory subunits, play essential roles in coupling cellular metabolism to membrane excitability, survival, and proliferation. Their expression in canine mammary gland tumours (cMGTs) remains uncharacterised. This study investigated the immunohistochemical expression of Kir6.1, Kir6.2, SUR1, and SUR2A in healthy canine mammary gland and in cMGTs.

Materials & Methods:
Forty-seven tissue samples were examined: 8 healthy mammary glands, 20 benign and 19 malignant cMGTs from female intact and spayed dogs. Immunohistochemistry was performed using antibodies against the above-mentioned subunits. These antibodies were selected for canine use on the basis of an in silico ClustalW alignment of the reported human (or murine) and canine amino acid sequences, which revealed high interspecific identity (>95%). Percentage positivity, staining intensity (scored 0–3), and distribution pattern were assessed for each subunit by two pathologists. The Kruskal–Wallis test with Dunn-Bonferroni correction was applied ($p < 0.05$).

Results:
Each subunit had a cytoplasmic labelling in all groups, with a predominantly multifocal pattern.
Mean percentage positivity (healthy, benign, malignant) was: Kir6.1 14.25%, 19.60%, 29.78%; Kir6.2 28.38%, 26.65%, 25.16%; SUR1 25.38%, 57.90%, 43.05%; SUR2A 45.0%, 67.75%, 54.89%. SUR1 positivity was significantly higher in benign tumours than in healthy tissue ($p = 0.042$). No significant differences were identified for the other markers.

Conclusion:
To the authors' knowledge, this is the first study to characterise the expression of K_{ATP} channel subunits in canine mammary glands. These data suggest that SUR1 may be upregulated in benign cMGTs relative to healthy mammary glands, identifying it as a candidate subunit that warrants further investigation to clarify its role in tumorigenesis.

Right Atrium Hemangiosarcoma in Dogs: necropsy and epidemiological findings for a tumour database guide in Portugal

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Topic: Cancer

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Background:
Canine cardiac tumours are rare, and right atrium haemangiosarcoma (RA HSA) is a highly malignant tumour arising specifically from the right atrium or auricle.

Materials & Methods:
From January 2023 until April 2026, 417 necropsies were performed on dogs at the National Reference Laboratory for Animal Diseases (INIAV, Oeiras, Portugal). 16 of these necropsies were associated with sudden death and resulted in a final diagnosis of RA HSA. The variables sex, age, breed, and metastatic sites were analysed to establish a database guide for this tumour.

Results:
During the study period, RA HAS accounted for 3,84% of all *post mortem* diagnoses. In these RA HAS cases, 43.75% were female and 56.25% were male, with no significant association with sex (OR 0.98; 95% CI 0.36–2.69; p=0.96). With regard to age, the sample was classified into 4 age- groups: 1 (<1 year), 2 (1 to 5 years), 3 (6 to 10 years), 4 (> 11 years). All cases occurred in dogs older than 6 years, with significant differences between the 6–10 and >11 year age groups ($\chi^2 = 18.0$, $p < 0.001$). Mixed-breed dogs showed the highest presentation, with other common breeds also affected, suggesting no specific breed predilection. The lung and liver were the main sites of metastasis, with macroscopic 'cannonball' appearance, consistent with haematogenous spread of this tumour.

Conclusion:
These results provide important epidemiological data on this neoplasm as a cause of sudden death in dogs, as it is difficult to establish a definitive *ante-mortem* diagnosis.

A case of clear cell odontogenic carcinoma in a dog

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Topic: Cancer

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Background:
Clear Cell Odontogenic Carcinoma (CCOC) is an uncommon malignant odontogenic tumour in Humans. CCOC is characterized by predilection for the mandibula. CCOC is composed of nests of clear cells, separated by hyalinized fibrous septa. BRAF mutation was described for some odontogenic tumours. An oral mandibular sample of a 5-years-old, male, Porcelaine breed dog was submitted for histopathology.

Materials & Methods:
The sample was fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin. Immunohistochemical detection of cytokeratins AE1/AE3, CK14, CK7, MelanA, PNL-2 and S100 was accomplished using a standard protocol. PCR to detect mutations in cBRAF gene was performed on formalin-fixed tissue.

Results:
Histopathology revealed an infiltrative mass consisting of neoplastic cells arranged in nests, in a fine hyalinized stroma. The cells were polygonal; had moderate amounts of clear, non-pigmented cytoplasm and excentric nuclei with vesicular chromatin. Anisocytosis and anisokaryosis were moderate. Up to 12 mitoses were visible per 2.37mm2. Lymphovascular and underlying bone invasion were seen. Atypical cells reacted to cytokeratin AE1/AE3 and CK14. They were negative for CK7, MelanA, PNL-2 and S100. Immunohistochemistry results support carcinoma; don't support melanoma. CCOC must be distinguished from other clear cell neoplasms. Negative clinical staging, negative CK7 and S-100 helped in the exclusion of other clear cells tumours. Mandibular localization, CK14 immunostaining pattern helped in the definitive diagnosis of CCOC. cBRAF PCR was negative.

Conclusion:
To the author's knowledge, this is the first report of oral Clear Cell Odontogenic Carcinoma in veterinary literature.

Immune Microenvironment Characterization of Canine Anal Sac Gland Adenocarcinomas and Its Association with Clinical Outcome

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Topic: Cancer

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Background:
Canine anal sac gland adenocarcinomas (ASACs) are aggressive tumors with a high metastatic rate. Tumor-infiltrating lymphocytes (TILs) and tumor-associated macrophages (TAMs) are increasingly investigated as prognostic markers in canine neoplasms. This study characterized the immune microenvironment of canine ASACs and evaluated its association with clinicopathological variables and survival.

Materials & Methods:
Forty-five canine ASACs with available clinical follow-up, tumor size, metastatic status, surgical margins, and stage were immunolabeled for CD3, CD20, FoxP3, IBA1, and CD204. Digital image analysis using QuPath quantified immune cells per mm² within intraepithelial (IE) and stromal (S) compartments. Immune cell populations were correlated with clinical variables and outcome.

Results:
IE FoxP3+ TILs and the FoxP3/CD3 ratio were significantly increased in stage IV tumors (p=0.02). Dogs presenting with metastasis at diagnosis had significantly higher IE CD3+ TIL densities (p=0.02). Stromal CD3+ and CD20+ TILs were significantly higher in dogs surviving at least one year after diagnosis compared with dogs that died within one year (548 vs 206 cells/mm² and 564 vs 322 cells/mm², respectively; p=0.04 and p=0.01). The stromal FoxP3/CD3 ratio was significantly higher in dogs surviving at least six months after diagnosis. IE CD204+ TAMs were markedly lower in dogs alive at six months, although not significantly. No association between surgical margins and other variables was observed.

Conclusion:
The immune microenvironment of canine ASACs is associated with tumor progression and survival. Increased stromal TILs infiltration correlated with improved outcome, whereas increased FoxP3+ infiltrates were associated with advanced-stage disease.

Cannabinoid receptors CB1R, CB2R and Orphan Receptor GPR55 in canine amelanotic oral melanoma: an immunohistochemical study

520

Topic: Cancer

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Background:
Canine amelanotic oral melanoma (CAOM) is a diagnostically challenging and highly aggressive neoplasm sharing biological and molecular features with human mucosal melanoma, positioning it as a valuable spontaneous model for translational oncology. The endocannabinoid system (ECS), through cannabinoid receptors 1 (CB1R) and 2 (CB2R), is implicated in tumor progression, apoptosis, and immune modulation across multiple cancer types. GPR55, an orphan receptor sometimes designated the "third cannabinoid receptor," has been increasingly associated with pro-proliferative and pro-metastatic signaling in oncogenesis. Expression of CB1R, CB2R, and GPR55 in CAOM remains uninvestigated.

Materials & Methods:
Twenty formalin-fixed, paraffin-embedded CAOM specimens were retrieved from a diagnostic archive. Indirect immunohistochemistry was performed using anti-CB1R (Abcam®, 1:100), anti-CB2R (Origene®, 1:200), and anti-GPR55 (Abcam®, 1:200) antibodies. Receptor expression in neoplastic cells was semi-quantitatively scored (0–3) by three independent blinded observers. Mucosal epithelium served as internal positive control.

Results:
CB1R expression was absent or minimal (score 0–1) in 62% of cases. CB2R showed moderate, diffuse cytoplasmic labelling (score 2) in all samples. GPR55 was positive in all cases, with the majority exhibiting moderate immunoreactivity (score 2); occasional nuclear membrane positivity of equivalent intensity was observed. Residual melanocytes containing melanin demonstrated intense GPR55 labelling (score 3).

Conclusion:
The consistent expression of CB2R and GPR55, contrasting with minimal CB1R immunoreactivity, suggests preferential involvement of these receptors in CAOM biology. Further studies are required, including tumours with different degrees of aggressiveness, to demonstrate the potential role of CB2R and GPR55 as biomarkers and candidates for therapeutic targets in this tumour

Collision primary squamous cell carcinoma and sarcoma in the skin of two cats

521
Topic: Cancer

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Background:
Collision tumors are defined as two or more concurrent, yet distinct neoplasms. Collision tumors are rare in veterinary medicine and, to the best of the authors´ knowledge, there is no previous description of these malignancies in the skin of cats. Here, collision tumors of squamous cell carcinoma (SCC) and sarcoma in the skin of two cats are reported.

Materials & Methods:
Two 10-year-old male neutered (case 1) and 13-year-old female neutered (case 2) European Shorthair cats were presented with proliferative lesions on both ears and at the muzzle, respectively. After surgery, the samples were submitted for routine histopathology diagnostics. Immunohistochemistry for cytokeratin and vimentin, as well as PCR testing for canine and feline papillomaviruses, bovine papillomavirus 1/2, and feline leukemia virus were performed on formalin-fixed, paraffin-embedded material.

Results:
Cat 1 had hard, ulcerated, and painful masses measuring 3x1.7x0.8 cm and 3.5x2.8x1.1 cm at both ears. From the mass of cat 2, two biopsies (5 and 7 mm) were submitted. Histologically, all samples consisted of two neoplastic populations: squamous epithelial cells immunolabelling for cytokeratin arranged in cords and nests, and bundles of vimentin immunolabelling spindle cells showing anisokaryosis and high mitotic activity. All PCR tests were negative.

Conclusion:
Feline SCC in combination with sarcoma is uncommon but possible. This should be considered if only biopsies from cutaneous masses are investigated, because biological behaviour and therapy may differ between the different tumor entities. Immunohistochemical examination may contribute to an accurate diagnosis, prognosis and treatment.

Concurrent ovarian and uterine lesions in female dogs with mammary masses

530

Topic: Cancer

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Background:
Mammary tumors are common in female dogs, with mastectomy and concurrent ovariohysterectomy frequently performed. However, reproductive tract specimens are seldom submitted for histopathology unless presenting gross lesions. Given limited data on synchronous/metachronous mammary and reproductive lesions, this study evaluated coexisting ovarian and uterine lesions in female dogs with mammary masses.

Materials & Methods:
One hundred and five intact female dogs (6–21 years; mean 9.7±2.4) with mammary masses submitted to simultaneous mastectomy and ovariohysterectomy. Ovariohysterectomy was routinely performed in females with mammary tumors, regardless of clinically suspected reproductive tract disease; thus, all eligible cases were included irrespective presence or absence of ovarian and/or uterine lesions. Mammary and reproductive tract tissues were histopathologically evaluated.

Results:
Mammary tumors were identified in 104/105 animals: 62 presented multiple tumors (59.6%), 75 (72,1%) presented ≥1 malignant tumor. A total of 253 mammary tumors were diagnosed, of which 131 (51.8%) malignant. Most common malignant subtypes were complex (n=27), carcinomas arising in benign mixed tumors/adenomas (n=21), papillary (n=18), ductal (13), and tubulopapillary (13) carcinomas. 101/105 animals showed reproductive tract lesions: 70 had ovarian lesions, including neoplastic lesions in 31 cases - 23 adenomas, 2 leiomyomas, 2 benign granulosa cell tumors, 3 carcinomas, and 1 dysgerminoma; and 99 presented uterine lesions, predominantly proliferative (n=83), hemorrhagic/vascular (n=37) and inflammatory/infectious (n=26), neoplasms were uncommon, with 4 leiomyomas identified.

Conclusion:
Synchronous mammary and reproductive lesions appear common in female dogs, with potential clinical and prognostic significance, but may be underdiagnosed when reproductive tissues are not routinely submitted for histopathological evaluation.

Biomarkers and prognostic factors in uterine neoplasia: a translational perspective for queens

534
Topic: Cancer

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Background:
Ki-67 is a well-established proliferation marker in human endometrial neoplasia; its increased expression is associated with high tumour grade and aggressive behaviour. Desmin and smooth muscle alpha-actin (SMA) are used to evaluate endometrial tumour environment in human oncology, contributing to the prognosis of these lesions. Despite being considered rare, recent research on feline endometrial adenocarcinomas (FEA) suggests they may be more prevalent than previously thought. This study aimed to determine if Ki-67, desmin and SMA in FEA may be used for markers of aggressiveness, such as in women endometrial cancer.

Materials & Methods:
The immunohistochemical expression of ki-67 (Mib-1, Dako, 1:50), Desmin (NCL-Desm, Novocastra, 1:100) and SMA (Novocastra, 1:200) was compared in 24 FEA and in 24 controls (normal feline uteri) at the level of the epithelial, stromal, myometrial and vascular compartments.

Results:
Ki-67 expression was significantly higher in the neoplastic endometrium compared to controls, indicating increased proliferative activity. Desmin expression facilitated the identification of myometrial invasion, a sign of tumour aggressiveness. The heightened expression of SMA in the FEA stroma indicated potential tumour microenvironment activation, suggesting an epithelial-

Conclusion:
Ki-67, Desmin and SMA provide complementary information regarding proliferation, invasion and stromal remodelling in FEA. Alike in woman uterine tumours, Ki-67, Desmin and SMA may provide valuable insights in FEA, a neoplasia for which research is scarce.

Diagnostic utility of CD138 immunohistochemistry for canine plasma cell tumors: a comparative study with MUM1 across round cell neoplasms

545
Topic: Cancer

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Background:
Multiple myeloma oncogene-1 (MUM1) is used to identify canine plasma cells but lacks specificity, labeling some B-cell and T-cell tumors and histiocytomas. CD138, a human plasma cell marker, may be useful in dogs. This study compared a heterologous CD138 with MUM1 in a range of canine round cell tumors.

Materials & Methods:
Formalin-fixed, paraffin-embedded sections from 15 plasma cell tumors, 6 T-cell lymphomas, 6 B-cell lymphomas, and 8 cutaneous histiocytomas were immunolabeled for CD138 and MUM1. Two blinded reviewers independently scored labeling intensity and tumor positivity on an ordinal scale. Interobserver agreement was assessed using Gwet's AC2. Sensitivity and specificity for plasma cell tumor identification were estimated using a cutoff of at least moderate consensus labeling.

Results:
Interobserver agreement was high for CD138 (0.77, 95% CI 0.61-0.94) and MUM1 (0.85, 95% CI 0.74-0.97). CD138 sensitivity (0.73, 95% CI 0.48-0.89) was lower than MUM1 (0.86, 95% CI 0.62-0.97). CD138 labeled one B-cell lymphoma and no T-cell lymphomas or histiocytomas (specificity 0.95, 95% CI 0.76-0.99) but often showed moderate background. In contrast, 6/8 histiocytomas were MUM1-positive, whereas B-cell and T-cell lymphomas were negative.

Conclusion:
Interobserver agreement was high for CD138 (0.77, 95% CI 0.61-0.94) and MUM1 (0.85, 95% CI 0.74-0.97). CD138 sensitivity (0.73, 95% CI 0.48-0.89) was lower than MUM1 (0.86, 95% CI 0.62-0.97). CD138 labeled one B-cell lymphoma and no T-cell lymphomas or histiocytomas (specificity 0.95, 95% CI 0.76-0.99) but often showed moderate background. In contrast, 6/8 histiocytomas were MUM1-positive, whereas B-cell and T-cell lymphomas were negative.

Recurrent mucinous mammary carcinoma in a cat: histopathological and immunohistochemical insights

551
Topic: Cancer

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Background:
Feline Mucinous Mammary Carcinoma (FMMC) is a tumour characterised by proliferation of luminal epithelial cells and large quantities of extracellular mucin. To date, its phenotype and biological behaviour remain poorly characterised. This study sought to further define this entity through immunohistochemical analysis.

Materials & Methods:
A 16-year-old female European Shorthair cat presented a well-defined soft nodule with exophytic growth (1.6x1.8cm) in the nipple of the third left mammary gland. Four months after surgery, local recurrence at the surgical scar was observed. Considering a panel of 17 antibodies (intermediate filaments, hormonal receptors, metalloproteinases, adhesion markers, and cellular proliferation), both, the primary and the recurrent masses were thoroughly studied.

Results:
The primary tumour was pseudolobulated, partially encapsulated yet focally infiltrative, and characterised by abundant extracellular mucin (Alcian blue pH 2.5^{+}, PAS-diastase^{+} and mucicarmine^{+}) with scattered epithelial acini floating within it. Neoplastic cells were predominantly cuboidal, with atypical nuclei and prominent nucleoli. Distinctive features of the recurrent tumour were a budding growth pattern and extensive necrosis. Immunohistochemically, tumour cells expressed epithelial markers (AE1/AE3, CK7, CK8/18, CK19 and CK20), vimentin, hormone receptors (oestrogen and progesterone), MMP9, and E-cadherin, lacked myoepithelial markers, and had a high proliferative index (Ki67: 55%).

Conclusion:
FMMC shares histological characteristics with subtype A pure mucinous carcinoma in women; however, its high proliferative index and expression of MMP9, support its aggressive biological behaviour. The recurrence observed four months after surgery is consistent with the clinical course reported for most malignant feline mammary tumours.

Characteristics and outcome of cutaneous epitrichial carcinomas in cats, a retrospective study of 86 cases (2020–2024)

553
Topic: Cancer

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Background:
Epitrichial carcinomas are among the most common cutaneous malignancies in cats, after squamous cell carcinomas and mast cell tumors, yet they remain poorly documented. This study aimed to characterize their histopathological features and clinical outcomes.

Materials & Methods:
This retrospective study included 86 cats with cutaneous epitrichial carcinomas (EpCas) diagnosed between 2020 and 2024 and treated by surgery alone, compared to 146 cats with cutaneous epitrichial adenomas (EpAds). Ceruminous and anal sac gland carcinomas were excluded. A two-year follow-up is currently available for 40 cats with EpAds and 32 with EpCas.

Results:
Mean age at diagnosis was 12.3 years for EpCas and 11.5 years for EpAds (not significant difference). EpCas were significantly larger than EpAds (mean tumor size: 17 ± 9 mm vs. 10 ± 5 mm, $p < 0.001$), more frequently multifocal (45% vs. 27%, $p = 0.03$), and exhibited a significantly higher mitotic count (26 ± 31 vs. 7 ± 8 mitoses per 2.37 mm², $p < 0.001$). The most common histological subtypes of EpCas were solid-cystic ductal (31%), simple tubulopapillary (23%), and solid (20%). EpCas displayed frequent central necrosis (found in 74% of EpCas) and cutaneous ulceration (55%), and less often atypical mitoses (31%), tertiary lymphoid structures (24%), lymphovascular invasion (17%), and positive (infiltrated) surgical margins (13%). Median overall survival for EpCas was 665 days (1.8 years).

Conclusion:
This study provides a comprehensive morphological characterization of feline cutaneous EpCas and will eventually allow identifying which histopathological criteria have a significant prognostic value for this tumor type.

Cardiac Tumours in Guinea pigs (Cavia porcellus)

556
Topic: Cancer

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Background:
Cardiac neoplasms in guinea pigs (*Cavia porcellus*) are rare and poorly characterized in the literature. This study aims to describe the gross, histologic, and immunohistochemical characteristics of myocardial tumors identified in guinea pigs at necropsy.

Materials & Methods:
Of a total of 937 guinea pig necropsies performed between 1.1.1996 and 1.4.2026, non-lymphomatous cardiac tumors were identified in five cases (0.5 %). For each case, cardiac tissue was collected and processed routinely to create hematoxylin and eosin-stained sections. Immunohistochemistry was performed using antibodies against Cytokeratin, Vimentin, Desmin, and α-SMA, each validated with several positive and negative control tissues.

Results:
Grossly, the tumors appeared as variably sized masses (2-14 mm in the greatest dimension) arising from the ventricular (N=4) or atrial (N=1) myocardium. Gross evidence of congestive heart failure was reported in 2 cases. Metastasis was not detected. Histologically, all five masses shared similar features, which included a mildly infiltrative growth pattern, comprising spindle-shaped to polygonal cells, separated by a moderate fibrous stroma. Tumor cells had poorly defined borders, contained oval nuclei and moderate amounts of eosinophilic cytoplasm. Tumor cells were uniformly immunopositive for Vimentin and focally immunopositive for Desmin and/or α-SMA antigen, while lacking expression for Cytokeratin.

Conclusion:
Histologic and immunohistochemical findings of all five guinea pigs support an undifferentiated mesenchymal tumor with myogenic differentiation. We propose that guinea pigs may develop a unique type of cardiac sarcoma that warrants further investigation.

Characteristics and outcomes of cutaneous epitrichial carcinomas in dogs: a retrospective study of 102 cases (2020–2024)

559
Topic: Cancer

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Background:
Cutaneous epitrichial carcinomas are uncommon adnexal malignancies in dogs and remain poorly characterized. This study aimed to describe the histopathological features and clinical outcomes of this tumor type.

Materials & Methods:
This retrospective study included 102 dogs diagnosed with cutaneous epitrichial carcinomas (EpCas) between 2020 and 2024 in two veterinary pathology laboratories, along with a control group of 70 dogs with cutaneous epitrichial adenomas (EpAds). Dogs with ceruminous or anal sac gland carcinomas were excluded. Two-year follow-up data are currently available for 25 dogs.

Results:
The mean age at diagnosis was 9 years for both EpCas and EpAds. EpCas were significantly larger than EpAds (22 ± 15 vs. 15 ± 7 mm; $p = 0.001$) and more frequently multifocal (18% vs. 3%; $p = 0.006$). The most common histological subtypes of EpCas were simple tubulopapillary (39%) and ductal (16%) carcinomas. EpCas exhibited significantly higher mitotic counts than EpAds (11±17 vs. 4±9 mitoses/2.37 mm²; $p = 0.002$), as well as more frequent cutaneous ulceration (36% vs. 3%; $p<0.001$), tertiary lymphoid structures (33% vs. 3%; $p <0.0001$), and infiltrated margins (19% vs. 4%; $p=0.016$). Additional features of EpCas included central necrosis (85%), moderate to severe peritumoral lymphoplasmacytic inflammation (62%), atypical mitoses (25%), and lymphovascular invasion (15%). The median overall survival of dogs with EpCas was 937 days (2.6 years).

Conclusion:
This comprehensive study of the histopathologic characteristics of canine cutaneous epitrichial carcinomas provides a foundation for an ongoing investigation into the prognostic factors of this tumor type.

Immunohistochemical Expression of CB1R, CB2R and GPR55 in Canine Lymphomas

567

Topic: Cancer

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Background:
The endocannabinoid system has been increasingly investigated in oncology due to its role in cell proliferation, apoptosis and immune regulation. However, information regarding cannabinoid-related receptor expression in canine lymphoid neoplasms remains limited. This study aimed to characterize the immunohistochemical expression of CB1R, CB2R and the orphan receptor GPR55 in canine lymphomas.

Materials & Methods:
Twenty-eight formalin-fixed, paraffin-embedded canine lymphomas were retrospectively selected, including 15 B-cell and 13 T-cell lymphomas. Indirect immunohistochemistry was performed using anti-CB1R, anti-CB2R and anti-GPR55 antibodies. Immunostaining was evaluated semi-quantitatively according to staining intensity, extension of positive neoplastic cells and nuclear immunoreactivity. Extension was semi-quantitatively categorized as low (0–10%), moderate (11–50%) or high (>50%).

Results:
CB1R demonstrated variable immunostaining patterns in both lymphoma subtypes, with low immunopositivity observed in 40.0% of B-cell lymphomas and high positivity identified in 53.8% of T-cell lymphomas. In contrast, CB2R showed predominantly high immuno-expression in both B-cell and T-cell lymphomas, observed in 86.7% and 69.2% of cases, respectively. GPR55 also exhibited predominantly high immunopositivity in both lymphoma subtypes, identified in 60.0% of B-cell lymphomas and 61.5% of T-cell lymphomas. Moderate to intense expression was more frequent for CB2R and GPR55 than for CB1R, whereas CB1R predominantly showed absent to weak intensity. Occasionally, nuclear immunoreactivity was also observed.

Conclusion:
CB2R and GPR55 showed high immuno-expression and more frequent moderate-to-intense positivity in both B-cell and T-cell lymphomas, whereas CB1R exhibited a more variable positivity pattern. These receptors may represent targets for further investigation in canine lymphomas.

TOPIC: FARM ANIMAL W/O Poultry

Infection dynamics of bovine respiratory disease pathogens during outbreaks at dairy farms in Southern Brazil

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Topic: Farm Animals w/o poultry

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Background:
Bovine respiratory disease (BRD) is a leading cause of illness and death in cattle. Although BRD pathogens are known, their infection patterns during outbreaks are less studied. This report examines pathogen dynamics during BRD outbreaks on 23 dairy farms in Southern Brazil.

Materials & Methods:
Nasal swabs (n=115) were collected from cows on 23 dairy farms in Southern Brazil and tested by multiplex qPCR for BRD-associated agents: BCoV, BRSV, BVDV, BoAlHV1, BPIV3, ovine gammaherpesvirus 2 (OvGHV2), *Mycoplasmopsis bovis*, *Histophilus somni*, *Mannheimia haemolytica*, and *Pasteurella multocida*. Samples with Cq ≤ 37 were considered positive. Statistical analyses evaluated clinical manifestations, assessed seasonality, pathogen frequency, and co-infections.

Results:
Cough (32.17%), fever (23.48%), respiratory distress (18.26%), nasal secretions (17.39%), and oral secretions (14.78%) were common clinical symptoms, and were more frequently observed in singular infections by H. somni (18.52%) and P. multocida (12.96%) than with other pathogens or combinations. At least one pathogen was detected in 70.43% (81/115) of samples. Single-pathogen detections were most common (43.48%; 50/115; $p < 0.001$), with additional dual, triple, quintuple, and sextuple patterns observed. *H. somni* was the most frequent agent overall (45.22%; 52/115) and in single detections (24.35%; 28/115), followed by *P. multocida* (30.43%; 35/115), BCoV (10.43%; 12/115), and BRSV (9.57%; 11/115). *H. somni* showed seasonality, being more frequent in winter (82.14%; 23/28) than in summer/autumn (7.14%; 2/28) or spring (3.57%; 1/28).

Conclusion:
These results demonstrate the multietiological nature of BRD and utilization of qPCR system is fundamental to understand pathogen dynamics of BRD.

Multicentric eosinophilic infiltration in a pot-bellied pig

85
Topic: Farm Animals w/o poultry

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Background:
Pigs are known to frequently exhibit eosinophilic infiltration in various tissues, most often only identified histologically. Such infiltrates are commonly associated with helminth infections (particularly Ascaris suum), nutritional factors, or immunological stimuli. We describe a case of multicentric eosinophilic infiltration in a 5-year-old pot-bellied sow in adequate body condition that died acutely.

Materials & Methods:
A complete necropsy was performed. Samples were collected and processed for histopathology and immunohistochemistry. Sections were stained with hematoxylin and eosin, Congo red and Giemsa. Immunohistochemical staining for CD3 and Ki-67 was performed.

Results:
Multiple superficial white nodules of variable size and number were observed in several internal organs. These lesions were most prominent beneath the epicardial surface of the heart. Additionally, small subcapsular nodules were identified in kidneys and spleen. Histologically, nodules consisted of superficial dense infiltrates of round cells with minimal associated tissue damage. The cells displayed prominent eosinophilic cytoplasmic granules and nuclei often without the typical binucleation, usually described for eosinophils. Congo red stain showed intense eosinophilic staining of the intracytoplasmic granules. Giemsa staining failed to identify mast cells. Immunohistochemistry for CD3 was negative, excluding T-cell origin. Ki-67 immunostaining showed a nuclear count (per 1 cm^2 grid area) of 39.8 in the kidney and 77.4 in the myocardium.

Conclusion:
Based on gross findings, histopathology, and immunohistochemistry, this case was classified as multicentric eosinophilic infiltration in an adult pot-bellied pig. Eosinophilic granulocytes were identified based on their morphology and intensely eosinophilic cytoplasmic granules. The cardiac involvement is considered the most likely cause of death.

Revisiting the etiopathogenesis of porcine ear necrosis through clinical, histopathological and microbiological evidence

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Topic: Farm Animals w/o poultry

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Background:
Porcine ear necrosis (PEN) is a frequent lesion affecting post-weaning piglets and characterized by crusting and necrotic dermatitis of the pinna tip, but its etiopathogenesis remains poorly understood. This study aimed to describe the clinical presentation and progression of PEN lesions to improve understanding of its etiopathogenesis.

Materials & Methods:
The study included 95 piglets (78 affected and 17 controls) from three farms. Clinical examinations were performed twice weekly and included macroscopic lesion scoring ranging from stage 1 (superficial lesions at the ear tip) to stage 4 (extensive lesions with tissue loss), as well as bacteriological and histological sampling.

Results:
Histological lesions included ulceration ($p<0.001$), deep neutrophilic dermal inflammation ($p<0.001$), and vasculitis ($p=0.01$), contrasting with the superficial perivascular lymphocytic infiltrates observed in control pigs. Histopathological features varied little across macroscopic stages, but two lesions appeared central to ulcerative progression: vascular lesions and dermal lymphopenia, whose co-occurrence markedly increased lesion severity ($p<0.001$) suggesting synergistic mechanisms driving tissue damage. An early peak of hyperthermia was observed in stage 1 piglets ($p<0.001$), and hyperthermic animals showed significantly more small-vessel vasculitis ($p=0.04$), but not dermal lymphopenia ($p=0.51$), suggesting a primary vasculitis occurring within an early systemic febrile and inflammatory syndrome. Opportunistic pathogens (mainly *Staphylococcus* spp*. and Streptococcus* spp.) were isolated from both affected and control piglets, providing limited support for primary bacterial etiology.

Conclusion:
These findings support a multifactorial pathogenesis of PEN involving early vascular and inflammatory alterations, with dermal lymphopenia potentially contributing to progression and severity of ulcerative lesions.

Detection of Bovine Polyomavirus-1 in an aborted calf in the United Kingdom

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Topic: Farm Animals w/o poultry

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Background:
A single fetal calf was submitted to investigate the cause of abortion from a herd of 1100 dairy cows. There were no gross lesions and routine tests for common abortifacient agents (including *Neospora caninum,* BVDV and bacteria) were negative. Histology on the kidney revealed a mild interstitial nephritis and intranuclear viral inclusions in renal tubular epithelium consistent with a viral infection.

Materials & Methods:
Formalin-fixed samples of kidney were prepared by routine methods for light microscopy and ultrastructural analysis. Frozen tissue samples of kidney, heart, lung and liver were subject to real time PCR analysis for bovine polyomavirus (BPyV) and one conventional PCR amplicon (530 nucleotides) was sequenced.

Results:
Histopathology revealed a mild interstitial nephritis, with swelling of renal tubular epithelium and large intranuclear viral inclusions. Electron microscopy demonstrated arrays of virus particles within nuclei of renal tubular epithelium. PCR analysis of kidney, heart, lung and liver confirmed high load of BPyV (Ct of 12 to 19) and highest sequence identity to BPyV-1 viruses, but microscopic lesions were present only in the kidney. Testing for all other common abortifacient agents of cattle was negative.

Conclusion:
BPyV-1 was detected in multiple tissues of an aborted calf foetus, including in association with typical lesions in the kidney. Abortion could not be attributed to any other cause, but the significance of the BPyV infection is uncertain. Infection with polyomaviruses in cattle populations worldwide is likely commonplace, but the occurrence of disease or pathology is rare.

Prognostic value of liver biopsy scoring and serum bile acids in donkeys

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Topic: Farm Animals w/o poultry

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Background:
Liver disease is common in donkeys, but there is limited published data on prognostic markers specific to this species. Therefore, disease parameters are partially extrapolated from the horse, which includes the Durham liver biopsy scoring system. This study investigates the prognostic value of liver biopsy and serum bile acids in donkeys in the context of liver disease.

Materials & Methods:
72 adult donkeys from the UK investigated for liver disease were selected. Liver biopsies, serum bile acid data (if collected within 15 days of biopsy) and outcome data, including reason for euthanasia in relation to liver disease was retrospectively collected. Liver biopsies were processed for routine histopathology with additional stains (Perl's Prussian blue and Masson's trichrome) and the samples were scored according to a published horse biopsy scoring system.

Results:
Weighted biopsy scores were associated with survival, and when a cut-off of ≥7 was applied, survival differed significantly (Kaplan-Meier analysis). The banded analysis (0, 2–6, 7–14) showed a graded survival pattern. Serum bile acids showed moderate discrimination for liver-related death, with ROC AUC = 0.768 and an optimal cut-off (Youden's index) ≈25 µmol/L (sensitivity 0.83; specificity 0.77).

Conclusion:
Both serum bile acids and histopathology provide meaningful prognostic information in donkeys with clinical signs of liver disease. A bile acids threshold of 25 µmol/L (moderate discrimination) and a weighted biopsy score >7 (significant discrimination) identified higher-risk cases for liver-related death. The horse scoring framework appears transferable to donkeys, although the small sample size prevented refinement of intermediate categories.

A case of pancreatic endocrine hypoplasia with severe alpha-cell depletion in a juvenile sheep

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Topic: Farm Animals w/o poultry

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Background:
Primary pancreatic endocrine disorders are poorly characterised in livestock, particularly in sheep, and are likely underreported due to their rarity, early culling and limited clinical recognition. We describe a case of pancreatic endocrine hypoplasia in a juvenile sheep.

Materials & Methods:
Thirty-one clinically normal two-month-old male Rasa Aragonesa lambs from the same lambing cohort were enrolled in an experimental study and maintained under identical conditions. One lamb developed early growth retardation and was excluded from the study but remained under observation until euthanasia at 17 months of age. Pancreatic tissue from all animals was examined histologically. Insulin and glucagon immunohistochemistry, followed by digital image analysis, was performed on the affected animal and one control lamb.

Results:
At 17 months, the affected lamb weighed 30.6 kg, compared with 50.71 ± 5.75 kg in the remaining animals. No lesions were identified in controls or in the pituitary gland of the affected animal. Pancreas of the affected lamb showed a reduction in islet number and area, with no inflammation, whilst the exocrine component appeared preserved. Immunohistochemistry demonstrated reduced insulin-positive area compared with the control (1.33% vs 2.02%; ~34% decrease), whereas glucagon-positive area was profoundly reduced (0.008% vs 0.25%; ~97% decrease), resulting in an increased insulin-to-glucagon ratio.

Conclusion:
Findings are consistent with pancreatic islet hypoplasia characterised by disproportionate α-cell depletion relative to β-cells, with juvenile onset. This may represent a previously undescribed developmental pattern in sheep that warrants further investigation. Pancreatic examination should be considered in sheep presenting with unexplained marked growth retardation.

Nasal tuberculosis in two free-roaming Black Nebrodi pigs with generalized Mycobacterium bovis infection in Sicily (Italy): pathological and strain characterization

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Topic: Farm Animals w/o poultry

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Background:
Nasal shedding of *Mycobacterium bovis* (MB) by infected suids constitutes a relevant route for bacterial spread and environmental maintenance. However, associated nasal lesions are rarely described.

Materials & Methods:
Two adult Black Nebrodi pigs (Pig#1: female, intact; Pig#2: male, castrated) were necropsied during routine abattoir inspection. Affected organs were collected and underwent microbiological (culture, RT-PCR, spoligotyping) and histopathological analyses.

Results:
Macroscopically, Pig#1 showed coalescing, caseocalcified granulomas affecting the right mammary parenchyma, multiple lymph nodes (prescapular, renal, mammary, inguinal), and nasal turbinates. Additionally, a 7x4x4 cm fibrocaseous granuloma (tuberculoma) bilaterally effaced the ethmoidal conchae and cribriform plate, extending into the frontal sinuses and compressing the olfactory bulbs. The adjacent dura mater exhibited a 3x3x0.5 cm, plaque-like granuloma. Pig#2 showed coalescing-to-miliary, caseocalcified granulomas affecting >50% of the nasal turbinates, as well as various lymph nodes (submandibular, retropharyngeal, tracheobronchial, renal, mesenteric, inguinal), tonsils, lungs, spleen, liver, parietal pleura, and omentum. Granulomatous spondylitis with bony lysis and spinal cord compression affected multiple vertebral bodies. Histologically, all granulomas featured mineralized-to-necrotic cores surrounded by epithelioid macrophages, scant Langhans' multinucleated giant cells, lymphocytes, and peripheral fibroblasts. No acid-fast mycobacteria were detected in multiple sections of all lesions stained with Ziehl-Neelsen and Fite-Faraco. Cultural and RT-PCR analyses confirmed MB in all tested samples, and spoligotyping identified spoligotypes SB1305 (Pig#1), SB0120 and SB2218 (Pig#2).

Conclusion:
Generalized tuberculosis in pigs may affect nasal tissues, with nasal granulomas potentially constituting relevant yet underreported shedding sites. Spoligotype SB2218 is unreported in Italy, suggesting complex MB transmission networks.

Molecular epidemiology and clinical associations of Histophilus somni in asymptomatic and dairy cattle with respiratory disease from Southern Brazil

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Topic: Farm Animals w/o poultry

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Background:
Although *H. somni* is a major bacterial pathogen of bovine respiratory disease (BRD), molecular and clinical dynamics data in dairy systems from Brazil are lacking. This study evaluated the clinical associations and molecular detection of *H. somni* in asymptomatic and sick dairy cattle from Southern Brazil

Materials & Methods:
Paired oral and nasal swabs were collected from vaccinated (n=23) and unvaccinated (n=67) dairy cattle for BRD. *H. somni* was detected using (quantitative PCR) qPCR. Epidemiological variables and clinical signs were assessed with Chi-square/Fisher's exact tests and multivariable logistic regression to identify independent predictors of detection.

Results:
*H. somni*was detected in 31.1% (28/90) of all animals. Diagnostic comparative analyses revealed that oral detection was significantly ($p<0.001$) superior to nasal detection, with detection probability being 96.4% (27/28) for oral and 39.3% (11/28) for nasal swabs. The bivariate analysis identified several significant variables, but only season and vaccination status were independent predictors of *H. somni* detection in the multivariable model. Pathogen detection during summer (AOR=50.1; CI:(95%)3.24–1530.93; $p=0.008$) and winter (AOR=22.3; CI:(95%)5.39–156.73; $p<0.001$) revealed significantly higher odds of positivity compared to autumn. Non-vaccinated cattle had significantly risk (AO=0.27; CI:(95%)0.07–0.92; $p=0.042$) of *H. somni* detection compared with vaccinated animals. Coughing was the only clinical sign associated ($p=0.026$) with *H. somni* detection.

Conclusion:
These findings demonstrated that *H. somni* circulated in these dairy herds and was associated with coughing. Additionally, oral sampling was comparatively more effective than nasal sampling for qPCR surveillance, detection, and diagnosis of *H. somni* in this population.

Meningeal abscess associated with Streptococcus lutetiensis in a goat

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Topic: Farm Animals w/o poultry

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Background:
Meningeal abscesses in goats are rarely reported and are associated with opportunistic bacteria spreading from primary infection sites via hematogenous, lymphatic, or retrograde axonal routes, as well as from otitis media. *Streptococcus lutetiensis*, a member of the *Streptococcus bovis/Streptococcus equinus complex,* is part of the normal colonic microbiota in humans, and has been described as a commensal in the rumen of cattle and intestines of cats. However, this bacterium is increasingly recognized as an emerging cause of bovine mastitis and has been once reported in neonatal meningitis and subdural abscess in humans.

Materials & Methods:
A 5-year-old female Saanen goat was presented for anorexia, pyrexia and circling behavior. Due to poor prognosis, the goat was euthanized and a necropsy was performed.

Results:
Upon opening the cranial cavity, a 0.5 cm diameter meningeal abscess was identified in the groove between the cerebrum and the cerebellum. Histologically, it consisted of a necrotic core containing numerous bacterial colonies of cocci arranged in pairs or chains, surrounded by degenerated neutrophils and macrophages. Multiple hemorrhagic foci were observed in the surrounding tissue, along with neutrophilic vasculitis, fibrinoid necrosis of blood vessels, and fibrin thrombi occluding small meningeal vessels. The remainder of the necropsy was unremarkable, except for 50 mL of serohemorrhagic pleural effusion and severe pulmonary edema. Bacterial culture of the meningeal abscess yielded *Streptococcus spp.,* and subsequent sequencing identified the isolate as *S. lutetiensis.*

Conclusion:
To our knowledge this is the first report of meningeal abscess associated with *S. lutetiensis* in a goat.

Molecular Detection Patterns of Bovine Respiratory Disease Pathogens in Vaccinated Feedlot Cattle from Brazil

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Topic: Farm Animals w/o poultry

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Background:
Breakthrough infections (BTI) and mixed-pathogen dynamics complicate the interpretation of bovine respiratory disease (BRD) in feedlots. This study assessed possible associations between BRD-compatible clinical signs and molecular detection of respiratory pathogens in vaccinated feedlot cattle from Brazil.

Materials & Methods:
Deep nasopharyngeal swabs were collected from 37 intact Nelore steers, from three feedlots in Brazil. All animals were immunized at entry with an inactivated vaccine containing *Histophilus somni*, *Pasteurella multocida*, *Mannheimia haemolytica*, BoAHV1, BPIV3, and BVDV (no unvaccinated comparator was included). PCR/RT-PCR targeted 10 BRD agents: BVDV, BoAHV1, BPIV3, BCoV, BRSV, OvGHV2, *M. haemolytica*, *P. multocida*, *H. somni*, and *Mycoplasmopsis bovis*. Clinical signs were evaluated using the modified DART system. Possible associations were determined by multivariable modelling.

Results:
Most cattle were negative for all pathogens (62.2%; 23/37), pathogen detected occurred in 37.8% (14/37). Single pathogen detection (24.3%; 9/37) was predominant , relative to dual (5.4%; 2/37) and triple (2.7%; 1/37) detections. BCoV was the most frequent (18.9%; 7/37), appeared in all detection patterns, including the only triple detection (BCoV + *M. haemolytica* + *H. somni*). Vaccine-included pathogens were detected (*P. multocida,* 8.1%; BoAHV1, *H. somni*, *M. haemolytica* each 5.4%), consistent with circulation and possible BTI. Profuse salivation and stunted growth predicted detection frequency ($p<0.05$). Strongest sign–pathogen links were salivation–*P. multocida* ($\beta=2.30$; $p=0.049$), stunted growth–BoAHV1 ($\beta=2.30$; $p=0.049$), and fever–BCoV ($\beta=2.98$; $p=0.010$).

Conclusion:
The vaccination protocols used were satisfactory. Molecular detection with standardized clinical evaluations supports pathology-oriented interpretation of pathogen-circulation and BTI in these feedlots.

Suspected systemic dendritic cell histiocytosis in a one-day-old piglet

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Topic: Farm Animals w/o poultry

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Background:
In human pathology, congenital systemic histiocytoses are well-characterized disorders that most commonly correspond to Langerhans cell histiocytosis, a proliferation of specialized dendritic cells displaying phenotypic and ultrastructural features of Langerhans cells, but reports in pigs remain rare. To date, the literature contains only one report of congenital cutaneous histiocytic proliferation in a piglet, with lesions confined to the skin and no systemic involvement identified.

Materials & Methods:
A one-day-old piglet was submitted to Labocea for investigation of multiple cutaneous lesions present at birth. A complete postmortem examination was performed, followed by ancillary testing, including bacteriological culture, metagenomics, histopathology, and immunohistochemistry for vimentin, desmin, S100, Iba1, and PU.1.

Results:
Necropsy revealed a multifocal nodular proliferative process involving the skin, subcutaneous tissue, skeletal muscle including myocardium, liver, kidneys, spleen, and meninges. Grossly, lesions appeared as multiple raised, whitish nodules, with cutaneous ulceration. Microscopically, the proliferation consisted of spindle-shaped and epithelioid cells exhibiting marked pleomorphism but no epitheliotropism. The neoplastic cells showed positive immunolabelling for histiocytic markers (Iba-1, PU.1, and vimentin) and were negative for desmin and S100. No bacteria or viruses were detected by bacteriology or metagenomics. Distinction between interstitial dendritic and Langerhans cell origins could not be assessed, as CD1a and langerin (CD207) were not evaluated. However, given the histiocytic phenotype and absence of epitheliotropism, a diagnosis of systemic dendritic cell histiocytosis is proposed.

Conclusion:
This case represents, to the authors' knowledge, the first reported occurrence of congenital systemic dendritic cell histiocytosis in a piglet.

Congenital fibropapillomas in a piglet

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Topic: Farm Animals w/o poultry

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Background:
A piglet less than 6 days old was presented with cutaneous growths on the plantar surface of the left hind limb, extending up to its pubic region. Papillomas are rare in swine, and only a few cases of congenital papillomas have been reported.

Materials & Methods:
A complete necropsy was followed by histopathology. Unfortunately, it was not possible to conduct supplementary immunohistochemical analyses or metagenomics.

Results:
Necropsy revealed multiple skin nodules, sometimes coalescing and cauliflower-like, extending from the base of the right tarsus on the inner side down to the abdomen, 1 to 10 cm in length. No other significant macroscopic findings were noticed. Histopathological examination revealed the presence of multiple tumoral growths originating from the epidermis, consistent with fibropapillomas.

Conclusion:
The histological lesions observed corresponded with the descriptions of previous clinical cases of congenital papillomatosis in piglets. In the absence of evidence of viral involvement, hamartoma cannot be ruled out as part of the macroscopic differential diagnosis. There are two reported cases of hamartoma in piglets in the literature. In those reports, histological analysis was decisive in establishing the diagnosis due to the presence of apocrine glands. In our case, however, a microscopic diagnosis of fibropapillomas was made. Furthermore, this affected piglet was the only one in its pen and in the herd, which suggests that viral involvement is unlikely, even if it could not be excluded.

Motor neuron disease with neurofibrillary accumulation in newborn Zwartbles lambs.

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Topic: Farm Animals w/o poultry

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Background:
Two one-day-old purebred Zwartbles lambs were submitted to investigate the cause of ataxia and weakness affecting multiple lambs since birth.

Materials & Methods:
The lambs underwent limited clinical and neurological examination, followed by humane euthanasia and necropsy. Samples of brain, spinal cord, nerve and skeletal muscle were fixed in formalin and processed for histopathology and electron microscopy.

Results:
Both lambs exhibited bilateral hindlimb paresis and ataxia, and one remained recumbent unless helped to stand. Mentation was normal and there were no cranial nerve deficits. Necropsy revealed no gross abnormalities. Microscopic examination of the brain and spinal cord of both lambs revealed degeneration of motor neurons in the brainstem, including the red and vestibular nuclei, and in the ventral horns of the spinal cord. Affected neurons were swollen and pale-staining, with dispersed Nissl substance (chromatolysis). There were no lesions in the spinal nerve roots, peripheral nerves or skeletal muscle. Ultrastructural examination of spinal ventral horn neurons revealed large interlacing bundles of neurofilaments displacing and surrounding small aggregates of rough endoplasmic reticulum and other organelles.

Conclusion:
The lesions in this case were consistent with motor neuron disease with neurofibrillary accumulation. The absence of neuronal necrosis and axonal degeneration reflects an early stage of disease. Motor neuron diseases have been described in Romney sheep and various breeds of dogs, cattle and pigs – generally with a heritable basis. Further investigations should focus on analysis of breeding records and genetic investigations to determine the genetic fault and mode of inheritance

Variability of calves' responses to poor barn air quality, and to an aerosolized inflammatory stimulus

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Topic: Farm Animals w/o poultry

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Background:
Bovine respiratory disease (BRD) risk factors promote subclinical inflammation that dysregulates subsequent responses to bacterial infection. Poor air quality is an important BRD risk factor, but the pathogenesis of this effect is unknown. This experimental study investigated calves' pulmonary inflammatory responses to reduced air quality and to an aerosolized inflammatory stimulus.

Materials & Methods:
One study involved 8 calves initially sampled in good quality room air, then ventilation was reduced (day 0), which resulted in increased particulate matter and ammonia concentrations. Bronchoalveolar lavage fluid (BALF) was sampled on days –1, 8, 13 and 14 relative to reduced ventilation; an inflammatory stimulus (lysate of killed Mannheimia) was aerosolized on d13. BALF was analyzed by differential counts, flow cytometry, and cytokine quantification.

Results:
Calves developed progressively higher BALF neutrophilia after reduction of ventilation. Of 8 calves, 5 developed BALF neutrophilia after reduced air quality (d8, d13) and had higher responses to aerosolized bacterial lysate (d14); 4/5 of these later (d17) developed clinical pneumonia. In contrast, 3 calves had minimal BALF neutrophilia after reduced air quality and had lower responses to aerosolized bacterial lysate; all 3 of these calves remained healthy. Two additional experiments yielded comparable findings.

Conclusion:
Although calf numbers are low, the study documents significant individual differences in responses to reduced air quality or aerosolized inflammatory stimuli, and suggest that calves mounting excessive responses are more likely to later develop pneumonia. The findings support the emerging concept that abnormal regulation of inflammatory responses in clinically healthy cattle promotes the later development of pneumonia.

Temporal changes in blood monocyte subsets in auction calves during high- and low-risk periods for bovine respiratory disease (BRD)

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Topic: Farm Animals w/o poultry

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Background:
Altered blood monocyte phenotype may contribute to dysregulated innate immune responses during BRD. This study evaluated temporal changes in monocyte subset percentage/activation in auction calves.

Materials & Methods:
Peripheral blood from two cohorts of mixed-breed auction calves in 2022 (n = 17 and n = 15) was analyzed by flow cytometry on Days 1 and 4 after arrival and on Day 32 after adaptation. Monocytes were classified as classical (cMo), intermediate (intMo), and non-classical (ncMo) based on CD14/CD16 expression. CD14 and MHC class II median fluorescence intensity (MFI), forward scatter (FSC), and side scatter (SSC) signal intensity were assessed as activation-associated readouts. Low BRD incidence (4/32 calves) precluded outcome-based analysis.

Results:
cMo increased in Cohort A from Day 1 to Day 4 (5.49 to 8.91; P=0.027), but decreased in Cohort B by Day 32 (8.51 to 6.89; P<0.001). intMo increased in Cohort B from Day 1 to Day 4/32 (0.20 to 0.42/0.48; P≤0.001). In cMo, CD14 MFI peaked at Day 4 in Cohort A (821/1135/395 on Days 1/4/32; P<0.001), whereas MHC-II MFI increased by Day 32 in Cohort B (171/187/276; P<0.001). cMo FSC decreased by Day 32 in both cohorts (P<0.001), and cMo SSC decreased mainly in Cohort A (40,930/34,120 to 28,344; P<0.001). intMo FSC decreased by Day 32 in both cohorts (P≤0.001), whereas intMo SSC decreased mainly in Cohort A (41,569/35,384 to 29,033; P<0.001).

Conclusion:
Auction calves showed temporal/cohort-associated heterogeneity, with reduced post-adaptation FSC/SSC and divergent CD14/MHC-II patterns suggesting altered monocyte activation, size/granularity, and antigen-presentation dynamics potentially reflecting increased innate immune responsiveness at arrival.

Non-tuberculous mycobacteria detected in alpacas in ireland

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Topic: Farm Animals w/o poultry

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Background:
The genus *Mycobacterium* is very large and comprises the *Mycobacterium tuberculosis* complex (MTC), which includes the commonest causative agents of the bovine tuberculosis -bTB- (*Mycobacterium bovis* and *Mycobacterium caprae*), and the non-tuberculous mycobacteria (NTM), which are typically free-living microorganisms widespread in nature and occasionally reported as opportunistic pathogens. In cattle, excluding *Mycobacterium avium* subsp. *paratuberculosis*, NTM infection has not usually been associated with clinical signs; however, they could interfere with bTB eradication.

NTM cases detected in alpacas through surveillance are here described.

Materials & Methods:
Alpaca cases submitted for post-mortem examination at DAFM (Ireland) during the period 2010-2026 were reviewed. Non-speciated NTM-cultured tissues were re-cultured, and the strains were then subjected to Ziehl-Neelsen staining, PCR, and MALDI-TOF MS.

Results:
Of a total of 265 submissions, eight cases were detected. *Mycobacterium avium hominissuis*, *Mycobacterium avium* subsp. *avium* (*Maa*), and *Mycobacterium kansasii* were associated, respectively, with multifocal pulmonary consolidation, chronic granulomatous enteritis, and granulomatous lymphadenitis in three alpacas. Pooled lymph nodes from two serologically TB-positive culled animals cultured NTMs (one cultured *Mycobacterium vaccae*; one did not re-grow); however, carcasses were unremarkable and had no gross bTB features. In a case with calcified hepatic abscesses, re-culture failed. Finally, *Mycobacterium spp*. different than MTC was diagnosed in other two carcasses having granulomatous lymphadenitis, and hepatitis/hepatic lymphadenitis, respectively.

Conclusion:
To the authors' knowledge, this is the first case-series of NTM infection described in alpaca. Maa should be included as a cause of granulomatous enteritis in this species. NTMs could complicate bTB detection in alpaca.

TOPIC: POULTRY

When Marek's Opens the Door: Concurrent Viral and Fungal Infections in Immunocompromised Poultry

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Topic: Poultry

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Background:
Marek's disease is an important lymphoproliferative disease of poultry associated with immunosuppression and increased susceptibility to secondary infections. This report describes concurrent lymphoid neoplasia, cutaneous avian pox, and pulmonary aspergillosis in commercial layer chickens.

Materials & Methods:
Approximately 300 birds from a commercial flock of approximately 6,000 layers developed proliferative cutaneous facial lesions with swelling, and ocular closure, with cumulative mortality of approximately 70 birds over one month. Five 25-week-old layers were submitted for diagnostic investigation. Complete necropsy and histopathological examinations were performed on major organs. Immunohistochemistry for CD3 and PAX5 were performed for lymphoid phenotyping.

Results:
Gross findings included multifocal proliferative cutaneous nodules, hepatomegaly, marked splenomegaly, renomegaly, pulmonary consolidation, and emaciation. Histologically, the skin lesions showed epithelial hyperplasia, ballooning degeneration, and eosinophilic intracytoplasmic inclusion bodies consistent with avian poxvirus infection. Multiple visceral organs, including liver, spleen, kidney, and lung, were infiltrated by pleomorphic to relatively monomorphic atypical lymphoid cells with frequent mitotic figures, consistent with multicentric lymphoid neoplasia suggestive of Marek's disease. The lungs additionally exhibited severe necrotising pneumonia containing abundant septate fungal hyphae with acute-angle branching, morphologically consistent with Aspergillus spp.

Conclusion:
This case demonstrates concurrent viral, neoplastic, and fungal diseases in commercial poultry. The findings support Marek's disease as the probable underlying condition associated with immunosuppression, predisposing affected birds to opportunistic infections including avian pox and aspergillosis.

A novel Single-Cell approach to investigate host responses to viral infection in avian species

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Topic: Poultry

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Background:
Usutu virus (USUV), an emerging mosquito-borne *Orthoflavivirus*, causes significant mortality in susceptible avian species, particularly passerines, whereas others display limited clinical impact. Characterizing these responses may improve our understanding of avian host–pathogen interactions and disease pathogenesis.

Materials & Methods:
We performed a comparative single-cell RNA sequencing analysis of immune responses in chicken and canary during experimental USUV infection. Samples were collected at multiple time points post-infection to characterize the dynamics of cellular and transcriptional responses in these two species displaying distinct infection outcomes.

Results:
Our analysis revealed marked interspecies differences in immune cell responses and transcriptional programs following infection. Chicken developed an early and controlled antiviral response, whereas canary exhibited a broader and less coordinated immune reaction associated with disease progression. In addition, variation in the basal expression of specific genes prior to infection suggests that constitutive host factors may contribute to the distinct response profiles observed. These findings identify candidate pathways involved in differential host responses to USUV infection.

Conclusion:
This study provides a descriptive single-cell atlas of USUV-induced immune responses in two avian species with contrasting infection outcomes. Given the interspecies nature of this comparison, the observed differences cannot be directly attributed to resistance or susceptibility mechanisms. However, they highlight candidate factors and pathways for further investigation. Future mechanistic studies within a single species, using targeted modulation approaches such as inhibitors, antibodies, or small interfering RNA, will be required to determine their causal role in protection or pathogenesis.

TOPIC: HORSES

Persistent pyrexia and thrombocytopenia associated with primary pulmonary adenocarcinoma and bone marrow metastasis in a horse.

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Topic: Horses

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Background:
A 15-year-old gelding was presented to the Department of Internal Medicine, Reproduction and Population Medicine of Ghent University with a history of persistent pyrexia (>1 week), lethargy, anorexia and progressive weight loss, unresponsive to therapy. Clinical examination revealed sweating and tachycardia while hematological analysis demonstrated thrombocytopenia. Due to progressive clinical deterioration and poor prognosis, euthanasia was elected.

Materials & Methods:
N.A.

Results:
Post-mortem examination revealed diffusely swollen and congested lungs with multifocal, small white nodules. Given the thrombocytopenia, the bone marrow was also evaluated and showed multiple small, gray mass-like lesions. Histological evaluation of both lungs and bone marrow revealed a neoplastic process composed of epithelial cells arranged in acinar and tubular-like structures, completely invading and effacing the normal parenchyma. Immunohistochemistry was positive for cytokeratin and in the absence of other primary masses, the findings were most consistent with a primary pulmonary adenocarcinoma with bone marrow metastasis.

Conclusion:
This case emphasizes the importance of including neoplasia in the differential diagnosis of horses with prolonged pyrexia of unknown origin and non-specific systemic signs. Furthermore, it highlights the diagnostic value of post-mortem evaluation of bone marrow in identifying disseminated malignancy.

Novel finding of Rustrela virus in Swedish horses with encephalitis

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Topic: Horses

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Background:
Rustrela virus (RusV; species *Rubivirus strelense*; family *Matonaviridae*) has been described in cases of encephalitis in a wide range of animal species, including a donkey, but so far not in horses. Recent research indicates RusV as the cause of feline Staggering disease, a serious neurological disease seen endemically in cats within the lake Mälaren region in Sweden. In this study we explored RusV as a potential etiology of encephalitis in Swedish horses.

Materials & Methods:
Formalin-fixed, paraffin-embedded brain and/or spinal cord tissues from 11 horses, comprising 6 cases with a diagnosis of non-suppurative encephalitis, encephalomyelitis or meningoencephalitis and 5 without, were evaluated. All horses were examined postmortem at the Swedish University of Agricultural Sciences, Uppsala, Sweden, between 2007-2021. From each horse, a minimum of two locations in the central nervous system were investigated for presence of RusV capsid protein using immunohistochemistry, and for RusV RNA using RT-qPCR. Additionally, RT-qPCR for Borna disease virus-1 (BoDV-1) was performed in encephalitic horses.

Results:
RusV capsid protein was detected in three encephalitic horses. Immunostaining was variably detected intracytoplasmically in brain and spinal cord neurons, and in occasional glial cell. RusV RNA was detected by RT-qPCR in all three cases. All encephalitic horses were negative for BoDV-1. Horses without encephalitis were negative for RusV antigen and RusV RNA.

Conclusion:
The results indicate RusV as a cause of encephalitis in Swedish horses. Further investigations into the pathogenesis, including transmission routes, are warranted.

Post-mortem diagnosis of hepatic haemochromatosis in a pony with chronic iron overload

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Topic: Horses

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Background:
We report the case of a 23-year-old gelded pony that died after progressive weight loss and diarrhoea, in a riding centre where several horses showed similar clinical signs and increased hepatic enzyme activities. This case was investigated to determine the cause of death and characterise the hepatic lesion.

Materials & Methods:
A full post-mortem and histopathological examination were performed. Liver tissue was examined using routine histology and Perls' Prussian blue stain to detect iron deposition.

Results:
The pony was severely emaciated with marked serous atrophy of adipose tissue. The liver was firm and macroscopically compatible with cholestatic hepatopathy. Histology revealed chronic periportal hepatitis with marked portal fibrosis, occasionally bridging, and abundant haemosiderin accumulation in macrophages, periportal hepatocytes and Kupffer cells. Perls' staining confirmed extensive hepatic iron deposition. These findings were consistent with hepatic haemochromatosis secondary to chronic iron overload.

Conclusion:
This case highlights chronic iron overload as an important differential diagnosis for progressive hepatopathy in horses. Post-mortem histopathology combined with Perls' staining is essential for confirming hepatic haemochromatosis and should prompt investigation of environmental iron exposure in affected herds

Immunohistochemical markers of myocardial damage in horses: a pilot study.

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Topic: Horses

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Background:
No immunohistochemical (IHC) markers of cardiac damage have been validated in horses. This pilot study aimed to describe the IHC features of cardiac troponin T (cTnT), desmin and connexin-43 (Cx43) in normal, fresh and autolysed, and damaged equine myocardium.

Materials & Methods:
Twenty-eight post-mortem equine hearts from the University of Liverpool were retrospectively divided into three groups: 8 fresh and 10 autolysed controls without concurrent cardiac pathology and 10 cases with chronic or acute myocardial lesions including fibrosis, haemorrhage, cardiomyocyte degeneration and necrosis. FFPE myocardial sections were immunolabelled for cTnT, desmin and Cx43. Labelling pattern and intensity were correlated with HE features.

Results:
In controls, desmin (7/8) highlighted a moderate granular sarcoplasmic and intercalated-discs (IDs) labelling. cTnT (8/8) showed strong sarcoplasmic fibrillary labelling. Cx43 (8/8) showed predominantly strong IDs' labelling with sparse membranous labelling. In all pathological hearts, IHC abnormalities were topographically associated with the corresponding HE changes: cTnT showed reduced sarcoplasmic and granular labelling. Desmin displayed irregular granular sarcoplasmic and scant IDs labelling. Cx43 showed reduced IDs labelling, with patchy sarcoplasmic and accentuated membranous labelling. All autolytic hearts showed cTnT and desmin diffuse loss of immunoreactivity (cTnT and desmin), while Cx43 showed reduced junctional labelling and variable mild cytoplasmic redistribution. Conduction fibres were better preserved than adjacent myocardium, with prominent membranous Cx43 and granular cTnT/desmin labelling.

Conclusion:
Cx43 IHC revealed a distinct staining pattern unique to pathological myocardium; whereas conduction fibre labelling was preserved in autolysed samples.

Uterine cytological, histopathological and immunohistochemical findings in native Sicilian donkeys

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Topic: Horses

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Background:
Pantesco and Ragusano donkeys are Sicilian native breeds with limited population sizes. Endometrial health in jennies is poorly understood, as is the role of macrophages and mast cells (MCs). This study aimed to evaluate endometrial cytological and histopathological findings in Pantesco and Ragusano jennies and to investigate the distribution of macrophages and MCs.

Materials & Methods:
Endometrial cytology and histopathology were evaluated in 23 Pantesco and 17 Ragusano jennies referred for reproductive examination because of fertility concerns, despite the absence of significant abnormalities on clinical and ultrasonographic evaluation. Histological lesions were classified according to the Kenney and Doig system. Immunohistochemistry was performed using macrophage marker (MAC387) and MC tryptase antibodies. Positive cells were quantified within luminal epithelium (LE), stratum compactum (SC) and stratum spongiosum (SP). Associations between macrophage and MC counts and histopathological severity were analysed using non-parametric statistics.

Results:
Cytology mainly identified acute inflammatory changes, whereas histopathology revealed chronic endometrial lesions. Histological categories included I (n=11), IIA (n=12), IIB (n=11) and III (n=6). Histopathological severity was not associated with age. Macrophage and MC counts were positively correlated with histopathological severity, particularly within SC and SP ($p < 0.0001$). Categories IIB and III showed significantly increased stromal inflammatory cell infiltration. No significant associations were identified with oestrous cycle phase.

Conclusion:
These findings may contribute to a better understanding of reproductive health in Sicilian native donkey breeds. Chronic endometrial lesions in jennies were associated with marked stromal macrophage and MC infiltration, supporting a potential role in endometrial fibrotic remodelling.

TOPIC: WILD LIFE WITHOUT FISH

Down the rabbit hole: Histological and immunohistological findings in European hares (Lepus europaeus) with myxomatosis

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Topic: Wild life w/o fish

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Background:
Myxomatosis is a well-known, often-fatal, Leporipox-virus disease in European rabbits. Recently, outbreaks of a recombinant myxomatosis strain were documented in Iberian hares in Spain and subsequently also in European hares in western European countries.

Materials & Methods:
Nine female and eleven male European hares, 15 shot and five found dead, with macroscopic lesions suggestive of myxomatosis were investigated histologically and immunohistologically to identify potential target cells (ABC method). Tissue samples included skin (lip, nose, eyelid, chest, pinna, anogenital region), liver, spleen, heart, lung, kidney, adrenal gland, gonads, bone marrow and brain. PCR was performed on skin samples.

Results:
Strain specific qPCR verified infections with recombinant Toledo-like MYXV (cq 12 to 21) In all hares, myxomatosis-like lesions were found in lips, nose and eyelid. Skin of the anogenital region, the chest and the pinna were affected in 16, eleven, and one cases, respectively. In inner organs, only minor lesions, most likely unrelated to viral infection, were present. Immunohistologically, viral antigen distribution in the skin aligned mostly histology, with 4 animals displaying small foci of immunopositive cells in the pinna and thoracic region without histomorphologic changes. Ten hares had antigen-containing cells in the testis, two in the lung, two in the ovary and one in the spleen.

Conclusion:
Myxomatosis in European hares shows similar morphologic changes as in European rabbits and Iberian hares, with generally little involvement of internal organs. Considering the often-fatal course of disease outbreaks and the recent adaption to new species, continuous monitoring of the disease is needed.

Avian influenza in a harbour seal (Phoca vitulina) in the Netherlands

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Topic: Wild life w/o fish

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Background:
Highly pathogenic avian influenza viruses (HPAIV) are of concern because of their One Health implications, including threats to wildlife conservation. As spillover hosts, marine mammals can be affected by these viruses and studying these will improve understanding of susceptibility and disease manifestation. This study details the pathologic findings from a fatal HPAIV H5N1 infection in a harbour seal (*Phoca vitulina*).

Materials & Methods:
A subadult female seal was found dead on a Dutch Wadden island in March 2025. During necropsy, samples were collected and analysed for histopathologic and microbiologic examination.

Results:
The main gross findings were a poor body condition and pulmonary congestion with oedema. Histologically, in the brain there was marked non-suppurative meningoencephalitis with malacia, and focal necrosis of peribronchial glands in the lungs. Associated to lesions, virus antigen was detected in cerebral neurons, ependymal cells, and epithelial cells of peribronchial glands. Influenza virus H5 was detected in the brain through RT-qPCR. At the national reference lab, brain tissue and nose swab tested positive for H5N1 virus of the DI.2 genotype. This genotype is closely related to HPAIV in wild birds, and could indicate that the seal was infected following exposure to infected wild birds.

Conclusion:
This case confirms the spillover of genotype DI.2 within Dutch marine mammals causing meningoencephalitis and inflammation of peribronchial glands and underscores the pathogenic potential of H5N1. HPAIV represent a continuing risk for expansion into new host species and increased pathogenicity, highlighting the need for continued surveillance of marine mammals.

Infectious disease patterns in a captive avian collection in Louisiana: a 10-year retrospective study with emphasis on nondomestic Anseriformes

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Topic: Wild life w/o fish

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Background:
Avian collections under human care are susceptible to a range of infectious diseases; however, long-term retrospective studies in nondomestic waterfowl collections are limited. The objective of this study was to characterise infectious diseases diagnosed in an avian collection under human care in Louisiana, USA, over a 10-year period, with emphasis on nondomestic Anseriformes.

Materials & Methods:
Diagnostic records from 449 avian submissions to the Louisiana Animal Disease Diagnostic Laboratory between 2012 and 2023 were reviewed. Cases included necropsies, "necropsy-in-a-jar" submissions, and biopsies. Major infectious disease categories and associated aetiologic agents were recorded based on histopathology and ancillary diagnostic testing. Descriptive statistics and multivariable logistic regression analyses were performed.

Results:
Anseriformes represented 70.2% (315/449) of cases. Infectious diseases were identified in 43.9% (197/449) of submissions, with bacterial disease being the most common category (37.6%, 95% confidence intervals [CI]:33.1-42.1), followed by fungal (13.8%,95% CI:10.6-17.0), viral (8.0% 95% CI:5.5-10.5), metazoan parasitic diseases (7.6%, 95% CI:5.2-10.0), and protozoal (6.0%, 95% CI:3.9-8.1). West Nile virus was the predominant viral disease, whereas *Aspergillus* spp. and *Mycobacterium* spp. were the most common fungal and bacterial pathogens, respectively. Viral diseases exhibited marked seasonality, with all cases occurring between July and November. Hatchling and nestling birds had significantly greater odds of bacterial infection compared with older birds.

Conclusion:
This study demonstrates the value of retrospective diagnostic pathology datasets for characterising infectious disease patterns in avian populations under human care and may assist future surveillance and population health management in nondomestic avian collections.

Sheep-associated malignant catarrhal fever in a Rusa deer (Cervus timorensis) from Southern Brazil

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Topic: Wild life w/o fish

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Background:
Ovine gammaherpesvirus 2 (OvGHV2) causes sheep-associated malignant catarrhal fever (SA-MCF) in Brazil affecting mammals both with and without contact with the asymptomatic carrier host (sheep). Most reported cases from Brazil involve cattle, with few in wildlife. Recent studies suggest that free-ranging wild boars (FRWB) may act as bridge-hosts for OvGHV2 dissemination. This report describes SA-MCF in wildlife without contact with sheep.

Materials & Methods:
A Rusa deer maintained in a propriety from Southern Brazil, with no sheep, and where OvGHV2-subclinically infected FRWB were sighted, died suddenly and was necropsied. Selected organs were processed for histopathology and PCR testing for common ruminant pathogens, including OvGHV2, bovine alphaherpesvirus 1, bovine viral diarrhoea virus, *Lyssa* virus, *Listeria monocytogenes*, and *Histophilus somni*. Spatial analysis assessed the potential role of FRWB in OvGHV2 dissemination.

Results:
Grossly, the gastrointestinal tract had multifocal erosions and ulcerations. Histopathology revealed erosive and ulcerative esophagitis, abomasitis, omasitis, and rumenitis; lymphoplasmacytic meningoencephalitis and interstitial nephritis; with lymphoplasmacytic necrotizing vasculitis in the lungs, liver, and brain. PCR detected the OvGHV2 tegument protein gene in the brain; all other assays were negative. Spatial analysis placed the FRWB within 5 km of the property.

Conclusion:
Pathology and molecular testing confirmed OvGHV2 infection, adding to the few SA-MCF cases in Brazilian wildlife. Spatial analysis suggests that FRWB, which can travel up to 80km daily, may disseminate OvGHV2 through incidental contact with susceptible ruminants. These findings indicate that SA-MCF can occur without sheep and highlight FRWB as a potential contributor to OvGHV2 epidemiology.

Thyroid development in asian houbara (Chlamydotis macqueenii)

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Topic: Wild life w/o fish

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Background:
The thyroid gland plays a critical role in metabolic regulation and seasonal adaptation in avian species. In Asian bustards, physiological variations remain poorly characterized. This study aimed to evaluate morphometric variations in the thyroid glands of Asian bustards and the influence of biological and seasonal factors.

Materials & Methods:
Thyroid samples (n=130) were collected during necropsy. Morphometric parameters (width, length, and weight) were recorded. Tissues were fixed in formalin, routinely processed, and stained with HE. Generalized linear models were developed to evaluate the influence of age, sex, season (winter and summer), and genetic origin (resident versus migratory populations) on thyroid morphology.

Results:
Thyroid gland morphology in Asian bustards showed age-dependent changes, with growth occurring until four years of age followed by a tendency for reduction in older birds. Length, width and weight showed consistent developmental patterns. Males exhibited slightly longer thyroid glands than females, while no sex-related differences were detected for width or weight. No significant seasonal or genetic effect was detected. Histologically, the gland showed typical avian organisation, with follicles of variable size lined by simple epithelium (cuboidal predominant in younger, mixture of cuboidal and flattened cells in adults), containing eosinophilic colloid and supported by vascularised interfollicular connective tissue within a thin capsule.

Conclusion:
Age was the main factor influencing thyroid morphology in Asian bustards, with limited sex-related variation and no significant seasonal or genetic effect detected. These findings contribute to the understanding of endocrine adaptation in this species and provide baseline morphometric data relevant for future studies.

Hypovitaminosis a in captive arabian bustards (Ardeotis arabs)

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Topic: Wild life w/o fish

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Background:
Hypovitaminosis A is a nutritional disorder affecting captive birds, associated with epithelial metaplasia, immunosuppression, and increased mortality. This study investigated a mortality event in Arabian bustards and evaluated the effectiveness of corrective measures.

Materials & Methods:
Ten 1-month-old Arabian bustards died in short period of time after presenting similar clinical signs. Complete necropsies were performed, and tissues were examined histologically (HE and Congo red). In addition, microbiological and PCR tests were conducted to exclude infectious causes.

Results:
Based on clinical and necropsy findings, hypovitaminosis A was diagnosed. Clinical signs included oropharyngeal plaques, cloacal fibrosis with fecal impaction, occasional neurological and ocular signs. Gross lesions included thickened cloaca, congested kidneys, and variably pneumonia, hepatitis, or enteritis. A consistent finding was an enlarged, thickened bursa of Fabricius with necrotic content. Histology revealed squamous metaplasia, lymphoid depletion, and secondary bacterial infections. The remaining flock received vitamin A supplementation and was clinically monitored.

Conclusion:
Mortality was attributed in eight of ten cases to secondary bacterial infections associated with epithelial squamous metaplasia. While the likely cause of squamous metaplasia of epithelia and cutaneous hyperkeratosis is hypovitaminosis A, other etiologic agents are possible including exposure to certain endocrine disruptors. Vitamin A deficiency can increase the susceptibility to bacterial infections due to failure of vitamin A-dependent, non-specific immune defense mechanisms. This deficiency may have impaired immune defenses, predisposing to infection. Histopathology was in these cases essential for diagnosis. Vitamin A supplementation resulted in clinical improvement, highlighting the importance of adequate nutrition in captive bustards.

Eyelid Amelanotic Signet-Ring Melanoma with Hepatic and Splenic Metastasis in a White Bengal Tiger (Panthera tigris tigris): A Case Report

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Topic: Wild life w/o fish

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Background:
A 13-year-old male white Bengal tiger (Panthera tigris tigris) presented with a slow-growing eyelid mass that had been present for three months. Subsequently, the tiger developed abdominal distension, generalised jaundice, and died.

Materials & Methods:
Routine necropsy, histopathological examinations and immunohistochemistry for vimentin, Melan-A, PNL-2, and pancytokeratin were performed.

Results:
At necropsy, the right medial canthus contained a 1-cm-diameter, sessile, pale tan, firm mass with an irregular surface. Generalised jaundice and haemorrhagic peritoneal effusion were noted. The liver and spleen contained multifocal-to-coalescing, variably sized, firm, mottled tan-to-brown, umbilicated multinodular masses with central necrosis. Histologically, the eyelid mass was composed of mixed spindloid and polygonal neoplastic cells that frequently contained intracytoplasmic eosinophilic globules and eccentrically placed nuclei, resulting in a signet-ring appearance. Fontana–Masson staining revealed sparse melanin pigment. Immunohistochemical findings were consistent with melanoma, characterised by positive membranous and cytoplasmic immunolabelling for vimentin, Melan-A, and PNL-2, and negative immunolabelling for pancytokeratin. Strong immunopositivity for the same markers was also detected within the intracytoplasmic globules. Additionally, neoplastic cells in the liver and spleen exhibited similar histological and immunophenotypic features.

Conclusion:
A diagnosis of amelanotic melanoma with signet-ring cell morphology was established in this case, and the eyelid was considered the most likely primary site, with hepatic and splenic metastases. Visceral metastasis of amelanotic signet-ring melanoma has rarely been reported in animals. This case highlights the importance of immunohistochemistry in establishing a definitive diagnosis of amelanotic melanoma and differentiating it from carcinoma with signet-ring cell morphology.

Two Cases of Bacterial Lower Urinary Tract Infection in Non-Human Primates

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Topic: Wild life w/o fish

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Background:
Non-human primates (NHP) are essential for the development of new drug modalities and hence are regularly used as a toxicity species in preclinical toxicity studies for the safety assessment of new medicines. The understanding of spontaneous pathology in NHPs is therefore fundamental. As data on lower urinary tract diseases are comparatively rare, the authors describe herewith two cases of bacterial cystitis of varying etiology.

Materials & Methods:
Two non-study animals underwent macroscopic examination. Tissues were processed to slide and stained with HE.

Results:
Case 1: A 2-year-old, female common marmoset (*Callithrix jacchus*) was euthanized due to painful abdomen and bloody malodorous vaginal discharge; microscopical examination revealed severe, diffuse, transmural, ulcerative and necro-hemorrhagic cystitis with bacterial colonies; cytologically, erythrocytes, neutrophils, high numbers of bacteria, and triple phosphate crystals were noted in the urine; microbiological examination revealed high content of *Proteus mirabilis*

Case 2: A 6.5-year-old, male cynomolgus monkey (*Macaca fascicularis*) was sacrificed due to poor general condition and vomitus; marked, diffuse, transmural, ulcerative and necro-hemorrhagic cystitis and urethritis were diagnosed microscopically; microbiological examination detected high contents of coagulase-negative staphylococci and *Staphylococcus arlettae*

Conclusion:
The present cases illustrate that cystitis caused by *Proteus mirabilis* and *Staphylococcus spp.* in NHP are clinically severe and show equal morphology characterized by fulminant ulcerative and necro-hemorrhagic inflammation. Both pathogens are known to cause infections in human; hence, the zoonotic potential must be considered.

Pathological Findings in Zoo-housed European Hamsters (Cricetus cricetus)

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Topic: Wild life w/o fish

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Background:
The Zoo Leipzig established a conservation breeding program for the European hamster (*Cricetus cricetus*) in 2022 to support reintroduction efforts in Central Germany. Due to the species' critically endangered status, this study was undertaken to identify common pathological lesions in this species, with the goal of supporting effective population management.

Materials & Methods:
Postmortem reports of 53 European hamsters (32 females, 21 males) necropsied between 2022 and 2026 were retrospectively evaluated. Pathological findings were systematically categorized by lesion type and organ system, and analysed in relation to age and sex. The main findings, associated with either the cause of death or the reason for euthanasia, were assessed.

Results:
The most common lesion type was inflammatory (52%), followed by neoplastic (30%) and toxic-degenerative (18%). Frequent inflammatory changes included interstitial pneumonia, periportal hepatitis, and subcutaneous abscesses or suppurative osteomyelitis affecting the head region. The most common toxic-degenerative lesion was renal tubular epithelial degeneration and necrosis accompanied by intranuclear acid-fast inclusion bodies. Neoplasia was the most frequent cause of euthanasia or death (50%) and predominantly affected the haemolymphatic, musculoskeletal, and integumentary systems. The most common neoplasms were thymomas or thymic carcinomas (28%), followed by soft tissue sarcomas (23%) and squamous cell carcinomas (13%). Neoplasia occurrence was significantly associated with age group, with the highest prevalence observed in senile individuals.

Conclusion:
Our findings reveal that neoplastic disease represents a major cause of mortality in zoo-housed European hamsters, with soft tissue sarcomas and thymic tumors being the most frequent.

Pathological findings in three Jaguars (Panthera onca) in Costa Rica: glaucoma, parasitic Infection, and metastatic mammary carcinoma

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Topic: Wild life w/o fish

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Background:
Jaguars (*Panthera onca*) are apex predators and keystone species in neotropical ecosystems. Despite their ecological importance, mortality events in wild and captive jaguars are rarely documented due to their elusive nature and low population density. Understanding underlying pathological causes is critical for conservation and wildlife management.

Materials & Methods:
Between 2023 and 2024, three adult jaguars were submitted to the Pathology service at the National University in Costa Rica. Jaguar A and B were found dead along the Pan-American Highway near Santa Rosa National Park (free-ranging). Jaguar C was confiscated from a private collection after being born in the wild but kept in captivity. Full necropsies, histopathology, and parasitological analyses were performed.

Results:
Jaguar A exhibited severe bilateral ocular disease, including keratoconus and advanced glaucoma in the right eye with loss of intraocular structures. A haemothorax was also present. Jaguar B had a severe traumatic liver laceration and confirmed parasitosis by *Oncicola venezuelensis.* Jaguar C, a female, had a tubulopapillary mammary carcinoma with metastases to the lungs and pancreas.

Conclusion:
These cases highlight that jaguar mortality can result from a combination of traumatic, infectious, and neoplastic diseases. While human-wildlife conflict is a major threat, underlying pathological conditions may predispose individuals to vulnerability and human interaction. This underscores the need for integrated wildlife health surveillance and early detection of disease in both free-ranging and captive populations.

Histopathological and Ultrastructural Detection of Suspected Microplastic Particles in European Brown Bear Gastric Tissues

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Topic: Wild life w/o fish

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Background:
European brown bears (*Ursus arctos*) are omnivorous wildlife species with extensive environmental exposure, making them sentinels for microplastic contamination. This study evaluated a digestion-free histopathological approach for detection and localisation of suspected microplastic particles in formalin-fixed paraffin-embedded (FFPE) stomach tissues from brown bears in Croatia.

Materials & Methods:
Archived gastric FFPE tissues from 51 free-ranging brown bears of the Croatian Dinara population were examined histopathologically using HE staining. Samples were obtained during necropsy following hunting- or traffic-related mortality. Histopathologically, gastritis was recorded. Particle-like structures morphologically consistent with suspected microplastic material were identified by polarised light microscopy (PLM) based on birefringence and morphology. As birefringence is not specific for microplastics, particles were classified as suspected microplastics. Six representative positive samples underwent scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS).

Results:
Suspected microplastic particles were identified in 29/51 (56.9%) samples. Particles were mainly localised within the lumen and mucosa, occasionally in deeper gastric layers. Chronic gastritis, predominantly lymphoplasmacytic, was present in 23/29 (79.3%) positive samples. SEM demonstrated morphological features consistent with microplastic material in four of six examined samples, while two were negative. All SEM-positive samples showed concurrent gastritis.

Conclusion:
Combined histopathology, PLM and targeted SEM/EDS analysis enabled detection and localisation of suspected microplastic particles in wildlife FFPE tissues. Although PLM alone cannot definitively distinguish microplastics from other birefringent materials, SEM/EDS increased confidence in particle identification. Preservation of tissue architecture enabled assessment of particle localisation and future investigation of tissue-particle interactions. The significance of associated inflammatory changes remains to be clarified.

Pathological findings in nine Humboldt penguins (Spheniscus humboldti) in Chile.

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Topic: Wild life w/o fish

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Background:
This study included nine Humboldt penguins stranded alive or dead along the Chilean coast between 2024 and 2026. All animals were donated under official authorization from SERNAPESCA, Coquimbo, Chile.

Materials & Methods:
Recorded data included ID, stranding number, condition at stranding, body condition score, and geographic location. Necropsies were performed at LiCiVet, Universidad del Alba, La Serena, Chile. A complete gross examination was conducted, and representative tissue samples were fixed in 10% formalin. Histological sections were stained with H&E, PAS, and Masson's trichrome when necessary.

Results:
The main lesions identified involved multiple organ systems. Renal lesions were the most frequent findings and included degeneration of the collecting tubular epithelium, cystic dilation, and intralesional trematodes associated with variable egg burdens (8/9). One moribund penguin exhibited traumatic lesions consistent with a dog bite, affecting the wings and pectoral musculature. The integument was characterized by a heavy ectoparasite infestation (genus *Philopteroides*) associated with eosinophilic dermatitis (3/9). Parasitic structures were observed throughout the gastrointestinal tract and were microscopically associated with marked heterophilic and eosinophilic enteritis (5/9). Additional findings included severe multifocal myocarditis with concurrent inflammation and tissue repair in one penguin, and a chronic transmural penetrating gastric lesion in another. Gastrointestinal plastic debris was identified in one individual.

Conclusion:
Lesions were diverse, with trematode-associated nephropathy representing the most frequent finding. Dog bite injuries are concerning given the species' conservation status. Although necrotizing myocarditis suggested a possible viral etiology, PCR testing for Influenza was negative. Expanded diagnostic panels are needed to improve infectious disease detection in Chilean wildlife cases.

Comparative Histopathological Patterns of CNS Lesions in Dogs, Cats, Foxes and Hedgehogs: A Three-Year Retrospective Study

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Topic: Wild life w/o fish

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Background:
Central nervous system (CNS) lesions comprise diverse pathological processes. This study compared CNS lesions in the animal species most frequently represented in our diagnostic archive.

Materials & Methods:
Histopathological examination was performed on 131 CNS samples from 39 dogs, 32 cats, 25 foxes and 35 hedgehogs collected over a three-year period. Clinical neurological signs were recorded in 22/39 dogs, 16/32 cats, 5/25 foxes and 30/35 hedgehogs. Poorly differentiated canine CNS neoplasms were evaluated immunohistochemically using GFAP, Olig-2 and vimentin.

Results:
In dogs, encephalitis/meningoencephalitis (6/39) and neoplasms (meningiomas (4/39), astrocytomas (2/39) and one oligodendroglioma) were identified. GFAP expression was detected in astrocytomas, vimentin positivity in meningiomas and Olig-2 immunoreactivity in oligodendroglioma. Oedema, hyperaemia and gliosis were frequent accompanying changes. In cats, encephalitic (12/32) and neurodegenerative lesions (11/32) predominated, accompanied by gliosis and cerebral oedema. Occasional neoplasms included two meningiomas and lymphoma. Foxes commonly exhibited neuronal degeneration (20/25), satellitosis, Purkinje cell degeneration (14/25), inflammatory infiltrates (12/25) and demyelination (9/25), accompanied by diffuse gliosis. Eosinophilic intracytoplasmic and intranuclear inclusions were observed in neurons and astrocytes. In hedgehogs, demyelination (16/35), often associated with wobbling hedgehog syndrome, predominated. One hedgehog had metastatic osteosarcoma involving the CNS, whereas primary CNS neoplasms were not identified.

Conclusion:
Distinct species-related patterns of CNS pathology were identified. Dogs were more frequently affected by inflammatory and neoplastic lesions, cats by encephalitic and neurodegenerative changes, foxes by inclusion-associated neurodegenerative lesions, and hedgehogs by demyelinating disorders. These findings provide comparative baseline data and highlight species-specific interpretation of neuropathological lesions.

Histopathological findings consistent with fatal avian malaria in an African penguin (Spheniscus demersus) during a concurrent HPAI outbreak

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Topic: Wild life w/o fish

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Background:
Avian malaria caused by *Plasmodium spp*. is a major cause of sudden death in captive penguins, which are highly susceptible to hemosporidian infections. Fatal cases may resemble other infectious diseases, including highly pathogenic avian influenza (HPAI). This report describes histopathological findings in an African penguin (*Spheniscus demersus*) found dead during an HPAI outbreak in Poland.

Materials & Methods:
In February 2026, an adult African penguin from a Zoo in Łódż, Poland, was found dead. The bird was housed separately from HPAI-positive birds. Complete necropsy was performed. Major organs were fixed in formalin, routinely processed, paraffin embedded, sectioned, and stained with hematoxylin and eosin. Selected tissues were examined immunohistochemically for HPAI antigen.

Results:
Gross lesions included splenomegaly, pulmonary congestion, and focal extensive hepatic necrosis. Histologically, lungs showed severe diffuse congestion with multifocal interstitial mononuclear infiltrates and numerous enlarged cells containing protozoal structures morphologically consistent with intra- and exoerythrocytic meronts/schizonts of *Plasmodium spp*. The liver showed focal hepatocellular necrosis with inflammatory infiltrates and similar parasitic stages. Cardiac tissue exhibited mild myocarditis with fibrinous intravascular aggregates containing protozoal developmental stages. No significant brain lesions were observed. Immunohistochemistry for HPAI antigen was negative.

Conclusion:
Histopathological features were consistent with fatal acute avian malaria. This case demonstrates that avian malaria should remain an important differential diagnosis for sudden death in captive penguins even during winter months and in the setting of simultaneous HPAI outbreaks. Regular surveillance and anti-malaria protocols should be considered in susceptible zoo collections.

Lung lesions in raccoon dogs (Nyctereutes procyonoides) from Lower-Saxony, Germany

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Topic: Wild life w/o fish

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Background:
Raccoon dogs are an invasive alien species (IAS) in Germany and other European countries and may carry zoonotic and non-zoonotic pathogens. Since reports of morphological changes are rare, this presentation aims to provide a more detailed characterisation of spontaneous pulmonary changes in raccoon dogs.

Materials & Methods:
22 raccoon dogs from various regions in Lower Saxony, northwestern Germany, were legally hunted as part of a larger study to assess the health status of IAS. The animals were examined postmortem using a standard protocol, followed by histology, histochemistry and immunohistochemistry using antibodies against CD3, CD20, PAX5, IBA1, CD31 and factor VIII-related antigen to characterize inflammatory infiltrations. Inflammatory lesions were scored semiquantitavely based on severity and distribution to assign a score to the lung health. Copromicroscopical examinations and molecular analysis of lung tissue have been initiated for the identification of parasites.

Results:
Among the 22 animals examined, 15 exhibited eosinophilic and/or granulomatous pneumonia. Of these, 8 had additional intralesional parasitic structures in accordance with larval and adult metazoic parasites. Some individuals presented different vascular lesions including lympho-histiocytic vasculitis, intimal fibrosis and thrombosis. High score inflammatory changes were clearly associated with the presence of metazoic parasites.

Conclusion:
Raccoon dogs are commonly affected by pulmonary inflammations. Based on the inflammatory profile and the detection of pathogens, the lung changes are primarily caused by parasites. Identifying the parasites will provide insights into the pathogenesis of the lesions and allow for an assessment of the health risk to wildlife, livestock and humans.

TOPIC: FISH AND OTHER JELLY

Hector's dolphin bycatch in New Zealand: fishing vessel incident reporting and necropsy findings

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Topic: Fish and other jelly

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Background:
Bycatch is considered a major threat to the survival of New Zealand's endangered Hector's dolphins, but definitive diagnosis is challenging in beachcast animals. Until recently, New Zealand relied on fisher self-reporting to monitor bycatch; in 2023, compulsory on-board camera monitoring was introduced.

Materials & Methods:
Hector's dolphin capture and necropsy records from 2006–2025 were reviewed to quantify and characterise known bycatch before and after camera deployment. Gross and histological findings in confirmed bycatch cases (n=18) were compared with a control group of non-bycaught beachcast dolphins (n=14). Associations between lesions and bycatch were assessed using Fisher's exact test.

Results:
Mean reported bycatch was 2.27 dolphins/year before camera deployment and 7.7/year after. Recovery rate for necropsy was 35% (12/34) and 78% (18/23) respectively. Lesions observed exclusively in bycaught dolphins included fresh ingesta in the stomach; skin lacerations with a finely wavy contour; chyle within intestinal lymphatics; encircling skin impressions; perirenal/renal capsular gas bubbles; and regurgitation. Lesions significantly associated with, but not exclusive to, bycatch included good body condition, pulmonary rib impressions, and nicks to the lips or leading edge of flippers. A composite lesion score incorporating wavy cuts, encircling lesions, fresh ingesta, and regurgitation strongly discriminated bycatch from controls; 17/18 bycaught dolphins had at least two of these lesions. No reliable histological indicators were identified.

Conclusion:
Mandatory camera monitoring has substantially increased detection and necropsy of bycaught Hector's dolphins. The resulting dataset supports a robust gross pathological framework for diagnosing bycatch in beachcast Hector's dolphins.

TOPIC: EXOTIC: PET-REPTILES, AMPHIBIANS, DOMESTIC BIRDS

Diagnostic challenges associated with atypical brucella spp. In amphibians

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Topic: Exotic: Pet-reptiles, amphibians, domestic birds

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Background:
Atypical *Brucella* spp. have recently been identified and associated with disease in amphibians in zoological institutions. Our general hypothesis is that atypical *Brucella* is present in captive and wild amphibian populations and that histopathology combined with immunohistochemistry constitutes a reliable and rapid diagnostic modality in formalin fixed tissues, especially when fresh / frozen tissue is not available for bacterial culture or molecular diagnostics.

Materials & Methods:
Medical records and paraffin tissue blocks from amphibians meeting certain inclusion criteria were requested from four zoological or academic institutions. Commercially available polyclonal and monoclonal antibodies against *Brucella* were separately assessed for their respective sensitivities and specificities in immunohistochemistry against positive and negative control cases (n=5 animals; 2 species). The polyclonal antibodies were then used against suspect cases (n=24 animals; 5 species). Eventually, control cases from other animal classes were also assessed for comparison using both antibodies for immunohistochemistry.

Results:
The polyclonal antibody demonstrated 100% specificity and sensitivity against *Brucella* in the amphibian control cases. However, when assessing the suspect cases, a lack of specificity was found as the antibody would react with numerous species of Gram-negative bacteria. The monoclonal antibody demonstrated almost no significant binding in any positive control case (low sensitivity).

Conclusion:
Two commercially available antibodies against *Brucella* spp. lack specificity or sensitivity for atypical forms and cannot be recommended in amphibians. When developing immunohistochemical tools, validation of the modality is needed, testing the antibodies for cross reactions against other pathogens in spite of the success of any previously published reports.

Clinical and pathological characterization of Usutu virus experimental infection in domestic canaries (Serinus canaria) and homing pigeons (Columba livia domestica).

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Topic: Exotic: Pet-reptiles, amphibians, domestic birds

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Background:
Background: Usutu virus (USUV) is a zoonotic arbovirus with a broad range of avian hosts. The comparison of natural infection among avian species suggests that different species respond differently to USUV infection. This project aims to characterize the clinical and pathological aspects of USUV infection in canaries (*Serinus canaria*) and homing pigeons (*Columba livia domestica*) and to clarify the potential role of different species in the transmission cycle of the virus.

Materials & Methods:
Materials and Methods: Canaries (n=9) and homing pigeons (n=9) were challenged with USUV lineage Africa3. Birds were monitored daily and sampled in compliance to the approved study plan. For each species, three groups of three infected individuals were euthanized at 3, 6 and 14 days post inoculation (d.p.i) respectively, and controls at 14 d.p.i. Birds were necropsied, and tissues were sampled for histopathology, immunohistochemistry and RT-qPCR.

Results:
Results: Pigeons showed no signs of general illness and developed very low viremia. Some infected canaries (5/9) showed mild clinical signs, and one animal spontaneously died at 2 d.p.i. Canaries developed higher levels of viremia and viral RNA and antigen were detected in multiple tissues. Mononuclear cells in skin, lymphoid organs and within circulation were the main targets of the virus. Small amount of USUV RNA was detected in tissue of pigeons at 3 d.p.i.

Conclusion:
Conclusions: Infected canaries develop viremia with mild disease. Mononuclear cells in the skin and lymphoid organs are an early target of USUV infection and may contribute to hematogenous systemic viral spread. Pigeons are non-competent hosts for USUV.

TOPIC: EXPERIMENTAL PATHOLOGY

Improving relationships between pathologists and non-pathologist scientists working with animal models; a pilot survey.

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Topic: Experimental Pathology

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Background:
Collaboration with pathologists can improve the reliability and applicability of pre-clinical research by non-pathologist scientists. Pathologists anecdotally report misunderstandings and challenges from non-pathologist scientists which impede productive collaboration. This study aimed to collect survey data from pathologists regarding their experiences and attitudes towards conducting research with non-pathologist scientists.

Materials & Methods:
Participants, recruited through snowball sampling, completed an online survey comprising multiple choice and free text questions. Responses were analysed using inductive content analysis for free text, and descriptive statistics.

Results:
Sixty-nine self-identifying pathologists completed the survey. Participants worked in academic (52%), governmental (10%) and commercial (38%) organisations with a median of >10 years' experience. Participants collaborated with non-pathologist scientists through histological interpretation (25%), data analysis (25%), study design (21%), training (16%) and ethical or regulatory review (10%) with a median frequency of monthly or more often. Misinterpretation of histology (25%), and inappropriate study design (19%) were the commonest areas of misunderstanding reported. Education in biological variation (26%) and awareness of when to consult a pathologist (37%) were the factors deemed most valuable for non-pathologist scientists to know. Participants recommended that pathologists should begin collaboration before the study design phase. Open communication (52%) was reported as the most effective tool to bolster productive collaboration.

Conclusion:
This study identifies knowledge gaps amongst non-pathologist scientists regarding histological sampling, tissue assessment and inappropriate study design. Facilitating communication and involving pathologists early in studies are recommended to improve productive collaboration in biomedical research.

Pyogranulomatous pneumonia due to Aspergillus fumigatus caused termination of a preclinical vaccination study in Syrian hamsters

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Topic: Experimental Pathology

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Background:
Aspergillus fumigatus infections are very rare in Specific Pathogen Free (SPF) laboratory animals. This report describes an outbreak of aspergillosis in Syrian hamsters during a long-term Duration of Immunity study for a SARS-CoV-2 subunit vaccine candidate. The severity of the outbreak necessitated premature study termination.

Materials & Methods:
Sixty individually housed male and female Syrian hamsters (12 weeks of age) received either an adjuvanted vaccine (n=50) or a mock vaccination (n=10) on day (D)0 and D21, with a third vaccination planned for D189. Necropsies were conducted on animals that reached a humane endpoint (n=2 on D123 and D127), failed to recover from anaesthesia (n=2 on D105 and D135), for diagnostic purposes (n=6 on D143) or upon study termination (n=50 on D161).

Results:
Clinical signs emerged between D50 and D161, affecting both vaccinated (n=5) and mock-vaccinated (n=2) animals. Initial signs included weight loss and perioral crusts or erosions. Gross and histopathological evaluations identified severe pyogranulomatous pneumonia with invasive fungal hyphae in n=6 hamsters. Additionally, granulomatous hepatitis was observed in n=4 animals. *A. fumigatus* was cultured from pulmonary tissue, but not from liver tissue and oral swabs. No alternative etiology was identified for the perioral lesions and granulomatous hepatitis. Despite extensive environmental sampling, the source of this outbreak was not identified.

Conclusion:
These findings demonstrate that *A. fumigatus* can cause disease in SPF Syrian hamsters, manifesting as pyogranulomatous pneumonia. This outbreak highlights the need for vigilance regarding opportunistic fungal pathogens in long-term rodent studies, even in the absence of known immunosuppression.

Goodbye manual scoring? Digital quantification of SARS-CoV-2 immunohistochemical staining

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Topic: Experimental Pathology

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Background:
Immunohistochemistry (IHC) enables assessment of viral antigen load in tissues in experimental COVID-19 models, providing key insights into immunopathogenesis of the disease. Although conventional immunohistochemical manual scoring remains the gold standard, digital analysis is increasingly adopted for its enhanced objectivity, reduced variability, and quantitative capabilities.

Materials & Methods:
In this retrospective study of experimentally infected mice, we applied a semiautomated, deep learning–based digital pathology approach using a commercial platform to quantitatively assess viral antigen burden in lung (n=248) and brain (n=106) tissues. Performance was benchmarked against conventional manual scoring and correlated with established virological and histopathological parameters.

Results:
Digital IHC quantification demonstrated near-perfect concordance with manual scoring in both lung ($r = 0.97$, $p < 0.001$) and brain ($r = 0.99$, $p < 0.001$), underscoring its high accuracy and reproducibility. In both tissues, quantitative outputs showed positive and significant correlations with viral RNA levels, infectious titers, and histopathological injury across disease stages, with the strongest associations at peak infection (5-7 days post infection). Vaccination did not modify the strength or pattern of these relationships, indicating that digital IHC scoring reliably captures the interplay between antigen burden, tissue damage, and virological parameters across distinct immune states.

Conclusion:
This work demonstrates that digital IHC scoring is a robust method for quantifying SARS-CoV-2 infection, offering a more reproducible, more objective and quantitative methodology poised to become the new gold standard.

Intestinal immune cell heterostasis in cystic fibrosis: Evidence from the neonatal pig model

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Topic: Experimental Pathology

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Background:
Cystic fibrosis (CF) is a detrimental autosomal recessive genetic disorder in humans, characterized by insufficient mucus hydration in the airways and intestines. Although recent therapeutic advances have restored mucus hydration, chronic inflammation persists, contrary to expectations. Possible explanations include congenital immune cell heterostasis unrelated to mucus hydration.

Materials & Methods:
Sequential intestinal segments from prenatal and early postnatal CF piglets as well as wild-type (WT) controls (n=4-6 per genotype) were subjected to histopathology, immunohistochemistry (CD3; S100-A9) and microbiome profiling using qPCR and 16S rRNA amplicon sequencing.

Results:
In contrast to WT piglets, early postnatal CF piglets had significantly increased S100-A9+ but not CD3+ cell counts orally to the canonical meconium ileus. This was accompanied by ingesta accumulation and intestinal distension. Furthermore, despite an increase in overall bacterial density, we observed a decrease in species richness, diversity and altered composition of the microbiota. No such differences were observed prenatally or early postnatally in CF intestinal segments affected by meconium ileus and beyond.

Conclusion:
Our observations in early postnatal CF piglets can likely be explained as a sequela of dysbiosis due to ileus-associated congestion and reactive inflammation. Contrary to the proposed alternative mechanism, our data fail to provide evidence of congenital immune cell heterostasis in the intestines.

Bovine mammary gland ex-vivo precision-cut tissue slices support high pathogenicity avian influenza H5N1 replication

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Topic: Experimental Pathology

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Background:
Following the recent spillover of high pathogenicity avian influenza (HPAI) H5N1 into dairy cattle in the USA, virus replication was detected in mammary gland epithelium, with high levels of viral RNA found in milk. We describe the development of a bovine mammary explant model to support further influenza virus research into routes of infection and pathogenesis and provide a practical alternative to *in vivo* animal models.

Materials & Methods:
Mammary tissue was freshly harvested from lactating dairy cattle at a local abattoir. The effect of temperature on tissue viability during transport in three different types of media was assessed, then precision cut tissue slicing was performed on core biopsies of superficial alveolar tissue. The effects of differing culture media on tissue slice viability were investigated, and viability determined by histopathology for three days post infection. Once optimal conditions were established, mammary tissue explants were infected using known recent avian and mammalian HPAI H5N1 clade 2.3.4.4b isolates. Reverse transcriptase (RT) PCR on the supernatant and immunohistochemistry (IHC) on formalin-fixed tissue were performed to identify active virus replication.

Results:
HPAI H5N1 was detected on RT-PCR, and IHC labelling was observed following infection using both isolates. Morphology remains viable for up to three days. Lectin labelling for sialic acid receptors was similar to that observed in normal bovine mammary tissue.

Conclusion:
This bovine mammary tissue explant model is suitable to evaluate the ability of influenza viruses to infect mammary tissue without the reliance on animal models as part of our 3R strategy.

Neuroendocrine cells in the upper respiratory tract of hamsters (Mesocricetus auratus) and mice (Mus musculus): marker distribution and morphology

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Topic: Experimental Pathology

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Background:
Neuroendocrine cells (NECs) constitute a quantitatively small but functionally significant cell population within the respiratory mucosa of the upper airways. They are predominantly found as single cells and only form clusters in specific locations. NECs are involved in sensory pathways, protective reflex-mechanisms and immune- and cell-modulatory processes.

Materials & Methods:
Neuroendocrine marker expression (calcitonin gene-related peptide (CGRP), serotonin (5-HT), protein gene product 9.5 (PGP)) as well as cell distribution, density and morphology of neuroendocrine structures in the upper respiratory tract (nose, larynx and trachea) of 8 adult hamsters and mice were evaluated using immunohistochemistry and semiquantitative analysis.

Results:
Neuroendocrine cells can be detected in all regions of the respiratory epithelium. They occur predominantly as single cells, however, bud-like structures are found in the mucocutaneous region of the larynx. NECs in the nose and mucocutaneus larynx are characterized by the expression of PGP and 5-HT. In the laryngeal and tracheal respiratory epithelium, NECs frequently show additional expression of CGRP. The marker profile in hamsters and mice is comparable.

Conclusion:
NECs occur as single cells throughout all regions of the respiratory epithelium and form bud-like structures in the larynx. NECs can easily be identified by their expression of neuroendocrine markers like 5-HT and PGP but, in the larynx and trachea, show additional expression of CGRP, which suggests region specific functions and tasks like e.g. modulatory effects on neighboring cells and the immune system. The differences between the species examined here were minor, suggesting a preserved functional pattern in hamsters and mice.

Mouse model of canine hereditary gastrointestinal polyposis: generation and phenotypic analysis

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Topic: Experimental Pathology

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Background:
Hereditary gastrointestinal polyposis (HGIP) is an autosomal dominant disease identified in Jack Russell Terriers in Japan, caused by a germline *APC* variant at codons 154 and 155 (c.[462_463delinsTT]) and characterised by the development of gastrointestinal tumours. Based on shared genetic and phenotypic features, HGIP is considered the canine counterpart of familial adenomatous polyposis (FAP) in humans, characterised by numerous colorectal adenomas. However, compared with FAP patients, dogs with HGIP develop fewer tumours but show a markedly higher incidence of gastric tumours.

Materials & Methods:
We generated *Apc*$^{\Delta 155/+}$ mice harbouring the same *Apc* variant as dogs with HGIP using the CRISPR–Cas9 system. Phenotypes were analysed at 8–20 weeks of age and compared with those of *Apc*$^{Min/+}$ mice carrying a germline variant at codon 850.

Results:
Macroscopically, *Apc*$^{\Delta 155/+}$ mice developed more tumours in the stomach and proximal small intestine than in the colorectum. Tumour numbers in these regions increased significantly with age. Histopathologically, most tumours were adenomas, while a small number of adenocarcinomas were detected in the small intestine. Compared with *Apc*$^{Min/+}$ mice, *Apc*$^{\Delta 155/+}$ mice showed a higher incidence and multiplicity of gastric tumours, but fewer total gastrointestinal tumours at 20 weeks. In addition, tumour distribution within the small intestine differed between the models, with tumours predominantly occurring in the distal small intestine in *Apc*$^{Min/+}$ mice and in the proximal small intestine in *Apc*$^{\Delta 155/+}$ mice.

Conclusion:
The germline *Apc* variant in the 5’ region results in fewer gastrointestinal tumours and preferentially affects the stomach and proximal small intestine.

Reactive Astrogliosis Following Ischemic Stroke in Mice

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Topic: Experimental Pathology

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Background:
Ischaemic stroke is a leading cause of death worldwide.Over the last decades,several animal models have been developed,such as middle cerebral artery occlusion (MCAO).Neural stem cells (NSCs) represent a heterogeneous population capable of differentiating into different cells.The main goal of our study was to induce transient MCAO in mice,transplant NSCs,and analyse reactive astrogliosis.

Materials & Methods:
We used two mouse strains as follows:wild-type and transgenic Thy1 YFP-16,both maintained on the same genetic background.Adult wild-type male mice were used for surgical procedures,while females were used for the NSCs isolation,which were proliferated *in vitro* and transplanted into the mouse brain 24 hours after surgery.Animals were divided in two groups:MCAO and control animals.Brain tissues were harvested two,four and eight weeks after surgery and analysed by immunohistochemistry,Western blotting and quantitative qPCR.

Results:
Our data showed extensive cell death in stroke-affected animals,with significant differences compared to controls.Cell death was analysed using TUNEL ($p < 0.0001$) and Cleaved Caspase-3 staining ($p < 0.0001$).Moreover,MCAO animals showed significantly higher expression of reactive astrocytes in both host tissues and grafted NSCs ($p=0.0004$).Furthermore,GFAP-immunolabelled astrocytes and IBA1-immunolabelled microglial cells exhibited a significantly higher cell number ($p < 0.0001$);these cells were larger and displayed more complex branching compared to the control group.Importantly,obtained results were confirmed with Western blotting and qPCR.Statistical analyses were performed using PRISM (GraphPad Software Inc.) with Student's T test as well as well as one-way ANOVA followed by Bonferroni's multiple comparisons test.

Conclusion:
We suggest that stroke induces astrogliosis in both the host tissue and the graft,which may contribute to the protection of surviving neurons within and around the stroke core.

New insights into the pathogenesis of Usutu virus infection of WT 129/Sv mice in the first weeks of life

446
Topic: Experimental Pathology

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Background:
Usutu virus (USUV) is an emerging mosquito-borne *Orthoflavivirus* closely related to West Nile Virus. Both are currently co-circulating all over Europe, causing seasonal episodes of mass mortalities in birds and sporadic neurological diseases in mammals, including humans. The underlying host-pathogen interactions have not been described yet, and the growing number of *Orthoflavivirus* infections reported in Europe recently undoubtedly represents a serious threat for public health. As infants and newborn mammals seem more susceptible, we aimed to study the factors underlying the age-dependent evolution of the outcome.

Materials & Methods:
We first developed our own model based on the dramatically different phenotypes observed in 9 (susceptible) and 15-day-old (resistant) pups following footpad infection. We then characterized the intrinsic differences in cell populations and transcriptome and their respective response to the infection combining bulk and single-cell transcriptomics, proteomics assay and flow cytometry and microscopic imaging techniques.

Results:
Interestingly, major dissimilarities were observed in various pre-existing immune cell populations in the skin, lymph node and spleen, and in their transcriptomic and cytokinic response to the infection between 9 and 15-day-old mice. As resistant pups show increased numbers of gamma-delta T cells, younger animals seem to preferably develop a more myeloid-oriented response with prominent interferon and pro-inflammatory pathways.

Conclusion:
The precocity of the differences spotted between our models suggested a very early determination of the phenotype following the viral inoculation. We highlighted a significant role of resident skin cell types as well as age-specific immune landscape in the susceptibility to the infection.

Keratinocyte-Derived Decellularized Extracellular Matrix Enhances Fibroblast Migration and Dermal Remodeling Signaling

515
Topic: Experimental Pathology

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Background:
Keratinocyte-derived decellularized extracellular matrix (HaCaT_dECM) has emerged as a promising biomimetic scaffold for regenerative medicine because it preserves bioactive extracellular matrix components involved in tissue remodeling. This study evaluated the effects of HaCaT_dECM on fibroblast behavior and its potential to modulate a regenerative dermal microenvironment.

Materials & Methods:
HaCaT_dECM was prepared using a detergent-based decellularization and lyophilization protocol designed to preserve extracellular matrix integrity. Human dermal fibroblasts (HDFs) were treated with HaCaT_dECM formulations to evaluate cellular responses, including migration using a scratch wound assay and expression of dermal remodeling markers by quantitative real-time reverse transcription polymerase chain reaction.

Results:
HaCaT_dECM significantly accelerated fibroblast migration compared with untreated controls, reducing the remaining wound area by approximately 65% at 24h and achieving near-complete closure by 48h ($p<0.05$). In addition, HaCaT_dECM treatment significantly upregulated *TGFB2* (2.5-fold, $p<0.01$) and *COL3A1* (1.4-fold, $p<0.05$) expression compared with controls, indicating the activation of dermal remodeling pathways associated with extracellular matrix reconstruction and tissue repair.

Conclusion:
Keratinocyte-derived dECM promotes fibroblast-mediated repair and enhanced regenerative gene expression in vitro. These findings suggest that HaCaT_dECM may contribute to restoration of the dermal microenvironment and represents a promising biomaterial for dermal regeneration and veterinary regenerative medicine.

KilliPATH: Development of a whole-body geropathology study of the African Turquoise Killifish, an innovative model for studying aging

541
Topic: Experimental Pathology

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Background:
As human life expectancy increases, so does the prevalence of age-related diseases, creating a public health challenge. However, progress in geroscience is hindered by the long lifespans of conventional animal models. The African Turquoise Killifish (ATK), or *Nothobranchius furzeri,* has emerged as an innovative, short-lived vertebrate model with a lifespan of six months. It exhibits the hallmarks of aging of vertebrates during its rapid aging process. Despite its growing use in aging research, the ATK remains poorly characterized histologically. A key question remains: What are the causes of ATK death?

Materials & Methods:
A large-scale histopathological study was performed on a FFPE biobank of ATK across the lifespan to identify age-related lesions; an original 'whole body' single-slide approach enabled the integrated evaluation of 17 organs, mimicking a microscopic necropsy; slides were stained with HE, complementary stains, or subjected to immunohistochemistry to highlight key aging biomarkers; the study aimed to identify age-related lesions and to develop a comprehensive geropathology grading system to compare chronological age with geropathology score (biological tissue age)

Results:
This geropathological study identified major age-related lesions similar to those observed in other vertebrates, including degenerative cardiac valvulopathy, glomerulopathy, and renal hematopoietic hypoplastic changes. Immunohistochemical quantification of proliferation biomarkers demonstrated an age-related decline across major organs.

Conclusion:
The whole-body geropathological study provides a standardized, integrated assessment of age-related lesions in ATK and putative insights into death causes. The geropathology grading system is a crucial tool for evaluating the impact of therapeutic interventions on biological age.

Development and Characterization of a Protocol for Plasma Extracellular Vesicle Isolation in Cats

546
Topic: Experimental Pathology

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Background:
Circulating extracellular vesicles (EVs) are promising biomarkers for metabolic and neoplastic diseases in companion animals, including cats, but their plasma isolation remains challenging. A major limitation is the co-isolation of lipoproteins, such as very-low-density lipoproteins (VLDL), which overlap in size with small EVs. Moreover, the adaptation of human-derived protocols and pre-analytical variables in veterinary practice may affect EV yield and purity. This study aims to develop an efficient protocol for EV isolation in cats.

Materials & Methods:
Blood samples (3 ml) were collected from 15 cats in sodium-citrate tubes. Clinical and pre-analytical variables were standardized and recorded. Plasma quality was assessed for platelet contamination and haemolysis. EVs were isolated by size-exclusion chromatography (SEC) and ultracentrifugation (UC), and characterized by Western blot, transmission electron microscopy (TEM), and nanoparticle tracking analysis (NTA). EV lysis and subsequent NTA were performed on pooled samples to assess the presence of non-EV particles.

Results:
The developed protocol enabled successful isolation of feline plasma EVs, confirmed by Western blot and TEM after SEC and UC. NTA showed comparable particle concentrations between obese and non-obese cats, but particle size was significantly smaller in obese cats ($p<0.05$). Lysis analysis revealed an approximately 30% reduction in particle number across samples.

Conclusion:
This study establishes a useful approach for feline plasma EV isolation and characterization, supporting future development of EV-based biomarkers for diseases such as cancer and metabolic disorders. However, co-isolation of non-EV particles remains a limitation. Combining size-based and non-size-based isolation methods could further improve EV sample purity.

TOPIC: TEACHING/TRAINING

Defining Core Competencies for ECVP Diplomates: Results of a Delphi-Based Consensus Study

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Topic: Teaching/Training

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Background:
The European College of Veterinary Pathologists (ECVP) conducted a survey to define core competencies expected of entry-level diplomates. The objective was to develop a framework to guide training programs, inform examination content, and support continuing education initiatives.

Materials & Methods:
A working group including experts from academia, diagnostic practice, industry, and veterinary education developed an initial competency framework based on literature and international guidelines. The framework was refined using a three-round Delphi methodology involving all ECVP diplomates. In Round 1, participants rated the importance of proposed sub-competencies using a four-point scale. Consensus was defined as ≥80% agreement. Round 2 re-evaluated items not reaching consensus and incorporated participant feedback. Round 3 sought justification for persistent disagreement.

Results:
Five domains were identified: macroscopic, microscopic, molecular, and research and evidence-based pathology, and professional skills. Most competencies achieved consensus as important/very important. Macroscopic and molecular pathology reached agreement after minor wording refinements. In microscopic pathology, technical and quality assurance competencies generated lower agreement, reflecting variability in roles and responsibilities; however, these were retained with revised wording emphasizing supervisory and contributory roles. Research and evidence-based pathology competencies were strongly supported, with refinement of stakeholder involvement language. Professional skills showed the greatest variability, particularly regarding wellbeing, diversity and inclusion, reporting responsibilities, and prioritization in complex settings, and were recommended for inclusion after minor re-wording.

Conclusion:
This study provides a consensus-based competency framework to support harmonized training and assessment of ECVP diplomates as well as curriculum development, professional skills and responsibilities.

Does artificial intelligence influence pathologists' decisions? A study on the anchoring bias in computer-assisted mitotic figure assessment by veterinary pathologists

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Topic: Teaching/Training

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Background:
Reliable mitotic figure identification is essential for mitotic count assessment in histological tumour sections but shows substantial observer variability. Artificial intelligence (AI) has been shown to improve reproducibility, but AI-generated suggestions may bias pathologists' interpretation. This study evaluated whether veterinary pathologists shift their judgements toward displayed AI-attributed confidence scores when assessing mitotic figures in canine cutaneous mast cell tumours.

Materials & Methods:
Fourteen pathologists scored 500 H&E image patches, each containing a mitotic figure candidate, over three rounds on a 0–100 scale. Scores >50 indicated a mitotic figure and scores <50 indicated no mitotic figure. Two unaided rounds assessed intra-rater variability. In the final round, AI-attributed confidence scores were displayed with the images. These scores were not calculated by a real AI model but were based on each participant's previous ratings and systematically shifted by +/-15, +/-25, or +/-35 points.

Results:
The two unaided rounds showed moderate agreement (intraclass correlation coefficient [ICC] = 0.648, 95% confidence interval [CI] 0.634–0.661), and median absolute intra-observer variability was 10 points. In the AI-supported round, participants shifted their ratings significantly toward the displayed AI-attributed values, with larger discrepancies causing larger shifts in the same direction (beta = 0.118, 95% CI 0.101–0.136, $p < 0.001$). However, this did not increase binary classification switches beyond unaided variability (22.8% vs. 20.3%).

Conclusion:
AI-attributed confidence scores caused a statistically significant but small anchoring

effect on veterinary pathologists' assessments. However, the results indicate that the AI-assisted approach did not negatively impact the pathologists' ability to interpret mitotic figure morphologies.

Use of 3D-Printed Hearts to Teach Cardiac Pathology to Undergraduate Veterinary Students

454
Topic: Teaching/Training

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Background:
3D-printed models are increasingly used to teach anatomy and clinical skills to veterinary students. However, systemic pathology is often taught using 2D images or fixed organs. Therefore, the aim of this study was to assess whether 3D-printed hearts showing endocardiosis or endocarditis could help students to differentiate between these lesions.

Materials & Methods:
This study included two cohorts of second-year veterinary students. One cohort (n=161) was taught cardiovascular pathology using 2D images and one cohort (n=169) was taught with the same 2D images, as well as 3D models of normal canine heart anatomy, endocardiosis and endocarditis of the left atrioventricular (mitral) valve.
Subjective feedback was sought from students and teachers on the appearance and usefulness of the 3D models. All students were asked to identify endocarditis or endocardiosis in their end of semester examination and the results between the groups were compared.

Results:
Subjectively, students felt the models aided their understanding of the lesions associated with endocardiosis and endocarditis. Teachers felt that the models could be more realistic. Use of the 3D models did not improve examination results. In the cohort with no access to the 3D models, 126/161 (78%) students answered the question correctly. In the cohort with access to the models, 129/169 (76%) students answered correctly.

Conclusion:
While students appreciated the 3D models, they were not associated with an improved examination result.

What's impacting our everyday diagnostic duties? A survey-based data collection on the pathologists' perception of factors influencing the diagnostic confidence.

503
Topic: Teaching/Training

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Background:
Accurate diagnosis in pathology is essential, as it directly influences patient care and clinical decision-making. Diagnostic confidence (DC) may be affected by multiple factors, including patient, sample, and pathologist-related factors. This study investigates perceived factors contributing to diagnostic uncertainty in veterinary pathology diagnostic practice.

Materials & Methods:
An online voluntary questionnaire distributed through national and international veterinary pathology organizations targeted board-certified and non-board-certified pathologists and residents. Respondents reported demographics, including experience (<5, 5–15, >15 years), selected factors most often influencing diagnostic confidence, and rated impact from 0 (none) to 5 (highest). Overall, 272 participants completed the survey.

Results:
Among patient-related variables, incomplete clinical information (84.9%) and incomplete signalment (69.1%) were most frequent, with 50.4% of respondents assigning high impact scores (4–5). Among specimen-related factors, small tissue samples (83.8%) and non-representative samples (83.1%) ranked highest, the latter receiving the highest impact scores (54.4%). Limited experience with the species (73.2%) or organ system (61.8%) led pathologist-related factors in both frequency and impact. Unavailability of immunohistochemistry (70.6%) and discordance between clinical and pathological findings (62.1%) were most frequent among other factors. Professional category and experience significantly influenced perceived impact of some factors, with less experienced pathologists rating ancillary test unavailability and limited organ expertise as more impactful.

Conclusion:
These findings underscore the multifactorial nature of diagnostic uncertainty in veterinary pathology, highlighting the need to improve sample collection training, strengthen clinicopathological communication, expand pathologists' exposure to diverse species and organ systems, and ensure support, particularly for less experienced pathologists, to enhance diagnostic confidence and accuracy.

Diving into Keratoconjunctivitis sicca (KCS) pathogenesis: AI-Based 3D Reconstruction and Deep Learning Analysis of the Canine Third Eyelid Lacrimal Gland

519
Topic: Teaching/Training

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Background:
Keratoconjunctivitis Sicca (KCS) is a common canine ocular disorder whose histopathological diagnosis is primarily based on the presence of lymphoplasmacytic infiltrates in the third eyelid lacrimal gland (TELG). The aim of this study is to explore the potential of AI tools combined with histology to investigate normal and pathological TELG anatomy specifically addressing KCS diagnosis.

Materials & Methods:
Third eyelids of three healthy dogs and a KCS subject were formalin-fixed and routinely processed for histology. Serial sections were cut at 5 µm thickness and collected at 20 µm intervals obtaining approximately 400 slides each. After HE staining, whole slide images (WSIs) were acquired using a digital slide scanner. WSIs were processed using Voloom software for 3D reconstruction and Medical Image Manager (MIM) for deep learning analysis.

Results:
Through the 3D reconstruction of TELG, unknown details such as the presence of additional independent glandular lobules were revealed. Moreover, the multi-class segmentation mask generated in MIM enabled a clear-cut distinction between acini, stroma, and lymphoplasmacytic infiltrate within the glandular tissue.

Conclusion:
This is the first morphometric study of TELG to generate a 3D reconstruction from histological sections, providing novel insights into its microanatomy and structural organization. Building on this, the MIM-generated mask demonstrates excellent performance in identifying inflammatory patterns of KCS. In continuation of this study, the mask will be tested on additional KCS cases to assess sensitivity and specificity, while exploring associations between annotated features, such as inflammatory infiltrates and vascularization, and their relationship with clinical manifestations of KCS.

POSTER ABSTRACTS ESVCP-ECVCP

TOPIC: BIOCHEMISTRY/ENDOCRINOLOGY

Predicting the need for sildenafil therapy by measuring serum big endothelin-1 concentrations in dogs with post-capillary pulmonary hypertension

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Topic: Biochemistry/Endocrinology

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Background:
Sildenafil can often improve clinical signs in patients with pulmonary hypertension (PH), however, it must be used carefully in post-capillary PH patients. Non-invasive assessment method can predict the need for sildenafil treatment in post-capillary PH dogs is warranted. Endothelin-1 (ET-1) is a potential biomarker for canine PH; as such, the present study investigates the diagnostic accuracy of serum big ET-1 determinations to predict the need for sildenafil therapy in dogs with post-capillary PH.

Materials & Methods:
Thirty-six dogs were retrospectively assigned to one of three groups: pre-capillary PH, post-capillary PH needs sildenafil therapy (Post-PH NS), and post-capillary PH need not sildenafil therapy (Post-PH NNS).

Dogs with PH without left-sided cardiac enlargement was assigned to the pre-capillary PH group. Dogs with PH with double-sided cardiac enlargement were assigned to the Post-PH NNS group if they demonstrated improved clinical signs due to standard therapy without sildenafil, while dogs with clinical signs that did not improve through standard therapy and required sildenafil were grouped as Post-PH NS. Receiver-operating characteristic curve analyses were performed.

Results:
The big ET-1 values of the pre-capillary PH and Post-PH NS groups were significantly higher than those of the Post-PH NNS group. To distinguish between dogs with Post-PH NS and dogs with Post-PH NNS, a big ET-1 cut-off value of 16.2 pmol/L (areas under the curve: 0.97) provided the best sensitivity (92.3%) and specificity (91.7%).

Conclusion:
Serum big ET-1 can be a clinical biomarker to predict the need for sildenafil therapy in dogs with post-capillary PH.

Interpreting biochemical biomarkers in Croatian population of griffon vultures (Gyps fulvus): the importance of tissue-associated enzymes

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Topic: Biochemistry/Endocrinology

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Background:
Assessing the health status of griffon vultures is difficult due to non-specific clinical signs and the limited specificity of common biochemical biomarkers such as AST, ALT and ALP. Additional biomarkers, including glutamate dehydrogenase (GLDH) and creatine kinase (CK), may better identify the source of enzyme elevation. This study evaluated associations among these enzymes to improve interpretation of biochemical findings in griffon vultures.

Materials & Methods:
Blood samples from 45 griffon vultures (22 females, 23 males; 30 juveniles, 15 adults) admitted to the rehabilitation centre on the island of Cres (Croatia) between 2023 and 2025 were analysed. Enzyme activities of GLDH, AST, ALT, ALP and CK were measured. Spearman's rank correlation coefficient was used to assess associations between variables. Analyses were repeated after exclusion of one or two extreme values exceeding Q3 + 5 × IQR.

Results:
High variability and right-skewed distributions were observed across all parameters.Statistically significant positive correlations were found between GLDH and AST ($\rho = 0.87$), ALT ($\rho = 0.62$) and CK ($\rho = 0.93$), and between CK and AST ($\rho = 0.94$) and ALT ($\rho = 0.67$).After excluduing outliers, correlations involving GLDH disappeared, while CK remained significantly associated with AST ($\rho = 0.65$, $p < 0.001$) and ALT ($\rho = 0.35$, $p < 0.05$).

Conclusion:
These findings indicate that elevated AST and ALT are more likely linked to muscle damage than primary liver disease, while ALP showed no clear association with liver- or muscle-related enzymes.Inclusion of GLDH and CK improved interpretation of enzyme activity patterns, although GLDH associations were influenced by outliers.

Inflammatory Profile and Obesity in Dogs: Blood Concentrations of IL-6, IL-10 and TNF-alpha in 39 Overweight and 46 Obese Dogs

153
Topic: Biochemistry/Endocrinology

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Background:
Canine obesity is a chronic multifactorial disease associated with low-grade inflammation, but circulating cytokine profiles remain poorly characterised. This study aimed to determine whether overweight and obese dogs display distinct inflammatory and endocrine profiles.

Materials & Methods:
Blood from overweight (body condition score 6-7/9; n=39 ; group OW) and obese dogs (body condition score 8-9/9; n=46 ; group OB) initially sent by practitioners to LabOniris for insulin, leptin, prolactin, Insulin-like Growth Factor 1 (IGF1), Thyroid Stimulating Hormone (TSH) and Total Thyroxine (TT4) assays, were retrospectively included in the study. Interleukin-6 (IL-6), interleukin-10 (IL-10) and tumour necrosis factor-alpha (TNF-alpha) were measured using a canine specific Luminex multiplex assay. Leptin and prolactin were measured by enzyme-linked immunosorbent assay, IGF1 and insulin by radioimmunoassay, and TT4 and TSH by chemiluminescence. Non-parametric statistical tests were used, including Wilcoxon, Kruskal Wallis and Spearman correlation tests.

Results:
TNF-alpha was significantly higher in OB than in OW (1.57 [0.70-2.81] versus 1.08 [0.70-2.51] pg/mL; p=0.004). IL-6 and IL-10 did not differ significantly between groups. Insulin was higher in OB (34.25 [7.50-387.50] versus 19.50 [3.50-113.00] µIU/mL; p=0.006), whereas TSH was lower in OB (0.20 [0.03-1.30] ng/mL versus 0.30 [0.05-2.90]; p=0.021). TT4, leptin, prolactin and IGF1 did not differ significantly between the 2 groups. IL-6 and TNF-alpha were positively correlated (r=0.73; p<0.001).

Conclusion:
In this study, canine obesity was associated with moderate inflammatory and endocrine changes rather than a distinct global profile. TNF-alpha and insulin were the most discriminating markers between overweight and obese dogs.

"Daisy-like crystals" in urine sediment of a dog with urinary tract infection

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Topic: Biochemistry/Endocrinology

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Background:
Urine sediment examination is a routine part of urinary diagnostics. However, some crystal types remain poorly described, including the rare "daisy-like crystals".

Materials & Methods:
N.A.

Results:
This case reports a 4-month-old male mixed-breed dog that presented with dysuria. Imaging revealed a urethral obstruction that was repositioned into the bladder. Initial urinalysis showed alkaline urine (pH 9.0), low specific gravity (1.015), marked pyuria, and rod-shaped bacteria. *Proteus spp*. was isolated, and antimicrobial therapy was initiated. Ten days later, repeat urinalysis demonstrated persistent alkaline urine (pH 9.0) and abundant leukocytes, along with three distinct crystal types: struvite, needle-like crystals, and numerous "daisy-like crystals." One week later, following treatment, urine pH decreased (5.5), bacteriuria resolved, and "daisy-like crystals" were no longer observed. Subsequent increases in urine pH did not result in their reappearance. During the next follow-up examination, the urinary stone was detected, removed, and submitted for analysis. Fourier-transform infrared spectroscopy (FTIR) identified the urolith as 80% dahlite and 20% struvite. Recurrent *Proteus spp.* infection and struvite crystalluria were later detected, while "daisy-like crystals" did not reappear. Further monitoring was discontinued due to loss of follow-up.

Conclusion:
This case describes a rare and transient occurrence of "daisy-like crystals" in canine urine associated with bacterial infection and alkaline pH. The crystals' inconsistent appearance and unclear relationship to a dahlite-rich urolith highlight the need for further investigation into their origin and diagnostic significance.

Use of blood from cavernous sinus for evaluation of nutritional status in wild red deer (Cervus elaphus)

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Topic: Biochemistry/Endocrinology

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Background:
Nutritional status in wildlife is commonly assessed through body weight, kidney fat index (KFI), and bone marrow fat percentage (%BMF). However, these methods lack standardization and are influenced by sex and age. Haematobiochemical analyses are easier to standardize but require high-quality blood samples, difficult to obtain postmortem in the field. In culled red deer, blood collection from the cavernous sinus (CS) of the encephalic *dura mater* has recently been validated (1), yielding abundant, less hemolytic plasma than major vessels. Aim: to correlate CS plasma hematobiochemical results with conventional nutritional indicators in wild red deer.

Materials & Methods:
Plasma from culled red deer was collected within 6 hours from the CS, excluding hemolytic samples. The following parameters were analyzed using the automated spectrophotometer BTC3500vet, Biotecnica Instruments, and instrument-specific reagents for the following measurements: total protein, albumin, urea, creatinine, triglycerides, cholesterol, NEFA, βOH-butyrate, calcium, and phosphorus. Results were correlated with KFI and %BMF using a generalized linear model including age class, sex and julian date as covariates.

Results:
221 samples from 122 adults, 37 subadults, and 62 calves (95 males,126 females) were evaluated. Deer with poorer nutritional status according to KFI and %BMF showed lower creatinine and cholesterol, higher urea, NEFA, and βOH-butyrate concentrations, an increased urea/creatinine ratio, and a decreased triglycerides/NEFA ratio.

Conclusion:
Results suggested negative energy balance and support CS blood as a valuable and objective matrix for assessing metabolism and welfare in wild red deer.

Comparative Analysis of Cerebrospinal Fluid and Plasma Biomarkers (WBC, Proteins, LDH, Neurofilament Light Chain, CRP) Across Various Canine Neurological Diseases.

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Topic: Biochemistry/Endocrinology

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Background:
CSF analysis is essential for diagnosing canine neurological diseases but struggles to differentiate inflammatory causes. Recently, the neurofilament light chain (NfL), a highly conserved protein across species, biomarker of axonal degeneration, has shown promising results. Our aim was to identify the most relevant biomarkers currently available for routine practice.

Materials & Methods:
Eighty-nine dogs were retrospectively divided into seven groups: controls (epilepsy, n=20), infectious causes (n=7), meningoencephalitis of unknown origin (MUO, n=13), neurodegenerative diseases (n=18), polyneuritis/cranial neuritis (n=12), steroid-responsive meningitis-arteritis (SRMA, n=15), and ischaemic accident or stroke (n=4). Diagnostic work-up included CSF analysis and MRI. Most dogs had LDH measurements on fresh CSF, serum CRP, and NfL (triplicates, microfluidic immunoassay, Ella platform) on frozen CSF and plasma stored at -20°C (one freeze-thaw cycle).

Results:
All tests were successfully performed using standard CSF volumes. Dogs with MUO, SRMA, and infectious diseases showed the highest CSF cell counts; however, WBC count, cytology, and protein levels alone did not always allow these three groups to be distinguished. Median NfL concentrations were highest for MUO in CSF (24,951pg/mL) and lowest in both matrices for controls (16.55pg/mL plasma, 536pg/mL CSF) and SRMA (25.10pg/mL plasma, 708pg/mL CSF). NfL variations were consistent across CSF and plasma, though more pronounced in CSF. Due to sample size, LDH and CRP could not differentiate the groups.

Conclusion:
Measuring NfL levels in CSF or plasma provides valuable additional information to discriminate between infectious causes, MUO, and SRMA. Larger studies are required to confirm these findings and identify further statistical differences.

Longitudinal dynamics of pseudotype neutralising antibodies in feline infectious peritonitis caused by novel FCoV-23 during antiviral treatment

504

Topic: Biochemistry/Endocrinology

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Background:
Feline infectious peritonitis (FIP),historically considered an inevitable fatal disease, is now treatable with antivirals, which dramatically improved clinical outcomes and survival rates. A novel feline coronavirus strain, FCoV-23, has recently emerged and may elicit different neutralisation antibody titres (NAT) compared with classical FIP virus strains. This study assessed pseudotype-based virus neutralisation assay (PVNA) across FCoV-23 FIP at presentation and during treatment.

Materials & Methods:
Residual sera (n=87) from cats with FIP (n=84) and non-FIP sick controls (n=3) collected during the Cyprus FCoV-23 outbreak were analysed using a previously described PVNA against FCoV-23 (short and long-spike variants) and classical FIPV strains DF2 and 79-1146. Neutralisation titres were determined by serial dilution, defined as the reciprocal serum dilution reducing infectivity by 90%. Nonparametric analyses compared groups and paired time points.

Results:
Ten contaminated samples were excluded, leaving 77 evaluable samples. Neutralising activity was undetectable in 19/77 (24.7%; 6 pre-treatment, 13 during treatment) samples, possibly reflecting immune heterogeneity, sampling timing, or assay variability. No significant differences in NAT were observed between FIP forms (neurological, wet and dry) or controls, although neurological FIP showed a trend towards higher titres ($p = 0.08$). NAT frequently reached the upper detection limit, indicating a ceiling effect. Longitudinally, NAT remained largely stable during treatment up to 20 weeks.

Conclusion:
NAT appear limited as a standalone biomarker for FCoV-23 FIP diagnosis or monitoring in this preliminary cohort. However, trends towards higher titres in neurological FIP may become significant upon evaluation in an expanded cohort.

Pre- and Postpartum Serum Changes in IL-6, TNF-α, leptin, insulin, and Lipid Profiles in Holstein Cattle

563
Topic: Biochemistry/Endocrinology

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Background:
The transition period in dairy cows involves negative energy balance and coordinated immune-metabolic adaptation as lactation begins. Inflammatory cytokines and metabolic hormones regulate these processes and interact with lipid metabolism. We hypothesized that peripartum cytokine, hormone, and lipid profiles would change significantly after calving. This study evaluated serum IL-6, TNF-α, leptin, insulin, and lipid profile to examine their relationship in cows 2 weeks before and 2 weeks following parturition.

Materials & Methods:
Fifty clinically healthy Holstein cows (parity~2; BCS~3/5), free of infectious or metabolic disease, were enrolled. Blood samples were collected from the same animals 2 weeks before and again after calving (Khouzestan, Iran). Serum was separated, stored at −20°C, and underwent one freeze-thaw cycle before analysis. IL-6,TNF-α, leptin, and Insulin were measured using bovine-specific ELISA kits (R&D Systems,USA); triglycerides, cholesterol, HDL, and LDL were measured by enzymatic colorimetric assay. Pre/post differences were assessed by paired t-test in SPSS; associations among variables were assessed by Spearman's correlation. Significance was set at $P<0.05$.

Results:
Serum IL-6, TNF-α, leptin, and insulin were significantly higher before parturition than after ($P<0.05$), while triglycerides, cholesterol, and LDL levels increased remarkably postpartum ($P<0.05$). Data analysis revealed a strong positive correlation between leptin and insulin levels. Significant positive correlations were observed among IL-6, leptin, and insulin levels (Spearman's correlation, $P<0.05$).

Conclusion:
The lower postpartum concentration of IL-6 and TNF-α may reflect resolution of the acute inflammatory response associated with calving, as sampling was performed 2 weeks after parturition. These findings highlight interactions between inflammatory mediators and lipid metabolism during the transition period.

TOPIC: HEMATOLOGY

Clinical Pathology Standard Parameters in Nude Rats: De Novo Generation Of Reference Intervals

6
Topic: Hematology

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Background:

Long term pharmacology and safety evaluation of innovative human-derived cell therapy products require the use of immunocompromised animals to avoid immune-mediated destruction of injected human-derived cells. Athymic Nude (RH-FoxN1rnu) rats represent an interesting alternative to broadly used Nude mice, particularly when implantation surgery is required and animals of larger size are preferred. Clinical pathology data obtained from this rat strain remain sparse in the literature.
Materials & Methods:

We sampled 34 untreated Nude male (N=16) and female (N=18) rats and analyzed a standard panel of hematology (Sysmex XN-1000V), biochemistry (Pentra C400) and coagulation (STA-Satellite Max) parameters routinely used in toxicology studies. Blood smears were prepared and examined. Data were qualitatively reviewed and compared with data obtained from conventional rat strains.
Results:

Whilst most biochemistry and coagulation parameters were overall unchanged, in hematology, white blood cell differential counts strongly differed from those recorded in other rat strains. Alterations in blood smear examination data were present and matched with hematology analytical findings.
Conclusion:

Full clinical pathology profiles of nude rats including blood smear examination enabled to characterize the Nude rat strain and to produce first reference intervals. Specific hematology changes were observed and consistent with the T-cell deficient phenotype of this rat strain. Those results should be taken into consideration when interpreting study results.

Customized acceptance limits are needed to ensure successful quality control monitoring for the Sysmex XN-V hematology analyzer

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Topic: Hematology

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Background:
With decreasing availability of veterinary hematology reference analyzers, there is a growing interest for the multispecies Sysmex XN-V analyzer. The quality control (QC) monitoring relies initially on manufacturer acceptation limits (AL_m), after what AL may be automatically adapted (AL_a) by the software, or freely customized (AL_c) by the user.

Successful QC monitoring relies on a probability of error detection (Ped) >90%. Ped is impacted by 1)the number N of QC levels (L1/L2/L3), 2)sigma, and 3)the elected QC rule. N and sigma being fixed, the only remaining moving piece to ensure sufficient Ped is the choice of the QC rule, meaning the choice of AL.

Materials & Methods:
QCM results for 25 analytes on 3 levels were collected over 1 month and Ped for AL_m were calculated; if non-satisfactory, AL_c for suitable Ped were generated.

Results:
AL_m-Ped was ~0% for 11 analytes in L1, 11 analytes in L2, and 15 analytes in L3; AL_m-Ped was >90% for only 2 analytes in L1 and 1 analyte in L3.

Conclusion:
Even very high sigma does not compensate for inappropriately broad AL. The latter results in minimal Ped, because the performance plateaus beyond sigma=6. AL_m should not be used, AL_a should be verified, and ideally AL_c should be generated in each laboratory. In a general manner, when N=3, AL_c of mean+/-3.5CV% (rule 1-3.5s) is recommended for analytes with sigma >6; otherwise, the lower sigma <6, the narrower AL_c should be (meaning the more stringent the QC rule should be) to maintain sufficient Ped.

Targeted alpha therapy in acute myeloid leukemia: preclinical evaluation of Actinium-225-labeled anti-CD47 antibodies in NSG mouse model

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Topic: Hematology

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Background:
Acute myeloid leukemia (AML) remains one of the most aggressive hematological malignancies in humans, with poor prognosis despite current conventional therapies. This work aims to evaluate efficiency and safety of an innovative targeted alpha-radionuclide therapy approach, leveraging tumor-specific anti-CD47 radio-immunoconjugate to selectively deliver cytotoxic alpha-radiation to AML cells.

Materials & Methods:
Both male and female NSG mice (9–18 weeks, N=56) were engrafted with human MOLM-14 AML cell line via caudal vein injection of 20,000 cells. Mice were treated at day 6 post-engraftment with four modalities: NaCl control (N=22), isotype-labeled actinium-225 radiotherapy control (N=10), unconjugated anti-CD47 antibody control (N=5) and actinium-225 anti-CD47 radio-immunoconjugate (N=10). Animals were monitored by weight and hematological follow-up at days 14, 26, 40, and 50, as well as biochemical assessment (alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen) and histological analysis of liver, spleen, and kidneys using a semi-quantitative scoring system at sacrifice.

Results:
Actinium-225 anti-CD47 radio-immunoconjugate significantly improved survival (median survival, MS not reached with 8/10 mice still alive at day 110 post-engraftment) compared to all control groups (MS=21-29 days, p=0.0005-0.0121). Early hematological toxicity was observed from day 14, with persistent RBC decrease and transient platelet and WBC suppression. No hepatic toxicity was detected. Transient weight loss and elevated blood urea nitrogen in groups treated with radiolabeled antibodies was not associated with significant renal histological damage, suggesting acceptable nephrotoxicity at this stage.

Conclusion:
This study demonstrates that actinium-225-based targeted-alpha-radionuclide therapy against CD47 represents a promising efficient and tolerable therapeutic strategy for AML.

Performance Evaluation of the Sysmex XN-1000V in Alpacas: Preliminary Results for Leukocyte Total and Differential Cell Counts

286
Topic: Hematology

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Background:
The Sysmex XN-1000V automated haematology analyser provides total and 5-part differential leukocyte counts. This study assesses the analytical performance of the Sysmex XN-1000V for total and differential WBC counts in alpacas by comparing results with the ADVIA 2120 and manual differential cell counts.

Materials & Methods:
Specific gates in the WNR and WDF channels were designed based on 10 preliminary alpaca cases and applied prospectively. Imprecision was assessed for Sysmex XN-1000V, ADVIA 2120 and manual differential counting. Linearity, interference and stability were evaluated for Sysmex XN-1000V. Method comparison was performed side-by-side using samples from 69 alpacas measured in duplicate.

Results:
Sysmex XN-1000V demonstrated good intra-assay imprecision for WBC (1.5%), neutrophils (1.9%), lymphocytes (2.8%) and eosinophils (3.6%), with higher imprecision for monocytes (6.2%) and basophils (9.0%). Linearity was confirmed across the manufacturer's range. WBC and differential counts remained stable for 24h and 48h at 24°C and 4°C, respectively. WBC count was affected by marked hyperbilirubinaemia. WBC in ADVIA 2120 and Sysmex XN-1000V were highly correlated (r=0.996) with significant proportional bias. The correlation between manual and Sysmex XN-1000V differential counts was high for lymphocytes (r=0.893), moderate to high for neutrophils (r=0.723), moderate for eosinophils (r=0.536), and basophils (r=0.575), and poor for monocytes (r=0.357). Methods were not interchangeable within the inherent imprecision limits.

Conclusion:
Sysmex XN-1000V demonstrated acceptable analytical performance for alpaca leukocytes. Differences between methods may reflect species-specific gating limitations and the high imprecision associated with manual differential counts. Manual gating is warranted where leukocyte clouds are not clearly separated.

Association Between Vitamin B12, Folate, and Erythrocyte Indices in Cats: A Retrospective Study

456
Topic: Hematology

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Background:
Vitamin B12 and folate deficiencies are typically associated with alterations in erythrocyte morphology, particularly macrocytosis, in humans. In cats, the hematological impact of these deficiencies remains incompletely characterized, and the clinical relevance of erythrocyte indices by automatic analyzers is uncertain. This study aims to evaluate the association between serum vitamin B12 and folate concentrations and erythrocyte indices in cats.

Materials & Methods:
This retrospective study included 82 cats with concurrent measurements of serum vitamin B12, folate, and complete blood count (CBC) (ADVIA 2120i). Correlation analyses (Pearson and Spearman) were conducted between vitamin concentrations and erythrocyte indices (RBC,HGB, HCT, MCV, MCH, MCHC,RDW, reticulocytes). Cats were categorized as low or normal according to the reference intervals (vitamin B12: <198.47pmol/L and 198.47–983.49pmol/L; folate: <25.23nmol/L and 25.23–49.09nmol/L, respectively), and comparisons were performed using Mann–Whitney tests. Group analyses were exploratory.

Results:
No significant correlations were identified between vitamin B12 or folate concentrations and any erythrocyte index ($p > 0.05$), with consistently weak associations ($|r| < 0.20$). Group comparisons revealed no significant differences in erythrocyte indices between cats with low (n=27) and normal (n=55) vitamin B12 concentrations. For folate, a nominally significant mild decrease in MCV was observed in deficient cats (low, n=26; normal, n=56; $p = 0.045$), while all other parameters remained non-significant.

Conclusion:
In contrast to other species, vitamin B12 and folate deficiencies in cats do not appear to produce clinically significant alterations in erythrocyte indices, suggesting that any potential effects on erythropoiesis are too subtle to be detected by CBC. Consequently, direct measurement of vitamin concentrations remains essential.

Monitoring of Coumarin Intoxication in Dogs using the Vitamin K1 Epoxide/Vitamin K1 Ratio

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Topic: Hematology

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Background:
Liquid chromatography-tandem mass spectrometry (LC-MS/MS) accurately and precisely determines vitamin-K1 and vitamin-K1-epoxide. Elevated vitamin-K1-epoxide concentrations, along with a markedly increased vitamin-K1-epoxide/vitamin-K1-ratio (<0.5 physiological), may indicate increased coumarin activity. This case series shows how absolute values of vitamin-K1-epoxide, vitamin-K1 and their ratio change under vitamin-K substitution and how therapy can be monitored.

Materials & Methods:
Long-term monitoring over 12-13 weeks was performed in three patients (Patient 1: female,spayed,5y; Patient 2: male,1y; Patient 3: female,5y) with confirmed coumarin ingestion and corresponding coagulopathy. Vitamin-K dosing was recorded. Serum vitamin-K1-epoxide- and vitamin-K1-concentrations were determined using a LC-MS/MS-method.

Results:
Patient 1: Initially, vitamin-K1-epoxide [ng/ml], vitamin-K1 [ng/ml], and the ratio were 3.32/<0.5 (-). Under vitamin-K substitution, concentrations changed to 6340/330 (19.2); 8070/324 (24.9); 4030/292 (13.8); 1870/259 (7.2); 1520/283 (5.3); and 134/144 (0.9) at the six consecutive therapy check-ups. Thirteen weeks after the initial measurement, values were 3.8/28.0 (0.1).

Patient 2: Initially, vitamin-K1-epoxide [ng/ml], vitamin-K1 [ng/ml], and the ratio were 3.02/0.8 (3.77). During vitamin-K substitution, concentrations changed to 7400/405 (18.3); 6280/691 (9.1); 6610/624 (10.6); and 3912/512 (7.6). Twelve weeks after the initial measurement, values were 1480/1000 (1.5).

Patient 3: No initial measurement prior to vitamin-K substitution was available. During therapy, concentrations were 33000/8710 (3.8); 61/10.3 (5.9); 6.2/25.6 (0.24), and 1.57/6.3 (0.24). Twelve weeks after the initial measurement, values were 3.5/20.1 (0.2).

Conclusion:
The vitamin-K1-epoxide/vitamin-K1-ratio provides insight into the residual activity of coumarin within the vitamin-K-cycle. It appears promising concerning patient-specific therapy adjustment. Further studies are required to define its utility in dose adjustment and treatment duration.

Coagulation profile, platelet function and platelet aggregation in Thoroughbred racehorses affected with Exercise-induced pulmonary haemorrage

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Topic: Hematology

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Background:
Exercise-induced pulmonary haemorrhage (EIPH) is a frequent condition in Thoroughbred racehorses, characterized by pulmonary bleeding during intense execise. It is typically diagnosed via bronchoalveolar lavage (BAL), and its pathogenesis remains unclear. This study aimed to compare the hemostatic profiles of EIPH-affected Thoroughbreds with healthy horses.

Materials & Methods:
A single blood sampling was executed from 27 Thoroughbreds diagnosed with varying grades of EIPH. Blood samples from 29 clinically healthy EIPH-negative horses (control group) were sampled monthly during their first three months training period. Blood was collected by jugular venipuncture into sodium-citrate tubes. Assessments included coagulation profiles (prothrombin time-PT, partial thromboplastin time-PTT, and fibrinogen levels), platelet function via Platelet Function Analyzer (PFA-100®), and platelet aggregation using Light Transmission Aggregometry (LTA) stimulated by ADP and collagen. Data were analyzed using one-way ANOVA.

Results:
Horses with EIPH exhibited significantly higher PTT values compared to healthy horses (60,05±5,07s vs T90 56,73±5,12s) and lower fibrinogen concentrations (130,4±19,65mg/dL vs T90 166,9±31,66mg/dl) Furthermore, EIPH-affected horses showed significantly decreased platelet aggregation (36,51±22,96% vs T0 62,83±7,455%; T30 63,2±10,17%; T60 59,72±10,47%; T90 62,22±9,771%) and prolonged PFA-100 closure times (114,4±28,45s vs T30 75,67±23,1s), indicating reduced platelet reactivity. PT values showed no significant differences between groups.

Conclusion:
The results suggest a systemic hemostatic impairment in EIPH-affected horses, characterized by a tendency toward hypocoagulability and platelet hypo-reactivity. This support the hypothesis that a predisposition to hemorrhage—potentially due to immune-mediated mechanisms, genetic factors, or coagulation deficits—plays a role in EIPH etiology. Despite study limitations, these results indicate that systemic hemostatic dysfunction may be a predisposing factor for Thoroughbreds.

Haematological reference intervals of Lacaune sheep using the Sysmex XN-1000V analyser and evaluation of haematological variation across reproductive states

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Topic: Hematology

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Background:
The Sysmex XN-1000V haematology analyzer is increasingly adopted in veterinary clinical pathology; however, reference intervals (RIs) specific for sheep remain unavailable. This study aimed to establish haematological RIs for Lacaune sheep and to evaluate the influence of reproductive state (lactating, dry, pregnant) on haematological variables.

Materials & Methods:
A total of 150 clinically healthy Lacaune dairy sheep were enrolled from a farm in central Italy (November–December 2025) and stratified as lactating (n = 51), pregnant (n = 56), or dry (n = 48). Animals were recruited within routine national surveillance programs; health status was confirmed by physical examination, biochemistry, serology, and coprological analysis. Blood was collected by jugular venepuncture into K3-EDTA tubes and processed within 3 hours. Complete blood counts were performed on the Sysmex XN-1000V analyser, and blood smears reviewed by microscopy. Outliers were identified using Tukey's test and visual inspection. Group-specific RIs with 90% confidence intervals (CIs) were calculated using a non-parametric method; comparability across groups was assessed by CI overlap and predefined clinical equivalence limits.

Results:
Lactating ewes showed lower RBC, haematocrit, and haemoglobin limits compared with dry and pregnant groups, alongside a higher MCHC upper limit and lower RDW upper limit. MCH was comparable across all groups. Pregnant ewes exhibited a higher MCV upper limit and lower platelet lower limit. Dry ewes had significantly higher white blood cell upper limits than both other groups.

Conclusion:
Haematological variables varied substantially across reproductive states, supporting stratification of RIs by physiological status for accurate clinical interpretation.

Attention messages for band neutrophils using Sysmex XN-1000v, ProCyte Dx and Element HT5+ in samples from dogs and cats.

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Topic: Hematology

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Background:
Attention messages for band neutrophils (bands) can improve workflow efficiency in clinical hematology. Instruments attention messages, and their diagnostic performance must be evaluated in samples from sick animals. The aim of this study was to assess the performance of Sysmex XN-1000v (Sysmex), ProCyte Dx (ProCyte) and ElementHT5+ (HT5+) regarding capability to correctly identify band neutrophils in blood samples from dogs and cats in a clinical setting.

Materials & Methods:
Surplus EDTA-blood from dogs and cats examined at a companion animal hospital was analyzed with Sysmex, ProCyte, and HT5+ within 24 hours. A blinded 200-cell manual differential count performed by an ECVCP diplomate served as reference for band identification (present or not). Instrument-generated attention messages in samples with bands present and samples where no bands present were compared between instruments.

Results:
Sixty-seven samples from dogs and 40 samples from cats were included. In dogs Sysmex (sensitivity (sens) 89%, specificity (spec) 96%, positive predictive value (PPV) 93%, negative predictive value (NPV) 76%, estimated (est.) AUC 83%) and HT5+ (sens 87%, spec 96%, PPV 98%, NPV 78%, est. AUC 91%) showed best performance compared to ProCyte (sens 70%, spec 85%, PPV 82%, NPV 74%, est. AUC 78%),) while in cats HT5+ (sens 90%, spec 100%, PPV 100%, NPV 91%, est. AUC 95%) performed better than Sysmex (sens 70%, spec 95%, PPV 93%, NPV 76%, est. AUC 83%) and ProCyte (sens 70%, spec 85%, PPV 82%, NPV 74%, est. AUC 78%).

Conclusion:
In this study, HT5+ most reliably identified band neutrophils.

Co-infection with Babesia odocoilei and Mycoplasma ovis in captive reindeer (Rangifer tarandus)

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Topic: Hematology

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Background:
Babesia odocoilei is a tick-borne protozoan parasite causing hemolytic anemia in captive reindeer, often characterized by hemoglobinuria and jaundice. Hemotrophic mycoplasmas, such as *Mycoplasma ovis*, are opportunistic bacteria that parasitize erythrocytes, potentially causing severe disease in young or immunocompromised animals. This report describes the clinical and diagnostic findings of *B. odocoilei* and *M. ovis* in two reindeer.

Materials & Methods:
Two 2-year-old female reindeer were referred to The Ohio State University Farm Animal Service. Case 1 presented with hematuria and sternal recumbency; Case 2 presented for suspected hematuria. CBC by Advia 2120i Hematology Analyzer, and blood smears stained with Wright-Giemsa were evaluated. EDTA whole blood samples were sent to North Carolina State University for PCR panel to detect *Anaplasma, Babesia, Bartonella, Ehrlichia, Hemotropic Mycoplasma* and *Rickettsia*. Any positive amplicons were sequenced for species identification.

Results:
Case 1 showed mild anemia (40% RI: 43-59) and blood smear examination revealed numerous pyriform-shaped organisms consistent with *Babesia* spp and occasional small blue cocci structures suspicious for *Mycoplasma* spp. Case 2 demonstrated mild anemia (28% RI: 43-59), leukopenia (1.82 $x10^9$/L RI: 2.8-8.1), and marked thrombocytopenia (28 $x10^9$/L RI: 166-602). In case 1, PCR amplified DNA from *Babesia odocoilei* using a qPCR targeting the LSU4 mitochondrial gene, and *Mycoplasma ovis* using a qPCR targeting the 16S rRNA gene. In case 2, only *M. ovis* was detected.

Conclusion:
Blood smear examination and PCR are critical for reindeers presenting with hematuria. These findings highlight the importance of screening for multiple hemopathogens in cervids with hemolytic disease.

Does anemia influence liver enzyme activity in dogs and cats?

569
Topic: Hematology

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Background:
Hypoxia has been described as a possible cause of secondary liver injury. However, whether mild liver enzyme elevations during anemia should be automatically attributed to hypoxia remains unclear. This retrospective study evaluated this association in dogs and cats.

Materials & Methods:
Medical records of dogs and cats (01/2023-04/2026, n=114) with hematocrit below 20% (Sysmex XN), were reviewed. Animals receiving specific therapy or hemolytic samples (Abbott) were excluded. The final dataset for statistics (MedCalc, $p<0.05$) included 48 dogs and 41 cats. Animals were divided according to ALT activity (Abbott; ≤60 or >61 U/L), severity of anemia (Hct≤10% or >11%), and disease course (acute or chronic).

Results:
No significant differences were found between low and high ALT groups in dogs (n=28 vs. n=20) for hematocrit (both 16%, p=0.59) or hemoglobin (48 vs. 52 g/L, p=0.74). Similarly, cats (n=23 vs. n=18) showed no differences in hematocrit (14% vs. 15%, p=0.80) or hemoglobin (both 48 g/L, p=0.78). In dogs, ALT (69 vs. 42 U/L, p=0.82) and AST (both 44 U/L, p=0.74) did not differ between Hct≤10% (n=6) and Hct>10% (n=42); the same applied in cats (n=10 vs. 31, ALT 54 vs. 56 U/L, p=0.72; AST 31 vs. 54 U/L, p=0.23). Acute and chronic anemia differed only in dogs AST activity (52 vs. 38 U/L, p=0.03).

Conclusion:
Anemia alone does not appear to cause ALT or AST changes in dogs and cats. Regarding significant differences in disease course, the highest AST activities were observed in dogs with blood loss anemia, likely reflecting tissue damage.

Reference Intervals for platelet parameters and Immature Platelet Fraction in healthy dogs measured using the Sysmex XN-2000V

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Topic: Hematology

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Background:
Accurate reference intervals (RIs) for platelet parameters are essential for interpreting haematological findings in dogs, particularly in thrombocytopenic disorders and bone marrow response. The Sysmex XN-2000V is an automated veterinary haematology analyser able to determine platelet count using impedance (I), optical (O), and fluorescence (F) methods. The fluorescence channel also allows evaluation of the Immature Platelet Fraction (IPF), an emerging marker of thrombopoietic activity.

Objectives
The aims of this study were to establish RIs for platelet parameters and IPF in healthy dogs and to evaluate the correlation among the three platelet counting methods.

Materials & Methods:
150 CBCs with corresponding blood smears from clinically healthy dogs, excluding Cavalier King Charles Spaniels, were evaluated. Platelet counts were measured using impedance, optical, and fluorescence methods. Mean Platelet Volume (MPV), Plateletcrit (PCT) Platelet Large Cell Ratio (P-LCR%), IPF% and absolute IPF (IPF#) were also assessed. RIs based on percentile methods were determined for all parameters. Correlation analysis among PLT-I, PLT-O, and PLT-F was performed.

Results:
Proposed RIs were: PLT-I 124-469 $\times 10^3/\mu L$; PLT-O 167-467$\times 10^3/\mu L$; PLT-F 169-471 $\times 10^3/\mu L$; MPV 8.9-12.7 fL; PCT 0.15-0.48%; P-LCR 15.6-48.9%; IPF 0-6%; and IPF# 2-13 $\times 10^3/\mu L$. Correlation between PLT-F vs PLT-I, PLT-F vs PLT-O and PLT-I vs PLT-O ($r=0.9477$, $P<0.0001$; $r=0.8935$, $P<0.0001$ and $r=0.8452$, $P<0.0001$, respectively) was strong.

Conclusion:
Canine-specific RIs for platelet parameters and IPF may improve the diagnostic interpretation of platelet abnormalities and support the clinical application of advanced platelet analysis in veterinary haematology. A strong correlation among platelet counting methods further confirms their analytical reliability.

TOPIC: CYTOLOGY

Black particulate material in septic peritonitis secondary to intestinal lymphoma-associated perforation in a dog after activated charcoal administration.

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Topic: Cytology

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Background:
Activated charcoal is commonly administered in suspected intoxications. When displaced outside the gastrointestinal lumen or aspirated, activated charcoal may act as persistent particulate material, potentially contributing to chemical or foreign-body inflammation. This report describes the cytological and histopathological detection of black particulate material consistent with activated charcoal in the effusion of a dog with intestinal perforation secondary to lymphoma.

Materials & Methods:
A dog with acute abdominal pain and vomiting received activated charcoal 24 hours before referral for clinical deterioration. Diagnostic imaging identified an intestinal mass with suspected perforation. Abdominal effusion was submitted for cytology and bacterial culture. The intestinal mass was surgically resected and submitted for histopathology.

Results:
Cytology revealed marked neutrophilic inflammation with intracellular bacteria, consistent with septic polymicrobial peritonitis. Numerous black granular particles, morphologically consistent with activated charcoal, were observed in the background and within neutrophils and macrophages. Bacterial culture confirmed polymicrobial infection. Histopathology revealed a perforation secondary to intestinal lymphoma, with concurrent acute fibrinopurulent inflammation. Charcoal-like particulate material extended from the ulcerated luminal surface through a fistulous tract to the serosal surface, admixed with alimentary debris, necrosis, and inflammatory exudate. Postoperative anastomotic dehiscence occurred, and the patient was euthanised.

Conclusion:
This case describes unusual cytological and histopathological findings of black particulate material in septic peritonitis secondary to intestinal lymphoma-associated perforation. The cytological and histopathological distribution, together with the recent administration of activated charcoal, supports its peritoneal dissemination following intestinal perforation and suggests a possible role in the severity and persistence of the peritoneal inflammatory process.

Cytological and microbiological diagnosis of equine fungal synovitis caused by Cyberlindnera fabianii

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Topic: Cytology

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Background:
Equine fungal infectious synovitis is rare. *Cyberlindnera fabianii* is a yeast capable of multiazole resistance that is considered an emerging pathogen in human medicine. We describe a case of equine synovitis caused by *Cyberlindnera fabianii*, and the laboratory techniques used for diagnosis and monitoring the infection.

Materials & Methods:
A 9-year-old mare was presented with suspected chronic septic synovitis, previously treated with intra-articular lavage and local perfusion with antibiotics and anti-inflammatory drugs. Arthrocentesis with synovial fluid cytological examination and microbiological culture was performed for diagnosis and follow-up. Cytological analysis included total nucleated cell count (TNCC) measured with a ProCyte Dx analyzer, and microscopic examination of direct and concentrated preparations stained with modified May-Grünwald-Giemsa, Grocott methenamine silver, and periodic acid–Schiff stains. Isolated microorganisms were identified by MALDI-TOF mass spectrometry.

Results:
Initial analyses demonstrated fungal synovial inflammation (TNCC of 15,820 cells/ µL) due to *Cyberlindnera fabianii*. Susceptibility testing was not performed. Systemic and intra-articular antifungal treatment was initiated. Serial analysis showed a decrease in inflammatory cells, with intermittent negative cultures; however, cytology consistently revealed yeast persistence, with higher detection sensitivity using Grocott stains. Four months after diagnosis the symptoms worsened, with synovial fluid showing moderate fungal synovitis and the patient was euthanized.

Conclusion:
This case highlights the diagnostic value of the combination of microbiological culture with cytology, including special stains, to avoid dismissing fungal growth as contamination and to detect low numbers of microorganisms in treated patients. Furthermore, accurate yeast identification is crucial to detect azole resistant species associated with poor treatment response.

Laboratory analysis of canine and feline body fluids, comparison of conventional and simplified Light's criteria

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Topic: Cytology

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Background:
Various classification systems exist for body cavity effusions; recently, lactate dehydrogenase (LDH) measurement has been added to routine analysis as part of simplified Light's criteria (SLC). This study compared the use of SLC with traditional categorisation in a large cohort.

Materials & Methods:
A retrospective study analysed 1193 canine and feline pleural and peritoneal fluid samples submitted to PraxisLab Veterinary Laboratory between 2024 and 2025. Total protein (TP), LDH activity, and total nucleated cell count (TNCC) were measured. Fluids were classified using conventional criteria (TNCC cutoff 3 G/L, TP cutoff 25 g/L) and SLC (TP and LDH cutoff 176 U/L). Agreement between methods was assessed through cross-tabulation and Cohen's kappa.

Results:
Exudates were the most common category in all fluids except for canine peritoneal samples, where high-protein transudates were equal. Classification differed in 16% of canine peritoneal samples ($\kappa=0.76$, $p<0.001$), 18% of canine pleural samples ($\kappa=0.57$, $p<0.001$), 47% of feline peritoneal samples ($\kappa=0.26$, $p<0.001$), and 29% of feline pleural samples ($\kappa=0.40$, $p<0.001$).

Conclusion:
Agreement between categorisations was good in canine peritoneal fluids, moderate in pleural fluids of both species, and only fair in feline peritoneal fluids. This difference may relate to feline infectious peritonitis (FIP) effusions, which are characterised by low cellularity but high LDH activity. Although SLC was originally developed for pleural fluids, its application to peritoneal effusions may yield diagnostic benefits. Incorporating the results of other diagnostic tests (FIP PCR, creatinine, cholesterol, and triglycerides) would clarify the actual clinical diagnostic utility of SLC.

From Cytospin to Cell Block: Diagnostic Utility of Nano NextGen Cell Blocking Compared with Shandon Cytoblock in Veterinary Effusion Cytopathology

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Topic: Cytology

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Background:
Cytological evaluation of effusions is important in the differential diagnosis of inflammatory and neoplastic lesions. Cytospin preparations support cytomorphological assessment, whereas cell block (CB) techniques enable histomorphological evaluation and ancillary testing. This study evaluated the diagnostic utility of Cytospin preparations and two CB techniques in feline and canine effusions.

Materials & Methods:
This retrospective study included 42 effusion samples (32 feline, 10 canine). Cytological preparations were obtained using a Shandon Cytospin 4 cytocentrifuge, whereas CB preparations were generated using the Shandon Cytoblock and Nano NextGen Cell Blocking kits (AV BioInnovation). Smears and CB sections were evaluated using routine cytological, histochemical, and immunohistochemical methods. Findings were interpreted with clinicopathological data.

Results:
Effusions were classified as neutrophilic/suppurative, pyogranulomatous, hemorrhagic, lymphocytic, and neoplastic. Diagnostic CBs were obtained only from samples yielding a visible cellular pellet after centrifugation. These sections showed a microbiopsy-like appearance and allowed serial sectioning for ancillary studies. Samples yielding diagnostic CBs generally had higher cellularity (mean: 284.4 cells/field); however, cellularity alone did not predict successful CB preparation. Samples lacking a visible pellet failed to yield diagnostic CBs, whereas Cytospin preparations provided sufficient cellularity for diagnosis. No significant differences were observed between the Nano NextGen Cell Blocking and Shandon Cytoblock methods.

Conclusion:
CB utility was associated primarily with successful cellular pellet formation and adequate diagnostic material rather than cellularity alone. While CBs were advantageous for ancillary testing, Cytospin preparations remained useful in samples with limited cellular material or poor sedimentation. Both CB methods showed comparable performance and may be considered complementary approaches in veterinary effusion cytopathology.

PARR as a unique diagnostic test in a case of erythrophagocytic low-grade splenic T-cell lymphoma in a cat

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Topic: Cytology

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Background:
While lymphoma is common in cats, the hepatosplenic form is rare. This case report describes a unique case of low-grade splenic erythrophagocytic T-cell lymphoma with peripheral blood involvement in a cat.

Materials & Methods:
A six-year-old neutered male cat was referred to the Veterinary Teaching Hospital of Perugia for severe chronic anaemia. When referred a complete blood count, biochemical analysis, abdominal ultrasonography, splenic and bone marrow cytology, splenic and blood flow cytometry, biomolecular test for Mycoplasma spp., Feline immunodeficiency virus (FIV), Feline Leukemia Virus (FeLV) and Feline Coronavirus, liver and spleen histology, splenic immunohistochemistry and PCR for antigen receptor rearrangements (PARR) were performed.

Results:
The patient presented severe non-regenerative anaemia (RBC 1.65 x10^6µL; Hb 4.0 g/dL; Hct 12.5%; MCV 75.8 fL; MCHC 32 g/dL), hyperbilirubinaemia, and splenomegaly. Erythrophagocytic mononucleated cells were seen at blood smear evaluation. Splenic cytology showed round-cells proliferation, exhibiting erythrophagocytosis and extramedullary erythropoiesis. Despite further diagnostic tests (biomolecular tests for infectious diseases, histology, immunohistochemistry and flow cytometry), a definitive diagnosis could not be achieved due to the presence of a polymorphic splenic infiltrate. PARR analysis identified a monoclonal T-cell population within the reactive background. A diagnosis of low-grade splenic T-cell lymphoma was made and the patient was treated with chlorambucil. The anaemia then progressively resolved and the cat ist still alive.

Conclusion:
To our knowledge, this is the first case of feline low grade erythrophagocytic splenic T-cell lymphoma. Furthermore, this case highlights the critical role of PARR in distinguishing low-grade lymphomas from reactive processes where conventional diagnostic tools may be inconclusive.

Cell block preparations from effusions and fine needle aspirates in canine mesothelioma: a pilot study

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Topic: Cytology

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Background:
Canine mesothelioma is diagnostically challenging due to overlap with reactive mesothelial hyperplasia and metastatic carcinoma. Cell block preparations enable ancillary testing on cytological specimens, but their quality remains unassessed in this tumour. Altered glycosylation is documented in mesotheliomas, with conserved N-linked glycosylation sites on canine mesothelin, yet lectin binding is unreported in this species. This pilot study evaluated CB adequacy, immunohistochemical findings and exploratory lectin binding in confirmed canine mesothelioma.

Materials & Methods:
CBs from canine mesothelioma cases were assessed, including body cavity effusions and fine needle aspirates of associated lesions, with diagnosis confirmed by tissue histopathology. CBs were processed by sequential ethanol-formalin fixation following centrifugation, paraffin embedding and HE staining. Quality was assessed using the Cytoblock Evaluation Score (CES, internal protocol). IHC for vimentin and multi-cytokeratin was performed on CB sections and tissue biopsies, and calretinin on CB sections. Lectin binding was assessed by confocal fluorescence microscopy on CB and tissue sections.

Results:
CES values were generally higher in FNA than in effusion preparations. Sections showed cohesive mesothelial clusters with vesicular nuclei, prominent nucleoli and peripheral cytoplasmic vacuolisation, consistent with epithelioid mesothelioma. Vimentin and multi-cytokeratin were positive in CB and tissue sections; calretinin was positive on CB sections. Variable lectin binding was demonstrated by confocal fluorescence on CB and tissue sections; without inhibition controls and comparison groups, these findings are preliminary.

Conclusion:
Cell block preparation yielded diagnostically adequate material from effusions and FNAs, supporting immunohistochemical characterisation, including calretinin, on cytological material. Lectin binding requires confirmation with inhibition controls and comparison groups before diagnostic interpretation.

Systemic metastatic calcification in a feline case with plasma cell neoplasia

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Topic: ESVP/ECVP Abstract

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Background:
Plasma cell neoplasia is a rare malignancy in cats. In human medicine, monoclonal hyperglobulinaemia-associated nephropathy can cause metastatic calcification. Here, we describe a previously unrecognised systemic calcification associated with feline plasma-cell neoplasia.

Materials & Methods:
The case was a 10-year-old neutered male domestic cat that presented with hyperglobulinaemia and severe renal failure (Day 1).

Results:
Splenic FNA revealed plasma-cell neoplasia (Day 1). A monoclonal peak was detected in the serum γ-fraction, and Bence-Jones proteinuria was positive. Hyperazotemia (elevated BUN/creatinine), hyperphosphatemia, and normocalcemia were also identified, and the calcium × phosphate product was 170.56 mg^2/dL^2. A bleeding tendency was observed on Day 2. Treatment with bortezomib, a proteasome inhibitor, resulted in a partial response. During the treatment course, calcification of the thoracic aortic arch was diagnosed using X-ray and CT scans; however, no abnormalities in cardiac function or blood pressure were observed. The patient died on Day 69. An autopsy performed on Day 72 revealed multiple myeloma, parathyroid hyperplasia, and systemic calcification, including in the gastric mucosa, lung, kidney, and vascular wall. Based on these findings, systemic metastatic calcification by multiple myeloma was suspected.

Conclusion:
The presence of Bence-Jones protein in the urine could be considered one of the factors that contributed to the worsening renal failure, and the life-limiting lesion in this case appeared to be secondary systemic calcification rather than a tumour. These novel insights from this case may help in recognising mineral abnormalities in cats with plasma cell neoplasia.

TOPIC: LAB QUALITY MANAGEMENT

Can the viscoelastographic test VCM®VET using citrate stabilized blood, correctly classify canine patients with regards to coagulability and fibrinolysis state?

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Topic: Lab Quality Management

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Background:
The Viscoelastic coagulation monitor (VCM®VET) has been validated for use in dogs and requires immediate analysis of native whole blood. Using citrate stabilized blood can give the clinician a longer time frame before VCM®VET analysis. The aim of this study was to evaluate VCM®VET using citrate whole blood and compare it to thromboelastography (TEG).

Materials & Methods:
A prospective study (January-May, 2025) was conducted at the Veterinary Diagnostic laboratory, University of Copenhagen, Denmark. Citrate-stabilized leftover whole blood samples submitted for TEG analyses from dogs were simultaneously analysed using VCM®VET. Coagulability was categorized as hypo-, normo-, or hypercoagulable based on TEG maximum amplitude (MA) and VCM maximum clot firmness (MCF) relative to reference values. Fibrinolysis status was categorized as normo- or hyperfibrinolytic using TEG lysis at 30 minutes (LY30) and VCM®VET lysis index at 30 minutes (LI30). Coagulability and fibrinolysis categories were analysed by Chi^2 and Fisher´s exact test, respectively.

Results:
25 paired samples from 20 dogs were included. TEG classified 4, 12 and 9 traces as hypo, normo- and hypercoagulable while VCM®VET classified 8, 10, and 7 traces, accordingly. For fibrinolysis, TEG identified 24 normofibrinolytic and 1 hyperfibrinolytic trace, whereas VCM®VET identified 20 and 5 traces, respectively. No statistical differences were detected between instruments for coagulability ($p=0.41$) or fibrinolysis ($p=0.19$).

Conclusion:
VCM®VET classified a higher proportion of samples as hypocoagulable or hyperfibrinolytic, overall agreement with TEG was observed. These initial data support using VCM®VET with citrate-stabilized canine blood. More data is needed particularly regarding fibrinolysis.

Analytical and clinical validation of the ProCyte Dx Haematology Analyser for body cavity fluid analysis

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Topic: Lab Quality Management

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Background:
The ProCyte Dx™ Haematology analyser is validated for hematologic testing across animal species, but its performance for body cavity fluid analysis was not fully described. This study aimed to analytically and clinically validate the analyser for total nucleated cell count (TNC) and red blood cell (RBC) count in canine, feline, and equine effusions.

Materials & Methods:
Abdominal, pleural, and pericardial effusions from dogs, cats, and horses submitted for routine diagnostics were included, representing a broad range of cellularity and classifications. Analytical validation included precision, recovery, reportable range/linearity, and method comparison. Precision for TNC was assessed using 10 intra-assay replicates at three decision levels per species, including a viscous feline effusion; RBC precision was evaluated at one level (canine). Method comparison was performed comparing two ProCyte Dx to a Sysmex XN ™ . Clinical validation assessed transudate versus exudate classification (TNC cutoff 5,000 cells/µL).

Results:
Performance met predefined criteria (CV ≤10% TNC, ≤5% RBC; bias ≤10% TNC, ≤5% RBC; total error ≤30% TNC, ≤15% RBC). Precision, recovery, and linearity were acceptable across species and fluid types. Method comparison showed minimal bias for TNC (−4.5%, n=96) and RBC (0.02x10^6/µl, n=66). The analyzer produced accurate results in viscous and chylous effusions. Agreement for transudate/exudate classification was 96.9% (κ=0.92).

Conclusion:
Unlike previous studies focused only on the method-comparison, this work provides an analytical and clinical validation of the ProCyte Dx for body cavity fluid analysis, demonstrating high accuracy and precision for TNCC and RBC counts and supporting its use in routine effusion evaluation.