2025 Accepted Abstracts – Platform and Poster Presentations

Clinical Pathology Focused Scientific Session I

Chairs: Samantha Evans, DACVP (CP); Lisa Schlein, DACVP (CP)

URINARY MIR-126 AS A NON-INVASIVE MARKER FOR CANINE IMMUNE COMPLEX-MEDIATED GLOMERULONEPHRITIS

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Background: Immune complex-mediated glomerulonephritis (ICGN) accounts for nearly 50% of biopsy-confirmed glomerular diseases in dogs. Renal biopsy is necessary to distinguish ICGN from other glomerular diseases, as immunosuppressive therapy is specifically recommended for ICGN. For dogs that are not suitable candidates for biopsy, urinary microRNA-126 (miR-126) may serve as a non-invasive biomarker, as suggested by our preliminary data.

Objective: To validate urinary miR-126 as a non-invasive marker for ICGN in 179 dogs.

Methods: Urinary miRNA was extracted from 1 mL of supernatant, and miR-126 expression was quantified by qRT-PCR and normalized to miR-151 and miR-28. Statistical significance ($p < 0.05$) was assessed using one-way ANOVA with Tukey-Kramer post hoc tests. Samples included dogs with renal (n=105), non-renal (n=40), and lower urinary tract diseases (n=24), and 10 healthy controls.

Results: Urinary miR-126 was significantly higher in dogs with ICGN (n=29) compared to healthy controls (p=0.01), dogs with renal disease [amyloidosis (n=13, p=0.004), glomerular basement membrane abnormalities (n=10, p=0.03), glomerulosclerosis (n=15, p=0.005), maldevelopment (n=15, p=0.04), and other (n=13, p=0.02)], dogs with non-renal diseases [gastrointestinal (n=8, p=0.02), liver (n=8, p=0.02), inflammatory (n=8, p=0.03), neoplastic (n=8, p=0.02)], and lower urinary tract diseases [bacteriuria (n=8, p=0.03) and pyuria (n=8, p=0.02)]. No significant differences were observed in the podocytopathy (n=10), metabolic disease (n=8), and hematuria (n=8) categories compared to ICGN.

Conclusions: These findings support urinary miR-126 as a promising non-invasive biomarker for canine ICGN, offering potential clinical utility in guiding immunosuppressive therapy when renal biopsy is not feasible.

ASSESSMENT OF PROTEINURIA DETECTION IN DOGS BY VISUAL AND AUTOMATED DIPSTICK ANALYSIS AND SULFOSALICYLIC ACID TEST COMPARED WITH THE URINE PROTEIN:URINE CREATININE RATIO

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Background: Discordant canine urine protein results between visual dipstick analysis and the sulfosalicylic acid (SSA) test occur. The Urisys 1100® (Roche, Laval, Quebec, Canada) is designed for automated dipstick analysis for human urine samples.

Objective: To determine the prevalence of discordant protein results between visual dipstick analysis and the SSA test, and to evaluate the reliability of visual dipstick analysis, the SSA test and Urisys 1100® in quantifying protein in canine urine.

Methods: Canine urine samples submitted to a diagnostic laboratory without marked pigmenturia were analyzed using visual dipsticks, the SSA test and Urisys 1100®. The agreement and correlation of each method with the urine protein:urine creatinine (UPC) ratio was determined using linear regression, Cohen's weighted kappa and Spearman rank correlation.

Results: Twenty-four percent of samples had a visual dipstick protein result > 0 and a SSA test result of 0 g/L. Goodness of fit (R^2) with $\log_{10}$UPC was strong for visual dipstick analysis and Urisys 1100®, and moderate for the SSA test. Agreement with UPC ratio based on Cohen's weighted kappa was weak to moderate for visual dipstick analysis, weak for Urisys 1100®, and very weak for the SSA test. Spearman rank correlation with UPC ratio was very strong for the SSA test, and strong to very strong for visual and automated dipstick analysis.

Conclusion: Visual dipstick analysis had the closest agreement with UPC ratio. Urisys 1100® was not superior in quantifying protein in canine urine samples over visual dipstick analysis.

PROTEIN BIOMARKERS IN URINE AND SERUM FOR EARLY DIAGNOSIS OF CANINE HIP DYSPLASIA

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Background: Canine hip dysplasia (CHD) is one of the most commonly diagnosed orthopedic disorders in dogs. According to the Orthopedic Foundation for Animals (OFA) database, the prevalence of CHD can be as high as 70% in certain breeds. Dysplastic dogs are often diagnosed in the irreversible phase (i.e., presence of osteoarthritis), which limits treatment options. Proteins in bodily fluids have been used as biomarkers for early diagnosis of several other disorders, providing potential for disease prevention and improving efficacy of treatment.

Objective: To identify protein biomarkers in serum and urine that differentiate dysplastic from normal dogs at an early age (i.e., prior to skeletal maturity).

Methods: Whole blood and urine were collected from 473 client-owned dogs (303 F, 170 M) between 4 to 6 months of age, and 6 biomarkers reflecting direct and indirect measures of joint health were measured. Radiographs were evaluated for joint pathology (62 CHD and 411 normal hips) based on OFA grading criteria at 1 and 2 years of age. Mann-Whitney U-test and single variable regression analysis were performed with significance set at $p<0.05$.

Results/Conclusions: Based on a subset of client-owned dogs (62 CHD and 62 normal hips; matched based on sex and breed type), urine concentrations of C2C ($p=0.012$), CTXII ($p=0.019$) and RANKL ($p=0.010$), and serum RANKL/OPG ($p=0.049$) were significantly different between normal and dysplastic dogs. Urine biomarkers have the potential to provide early, accurate diagnosis of CHD in puppies. Ongoing analyses on samples from 349 client-owned dogs will validate this method for clinical use.

SAMPLING SCHEME AND CORRECTION FORMULA DETERMINATION FOR IOHEXOL-DETERMINED GLOMERULAR FILTRATION RATE IN PUPPIES WITH AND WITHOUT X-LINKED HEREDITARY NEPHROPATHY

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Background: Iohexol plasma clearance is a common method for estimating glomerular filtration rate (GFR) to assess kidney function in dogs. To most accurately determine GFR, an 8-point sampling method is required. Sampling schemes that require fewer post-iohexol blood draws are possible, but this requires application of a correction formula. Sampling schemes with associated correction formulas have been previously developed for adult dogs but not for puppies.

Objective: To determine the best sampling schemes and correction formulas in a population of puppies with and without X-linked hereditary nephropathy (XLHN).

Methods: GFR was determined post-iohexol administration based on 8-point sampling measurements (5, 15, 30, 60, 120, 180, 240, and 360 minutes) using WinNonlin and a non-compartmental analysis, wherein the trapezoidal rule was applied to determine the area under the curve. GFRs were normalized to dog (mL/min/dog). Using the 8-point GFR as the response variable, multiple potential correction formulas were generated via linear regression models, where uncorrected GFR determinations were the explanatory variable (1-, 2-, 6-hour; 1-, 4-, 6-hour; 1- and 6-hour; and 2-, 3-, 4-hour).

Results: For GFR determination in these puppies, all population-specific correction formulas out-performed previously generated correction formulas for adult dogs. Sampling schemes varied by age; however, the widely accepted 2-, 3-, and 4-hour sampling scheme consistently produced GFRs that corresponded the least well with the 8-point WinNonlin-generated GFRs.

Conclusions: This study highlights the importance of using population-specific correction formulas to attain the most accurate GFR and that the standard sampling scheme might not be ideal for every population.

THE EFFECTS OF ANTICOAGULANTS ON HEMATOLOGIC VALUES IN CAPTIVE CHILEAN FLAMINGOS (PHOENICOPTERUS CHILENSIS)

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Background: The Chilean flamingo (Phoenicopterus chilensis) is a common bird species under human care facing declining wild populations, highlighting the need for effective conservation and health monitoring. The use of anticoagulants allows for delayed evaluation of hematologic parameters, which is essential for clinical health assessment. Dipotassium ethylenediaminetetraacetic acid (EDTA) is generally recommended as the preferred anticoagulant in most birds; however, lithium heparin (LH) has been found to be superior in some species. The effects of anticoagulants on hematologic parameters of the Chilean flamingo are unknown.

Objective: Determine the effects of EDTA and LH on hematologic values and cellular morphology in Chilean flamingos.

Methods: Blood samples were collected from 20 Chilean flamingos and divided into vials containing EDTA and LH. For each sample, packed cell volume (PCV), refractometric protein, and complete blood counts (CBC) were performed.

Results: EDTA samples showed significantly higher PCV, refractometric protein, and leukocyte and thrombocyte slide estimates and decreased numbers of lysed cells per 50x objective field when compared to LH. Leukocyte counts and monocyte counts determined using the Phloxine B method were also significantly decreased in LH samples. Anticoagulant selection created constant and proportional bias for multiple analytes. Subjectively, there were no apparent differences in leukocyte staining or morphology between the anticoagulants. Gross hemolysis was not observed in blood samples with either LH or EDTA.

Conclusion: The results indicate that EDTA may be the preferred anticoagulant in Chilean flamingos for hematologic testing and for the quantitative assessment of PCV, refractometric protein, and leukocyte and thrombocyte mass.

IMMUNOHISTOLOGICAL IDENTIFICATION OF ERYTHROID PRECURSORS IN CANINE AND FELINE BONE MARROW CORE BIOPSIES

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Background: Accurate identification of erythroid precursors, especially rubriblasts, is challenging when relying on morphology alone or if dyserythropoiesis is present. In human medicine, immunohistochemical markers like transferrin receptor (CD71) and

alpha-hemoglobin stabilizing protein (AHSP) aid in confirming erythroid lineage in bone marrow biopsies. Together, they offer complementary identification across erythroid maturation.

Objective: Evaluate CD71 and AHSP immunohistochemistry for identifying erythroid precursors in formalin-fixed, paraffin-embedded bone marrow.

Specific Aims:

1. Identify and characterize erythroid precursors

2. Differentiate erythroid precursors from other hematopoietic lineages

3. Support the diagnosis of erythroid hyperplasia, hypoplasia, or neoplasia

4. Enhance accuracy of diagnosing various bone marrow disorders

Methods: Formalin-fixed, paraffin-embedded bone marrow biopsies from 30 dogs and cats were retrospectively selected to represent a range of hematologic conditions—reactive (e.g., erythroid hyperplasia/hypoplasia, immune-mediated anemia, inflammation) and neoplastic (e.g., myeloid, erythroid, lymphoid leukemias, myelodysplastic syndrome)—to assess marker performance across diverse marrow pathologies.

Tissue sections were immunohistochemically stained for CD71 and AHSP after protocol optimization. Immunoreactivity was evaluated in erythroid precursors, mature erythrocytes, myeloid cells, megakaryocytes, blasts/neoplastic cells, and stromal tissue. For each marker, staining intensity, distribution, and cell-specific localization were recorded.

Results: Early erythroid precursors (rubriblasts, basophilic rubricytes) showed strong CD71 expression. Polychromatophilic rubricytes stained moderately, with weak or absent staining in later stages and none in mature erythrocytes. CD71 localized to membrane and cytoplasm with punctate and diffuse patterns. Nuclear staining was absent, though the membrane was positive. AHSP showed interfering background staining. CD71 reliably marked erythroid precursors, aiding lineage, maturation, and marrow response assessment.

DGGR-LIPASE ACTIVITY IN 1,000 DOGS: HIGH CORRELATION WITH SEVERITY OF INCREASE IN HEPATOCELLULAR, BILIARY, CARDIAC, AND LIPID BIOMARKERS, AND AGE

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Background: We previously demonstrated DGGR-lipase (DGGR) is an accurate biomarker for canine pancreatitis, and determined its reference range (RR) based on Receiver Operator Curves with the gold-standard, pancreatic lipase immunoassay, and on statistical assessment of 10,000 canine cases. Here, we assessed DGGR in 1,000

dogs for association with standard, clinical biochemistry biomarkers, and age. **Methods**: Cases were divided into 5 DGGR progressively-increasing, activity-groups (U/L): Low: 0-20 (16% cases); RR: 21-80 (57%); mild: 81-150 (13%); moderate: 150-499 (11%); marked: >499 (3%). Log mean values were assessed by linear regression analysis to determine correlation coefficient and F-statistic. Comparisons to RR were by t-test. 30 cases (20 positive) by DGGR were confirmed by immunoassay. All $p<0.05$. **Results**: Linear correlation was high (r=0.91-0.99) for only 8 parameters (ranked by highest F-statistic): alkaline phosphatase (ALP), age, triglyceride (TG), amylase, glutamate dehydrogenase, alanine transaminase, gamma-glutamyl transferase, and cardiac troponin I (cTnI). Only with marked DGGR, were glucose, electrolytes, phosphate, and urea values abnormal, but total calcium, aspartate transaminase, creatine kinase, and cholesterol were unaffected. Compared to RR: a) low DGGR had 31% lower ALP, 46% lower TG, and 50% lower GLD; b) mild DGGR had increases of 360% in cTnI, 125% in GLD, 76% for ALP, 60% for ALT. **Conclusion**: Mild, moderate and marked pancreatitis occur in 13%, 11% and 3% hospital canine-cases, respectively, with its severity correlated with severity of hepatocellular injury, hepatobiliary disorder, myocardial injury, hypertriglyceridemia and age. DGGR below RR is surprisingly associated with lower than RR values of ALP, TG and GLD.

OPTIMIZATION OF STORAGE CONDITIONS TO PRESERVE CANINE HEPATOCYTE MORPHOLOGY PRIOR TO EMBEDDING INTO AGAROSE CELL BLOCKS

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Cell blocks have potential to improve the diagnostic utility of aspirated samples by enabling histopathologic techniques to aspirated cells. Currently, the equipment, reagents, and technical training limit its use to academic settings. Therefore, for private practitioners to offer this modality to their clients, aspirated material must be stable for 1-3 days post-collection. The objective of this study was to investigate the effect of different storage media on cellular morphology of canine hepatocytes, the model solid tissue, for up to 72 hours post-collection. Liver aspirates collected from fresh canine cadavers were either processed immediately into cell blocks, or distributed into each of 6 different storage conditions: Hanks' buffered salt solution (HBSS), isotonic saline, 100% fetal bovine serum (FBS), 100% canine serum, 10% FBS in HBSS, or 10% canine serum in isotonic saline. Samples were stored at 4°C for either 24 or 72 hours before being embedded into 3% agarose gels to make the cell blocks. Cell blocks were then fixed in 10% neutral buffered formalin, followed by paraffin embedding, sectioning, and hematoxylin and eosin (H&E) staining for microscopic evaluation. Slides were evaluated for cellularity, quality of H&E staining, cellular morphology, cellular preservation, and presence of architecture. We found that the samples stored in solutions containing serum had better preservation of cellular morphology compared to samples stored in saline solutions alone. Morphology was maintained for up to 72 hours post-collection. These findings offer guidance for optimizing the diagnostic quality of samples transported to a laboratory for cell block preparation.

Diagnostic Pathology Focused Scientific Session I

Chairs: Ian Hawkins, DACVP (AP); Pedro Pinczowski, DACVP (AP)

UNIQUE GROSS AND HISTOPATHOLOGIC APPEARANCE OF A FATAL MEDICATION ADMINISTRATION ERROR

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An 8-year-old neutered male Great Pyrenees was presented to MSU-CVM Diagnostic Laboratory for postmortem examination following sudden death during hospitalization for severe melena and anemia. During hospitalization, the dog was diagnosed with a gastric ulcer and received oral barium sulfate every six hours. On postmortem exam, the coronary vessels were diffusely prominent, raised, stark white, and firm. The right auricle was markedly distended and contained pasty, white material that was impacted between the pectinate muscles and adhered to a postmortem clot. All observed postmortem clots throughout the body were coated with similar material. The liver had fine, thread-like, arborizing, white vessels entering and exiting the centrilobular regions. Within the stomach, at the level of the pylorus, there was a 3x2cm chronic ulcer. Notably, there was no barium within the stomach. The predominant histopathological finding was occlusion of vascular spaces by numerous, rhomboidal, clear, birefringent bright white, <1-20um crystals with rough edges in all major organs. Additionally, there was widespread thromboembolism consistent with disseminated intravascular coagulopathy (DIC). Following gross and microscopic findings, radiographs were taken of the heart, liver, and lungs, which revealed diffuse, radiopaque vascular patterns in each organ. Sections of liver were sent to Michigan State University for GC/MS which was negative for all compounds. A mineral analysis specific for barium was also performed, and barium levels were measured at 230.52 µm/g. The cause of death was attributed to intravascular administration of barium sulfate, which led to vascular occlusion and acute cardiovascular collapse.

****Content based on forthcoming publication****

MYCOPLASMA BOVIS LIMB CELLULITIS IN DAIRY CATTLE: CASE INVESTIGATION AND A RETROSPECTIVE STUDY

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Mycoplasma bovis is a well-known bovine pathogen primarily associated with bronchopneumonia and otitis. Its involvement in cellulitis has not been previously described. Our study reports several cases of *M. bovis* limb cellulitis in cattle, based on

a case investigation and a ten-year retrospective study. In the case investigation, four dairy cows from a single herd were presented with forelimb swelling and lameness. Postmortem examination revealed chronic necrosuppurative cellulitis and tenosynovitis. *M. bovis* was detected in affected tissues of all 4 animals by culture and immunohistochemistry, while no other bacteria or fungi were isolated. A retrospective study of bovine cases submitted to the California Animal Health and Food Safety Laboratory between 2014 and 2024 identified 12 additional cases of *M. bovis* infection associated with cellulitis, primarily in the limbs and also in the prepuce (n=1) and soft tissues surrounding lymph nodes (n=1). In these 12 cases, *M. bovis* was identified by immunohistochemistry, culture, and/or PCR. Concurrent bronchopneumonia (n=6), otitis media (n=2), and mastitis (n=1) were observed. This study provides evidence of a novel role for *M. bovis* as a cause of limb cellulitis in cattle. Findings support its inclusion as a differential diagnosis in cattle with lameness and subcutaneous inflammation. Further studies are needed to elucidate the pathogenesis, potential routes of infection, and the impact of co-infections.

POLYNEURITIS EQUI-LIKE SYNDROME IN AN ADULT ALPACA

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Background: Polyneuritis Equi (PNE) is an infrequent progressive neurological disease characterized by nonsuppurative neuritis of the sacrocaudal and/or cranial nerve roots in equids and other animals, likely of immune-mediated origin. PNE has not been previously reported in alpacas. This report describes a PNE-like syndrome in this species.

Objective: To describe the clinical and pathological findings of a progressive hindlimb paralysis resembling PNE in an adult alpaca previously challenged to produce SARS-CoV-2 antibodies as part of a research study.

Methods: A 10-year-old female alpaca with progressive non-ambulatory paraplegia, urinary/fecal incontinence, and perineal hypoesthesia underwent clinical, neurological, radiographic, and laboratory examinations. Following euthanasia, a complete autopsy and histopathology were performed.

Results: Clinically**,** neurological deficits were localized to a diffuse lesion of the lumbosacrocaudal (L6-Cd5) spinal cord and/or associated spinal nerves roots and spinal nerves. Radiographic examination of this segment was unremarkable. A mild elevation in CK was noted. At necropsy, there was a moderate and asymmetric swelling of many nerve roots at the lumbosacral region. Microscopically, lumbosacral nerve roots were heavily infiltrated with large numbers of lymphocytes and plasma cells, and moderate numbers of histiocytes, with myelin dilation, swollen axons (spheroids) and multifocal areas of hemorrhage. The inflammatory process extended to the meninges of the spinal cord, perivascular spaces and into the associated skeletal muscle.

Conclusions: Progressive polyradiculoneuritis and meningomyelitis are findings consistent with PNE in horses. To the best of our knowledge, this is the first description of a PNE-like case in an alpaca, presumably associated with an immune-mediated component.

SYSTEMIC FIBRINOGEN A ALPHA-CHAIN AMYLOIDOSIS IN AGED MANCHESTER TERRIERS

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Background: Amyloidosis in dogs comprises well-known entities such as familial systemic AA amyloidosis in Shar-Peis and senile cardiovascular Fibrinogen Aα-chain (FibA) amyloidosis. We identified a novel systemic amyloidosis in Manchester Terriers.

Objective: To elucidate the pathological characteristics of systemic amyloidosis in Manchester Terriers.

Materials and Methods: A retrospective analysis of the University of Missouri's Veterinary Medical Diagnostic Laboratory database from 2010–2019 identified 12 Manchester Terriers diagnosed with amyloidosis, all dogs originated from a single client. FFPE samples were available for five cases diagnosed since 2015. Histological analysis (H&E and Congo red staining) and immunohistochemistry for serum amyloid A (SAA) and fibrinogen Aα-chain (FibA) were performed. LC-MS/MS was conducted on amyloid deposits in the kidney and/or heart in four cases.

Results: All dogs were aged (mean age: 14.58 ± 2.12 years, range: 9–17 years). Amyloid deposits were most prominent within the kidneys (12/12), with additional involvement noted in the small intestinal submucosa (9/12), spleen (6/12), intramyocardial arterial walls (5/8), atrioventricular valves (2/3), and the intrahepatic arterial wall (1/12). In the kidneys, amyloid was deposited exclusively in the glomeruli. LC-MS/MS confirmed FibA in high score in all four analyzed cases. Immunohistochemistry showed positive for FibA and negative for SAA in all amyloid deposits.

Conclusion: We identified a novel, likely familial form of systemic FibA amyloidosis in aged Manchester Terriers. This study expands the spectrum of canine breed-specific amyloidosis and underscores the utility of immunohistochemistry and proteomic analysis for definitive amyloid typing, which is essential for accurate diagnosis and understanding of disease pathogenesis.

SKELETAL-EXTRASKELETAL ANGIOMATOSIS IN A FEMALE BOXER PUPPY

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A 3-month-old, female, Boxer puppy presented for bilateral inguinal lymph node enlargement, vulvar swelling, and hip pain. Pelvic radiographs revealed moth-eaten to permeative lytic lesions affecting the right hemipelvis and right femoral head and neck. An incomplete pathologic fracture of the cranial proximal right femur, an impending right femoral neck fracture, and a suspected folding fracture of the right ischium were also noted. The dog was euthanized due to a poor prognosis. The fractures were confirmed at necropsy and were surrounded by extensive dark red areas in the skeletal muscle and subcutaneous tissues. Additional gross findings included dark red and enlarged inguinal and iliac lymph nodes, dark red foci in the lungs, and a swollen vulva. Histopathology revealed an infiltrative proliferation of vascular channels lined by bland to mildly hypertrophied CD31-positive and variably PROX1-positive endothelial cells. Affected tissues included the right femur and ischial arch, adjacent gluteus medius muscle, multiple lymph nodes, lungs, and reproductive tract. The findings are consistent with skeletal-extraskeletal angiomatosis, a rare proliferation of vascular structures involving both the bone and soft tissues. In children, this condition is thought to represent a vascular malformation. The few reports of this entity in dogs have all involved the distal phalanx and surrounding soft tissue, with this case being the first report in the femur and visceral organs, to the best of our knowledge.

SYSTEMIC TOXOPLASMOSIS IN A BRUSH RABBIT IN LOS ANGELES COUNTY, CALIFORNIA

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An adult, male, brush rabbit found recumbent and lethargic was euthanized and submitted for necropsy. On postmortem examination, the lungs were red, had pinpoint white foci and oozed red watery fluid on cut section. The liver had a single ~1 cm diameter round, white nodule. In the skeletal muscle of the right hindlimb, there were many 1–2 cm in diameter, irregular, white, foci. On histopathology, there were numerous, randomly scattered ~15 µm diameter cysts containing numerous ~1 µm elongate basophilic zoites in the liver, lung, spleen, lymph nodes, and heart. The cysts were admixed with large amounts of necrotic debris, lymphocytes, macrophages, and plasma cells. Electron microscopy of the liver showed single cysts containing numerous individual zoites, which measured approximately 4.88 µm by 1.53 µm. The zoites had an apical complex consisting of long conidia, a nucleus, glycogen, mitochondria, lipid droplets, and vacuoles. *Toxoplasma* spp. was detected by immunohistochemistry in the lungs, liver, and spleen. *T. gondii* DNA was identified by conventional PCR at the ITS-1 locus. DNA sequencing of the B1 and GRA6 loci showed 100% sequence identity with *Toxoplasma gondii* Type X. *T. gondii* is a serious concern due to its potential to affect other intermediate hosts, including humans and other animals, and can cause abortions and be fatal in immunosuppressed hosts.

NECROTIZING ATROPHIC RHINITIS AND ESOPHAGITIS CAUSED BY GEOTRICHUM CANDIDUM IN A TAMMAR WALLABY (MACROPUS EUGENII)

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A 4.5-year-old tammar wallaby with a 2-month history of dental disease, facial abscesses, and atrophic rhinitis was submitted for necropsy. Grossly, there was bilateral atrophy of the nasal turbinates with abundant caseous, firm, yellow/tan material throughout the nasal cavity. The left lung was markedly swollen, non-collapsed, and had multifocal to coalescing white/yellow purulent material in the airways. There was also focally extensive fibrinous pleuritis and pericarditis. Multifocal to coalescing white to yellow plaques were also noted along the esophageal mucosa. Diffusely, the liver had white to yellow foci surrounding the blood vessels. Histologically, there were numerous fungal hyphae characterized by ~0.3 µm wide segmented and parallel walls, admixed with large amounts of eosinophilic necrotic debris, bacterial colonies, and lining the surface of the necrotic mucosal epithelium, the nasal turbinates and esophagus. Additionally, there was evidence of necrotizing bronchopneumonia with intralesional bacteria, and perivascular foci of hepatocellular necrosis and degeneration within the liver. *Geotrichum candidum* was isolated and identified by MALDI-TOF in samples from nasal turbinates. This fungus has been associated with immunocompromised hosts, including human patients with HIV and diabetes mellitus, dermatomycosis in horses, tonsillitis in a pig, and nasal granuloma(s) in cattle. Reports of nasal lesions in wallabies are uncommon and, to the best of our knowledge, this is the first report of necrotizing atrophic rhinitis associated with *Geotrichum candidum* in this species.

FIRST REPORT OF PHASIANUS CHAPHAMAPARVOVIRUS-1 ASSOCIATED WITH HEPATIC NECROSIS IN PHEASANTS (PHASIANUS COLCHICUS) IN THE UNITED STATES

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Parvoviruses are small, icosahedral, non-enveloped, single-stranded DNA viruses. Phasianus chaphamaparvovirus-1 (PhChPV-1) has been associated with hepatic necrosis, enteritis, and nephritis in young pheasants in countries other than the US. We describe a mortality event associated with PhChPV-1 in pheasants in Northern California. A total of 118 out of 1,500 young pheasants died following a history of anorexia on a rearing farm (8% mortality). Eight carcasses (two males and six females)

were submitted for necropsy. Gross findings included hepatic pallor and random, red, pinpoint foci throughout the liver in all birds. Similar foci were observed in the heart in 2 of 8 cases. Histologically, there was multifocal and often pan-lobular hepatocellular degeneration and necrosis, moderate ductular reaction, and mild histiocytic and lymphoplasmacytic portal infiltrates. Occasional hepatocellular karyomegaly with intranuclear inclusion bodies was observed. Additional findings included lymphoplasmacytic nephritis and myocarditis with hemorrhage and rare necrosis. Ultrastructural examination revealed Parvoviridae viral particles in the liver of all birds. Whole-genome sequencing of liver samples identified a chaphamaparvovirus with 94.4% nucleotide identity to PhChPV-1. To our knowledge, this is the first report of chaphamaparvovirus-like causing hepatic necrosis in pheasants in the United States. Given that these pheasants were reared for hunting, the potential spread of PhChPV-1 into wild populations warrants consideration.

PATHOLOGIC CHARACTERIZATION AND CLINICAL SIGNIFICANCE OF LYMPHOVASCULAR INVASION IN CANINE THYROID CARCINOMAS

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Background/Objective. Microscopic lymphovascular invasion (LVI) is recognized in canine thyroid carcinoma (cTC). Associations between LVI and clinical outcomes have been poorly characterized, and a standard definition of LVI is lacking. This study morphologically characterizes LVI and evaluates the relationship between LVI and outcomes.

Methods. Clinical and histopathologic data were collected from 30 thyroidectomized dogs with 35 cTC from the University of Pennsylvania (2011-2020). Submission forms and follow-up questionnaires supplied clinical data. Histologic parameters included cTC subtype, nuclear pleomorphism, mitotic count, necrosis, mineralization, capsular invasion, and margins. Extracapsular or capsular LVI was categorized as: nonendothelialized LVI with adherent fibrin, three morphologic variations of endothelialized LVI (bulging, adhered, floating), pseudoinvasion, or absent. CD31 and PROX-1 immunohistochemistry determined blood or lymphatic vascular origin, respectively. Descriptive statistics included means with standard deviations or medians with ranges, and categorical variables were summarized using frequencies and percentages. Survival analysis was conducted using univariate and multivariate Cox proportional hazards models.

Results. Endothelialized LVI were identified in 26 samples; ten were within extracapsular vessels. On multivariate analysis, endothelialized/extracapsular LVI (ET/EC) was associated with a 3-fold increase in the likelihood of death (HR 3.15, 95% CI 1.27-7.83, P=0.013). Endothelialized LVI were most common in blood vessels (CD31+; n=17), and one case of PROX-1+ lymphatic vessels. No histopathologic or LVI parameters were associated with tumor metastasis or recurrence.

Conclusion. ET/EC is associated with worse outcomes in cTC, whereas other morphologic variations of LVI are not. Further study is warranted on a larger population of dogs with standardized treatment and follow-up.

MOLECULAR AND MORPHOLOGICAL CHARACTERIZATION OF EMERGING HEMOPROTOZOAL INFECTIONS IN IMPORTED REPTILES IN TAIWAN

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Hemoprotozoa are blood-parasitic microorganisms with complex life cycles. Despite their high prevalence, little is known about their diversity and interactions with reptilian hosts. This study investigated samples from 9 reptiles across 5 species imported to Taiwan as pets, employing molecular, clinical, and anatomical pathological techniques. In *Ctenosaura* spp., hemococcidian sporozoites were identified in both *C. quinquecarinata* and *C. similis*. Phylogenetic analysis of 18S rDNA and cytochrome c oxidase subunit 1 (*COI*) sequences revealed that all strains to belong to *Lankesterella* sp. In contrast, *Hepatozoon* sp. gametogenic stages in *C. quinquecarinata* were morphologically distinct from *H. gamezi* in *C. similis*. Phylogenetic analysis revealed that these sequences are closely related to *Hepatozoon* species from snakes. Further, novel *Hepatozoon* sp. gamonts were observed in blood smears from the blue tree monitor, with various developmental stages identified in hepatic tissue. Given the phylogenetic divergence from GenBank 18S rDNA sequences, we propose a new species, *Hepatozoon macraei* sp. nov. In the anemic plumed basilisk, multiple *Plasmodium* sp. trophozoites were observed within erythrocytes, and a meront was present in the thrombocyte cytoplasm. After molecular cloning and phylogenetic analysis of cytochrome *b*, *COI*, and 18S rDNA genes, the newly obtained sequences formed a distinct monophyletic clade, which was phylogenetically distant from sequences available in GenBank. This study provides molecular and morphological evidence of hemoprotozoan diversity in reptilian pets in Taiwan, highlighting the need for further research into host-pathogen interactions and their impact on native fauna in the context of the global exotic pet trade.

MYELOLIPOMA IN DOGS AND CATS

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Myelolipomas are benign neoplasms composed of hematopoietic and adipose tissue. They are reported in dogs and cats and are mostly found in the spleen and liver. The clinical signs are non-specific. Breed or sex predisposition has not been established due to the limited number of reported cases. Splenic hematomas have not been associated with myelolipomas. A multi-institutional retrospective study was performed to better describe the pathological findings, demographic trends, and clinical significance of myelolipomas in dogs and cats. Our preliminary results concluded that myelolipomas are more common in dogs than cats (95.6%; 801/838). In dogs, the average age at the time of diagnosis was 11.4 years (SD ±2.0 years), the average size without hematoma formation was 5.5 cm (SD ±4.0 cm), 14.6% (141/801) of the cases presented multiple lesions, 51.5% occurred in males and Labrador Retrievers were the most common affected breed (13.8%;111/801). The spleen was the most common anatomical location in dogs (98.1%;786/801), followed by the adrenal gland (1.2%; 10/801). Only one case was identified within the liver, omentum, and epidural spinal canal. Multiorgan myelolipomas (spleen and liver) were described in two dogs. Clinical signs associated with myelolipomas included lethargy, anorexia, abdominal distension, vomiting, and increased liver enzymes. The most common lesions associated with splenic myelolipomas were hematoma formation (39.2%; 314/801), splenic rupture (7.0%; 56/801), and hemoabdomen (6.6%; 53/801). None of these lesions were identified in feline splenic myelolipomas. Our findings emphasize the formation of splenic hematomas caused by myelolipomas and the predisposition of Labrador Retrievers.

MICROBIOLOGICAL, MOLECULAR AND HISTOPATHOLOGICAL DETECTION OF BACTERIAL ZOONOTIC PATHOGENS IN GUINEA PIGS FOR HUMAN CONSUMPTION IN COLOMBIA

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Background: The guinea pig is a hystricomorph mammal raised in some South American countries for human consumption. There is no current information on zoonotic bacterial agents affecting guinea pigs intended for human consumption in Colombia.

Objective: Detect and characterize zoonotic bacteria in guinea pigs from slaughterhouses using microbiological and molecular tests, and histopathological evaluation.

Methods: A cross-sectional observational study was conducted in which 60 animals that presented macroscopic changes suggestive of bacterial infection were collected.

Swabs and tissues were collected for microbiological, molecular, and histopathological tests.

Results: *Salmonella enterica*, *Streptococcus equi* subsp*. Zooepidemicus* and *Yersinia pseudotuberculosis* were isolated from the liver and lymph nodes. Histologically, granulomatous inflammation was present, with immunohistochemical labeling positive for *Y. pseudotuberculosis.* Also, a widespread phenomenon of multidrug resistance was found, mainly to antimicrobials of the beta-lactam, quinolone, and macrolide families.

Conclusions: The present study demonstrated the presence of infectious agents, of which *Salmonella* spp. and *Streptococcus* spp. have not been previously reported in this species in the country and are associated with serious disease in humans and animals. The results highlight the need for monitoring bacterial infections in guinea pigs, to establish good practices in the use of medicines and biosafety protocols in slaughter and sale in commercial establishments.

ASSESSMENT OF A PAN-MYCOBACTERIUM IN SITU HYBRIDIZATION FOR THE DIAGNOSIS OF PARATUBERCULOSIS (MYCOBACTERIUM AVIUM SSP. PARATUBERCULOSIS) IN GOATS

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Paratuberculosis (*Mycobacterium avium* ssp. *paratuberculosis,* MAP) is a chronic infection causing reduced milk production, weight loss, and mortality in goats, impacting agricultural economies. MAP culture is the gold standard for diagnosis, but it is slow (8-16 weeks) and challenging. Recently, in situ hybridization (ISH) has proven sensitive and specific in detecting mycobacteria in formalin-fixed paraffin-embedded (FFPE) tissue. In this study, archived intestinal cases of goat paratuberculosis were reviewed. An RNA scope-based chromogenic pan-*Mycobacterium* ISH was performed in selected cases (n = 20) and five negative controls (culture and PCR negative for MAP). Cases included multibacillary (n = 11) and paucibacillary (n = 9) infections, identified on Ziehl-Neelsen (ZN) stain, including 2 with postmortem autolysis. Results were compared with ancillary testing at the time of diagnosis (MAP culture and/or PCR), ZN, and pan-*Mycobacterium* PCR from FFPE tissues. Ten cases had been cultured, with MAP confirmed in 9 (9/10, 90%). MAP PCR had been performed in 7 cases and detected in all (7/7, 100%). *Mycobacterium* spp. PCR from FFPE tissue was positive in 18 cases (18/20, 90%); the remaining two were confirmed by culture and PCR. Hybridization signal was seen in 19 cases (19/20, 95%); the one lacking hybridization signal had severe autolysis. Hybridization signal was seen in 8 paucibacillary cases (8/9; 89%). No hybridization signal was seen in negative controls. These results support that ISH is more sensitive than ZN, can reduce diagnosis uncertainty, and contribute to the diagnosis of paratuberculosis in goats, especially when fresh tissue is unavailable for culture.

T-CELL RICH B-CELL COLONIC LYMPHOMA IN A NORTH AMERICAN PORCUPINE (ERETHIZON DORSATUM)

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Lymphoma is an uncommon diagnosis in captive porcupines, with only a single prior case in the literature describing disseminated T-cell lymphoma involving multiple organ systems in a Brazilian porcupine (Coendou prehensilis). Moreover, T-cell rich B-cell lymphoma (TRBCL) is an unusual and often more aggressive variant of diffuse large B-cell lymphoma. We report a novel case of TRBCL localized to the colon of a captive adult, intact female North American porcupine (Erethizon dorsatum). Necropsy revealed two adherent, black, firm, raised nodules on the serosal surface of the orad large intestine, with no significant lesions in other organs. Histopathologic evaluation demonstrated dense sheets of neoplastic small round cells effacing the submucosa, tunica muscularis, and serosa. Within the population of small round cells, a smaller number of intermediate to large neoplastic lymphocytes were identified. Immunohistochemistry revealed strong nuclear PAX5 immunoreactivity in the intermediate to large lymphocytes, confirming B cell lineage, while approximately 60% of the smaller cells were CD3-positive T lymphocytes. This case represents the first documentation of a localized, colonic, T-cell rich B-cell lymphoma in this species. Recognizing and characterizing a spontaneous disease, an unusual form of lymphoma with a unique presentation, broadens our understanding of novel hematopoietic neoplasia in wildlife species, helps with early detection, guides management decisions, and supports baseline health.

CANINE TONSILLAR ADENOCARCINOMA WITH SQUAMOUS DIFFERENTIATION

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A 10-year-old female spayed Chihuahua presented for a comprehensive oral health assessment and dental prophylaxis. A large mass was identified in the region of the left palatine tonsil. A scraping of the mass revealed a heterogenous population of small to intermediate sized lymphocytes and several atypical nucleated squamous cells with finely granular chromatin, multinucleation, cytoplasmic vacuolation, and moderate anisocytosis. The mass was excised, and microscopic evaluation showed a large ulcerated, pedunculated and papilliferous mass predominantly expanding the tonsillar submucosa. Approximately 70-80% of the mass was composed of epithelial cells arranged in anastomosing trabeculae and acinar structures containing a moderate amount of proteinaceous fluid. Acini were primarily lined by one layer of polygonal cells with a moderate amount of basophilic, glassy cytoplasm and one to three prominent nucleoli. Multifocally, polygonal cells displayed keratinizing squamous differentiation characterized by hypereosinophilic, homogenous cytoplasm and faint to absent nuclei. Based on these histologic findings, the diagnosis of primary tonsillar adenocarcinoma with squamous differentiation was made. Tonsillar neoplasms are uncommonly reported in the veterinary literature, with the most common malignant variant being squamous cell carcinoma, followed by lymphoma. These two variants account for 72% of all malignant tonsillar neoplasms in canines, while tonsillar adenocarcinoma is reported to constitute only 1.2% of cases. This case describes the cytologic and histologic features of a rare tonsillar neoplasm in a dog.

SALMONELLOSIS IN BEARDED DRAGONS (POGONA VITTICEPS)

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Background: *Salmonella* spp., members of the *Enterobacteriaceae* family, are part of the normal microbiota in both free-ranging and captive reptiles, with clinical disease rarely reported.

Objective: To retrospectively evaluate the gross, histologic, and bacteriological findings in seven cases of salmonellosis in Bearded Dragons (*Pogona vitticeps*).

Methods: Gross and histopathological examinations, bacterial culture, and serotyping were performed.

Results: Seven Bearded Dragons (median age: 2.25 years, range: 4 weeks to 8 years) died or were euthanized following a clinical course characterized by lethargy, anorexia, weakness, dehydration, and weight loss. Gross lesions were observed in 5/7 cases, with multiple dark red foci in the liver and lungs (4/7 cases), hepatomegaly (1/7), jaundice (1/7), and colonic dilation with intraluminal blood clots (1/7). Histopathology revealed fibrinonecrotizing to heterophilic/lymphocytic enterocolitis with intralesional short rods (5/7), and rare colon ulceration (1/7); heterophilic/necrotizing hepatitis (4/7), rarely accompanied by granulomatous inflammation (1/4); lymphohistiocytic bronchopneumonia (2/7) and heterophilic interstitial pneumonia (1/7). Other findings included heterophilic/lymphocytic tubulointerstitial nephritis with tubular necrosis and intratubular rods immunolabeled with an anti-*Salmonella* sp. antibody (1/7), necrotizing cloacitis (1/7), lymphohistiocytic coelomitis (1/7), lymphocytic rhinotracheitis (1/7), and lymphocytic epicarditis (1/7). *Salmonella* spp. were cultured from the liver (4/7), lung (2/7), kidney (1/7), feces (1/7), coelom (1/7), and skin (1/7). Serotyping (6/7) identified *S. enterica* subsp. *houtenae* (3/6), *S. enterica* subsp. *diarizonae* (1/6), and serovars Rissen (1/6) and Cotham (1/6).

Conclusions: Salmonellosis in Bearded Dragons is considered an opportunistic infection, potentially linked to immunosuppression or other comorbidities. Its zoonotic potential highlights the importance of awareness in reptile management.

EXTRACRANIAL CUTANEOUS MENINGIOMAS IN EIGHT RAGDOLL CATS

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Background: Meningiomas are the most common neoplasm of the central nervous system in cats typically located in the dural region of the brain or spinal cord. Meningiomas can occur in extracranial sites, but there is limited information in veterinary medicine. To the best of our knowledge, these are the first reports of cutaneous meningiomas in cats with a specific breed predilection and presumed hereditary component.

Objective: To investigate extracranial cutaneous meningiomas in eight Ragdoll cats, some with known familial relations

Methods: Masses from eight Ragdolls, median age of one year, were submitted for histopathologic review at Finn Pathologists and the Colorado State University Veterinary Diagnostic Laboratory. When available, pedigree analysis was also performed.

Results: Grossly, all cats presented with a singular cutaneous mass along the dorsal or cranial aspect of the head. Histologic assessment demonstrated unencapsulated, solitary, dermal masses composed of neoplastic spindloid cells arranged in streams and whorls around dermal collagen. Two of the eight masses had regions of mineralization. All masses were positive for vimentin and E-cadherin, variably positive for cytokeratin, and had rare reactivity for GFAP. A small subset of cats are related to each other.

Conclusions: Gross, histologic, and immunohistochemical findings closely resemble cutaneous meningioma cases in human and veterinary literature. The appearance early in life and familial relations of these Ragdolls suggest a congenital, and possibly hereditary, component to disease pathogenesis. Heritable genetic mutations are being evaluated. A cutaneous meningioma should be a differential for a craniodorsal, cutaneous head mass in young cats, particularly Ragdolls.

UNDIFFERENTIATED SPLENIC STROMAL SARCOMA IN A DOG PRESENTING AS HYPERGLOBULINEMIA

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A 10-year-old female spayed mixed large breed dog presented for routine annual examination at which time serum biochemistry testing revealed a moderate hyperglobulinemia. Whole body computed tomography identified an ~ 9 cm x 9 cm x 8 cm soft tissue splenic mass. A fine needle aspirate of the mass was obtained, and cytology identified an atypical mesenchymal cell proliferation concerning for sarcoma accompanied by plasma cell and lymphoid hyperplasia. Serum protein electrophoresis revealed a restricted polyclonal gammopathy. Splenectomy was elected and histopathology yielded a diagnosis of undifferentiated splenic stromal sarcoma. By three weeks post-splenectomy, serum globulin levels returned to within reference interval. Doxorubicin chemotherapy was well tolerated however a hepatic mass concerning for metastasis was identified during re-staging at the six-month time point. Bloodwork again revealed a hyperglobulinemia. A liver lobectomy was performed, histopathology confirmed metastasis of the stromal sarcoma, and resolution of the hyperglobulinemia was observed. At the one-year time point, a hyperglobulinemia was again detected. Re-staging revealed multiple nodules within the liver, a single mass within the pancreas, and multiple enlarged abdominal lymph nodes. Human euthanasia was elected, and an autopsy was performed confirming metastasis of the undifferentiated stromal sarcoma. Immunohistochemistry was performed and neoplastic cells exhibited weak immunolabeling with vimentin and patchy but strong immunolabeling with Iba1. In all locations, neoplastic stromal cells were accompanied by lymphoid follicular hyperplasia

and plasma cell infiltrate. This case report provides an in depth diagnostic description and clinical work up of a complex disease entity with a unique presentation.

SYSTEMIC TOXOPLASMOSIS IN AN ALPACA (VICUGNA PACOS)

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An 11-year-old female alpaca with a history of sternal recumbency for 3 days prior to death was submitted for necropsy. Hydrothorax, ascites, hepatomegaly, fibrinous pleuritis, cranioventral bronchopneumonia, and pulmonary atelectasis were observed grossly. Significant microscopic findings included chronic histiocytic, lymphocytic, and necrotizing hepatitis with portal bridging fibrosis, thrombosis in thoracic and abdominal adipose tissue, fibrinosuppurative pleuritis, pyogranulomatous pneumonia, and ulcerative gastritis in C1. Intracellular and extracellular clusters of 1 µm diameter basophilic protozoal organisms were observed in liver, thoracic and abdominal adipose tissue, pleura, lungs, and C1. Toxoplasma gondii was detected in the liver by quantitative PCR, and the presence of bradyzoites and tachyzoites within areas of inflammation and necrosis was confirmed by immunohistochemistry. Systemic toxoplasmosis is most commonly observed as an acute disease in neonatal or immunocompromised animals. A predisposing cause was not identified in this alpaca. While seropositivity for T. gondii in camelids is common, clinical disease is extremely rare. Chronic hepatitis is an exceedingly rare lesion of toxoplasmosis but was described in the only other report of systemic toxoplasmosis in a South American camelid, an adult llama. To the author's knowledge, this is the first case of fatal toxoplasmosis in an adult alpaca.

NECROSUPPURATIVE BRONCHOPNEUMONIA WITH FUSOBACTERIUM NECROPHORUM INVOLVEMENT IN TWO MACROPODS WITHOUT ORAL DISEASE: EXPANDING THE ROLE OF MPPD-RELATED ANAEROBES

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Background: Anaerobic bacteria, such as *Actinomyces*, *Bacteroides*, *Porphyromonas* and *Fusobacterium* genera are involved in macropod progressive periodontal disease (MPPD, "lumpy jaw"), which is a necrotizing, polymicrobial oral disease, with *F. necrophorum* being the most frequently isolated pathogen.

Objective: The aim of this study is to describe the pathological features of sudden death in two adult macropods, one agile wallaby (*Macropus agilis*) and one red kangaroo (*Osphranter rufusin*), in which MPPD-related anaerobes were isolated from lung tissue in the absence of oral lesions.

Methods: Postmortem examination, histopathology with Gram staining, aerobic and anaerobic cultures, and *Toxoplasma gondii* PCRs were performed on lung tissue from both animals.

Results: Histological examination revealed a severe necrosuppurative bronchopneumonia with marked pleuritis and mixed bacterial aggregates composed predominantly of filamentous Gram-negative bacilli. Anaerobic culture yielded heavy growth of *F. necrophorum* and *Prevotella spp* in both cases, with heavy growth of *B. pyogenes* in one case, while aerobic culture yielded heavy growth of mixed bacteria including *Staphylococcus aureus* in the wallaby case and few low-virulence microorganisms in the kangaroo case. PCRs were negative for *T. gondii*. No gross evidence of oral disease was present.

Conclusion: These cases describe the isolation of MPPD-related anaerobes in two cases of macropods bronchopneumonia without concomitant oral lesions. The findings expand the pathogenic potential of anaerobes and underscore the importance of including anaerobic culture and molecular diagnostics to reach a final etiological diagnosis in such cases.

EVALUATION OF THE MIRNOME OF CANINE PITUITARY ADENOMAS USING NEXT-GENERATION SEQUENCING

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Background: Pituitary adenomas are a leading cause of hyperadrenocorticism in dogs, yet their molecular drivers remain poorly characterized. MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression and hold potential as diagnostic and therapeutic targets. This study aimed to define the miRNA transcriptome of canine melanotroph pituitary adenomas and identify candidate reference genes for downstream quantification.

Methods: Formalin-fixed, paraffin-embedded (FFPE) pituitary tissues from dogs with adenomas (n=15) and controls (n=5) were analyzed using Qiagen's QIAseq miRNA platform. Differential expression analysis identified 70 significantly altered miRNAs (FDR < 0.05).

Results: Among the most dysregulated were cfa-miR-223 ($log_2FC = 4.40$, FDR = 1.49e-12), cfa-miR-8908b ($log_2FC = 4.91$), and cfa-miR-202 ($log_2FC = 3.78$), all previously linked to endocrine function or tumorigenesis. Based on predicted target pathways, top-ranking DE miRNAs included cfa-miR-34b (MET, BCL2), cfa-miR-10b (PTEN, NF-κB), and cfa-miR-326 (SMO, MAPK1), implicating roles in apoptosis, neuroinflammation, and tumor progression. For reference gene development, utilizing droplet digital PCR (ddPCR) and an expression stability algorithm (Normfinder), we prioritized stably expressed miRNAs with low variance and minimal fold change across groups. Candidates such as cfa-miR-7, cfa-let-7a, cfa-miR-335, and cfa-miR-191 met these.

Conclusion: This pilot study provides the first miRNA expression profile of canine melanotroph pituitary adenomas, identifies novel miRNAs associated with tumor

biology, and identifies reference gene candidates for future ddPCR-based quantification studies in veterinary neuroendocrine research.

CLINICOPATHOLOGIC FEATURES OF ORAL AND CUTANEOUS EPITHELIAL LESIONS IN AFRICAN PYGMY HEDGEHOGS (ATELERIX ALBIVENTRIS)

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Background: African pygmy hedgehogs (*Atelerix albiventris*) are increasingly popular exotic pets and are prone to developing various pathologies, particularly dermatological alterations and neoplastic processes. Among epithelial tumors, oral squamous cell carcinoma (SCC) has emerged as a significant cause of morbidity and mortality in this species.

Objective: This study aimed to describe and characterize the clinicopathologic features of 15 epithelial lesions diagnosed in *Atelerix albiventris* in Chile.

Methods: A retrospective analysis was performed on 15 hedgehogs with histopathologic diagnoses of oral or cutaneous masses. Data regarding age, sex, lesion location, and local infiltration were collected. All tissue samples were fixed in 10% buffered formalin and routinely processed for histopathological evaluation using hematoxylin and eosin staining.

Results: 15 cases (including biopsies and necropsies), 9 (60%) were diagnosed as SCC, 4 (26.7%) as epithelial dysplasia, and 2 (13.3%) as dermal cysts. SCC cases (6 males, 3 females) had a mean age of 3.6 years; 7 were oral in location, and all showed local infiltration. 4 cases of epithelial dysplasia (1 male, 3 females) were exclusively oral, with a mean age of 4 years and no infiltration. Dermal cysts (1 male, 1 female) were cutaneous, non-infiltrative, and had a mean age of 4.5 years.

Conclusions: SCC was the most common malignant lesion, noted for its local aggressiveness. The presence of epithelial dysplasia highlights a potential preneoplastic stage. The identification of benign lesions like dermal cysts underscores the necessity of histopathologic evaluation to distinguish between benign, preneoplastic, and malignant conditions for accurate prognosis and management.

CUTANEOUS APOCRINE GLAND NEOPLASIA IN AFRICAN PAINTED DOGS (LYCAON PICTUS) IN UNITED STATES ZOOLOGICAL INSTITUTIONS

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African painted dogs (*Lycaon pictus*; APD) are endangered canids from sub-Saharan Africa. There are approximately 190 APDs under managed care at 34 US zoological institutions. Cutaneous apocrine gland tumors have been sporadically reported in APDs from US, South African, and European zoos. To date, the pathogenesis and clinical significance of these tumors are not fully elucidated. A survey of nearly 400 pathology reports, including 206 necropsies and 185 biopsies, from 214 individuals (108 male, 99 female, 7 sex undetermined) submitted to the Species Survival Plan database between 1996 and 2024 revealed 40 cases of apocrine gland neoplasia from 26 different individuals. Age of affected individuals ranged from 7-15 years with a median of 11.6 years. A majority of tumors were reported in females (17; 65.3%). Of the 35 total tumors described, 18 were classified as carcinomas (52%), and 17 were classified as adenomas (48%). Most apocrine gland tumors were located along the dorsal midline either as a single mass, small clusters of closely apposed masses, or extensive chains of coalescing masses. Masses were raised, hairless, multilobulated, firm, and variably ulcerated. Histologically, masses were comprised of islands and tubules of neoplastic epithelial cells with distinct cell margins and occasional apical blebs. In more aggressive tumors, neoplastic cells extended into adjacent subcutaneous tissues and skeletal muscle, though lymphovascular invasion and metastases were rare (2; 5%). Investigations of potential hormonal and genetic influences are in progress to develop appropriate treatment and management strategies for apocrine gland tumors in APDs.

ACUTE MASTITIS IN FREE-LIVING RED FOXES (VULPES VULPES) IN ENGLAND

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Three free-living, adult, female red foxes from the south west or east of England were either found dead or were euthanised on welfare grounds in spring of 2023 and 2024. Post mortem examinations were performed for disease surveillance. Macroscopically, the mammary glands were enlarged. In case 1, the mammae were also firm and oedematous, with purple discolouration of the overlying skin and thick, yellow secretion. In case 2, milk was not expressible. In case 3, the mammary glands were hard and exuded pus on incision. All foxes were in poor body condition with minimal content in the gastrointestinal tract. A very heavy pure growth of *S. intermedius* was cultured in both case 1 and case 3. The isolates showed evidence of resistance to either tetracycline (case 1) or penicillin (case 3). Microbial culture was not undertaken for case 2. Histologically, the mammary gland tissue in all cases exhibited extensive coagulative necrosis with numerous intralesional Gram-positive cocci consistent with *Staphylococcus* spp.. There are currently limited published reports of mastitis in wild red foxes. Previous studies have identified foxes as reservoirs for antimicrobial resistant *Staphylococcus* spp., *Escherichia coli* and enterococci, primarily isolated from oral mucosa, rectum, or skin. Here we document three cases of acute mastitis considered to be the cause of death or euthanasia in free-living foxes. We suggest that *S. intermedius*

should be considered as a potential causative agent in such cases and note that, in our cases, both *S. intermedius* isolates showed evidence of antimicrobial resistance.

DIVERGENT DIFFERENTIATION AND HISTOLOGIC SUBTYPING IN CANINE UROTHELIAL CARCINOMA

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Abstract: Invasive urothelial carcinoma (InvUC) is an important neoplasm in dogs due to its aggressive behavior and high mortality rate. Canine InvUC is also an important naturally occurring model for human high grade InvUC. Divergent differentiation and histologic variants of InvUC are well-described in humans, and some variants have prognostic significance. There is limited knowledge of divergent differentiation and histologic variants in canine InvUC. The purpose of this study is to describe the occurrence of histologic variants in canine InvUC. Cases evaluated to date were selected through histologic screening of research samples collected from 2006-2021 from dogs with known final outcomes of metastasis (36) or no metastasis (16). Preliminary results indicate that 29 of 36 metastasis group dogs and 14 of 16 no metastasis group dogs had areas of divergent differentiation or histologic variants in at least one sample. Patterns include glandular (23/36 and 11/16) and squamous differentiation (0/36 and 3/16) and nested (10/36 and 3/16), villoglandular (9/36 and 5/16), and microcystic (3/36 and 3/16) variants. Multiple variants or areas of divergent differentiation were seen in 13/36 and 8/16 dogs, either within the same sample or across multiple timepoints, while 6/36 and 2/16 were sampled at multiple timepoints with areas of divergent differentiation or variants seen in only one sample. Our data further confirms that histologic variants exist within canine InvUC and shows that glandular differentiation may be more prevalent than previously described. Further analysis is underway to determine prognostic significance of these variants.

Diagnostic Pathology Focused Scientific Session II

Chairs: Ian Hawkins, DACVP (AP); Pedro Pinczowski, DACVP (AP)

TRYPANOSOMA CRUZI IN CEREBROSPINAL FLUID FROM A DOG: A RARE PRESENTATION IN A NON-ENDEMIC REGION

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Background: Dogs infected with *Trypanosoma cruzi*, a zoonotic protozoal parasite endemic to Latin America and parts of the southern United States, classically present with cardiac disease. Primary central nervous system involvement diagnosed post-mortem has rarely been reported.

History: A 5-year-old male castrated mixed-breed dog living in Wisconsin had an abnormal gait since adoption from Texas at 10 weeks of age. The patient presented to the Neurology service due to progressive worsening of gait abnormalities and development of urinary and fecal incontinence.

Findings: Neurologic deficits were limited to pelvic limbs with moderate paraparesis, proprioceptive ataxia, absent paw placements, and reduced withdrawal reflexes bilaterally. Cardiac auscultation, thoracic radiographs, and MRI of thoracolumbar spine were unremarkable. Lumbar and cisternal cerebrospinal fluid (CSF) analysis revealed mild-moderate lymphocytic pleocytosis and mildly increased protein. Rare organisms consistent with trypomastigotes were present on lumbar CSF cytology; a single motile suspect trypomastigote was observed in the cisternal CSF hemocytometer counting chamber. No organisms were found on blood smear review. *Trypanosoma cruzi* is not endemic to Wisconsin nor commonly associated with neurological deficits, and was not considered a differential diagnosis prior to the diagnostic work up. Serum indirect fluorescent antibody testing was positive at the maximum dilution (1:1280), consistent with *Trypanosoma cruzi* infection.

Conclusion: This case highlights an unusual presentation of Trypanosoma cruzi infection manifesting as progressive neurologic deficits without overt cardiac disease. It also raises awareness of potential for Trypanosoma cruzi infection in non-endemic areas in animals that originated from or have been traveling to endemic areas.

FELINE PULMONARY CARCINOMA WITH SYSTEMIC METASTASES AND AORTIC THROMBOSIS PRESENTING AS PARAPLEGIA

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A 12-year-old, female spayed, American Shorthair cat exhibited acute onset paraplegia, tachypnea, and a history of anorexia and constipation. Neurological examination revealed absent hindlimb pulses and pain sensation. Supportive care with anticoagulants resulted in transient pulse return. Abdominal and cardiac ultrasound excluded cardiac disease but identified bilateral renal masses and an abdominal mass.

Thoracic radiographs showed alveolar and interstitial patterns. Despite therapy, impairment of function of the hindlimbs was noted and euthanasia was performed due to poor prognosis.

Grossly, multiple firm white to beige masses were noted in lungs, kidneys, pancreas, and mediastinal lymph nodes. The lumen of abdominal aorta was occluded by a firm, red thrombus. Histopathology revealed carcinoma characterized by neoplastic epithelial cells forming tubular, papillary, and sheet-like structures with necrosis and suppurative inflammation in lung, kidneys, pancreas, mediastinal lymph nodes, and choroid of the right eye. Immunohistochemistry the neoplastic cells were positive for cytokeratin, and TTF-1, suggestive of a pulmonary origin of the neoplasm.

This case highlights a feline pulmonary carcinoma with aggressive metastases to multiple organs and aortic thrombosis leading to ischemic hindlimb paralysis. The findings are consistent with the reported “Feline MODAL syndrome” (muscle/ocular/digit/aorta/lung) pattern of metastatic spread. The aortic thrombosis is likely contributed to clinical signs in hindlimb and might be caused by paraneoplastic hypercoagulability.

UTERINE CHORIOCARCINOMA IN A DOG

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Choriocarcinomas are malignant tumors of trophoblastic cells described in humans, with rare reports in domestic animals, laboratory rodent species, and non-human primates. After a routine spay, the uterus of a 4-year-old mixed breed dog was submitted for evaluation due to the discovery of an approximately 3 cm diameter, elevated, grey-green, firm nodule on the left uterine horn. The dog was from a shelter with no known history. Histologically, the endometrium, myometrium, and serosa were infiltrated by individual to aggregated, large, pleomorphic, polygonal cells with vacuolated cytoplasm, marked anisokaryosis with karyomegaly, and frequent multinucleation. The surrounding tissues were multifocally expanded by hemorrhage and infiltrated by numerous neutrophils, macrophages, lymphocytes, and plasma cells. Neoplastic cells were diffusely positive for pancytokeratin and relaxin (RLN), while negative for vimentin, smooth muscle actin (SMA), and CD204. Neoplastic cells were diffusely positive for cyclooxygenase 2 (COX2), suggesting invasive behavior. Collectively, these results supported the diagnosis of choriocarcinoma. In humans and other species, human chorionic gonadotropin (hCG) can be used as a marker for trophoblasts and trophoblast-origin neoplasia; however, canine trophoblasts do not produce chorionic gonadotropin. Instead, RLN hormone is produced by canine trophoblasts and is a reliable marker of pregnancy in dogs. The neoplastic cells were negative for hCG and positive for RLN in our case. To our knowledge, this is the first reported case of uterine choriocarcinoma in a dog and the first case using RLN immunohistochemistry in canine choriocarcinomas.

RENAL NEUROENDOCRINE CARCINOMA WITH PULMONARY METASTASIS IN RICHARDSON'S GROUND SQUIRREL (UROCITELLUS RICHARDSONII)

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Background and Objective: Primary neuroendocrine carcinoma (NEC) of the kidney is extremely rare in domestic animals. This study describes the histopathological and immunohistochemical characteristics of renal NEC with pulmonary metastasis in a Richardson's ground squirrel (*Urocitellus richardsonii*).

Signalment: A 2-year-old female Richardson's ground squirrel weighing 455 g presented with anorexia. Abdominal ultrasound examination revealed a vascular-invasive mass. The animal died two days later, and a necropsy was performed by a clinician. Representative organs were submitted for histopathological examination.

Results: Grossly, an abdominal mass measuring approximately 7 cm in diameter was observed, replacing most of the right kidney parenchyma, and a cyst was observed in the renal cortex. Histologically, the mass was poorly demarcated, infiltrative, and composed of polygonal cells arranged in sheets, nests and occasional pseudo-glandular structures within eosinophilic fibrous stroma. Approximately 70% of neoplastic cells were positive for synaptophysin, focally positive for cytokeratin AE1/AE3 and vimentin, and negative for PAX8, Iba-1, and neurofilament. Grimelius stain showed partial positivity, indicating the presence of intracytoplasmic argyrophilic granules. These findings supported the diagnosis of neuroendocrine carcinoma. In addition, metastatic lesions were observed in the lungs.

Conclusion: This is the first case of primary renal NEC with pulmonary metastasis in Richardson's ground squirrel. Renal NEC is composed of various tissue forms, requiring comprehensive evaluation using histology and immunohistochemistry for diagnosis. The origin and distribution of neuroendocrine cells in the kidney are unclear, therefore, further accumulation of cases across animal species and comparative studies is needed to clarify the pathogenesis of renal NEC.

SUSPECTED CALCIUM OXALATE CRYSTAL DEPOSITS IN THE RENAL CORTEX OF AN AQUARIUM-MAINTAINED LINED SEAHORSE

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A female lined seahorse (Hippocampus erectus) of unknown age was euthanized due to poor quality of life after reportedly having multifocal white spots on its abdomen and head and an immobile mouth, resulting in the animal being unable to eat. This seahorse had resided in a commercial aquarium with a variety of other fish, none of which were observed to have any concerning symptoms of disease. On gross necropsy, no significant abnormalities were identified, but microscopic examination of the kidneys revealed that many renal tubules contained crystals which exhibited strong birefringence under polarized light. The gross appearance of these crystals was

suggestive of calcium oxalate, and they exhibited mild positive peripheral staining with von Kossa calcium stain. To our knowledge, there have been no previously reported cases of renal tubular calcium oxalate crystals in seahorses or other syngnathids. The etiology of this lesion in the examined seahorse is yet unknown.

ADRENOCORTICAL CARCINOMA WITH MULTIPLE ORGAN METASTASES IN A RABBIT

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A female spayed rabbit was presented with a one-year history of epiphora and conjunctival white spots in the lower eyelids, which initially improved with topical treatment. In March 2024, bilateral nasolacrimal duct obstruction was diagnosed, followed by anorexia, lethargy, and neurological signs including head tremor. Despite supportive care, the rabbit experienced sudden collapse and shock, leading to death.

Necropsy revealed the ruptured Lt. adrenal gland replaced by a large mass of yellow to dark red friable material. Multifocal yellow plaques on the surface of liver, pale areas in kidneys, diffuse splenic congestion and meningeal vessel dilation were noted. Histopathology identified an adrenocortical carcinoma (ACC) replacing ~90% of the adrenal tissue with sheets of polygonal neoplastic cells showing marked anisocytosis, anisokaryosis, frequent binucleation, karyomegaly, and a mitotic count of 11/10 HPF. Similar neoplastic proliferation was observed in the liver, spleen, lung, kidney, and a peripancreatic lymph node, confirming widespread metastases. Immunohistochemistry demonstrated Melan-A positivity in the tumor cells of liver, supporting adrenocortical origin.

This case highlights a rare occurrence of rabbit ACC with aggressive behavior and extensive metastases leading to multi-organ failure and internal hemorrhage. The tumor's high malignancy is evidenced by massive necrosis, hemorrhage, and destruction of normal organ structures. Although functional status was not confirmed, unilateral adrenal involvement in the present case may suggest potential functionality and the atrophy of contralateral adrenal gland.

C-KIT–DRIVEN ANGIOINVASIVE SEMINOMA IN A SENIOR QUARTER HORSE: A RARE EQUINE GERM-CELL MALIGNANCY

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Background: Seminoma is the most frequently recorded equine testicular neoplasia, yet overall prevalence is low and true malignancy—defined by confirmed vascular/lymphatic invasion or metastasis—is rarely documented in older equines. Classical seminomas typically express the stem-cell receptor c-KIT, a feature that aids discrimination from embryonal carcinoma and other germ-cell tumors.

Case Presentation: A 21-year-old Quarter Horse presented with progressive scrotal enlargement and discomfort. Ultrasound revealed a heterogeneous, highly vascular left testis; unilateral closed castration was elected. The excised testicle measured 20 × 15 × 7 cm and weighed 3.8 kg. On cut section, the parenchyma was replaced by multilobulated grey-white tissue with cavernous blood-filled spaces. Histologically, sheets and nests of large round-to-polygonal germ cells with abundant pale cytoplasm replaced and expanded seminiferous tubules. Numerous anisokaryotic mitoses, 40 per 2.37 square mm were documented including bizarre mitoses. Neoplastic cells dissected and filled tunical veins and lymphatics, confirming angioinvasion.

Ancillary Testing: Immunohistochemistry demonstrated intense, diffuse membranous and cytoplasmic labelling for c-KIT (CD117) in > 90 % of neoplastic cells, consistent with classical seminoma.

Significance: While most equine seminomas are reported as benign, malignant behavior—including documented abdominal metastasis—has been described and appears more common in horses than in other species. The present case in a senile horse adds to only a handful of angioinvasive, c-KIT–strong seminomas in the literature, emphasizing the value of vascular invasion and immunophenotype in prognostication. Early unilateral orchiectomy remains the treatment of choice; long-term follow-up is recommended because metastasis may occur months to years, post-surgery.

ATYPICAL SUPRASELLAR NEUROEPITHELIAL NEOPLASM IN THREE DOGS

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This case series describes an atypical suprasellar neuroepithelial neoplasm in three dogs. Cases were acquired from three institutions over a 19-year period (2005-2024). Represented breeds were Boxer (case 1), Labrador Retriever (case 2), and Golden Retriever (case 3). Ages spanned from 3 to 6 years. Cases 1 and 3 were female spayed; case 2 was male neutered. Clinical signs included but were not limited to weakness, anisocoria, and generalized seizures; duration ranged from one day to 10 months. Grossly, all cases had well-demarcated, suprasellar masses extending from the ventral telencephalon to the thalamus with secondary mild to moderate hydrocephalus. Case 1 also demonstrated severe syringohydromyelia. Histopathology revealed two populations: 1) irregular islands of round to polygonal cells with variably distinct cell borders, scant eosinophilic cytoplasm, and large, round to ovoid, vesicular nuclei with indistinct nucleoli and 2) a neuroparenchymatous stroma intercalated between the islands of cells. Immunohistochemistry revealed that the islands immunolabeled for cytokeratin and MAP2 and lacked labeling for GFAP, NSE, Olig2, synaptophysin, CK20, vimentin, CNPase, CD117, and desmin. The stroma was immunolabeled for NSE, Olig2, vimentin, and CNPase and lacked labeling for cytokeratin, CK7, CK20, and CD117. PGP9.5 and CK7 labeling varied in the islands, whereas GFAP, PGP9.5, and synaptophysin labeling varied in the stroma. Based on

the tumor location, morphology, and immunohistochemistry results, the authors propose a diagnosis of atypical suprasellar neuroepithelial neoplasm. Given that this tumor has not been previously reported in any species, it may represent a rare entity that is unique to the dog.

AN UNUSUAL CASE OF PAPULASPORA EQUI FUNGAL KERATITIS AND UVEITIS IN A MINIATURE HORSE

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A 15-year-old miniature horse gelding was undergoing treatment for a corneal ulcer when he had acute onset of colic, diarrhea, and eventually disseminated intravascular coagulation, and was euthanized. At the time of necropsy, there was a temporal paraxial brown to gray depression on the left cornea. Histologic evaluation revealed complete loss of corneal epithelium across 90% of the cornea. Infiltrating the superficial cornea and extending approximately 25-70% into the stroma were basophilic to brown-red, parallel walled but occasionally globoid, regularly septate, acute angle branching fungal hyphae. These hyphae infiltrated into the inferior sclera, iris leaflet, and ciliary body and accumulated in the trabecular meshwork and the posterior chamber. Panfungal PCR by the Molecular Fungal Identification Lab at University of Florida was consistent with *Papulaspora equi*. While fungal organisms are fairly common in cases of equine ulcerative keratitis, there are few reports of *P. equi* keratitis in both humans and horses, only some of which had a history of ocular trauma. In addition to isolation of an uncommon organism, this case features an unusual distribution of fungal hyphae: not only within the cornea, but also within the anterior and posterior chambers and anterior uveal stroma.

NASAL CARCINOMA WITH PERIORBITAL AND CRIBRIFORM PLATE INVASION, VON WILLEBRAND DISEASE, AND PRESUMPTIVE FIBROCARTILAGINOUS EMBOLISM IN A 7-YEAR-OLD ROTTWEILER

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Background: Nasal tumors in dogs, such as carcinoma, frequently cause epistaxis and can be locally invasive. Von Willebrand Disease (vWD) can exacerbate bleeding tendencies. Fibrocartilaginous Embolism (FCE) is an uncommon cause of acute myelopathy.

Objective: To describe the clinical findings, diagnostics, management, and outcome of a Rottweiler with extensive nasal carcinoma, Von Willebrand factor (vWF) deficiency, and a presumptive FCE.

Methods: A 7-year-old male Rottweiler was evaluated for epistaxis. The initial diagnostic protocol included blood analysis (with vWF measurement), rhinoscopy with biopsy sampling, for histopathology and immunohistochemistry analyses (Ki67 antigen, monoclonal mouse, anti-human, clone MIB-1, Agilent Technologies Singapore, 1:100).

Subsequently, a head computed tomography (CT) scan was performed to assess tumor extent and prognosis. Acute neurological deficits post-CT were investigated.

Results: Blood tests showed vWF deficiency (40.5%). Rhinoscopy revealed a left nasal mass; histopathology confirmed nasal carcinoma (adenomatous/solid pattern). Head CT imaging suggested a malignant infiltrative process (differentials: Chondrosarcoma, Carcinoma, Sarcoma), with extensive bone lysis invading the periorbital region, cribriform plate (meningeal/olfactory bulb contact), nasopharyngeal meatus, and left choanae. The left mandibular lymph node was reactive (metastasis vs. inflammation). Post-CT, acute posterior paresis (clinical FCE) occurred; physiotherapy was initiated. Analyzed samples showed marked immunopositivity for Ki67 in the nuclei of neoplastic epithelial cells.

Conclusion: This case illustrates a locally highly invasive nasal carcinoma with a guarded prognosis confirmed by CT, complicated by vWD. The marked Ki67 positivity suggests a high replicative capacity. The presumptive FCE further worsened the condition. Intractable epistaxis, aggravated by vWD and advanced malignancy, led to euthanasia.

CUTANEOUS PLASMACYTOSIS WITH ATYPICAL CLINICAL BEHAVIOR IN TWO DOGS

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Background

Canine cutaneous plasmacytosis (CP) is a rare condition characterized by multiple cutaneous plasmacytomas without multiple myeloma. While no pruritus or spontaneous regression occurs in typical CP, its biologic behavior remains poorly understood due to its low incidence. Here, we describe two dogs with CP showing atypical biologic behavior.

Results

Case 1: A 12-year-old female pug presented with progressive facial and limb nodules, ulcers, and pruritus. Histopathology revealed a sheet-like neoplastic proliferation of round cells with moderate, weakly eosinophilic cytoplasm and large ovoid nuclei, featuring prominent nucleoli and anisokaryosis, with 8 mitotic figures per 2.37 mm², suggestive of lymphoid or histiocytic neoplasia. Immunohistochemistry (IHC) revealed that the tumor was negative for CD3, CD20, granzyme B, IBA-1, PAX5, CD204, and c-kit, but positive for MUM1 and lambda light chain, leading to a diagnosis of CP.

Case 2: A 12-year-old male Golden Retriever with multiple skin nodules on the back and abdomen. Histopathology revealed medium to large round neoplastic cells infiltrating from the superficial dermis to the subcutaneous adipose tissue. The

neoplastic cells have scant to moderate eosinophilic or vacuolated cytoplasm, large nucleoli, anisokaryosis, and a high N/C ratio, with 12 mitotic figures per 2.37 mm², suggesting possible epitheliotropic cutaneous lymphoma. Immunohistochemistry showed MUM1 positivity, leading to a diagnosis of CP. The lesions regressed spontaneously within 28 days without any additional treatment and no recurrence to date.

Conclusion

Our cases suggest that CP can exhibit variable clinical behavior, which may be difficult to differentiate from cutaneous lymphoma based on histopathology.

IMMUNOHISTOCHEMICAL EVALUATION OF PHOSPHORYLATED STAT3 AND STAT5 EXPRESSION IN CATS WITH LYMPHOPLASMACYTIC ENTERITIS AND LOW-GRADE INTESTINAL T-CELL LYMPHOMA

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Differentiating low-grade intestinal T-cell lymphoma (LGITL) from lymphoplasmacytic enteritis (LPE) in cats remains challenging due to overlapping histopathology and limited specificity of current diagnostics. Conventional immunohistochemistry (IHC) markers (CD3, CD20, Ki-67) and molecular testing (PARR) offer limited discriminatory value. This study investigated phosphorylated STAT3 (pSTAT3) and STAT5 (pSTAT5), proteins associated with cell proliferation and tumorigenesis, as potential markers to distinguish LGITL from LPE. Archived formalin-fixed paraffin-embedded duodenal tissues from 7 healthy cats, 12 with LPE, and 15 with LGITL were evaluated. IHC for pSTAT3 and pSTAT5 was performed using an automated stainer. Stained slides were scanned, and at least five 0.38 mm^2 representative mucosal fields per case were selected. Image analysis was performed using a custom Python-based script on RGB images. For each image, pixels were classified into DAB-positive (brown), hematoxylin-stained (blue), and unstained regions after excluding white background pixels. The script quantified total tissue area, percentage of brown, blue, and unstained regions, the proportion of brown among stained areas, and the brown-to-blue ratio. Both pSTAT3 and pSTAT5 showed cytoplasmic and nuclear labeling in lymphocytes and variable cytoplasmic labeling in enterocytes. Weak, infrequent labeling was observed in healthy cats, while moderate to strong, multifocal to diffuse labeling was seen in LPE and LGITL for both markers. Although labeling of pSTAT3 and pSTAT5 significantly differed between healthy, LPE, and LGITL ($p<0.001$, Kruskal-Wallis with Dunn's post hoc tests), no statistical differences were found between LPE and LGITL. These findings suggest that pSTAT3 and pSTAT5 alone are insufficient to distinguish LPE from LGITL.

EMERGING HEPATIC ECHINOCOCCOSIS IN A DOMESTIC DOG FROM MISSOURI: RARE CONFIRMED HEPATIC INFECTION OF ECHINOCOCCUS MULTILOCULARIS IN A NON-ENDEMIC REGION

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Background: Echinococcosis is a zoonotic, parasitic disease caused by the larval stages of *Echinococcus* spp., most commonly *E. granulosus* and *E. multilocularis*. While endemic areas, namely North-central and Arctic regions in North America, are generally well-documented, sporadic cases have been confirmed outside traditional regions. This case describes a rare presentation of hepatic echinococcosis in a domestic dog from Missouri, highlighting the importance of awareness even in non-endemic areas.

History: A 2.5-year-old spayed female mixed-breed dog with a known history of predation ingestion was evaluated for intermittent coughing, mild lethargy, and exercise intolerance. Thoracic radiographs revealed multiple cystic lesions within the liver. Liver was mottled by pale, raised nodules with significant fibrinous capsulitis and capsular sclerosis. Surgical biopsy was submitted to Kansas Veterinary Diagnostic Laboratory as a tularemia suspect.

Subsequent histopathology demonstrated chronic granulomatous hepatitis centered on large, multilocular cysts containing laminated eosinophilic walls and protoscolices consistent with a *Echinococcus* spp. infection. "PCR analysis of the cyst material using *nad1* gene for *E. multilocularis* identification confirmed *Echinococcus multilocularis*. No travel history outside of Missouri was reported, raising concern for possible local transmission
This case represents a rare but significant diagnosis of hepatic echinococcosis confirmed in a young dog from a non-endemic area. It underscores the importance of including parasitic infections in differential diagnoses for cystic hepatic lesions, regardless of geographic expectations. This finding also raises potential public health implications, particularly considering the zoonotic nature of *Echinococcus* spp. and their life cycle involving wildlife and domestic hosts.

MULTICENTRIC POORLY DIFFERENTIATED NEUROENDOCRINE TUMOR WITH INTRACRANIAL METASTASIS IN A 2-YEAR-OLD GERMAN SHORTHAIRED POINTER

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Neuroendocrine tumors (NETs) are rare neoplasms arising from various neuroendocrine cells. Non-epithelial NETs, including pheochromocytomas and extra-adrenal paragangliomas, originate from neuroectodermal tissue. NETs may be locally invasive in pheochromocytomas but rarely exhibit widespread metastasis, which is more common in malignant paragangliomas. Intracranial metastasis of non-epithelial NETs has not been previously reported in veterinary medicine. A 2-year-old intact female German Shorthaired Pointer was presented to the Athens Veterinary Diagnostic Laboratory with a clinical suspicion of infectious pneumonia, based on severe leukocytosis and multiple nodules seen on thoracic radiographs. On gross examination, multiple pale tan, firm, umbilicated nodules were observed in the subcutaneous

adipose, skeletal muscles, peri-tracheal and aortic tissues, lungs, heart, liver, kidneys, adrenal glands, intestines, lymph nodes, uterus, and cerebral cortex. Microscopically, these nodules were composed of pleomorphic neoplastic cells—polygonal, spindle, and round—arranged in trabeculae, short streams, styliform forms, or sheets. Multifocal areas of extensive geographic necrosis were bordered by a neoplastic population exhibiting marked cellular atypia, a high mitotic activity, and containing scattered multinucleated cells. The neoplastic cells contained variable numbers of argyrophilic, intracytoplasmic granules and showed strong to moderate cytoplasmic immunolabeling for vimentin and PGP9.5, indicating a neuroendocrine origin. Marked pleomorphism and extensive dissemination make identifying the tissue of origin challenging; however, a large, discrete mass adjacent to the aorta, without involvement of other neuroendocrine glands, suggests a paraganglioma. This is the first report of a non-epithelial neuroendocrine tumor, most likely a paraganglioma, with intracranial involvement, thereby expanding the differential diagnosis of non-neuronal intracranial neoplasms.

NASOPHARYNGEAL AND ORAL PLASMA CELL TUMORS IN CATS

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Plasma cell tumors (PCT) in the nasal and oral cavities of cats are rarely diagnosed neoplasms, and their biologic behavior is not well characterized. The aim of this study was to retrospectively assess the histologic and molecular characteristics and the clinical behavior of PCT arising from the nasopharynx and oral cavity of cats. Twenty-one feline PCT were evaluated, including 17 from the nasopharynx and 4 from the oral cavity. The neoplasms were assigned to one of six morphologic categories, with 15/21 neoplasms demonstrating features of the mature type. Twelve cases had extracellular material consistent with amyloid. Degree of mitotic activity, karyomegaly, and multinucleation were assessed. Neoplastic cells within the oral cavity demonstrated a higher degree of malignant criteria than those in the nasal cavity. In 20 cases with PCR for antigen receptor rearrangement analysis, all cases demonstrated a clonal immunoglobulin gene rearrangement. Metastatic spread to the mandibular lymph node was confirmed in one case, and three cases had evidence of lymphadenopathy or pulmonary nodules on advanced imaging. Survival time was available for ten cases, and there was a median survival time of 286 days. Cats treated with chemotherapeutics had the longest survival when compared to other therapeutic modalities. Cats with evidence of bony lysis in surrounding tissues had shorter median survival times when compared to those without. This study demonstrates PCT in the nasopharynx and oral cavities of cats often display a mature histologic morphology and can be locally aggressive with metastasis to regional lymph nodes or the lungs.

METASTATIC EXTRASKELETAL OSTEOSARCOMA ASSOCIATED WITH GOSSYPIBOMA IN A DOG, CONFIRMED BY SATB2 IMMUNOHISTOCHEMISTRY

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Background: Gossypiboma refers to a chronic inflammatory reaction to a retained surgical sponge. While rare, there are a few reports of sarcomas arising from gossypibomas; however, metastatic progression has not been reported in dogs.

Objective: To describe an unusual case of metastatic osteosarcoma originating from a gossypiboma in a dog, with immunohistochemical confirmation using SATB2.

Methods: Gross and histopathologic evaluation and immunohistochemistry for IBA-1 and SATB2 were performed.

Results: A 10-year-old spayed female Mi-Ki dog died following a week of hyporexia and dyspnea. Postmortem examination revealed metastatic anaplastic sarcoma with large numbers of multinucleated cells in the liver, heart, and lungs. A firm, 5.2 × 2.2 × 3.0 cm mesenteric mass was located adjacent to the ileocecal junction, tightly adhered to the duodenum, ileum, and colon. On microscopic examination, this mass was identified as a gossypiboma with osseous metaplasia, based on fibrillar, birefringent foreign body fibers (up to 550 × 10.0–12.5 µm) surrounded by granulomatous inflammation. Regionally within the gossypiboma, atypical neoplastic osteoblasts formed solid sheets or were embedded in abundant osteoid with multiple neoplastic emboli within blood vessels. The neoplastic cells in the visceral organs were more pleomorphic than those in the mesenteric mass. Neoplastic cells in the liver did not express IBA-1, excluding histiocytic sarcoma, and had moderate nuclear labeling for SATB2, supporting osteoblastic origin. The mesenteric osteosarcoma had strong nuclear labeling for SATB2.

Conclusions: This case represents a rare instance of metastatic osteosarcoma arising from a gossypiboma, confirmed by SATB2 immunohistochemistry.

DISSEMINATED GRANULOMATOUS DISEASE CAUSED BY PAECILOMYCES SP. IN A PRESUMPTIVELY IMMUNOCOMPETENT DOG

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A 3-year-old spayed female Giant Schnauzer was presented multiple times over two months for evaluation of lethargy, hind limb weakness, and decreased appetite. A 4DX SNAP Test Plus was positive for *Anaplasma* spp. antibodies, and no abnormalities were observed on radiographs. The patient was discharged on doxycycline, meloxicam, and gabapentin. Two months after initial presentation, the patient's clinical signs worsened and were accompanied by vomiting and jaundice. Abdominal ultrasound revealed diffuse splenomegaly, hepatomegaly, and abdominal lymphadenopathy. Fine-needle aspirates of the liver and spleen showed moderate to marked pyogranulomatous inflammation with numerous round-to-oval yeast-like organisms and a few septate hyphae. Whole blood PCR was negative for *Candida* spp., *Coccidioides* spp., *Cryptococcus* spp., *Blastomyces* spp., and *Histoplasma capsulatum*. Supportive therapy

was elected, and the patient was discharged; however, two weeks later, the patient was presented for euthanasia. Necropsy showed disseminated PAS- and GMS-positive, non-pigmented fungal structures in the spleen, lungs, liver, lymph nodes, heart, kidneys, pancreas, and bone marrow. Fungal culture of a splenic sample yielded *Paecilomyces* spp. These organisms are environmental molds from the phylum Ascomycota, distributed worldwide and commonly found in soil, air, and decaying organic matter. Though not typically pathogenic, *Paecilomyces* can cause hyalohyphomycosis and has been associated with both localized and systemic infections in dogs. While such infections generally occur in immunocompromised hosts, this case highlights the potential for disseminated *Paecilomyces* infection in an apparently immunocompetent animal, underscoring the importance of including opportunistic fungi in the differential diagnosis, even in the absence of recognized predisposing conditions.

ASSESSMENT OF HEPATIC IRON STORAGE USING HALO® QUANTITATIVE IMAGE ANALYSIS SOFTWARE AND PERLS'S PRUSSIAN BLUE STAIN

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Exogenous iron administration in neonatal swine is standard practice for prevention of iron deficiency anemia. Iron dextran is a source of ferric iron analogous to that found in ferritin, the iron storage complex readily available for cellular processes. Excess cellular iron is stored as hemosiderin and is visible with light microscopy. Classically, erythrocyte indices are measured to assess anemia, and liver iron stores are quantified by mass spectrometry (ICP-MS).

An alternative and cost-effective way to evaluate hepatic iron stores is microscopic image quantification of ferric iron deposits highlighted by Perls's Prussian Blue (PPB) staining of formalin-fixed tissue. In an experiment assessing various iron dextran dosages, HALO® (v3.4.2986, Indica Labs, Albuquerque, NM) software was used to objectively quantify the percentage of liver tissue stained by PPB. Piglets were administered 0 mg, 200mg, 400mg, or 800mg of Uniferon® 200 (Pharmacosmos†, Holbæk, Denmark). Representatives from each group were terminally sampled at 7, 14, 21, and 28 days post injection for blood and liver sample collection.

Subjective hemosiderin scores assigned by a pathologist and liver iron levels measured by ICP-MS were higher for the 400mg and 800mg groups than those for the 200mg group. Importantly, HALO® quantification of PPB area coverage reflected these trends as well. In fact, there was a strong positive correlation (r^2= 0.5781) between PPB area coverage and liver iron by ICP-MS. Therefore, we assert quantification of iron stores with HALO® is an accurate continuous variable assessment of hepatic iron storage that can be used to support ICP-MS data.

A RETROSPECTIVE STUDY OF CUTANEOUS FOLLICULAR TUMORS IN 388 CATS (2001–2025)

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Cutaneous follicular tumors are commonly diagnosed in dogs and less commonly in cats. The objective of this study was to characterize the frequency and types of cutaneous follicular tumors in cats. A retrospective search was performed at the Athens Veterinary Diagnostic Laboratory biopsy archives for cutaneous follicular tumors in cats between 2001 and 2025. A total of 388 biopsies were retrieved. Diagnoses included basal cell tumors or BCTs (260 cases), trichoblastomas (77 cases), trichoepitheliomas (24 cases), tricholemmomas (12 cases), pilomatricomas (9 cases), and trichofolliculomas (6 cases). The age of affected cats ranged from 1 to 20 years (median age = 11 years). Tumors affected 199 males (176 castrated and 23 intact) and 189 females (161 spayed and 28 intact). Domestic Shorthair cats were most frequently affected (228 cases), followed by Domestic Longhair (67 cases) and Domestic Medium-Hair cats (37 cases). The most common sites for BCTs were the neck (63 cases), thorax (61 cases), limbs (57 cases), and head (46 cases). For trichoblastomas, the most common sites were the neck (20 cases), limbs (17 cases), thorax (14 cases), and head (12 cases). The anatomic location of follicular tumors did not differ significantly among sex (Chi-square test, P=0.097), breed (Chi-square test, P=0.461), or age (Chi-square test, P=0.194). In 7 patients, multiple follicular tumors were diagnosed. In conclusion, BCTs and trichoblastomas were the most common follicular tumors in our cats. While other retrospective studies describe feline tricholemmomas as rare, we found that they had a similar frequency as pilomatricomas.

CONGENITAL HEPATIC FIBROSIS IN A CALF

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A four-week-old crossbreed heifer calf was submitted for necropsy following a brief history of failure to thrive. The gross and histopathologic evaluations of the liver were consistent with severe bridging fibrosis with marked ductular reaction and moderate cholestasis. These lesions combined with low ductular replication confirmed via immunohistochemistry with Ki67 are supportive of a diagnosis of congenital hepatic fibrosis. Congenital hepatic fibrosis is a rare disease in veterinary medicine that results from abnormal remodeling of the ductal plate. It is most commonly reported in dogs and cats but is rarely reported in other species. Congenital hepatic fibrosis has been linked to PKHD-1 gene mutations in some species; however, future research is required to identify additional genetic predispositions or environmental factors that may contribute to this disease. This report serves to highlight an uncommon diagnosis that can expand the list of differential diagnoses that veterinarians consider when faced with similar signs in calves.

CLOSTRIDIUM PERFRINGENS TYPE C-ASSOCIATED EMPHYSEMATOUS GASTRITIS IN A DOG WITH PANCREATIC DISEASE

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A 7-year-old dog presented to the Veterinary Medical Teaching Hospital for anorexia and icterus of several days. The dog was hospitalized for 12 days and clinically diagnosed with refractory ileus, pancreatitis, exocrine pancreatic insufficiency (EPI), and an extra-hepatic bile duct obstruction. The dog did not improve despite aggressive medical management and was euthanized. An autopsy was performed approximately 1 hour after death, and showed chronic pancreatitis, a common bile duct obstruction, and severe emphysematous gastritis. Anaerobic culture of the stomach identified 3+ growth of *Clostridium perfringens*, and toxin typing rtPCR identified type C with beta2 toxin, based on the presence of *cpa*, *cpb*, and *cpb2* genes. Histologically, the stomach had an emphysematous and necrohemorrhagic gastritis with intralesional gram-positive bacilli. Fluorescent in situ hybridization (FISH) using a *C. perfringens* 16S rRNA probe demonstrated positive bacilli associated with the gastric mucosa and submucosa emphysema. *C. perfringens* type C is a well-documented cause of necrotizing and hemorrhagic enteritis in humans and young pigs, ruminants, and equids. The bacterial toxins are usually inactivated by trypsin and causes disease in individuals with diets high in trypsin antagonists or low levels of trypsin. The dog's clinically documented low trypsin concentration due to EPI likely contributed to excessive *C. perfringens* type C toxins due to lack of toxin inactivation. This represents the first case of *C. perfringens* type C associated gastrointestinal disease in a dog with pancreatic insufficiency.

The authors declare that the content of this abstract is based on a forthcoming article.

INFLUENZA A-ASSOCIATED RHABDOMYOLYSIS IN A MYHM HETEROZYGOUS QUARTER HORSE: A CASE REPORT

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Rhabdomyolysis is a serious clinical syndrome with high morbidity and mortality and a wide range of reported triggers. While Influenza A-associated rhabdomyolysis is documented in humans, it is rarely reported in veterinary species. We describe a case in a 4-year-old male castrated Quarter Horse that presented with acute respiratory signs and fever, which rapidly progressed to pigmenturia and recumbency. Due to the grave prognosis, humane euthanasia was elected.

Postmortem examination revealed extensive monophasic rhabdomyolysis affecting all major skeletal muscle groups, accompanied by renal tubular necrosis, intratubular casts, and pigmenturia. There was also necrotizing bronchitis and bronchiolitis, and epithelial necrosis within the guttural pouches. The lungs were PCR positive for Influenza A. Accordingly, the widespread rhabdomyolysis was attributed, in part, to Influenza A infection.

Given the horse's breed, we also explored the possibility that genetic factors contributed to its susceptibility to rhabdomyolysis. Periodic acid-Schiff (PAS) staining revealed no abnormal polysaccharide accumulation within the muscle, and genetic testing confirmed

the absence of the Polysaccharide Storage Myopathy type 1 (PSSM1) allele. However, the horse was identified as a heterozygous carrier of Myosin-Heavy Chain Myopathy (MYHM), a disease associated with two distinct clinical presentations: immune-mediated myositis and non-extertional rhabdomyolysis. MYHM requires immune triggers, such as infection or vaccination, to manifest clinically. In this case, Influenza A likely served as the trigger, exacerbating disease severity.

This case highlights the importance of including Influenza A as a differential diagnosis or potential instigator for rhabdomyolysis in horses, particularly those with a known genetic predisposition.

AN AI-BASED APPROACH FOR HISTOLOGIC GRADING OF HE STAINED CANINE MAST CELL TUMORS

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Background:
Mast Cell Tumors (MCTs) are the most common malignant cutaneous neoplasms in dogs, exhibiting a broad spectrum of clinical behaviors. Accurate tumor grading is critical for
determining prognosis and guiding treatment strategies, and is influenced by factors such as tumor location, histopathological characteristics, immunohistochemical markers, and underlying genetic alterations. Traditional grading systems, such as the Patnaik (three-tier) and Kiupel (two-tier) classifications, rely on manual assessment of 10 high-power fields. We introduce an AI-driven image analysis system that utilizes whole-slide images (WSIs) to automatically identify relevant tumor regions and evaluate key histological parameters in accordance with the Kiupel grading system.
Materials and Methods:
For automated tumor grading, we used 80 WSIs; 30 for training, 50 for validation. Deep learning models were developed to detect key features: tumor regions, mitotic figures, anisokaryosis, multinucleated cells. These features served as the basis for automated tumor grading. Algorithm grading was benchmarked against pathologist assessments via the Matthews correlation coefficient.
Results:
The tumor region segmentation algorithm achieved a Dice coefficient of 0.95. Detection performance for individual histopathological features was also strong, with a precision of
0.90 ± 0.03 and a recall of 0.87 ± 0.05. Moreover, the final algorithmic tumor grades showed a strong correlation with the two-tiered grading assignments independently determined by a panel of board-certified veterinary pathologists.
Conclusion:
By implementing automated whole-slide image analysis within an optimized workflow, diagnostic and treatment processes can be significantly expedited. Furthermore this approach aims to enhance the objectivity, consistency, and efficiency of MCT grading in veterinary pathology.

INTER-PATHOLOGIST AGREEMENT REGARDING RECOGNITION AND REPORTING OF HISTOLOGIC FEATURES IN CANINE INTEGUMENTARY MAST CELL TUMORS

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Background: Canine integumentary mast cell tumors (CIMCTs) are common, but their clinical behavior varies significantly. Histologic grading systems are widely implemented to predict their biological behavior. Acceptable agreement amongst pathologists has been established with some grading systems but studies have shown poor agreement for single features, such as mitotic count. For most histologic features, no thorough investigation on agreement exists.

Objective: To evaluate the inter-pathologist agreement for morphologic diagnoses (cutaneous vs. subcutaneous) and individual histologic features commonly evaluated in CIMCTs.

Methods: A survey was developed that asked participants to report the morphologic diagnosis and evaluate 31 histologic features of 20 CIMCTs. The survey included both multiple choice and open numerical questions. Whole-slide images of a single representative section from each CIMCT were reviewed using an online slide viewing platform. Twenty-three anatomic pathologists located in eight countries completed the survey without guidance on survey questions. Statistical analysis, including calculating Krippendorff alpha values, was performed for each feature across all cases.

Results: Agreement on morphologic diagnosis was poor (alpha = 0.452). For histologic features, alpha values ranged from –0.00803 (for granularity) to 0.974 (tumor depth in mm). The presence of ulceration, depth and width of tumor (in mm), and deep histologic tumor-free distance had good agreement (alpha >0.80). Twenty-six features had poor agreement (alpha <0.67).

Conclusions: There is poor agreement amongst pathologists in assigning morphologic diagnosis and evaluating most histologic features of CIMCTs. Future work includes investigating how inter-pathologist agreement may change when definitions are provided for each histologic feature.

BETA-ADRENERGIC RECEPTORS 1, 2 AND 3 IMMUNOHISTOCHEMICAL EXPRESSION IN CANINE VISCERAL HAEMANGIOSARCOMA - A POTENTIAL THERAPEUTIC TARGET?

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Canine visceral hemangiosarcomas (HSA) are aggressive tumours with a poor prognosis. In humans, beta-adrenergic receptors (β-AR) are expressed in various

tumours, including malignant vascular tumours. The use of β-adrenergic antagonists (beta-blockers) is correlated with reduced progression in many cancers. Beta-blockers have also been used, with chemotherapy, as a potential treatment for angiosarcomas in humans. In dogs, HSA therapies rely mostly on surgery and/or chemotherapy. Beta-blockers may represent a potential inexpensive and easy-to-administer co-therapy.

This study investigated the expression of β-AR1, 2 and 3 in non-neoplastic, primary and metastatic neoplastic tissue in canine visceral HSA, comparing primary neoplasms with metastasis and adjacent unaffected tissues. Tissues were collected during diagnostic necropsies. Expression was assessed by immunohistochemistry and tumours were given a semi-quantitative expression score. A total of 193 tumour sections, from 43 animals, were assessed - 62 for β-AR1, 66 for β-AR2 and 65 for β-AR3. Most (82.90%) of the examined tumour sections displayed positive immunolabelling. Variable expression of the three receptor subtypes was observed but no statistically significant patterns were identified comparing location or primary and metastatic tumour. There was a significant difference in the expression score for sex (higher expression of β-AR1, $p=0.02$, and β-AR3, $p=0.04$ in males) and neutering status (neutered animals representing a larger proportion of β-AR1 expressing tumours than statistically expected, $p=0.02$).

This study evidenced expression of β-AR1, 2 and/or 3 in more than 80% of canine visceral HSA, and may serve as a platform to explore the possible use of beta-blockers as a co-treatment for HSA.

SPONTANEOUS OVARIAN EPITHELIAL NEOPLASMS IN GUINEA PIGS

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Background: Ovarian epithelial neoplasia in guinea pigs is rarely reported.

Objective: Categorize ovarian epithelial neoplasms in guinea pigs.

Methods/Results: Pathology archival searches identified 15 guinea pigs (1-5-years-old) with 22 ovarian epithelial neoplasms. Eleven were from the rete ovarii and 11 were from the ovarian surface epithelium. Rete neoplasms were within the rete ovarii and had arborizing papillae, cords, or tubules. They had minimal atypia and were considered adenomas (11/22); nine were cystic. Ovarian surface epithelium (OSE) tumors were exophytic and either well differentiated and focal (6/22), had criteria of malignancy including one or more of the following criteria - mitotic figures, invasion into the ovarian stroma or ovarian bursa, and/or implantation metastasis (3/22) or borderline tumors (2/22) which were diffuse well differentiated OSE proliferations. PAX8 nuclear immunoreactivity was present in all rete neoplasms and negative in all OSE neoplasms.

Conclusions: Rete neoplasms had a well differentiated histological appearance. Tumors of the OSE had variable atypia with features of adenoma, carcinoma and

borderline tumors. Unfortunately, no outcome information was available, so the prognosis of these neoplasms is unknown. PAX8 expression is helpful to confirm rete ovarii verses OSE origin in neoplasms where architecture is obscured.

Industrial and Toxicologic Pathology Focused Scientific Session

Chair: Laura Hoon-Hanks, DACVP (AP)

AI-DRIVEN HISTOLOGICAL ANALYSIS OF RAT KIDNEYS: INVESTIGATING THE IMPACT OF REPEATED GLYPHOSATE AND HEAT EXPOSURE IN THE DEVELOPMENT OF CHRONIC KIDNEY DISEASE OF UKNOWN ETIOLOGY (CKDU)

Jie Zhou[1], Shaolin Xiang[2], Daniel Verdugo[3], Kathryn Greenberg[1], Jeanine Escandor[1], Yang Yang[2], Nancy Denslow[1], Abdel Ali[1], Chris Vulpe[1], Valentina Stevenson[3]
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Advancements in digital pathology have led to significant developments in Whole Slide Imaging (WSI) analysis, particularly through the integration of Artificial Intelligence (AI) and deep learning algorithms. These innovations offer new opportunities to investigate complex diseases such as Chronic Kidney Disease of unknown etiology (CKDu), an urgent health issue with increased mortality among farmworkers across the globe, for which the underlying cause(s) remain unclear. Here, we established a controlled exposure system in male Sprague-Dawley (SD) rats to model the effects of glyphosate and heat stress exposure on the development of CKDu. To improve the objectivity and precision of histopathological analyses, we employed an AI-based analysis platform (HALO AI) that used customized algorithms to analyze rat kidneys. Briefly, an initial phase involved manual annotations supervised by a trained anatomic pathologist to guide AI in identifying key structures. A second algorithm was designed to combine structural analysis with special stains and immunohistochemistry markers, including PAS and IBA-1, allowing for precise assessment within the different regions of the kidney. Our analysis suggested increased PAS-positive material in glomeruli and decreased cortex area size in animals exposed to glyphosate and/or heat. Moreover, these animals had significantly higher macrophage count and intratubular protein area compared to the age-matched control group.

Together, these AI-driven algorithms enable the objective quantification of subtle histopathological changes that may be difficult to discern through traditional pathology. Future work will focus on expanding the application of these tools to provide reproducible and accurate assessments of kidney disease in clinical and research settings.

PATHOLOGY OF A 26-WEEK CATHETER OVER NEEDLE (OTN) TAIL VEIN INTERMITTENT INFUSION STUDY IN CD-1 MICE

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There is increasing demand for intravenous (IV) infusion studies of up to 26-week duration in mice. Intermittent IV infusion is necessary to mitigate toxicity noted with bolus IV dosing. Traditional methods of through the needle (TTN) tail vein catheters and surgically implanted jugular vein catheters are fraught with complications resulting in

loss of patency and procedure-related morbidity/mortality. As an alternative, we investigated the feasibility of conducting infusion studies using a recently available 26-gauge ½ inch over the needle (OTN) pediatric catheter. A series of non-GLP pilot studies dosing CD-1 mice for up to 13 weeks with 0.9% sterile saline once weekly via tail vein OTN catheter resulted in greater than 95% IV patency and few procedure-related unscheduled deaths. Based on positive results of these in-house studies and an 8-week dose range finding study, a 26-week GLP study was conducted administering vehicle or the test article once per 4 weeks via OTN tail vein infusion to male and female CD-1 mice with a dose volume infusion rate of 50 mL/kg/hour for 30 minutes. There were no test article- or procedure-related unscheduled deaths. Macroscopic observations at the infusion site consisted of red discoloration and tail scab which correlated microscopically with dermal inflammation/necrosis and ulceration, respectively. Microscopic tail vein changes consisted of minimal to mild perivascular hemorrhage, vascular/perivascular mixed cell inflammation and/or fibrosis, vascular medial thickening, and thrombus. Thus, the OTN catheter is an effective alternative to traditional surgical murine models of IV infusion in studies of up to 26 weeks duration.

INNOVATIVE IN VITRO-IN SILICO PLATFORM FOR DOSE-RESPONSE IN CANINE BLADDER CANCER: A 3D ORGANOID- AND MATHEMATICS-BASED APPROACH

Mackenzie Long, Yvonne Peng, Saumya Gade, Hannah Nicholson, Michael Catucci, Christopher Zdyrski, Aleksandra Pawlak, Andrew Woodward, Karin Allenspach, Jonathan Paul Mochel
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Approximately 25% of urothelial carcinoma (UC) cases progress to high-grade, muscle-invasive bladder cancer (MIBC), for which treatment response remains difficult to predict. Current preclinical models, including traditional 2D cell cultures and murine systems, often fail to replicate the biological complexity and heterogeneity of human tumors. In contrast, canine UC closely mirrors human MIBC at the histological, molecular, and clinical levels, positioning it as a highly relevant comparative model. To address the need for more predictive platforms, we are developing an integrated *in vitro–in silico* system combining 3D canine bladder cancer organoids with advanced dose-response modeling. In this study, we established the foundation of this platform by optimizing a chemoradiation assay in canine-derived UC organoids. Tissue and urine derived organoids from four canine UC patients were irradiated (0, 5, 9, or 15 Gy) 24 hours after dissociation and plating (10,000 cells per well). At 72 hours post-plating, cisplatin (0, 10, 25, 50, or 100 μM) was administered. Cell viability was assessed one week after plating using a PrestoBlue metabolic activity assay. Combined effects of radiation and cisplatin were estimated simultaneously using a two-dimensional Hill model, which showed inter-patient variability in response to both radiation and cisplatin, with varying potency for the radiation dose (posterior median ED_{50}: 0.55-7.6 Gy) and the cisplatin concentration (posterior median EC_{50}: 40-174 μM). By integrating 3D organoid culture with *in silico* modeling approaches, this study lays the foundation for the future development of personalized and optimized chemoradiation strategies in canine and human bladder cancer.

DEVELOPMENT OF A DEEP LEARNING MODEL FOR SEGMENTATION OF HEPATIC VASCULAR AND BILIARY STRUCTURES FOR QUANTITATIVE ANALYSIS IN VIRAL HEMORRHAGIC FEVERS

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Background: Viral hemorrhagic fevers (VHFs), such as Ebola virus and Lassa virus infections, often cause hepatocellular degeneration, necrosis, and inflammation. However, reports on the zonal distribution of these lesions vary. Mapping lesions relative to bile ducts, arteries, and veins provides a reproducible analysis framework, yet quantifying these structures remains challenging due to morphological complexity and interobserver variability. **Objective:** To develop a deep learning model that accurately segments hepatic vascular and biliary structures in VHF-infected liver tissue, supporting objective spatial lesion mapping. **Methods**: A U-Net–based model was trained to segment bile ducts, arteries, and veins in H&E-stained liver whole slide images (n=86) from VHF-infected non-human primates. Expert annotations served as ground truth. Data were split into training (70%), validation (20%), and test (10%) sets with class balancing by oversampling and random spatial augmentations to increase the diversity of the training data. Loss metrics were tracked across 100 epochs. **Results:** The model showed effective convergence with validation performance stabilizing after approximately 40 epochs. The specificity exceeded **99.5%**, and negative predictive value surpassed **99%** for all structures. The positive predictive value ranged from **81–93%**, with a pixel-wise sensitivity near **70%**. **Conclusion:** This deep learning model reliably segments hepatic structures in infected liver tissue, providing an automated and reproducible method to map lesion distribution in VHFs. Future work will integrate lesion detection (e.g., necrosis), immunohistochemistry, and cross-species applications to refine spatial pathology insights.

CHARACTERIZATION OF THE PORTOSYSTEMIC SHUNT PHENOTYPE AND ITS AGE-ASSOCIATED EFFECTS IN C57BL/6J MICE

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Portosystemic shunts (PSS) in C57BL/6J mice are associated with altered hepatic and neurologic physiology and morphology. This study defines the clinicopathologic features of PSS and assesses the impact of PSS on age-related changes in the liver of C57BL/6J mice. Gross images and HE-stained sections of liver and brain from 45 C57BL/6J mice with PSS (1 – 21 months, median 5 months) and 48 controls (1 – 20 months, median 5 months) were reviewed, and brains were subjected to anti-GFAP and Iba-1 immunohistochemistry. To determine whether PSS increased age-related changes, livers from aged C57BL/6J mice with PSS were histologically scored and

compared to controls. Presenting complaints included lethargic, female breeders or aged males exhibiting seizure-like activity. Grossly, livers were small and light brown with pitted capsular surfaces. Histologically, portal areas had portal vein hypoperfusion, hepatic arteriolar reduplication, bile duct hyperplasia, lymphangiectasia, and lobular atrophy. In the brain, Alzheimer type II cells, defined as glial cells with nuclei at least twice the size of adjacent glial cells, greater than 75% chromatin clearing, and chromatin peripheralization, occurred with greater prevalence in PSS mice ($p < 0.0001$) and were more likely to be in the hippocampus than the cerebral cortex ($p = 0.0015$) or basal ganglia ($p = 0.0121$). Iba-1 immunoreactivity was equivalent between groups. GFAP immunoreactivity was significantly reduced ($p = 0.006$) in the brains of PSS mice. In aged C57BL/6J mice, PSS mice had significantly higher hepatic geropathology scores ($p <0.0001$), regardless of sex (females $p = 0.0001$; males $p = 0.006$).

DEVELOPMENTAL EXPOSURE TO TRICHLOROETHYLENE DISRUPTS DNA METHYLATION AND INDUCES MORPHOLOGIC ALTERATIONS IN ZEBRAFISH (DANIO RERIO)

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Background: Trichloroethylene (TCE), a volatile organic solvent and legacy environmental toxicant, may induce epigenetic toxicity in developmental stages by altering DNA methylation.

Objective: This study assessed whether TCE exposure during zebrafish development affects DNA methyltransferase expression, transgenerational DNA methylation and morphologic traits.

Methods: Zebrafish embryos were exposed to 0, 5, 50, or 500 ppb TCE from 1-120 hours post fertilization (hpf). Larvae were evaluated for morphologic alterations at 120 hpf and then rinsed, pooled, and processed for RNA extraction and cDNA synthesis or genomic DNA isolation. Expression of seven DNA methyltransferase genes was quantified via qPCR and DNA methylation (5-hmC) levels were measured in F0, F1, and F2 generations using ELISA.

Results: Significant changes in gene expression were demonstrated with increased relative expression of *dnmt1* in the 5 and 50 ppb groups and decreased relative expression of *dnmt3bb.2* in the 500 ppb group. 5-hmC DNA quantification indicated significantly lower levels of global DNA methylation in the F0 generation for all exposure concentrations, while the F2 500 ppb had significantly higher 5-hmC than the F0 500 ppb. Significant morphology alterations were observed in the F0 generation with morphology alterations persisting in the F1 and F2 generations, especially in the 50ppb and 500 ppb groups.

Conclusion: Developmental exposure to TCE does change gene expression involved in DNA methylation, with significantly reduced 5-hmC levels at the exposed F0

generation and tendency to transgenerational epigenetic compensation, and morphologic changes at higher concentrations.

Natural Disease Focused Scientific Session I

Chair: Mason Jager DVM PhD DACVP (AP)

EMERGING MESOMYCETOZOA SPHAEROTHECUM DESTRUENS IN STRIPED SHINERS IN THE SOUTHEASTERN UNITED STATES

Abigail Armwood[1], Samantha Oakey[1], Chareerut Phruksaniyom[1], Shay Kashey[2], Sonya Carlson[2], Lori Westmoreland[2], Emily Christiansen[2], Bridgette Gunn[3], John Leary[3], Al Camus[3]

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Background: *Sphaerothecum destruens* is an emerging mesomycetozoal fish pathogen, which classically affects salmonids and now is associated with high mortalities in cyprinids in Europe. In 2023, *S. destruens* was detected in a wild-caught, aquarium-housed warpaint shiner in Georgia, representing the first documented case in a North American cyprinid.

Objective: The study objective was to confirm and characterize an *S. destruens* outbreak in wild-caught, aquarium-housed striped shiner juveniles (*Luxilus chrysocephalus*), reared in North Carolina as host fish for federally threatened long-solid mussels.

Methods: Submitted shiners were assessed utilizing gross examination, histopathology, special stains, quantitative PCR, and *in situ* hybridization. Quantitative PCR and histopathology were additionally performed on surviving fish approximately one-year post the initial diagnosis.

Results: Grossly, fish exhibited marked kyphosis and scoliosis. Histopathologic examination revealed widely disseminated discrete granulomas throughout numerous tissues. Granulomas contained 1-3 µm, brightly eosinophilic, round, intracellular spores with basophilic nuclei and scant cytoplasm. The organisms stained positively with Grocott-Gomori's methenamine silver (GMS) and periodic acid Schiff (PAS) stains and negatively with Ziehl-Neelsen acid-fast and Gram stains. Spinal deformities correlated with granulomas. Quantitative PCR detected *S. destruens* in the initial group of fish and those submitted approximately one year later. RNAscope® *in situ* hybridization probes designed for *S. destruens* strongly labeled the spores.

Conclusions: Pathologists reviewing fish diagnostic cases should remain aware of this emerging agent in North American cyprinids given potential threats to ecosystems, aquaculture, and aquaria. Additional work will include sequence analysis to assess the haplotype and a potential source.

ENDOLIMAX AMOEBIASIS IN GOLDFISH

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Amoebae from the genus *Endolimax* are best characterized as components of the human gastrointestinal microbiota. However, *Endolimax* spp. have also been identified in feces from non-mammalian hosts, including amphibians, birds, reptiles, insects, and fish. Recently, two *Endolimax* species, *E. carassius* and *E. piscium*, have been reported in association with systemic granulomatous disease in goldfish (*Carassius auratus*) and farm-raised sole (*Solea senegalensis*), respectively, in Europe. Multiple goldfish (*C. auratus*) from a backyard pond in the southeastern United States presented with a history of circling behavior and lying on the bottom of the pond. Two fish were submitted for postmortem examination, and microscopically, both fish exhibited disseminated granulomatous inflammation characterized by variably sized granulomas containing necrotic cores and large numbers of 3-4 µm diameter protozoa with a single, central to paracentral nucleus. Polymerase chain reaction designed to amplify a short segment of the 18S rRNA gene of *Endolimax* spp. and other closely related archamoebae produced an amplicon of the expected size, exhibiting 99.1% nucleotide identity over a 330 bp region to *E. carassius* previously reported in Europe. The identification of this organism in a nondescript backyard pond suggests that *Endolimax* amoebae may be an underdiagnosed cause of disease in at least goldfish in North America, and pathologists should be aware of this organism as a differential for granulomatous disease in goldfish.

MYCOBACTERIUM ULCERANS ECOVAR LIFLANDII OUTBREAK IN A CAPTIVE-BRED XENOPUS LAEVIS COLONY

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Background: The African clawed frog (*Xenopus laevis*) is a widely used model in biomedical research but remains susceptible to opportunistic and emerging infections.

Objectives: To characterize the pathological findings of a mortality event in a colony of *Xenopus laevis.*

Methods: Seven frogs underwent necropsy with histopathology (H&E, Gram, Ziehl–Neelsen) and cytology performed. Swabs (coelom, liver, spleen) and environmental samples (sump sludge, feces) were cultured (aerobic/anaerobic bacterial and fungal). Pooled tissues (liver, spleen, ovary) and environmental samples (sump biofilm, skin, feces) were tested by PCR (Xenopus Essential Panel, IDEXX).

Results: Clinical signs included lethargy, anorexia, and coelomic distension. Gross lesions comprised splenomegaly, coelomic effusion, hepatomegaly with multifocal tan-yellow nodules, and severe cardiomegaly. Histopathology revealed necrotizing to granulomatous hepatitis, nephritis, splenitis, oophoritis and salpingitis,

bronchopneumonia, pancreatitis, coelomitis, and pancarditis. Intralesional acid-fast bacilli were identified in liver, spleen, and heart. PCR testing of environmental samples during quarantine was negative. At four-month follow-up, sump biofilm tested PCR-positive for *M. ulcerans*, while skin swabs and feces remained negative. PCR on pooled tissues confirmed the diagnosis.

Conclusions: Detection of *M. ulcerans Liflandii* in sump biofilm only after clinical disease highlights the limited sensitivity of environmental and superficial sampling. Postmortem analysis was essential for diagnosis. Infection in captive-bred frogs housed under controlled conditions suggests persistence from endogenous colony reservoirs and granulomatous oophoritis and salpingitis raise concern for possible vertical transmission, as seen in other aquatic species. A multimodal diagnostic approach is critical to ensure colony welfare, minimize potential zoonotic risk, and preserve the integrity of scientific research.

RANAVIRUS RANA1 (FROG VIRUS 3)-ASSOCIATED LESIONS, VIRAL TROPISM, AND GENOMIC ANALYSIS DURING A NATURAL OUTBREAK IN EASTERN BOX TURTLES (TERRAPENE CAROLINA CAROLINA)

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Rationale: Ranavirus rana1 (formerly Frog virus 3 [FV3]) is an emerging pathogen causing mass mortality in ectothermic vertebrates. Viral pathogenesis and lesion distribution have not been extensively investigated in reptiles compared to amphibians. This study aimed to perform detailed histological evaluations, correlate lesion distribution with viral tropism, and perform genomic characterization of a Ranavirus rana1 outbreak in eastern box turtles (Terrapene carolina carolina) in Louisiana.

Methods: Five eastern box turtles with lethargy, inappetence, nasal discharge, and periocular swelling were clinically examined, and three were submitted for postmortem examination (Turtles #1-3). Pan-ranavirus qPCR and ISH targeting the major capsid protein (Orf90R) were performed in addition to virus isolation, transmission electron microscopy, and whole genome sequencing.

Results: Turtles #1 and #2 had severe multisystemic, necrotizing inflammation, while in #3, mild to moderate necrotizing lesions restricted to the upper gastrointestinal tract (GIT). Lesions were associated with ranavirus DNA, within situ, DNA signal abundant in epithelial, endothelial, and histiocytic cells across organs of #1 and #2 (correlating with higher viral load), and in the upper GIT in #3. Phylogenetic analysis following whole genome sequencing determined this isolate was closely related to Frog Virus 3 isolate OP/2025/Netherlands, with a major and minor parent derived from isolates identified in Canada and China, respectively.

Conclusion: Ranavirus rana1 is a multisystemic viral disease with high mortality. In this outbreak, lesion severity corresponded to increased viral load with abundant in situ DNA signal. The Ranavirus rana1 isolate from this outbreak derives from Canadian and Chinese parents.

ALIMENTARY MYCOSIS IN SLOTHS

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Alimentary mycosis is seldom reported in sloths. Through a multi-institutional retrospective study, we described the histological features of fungal infections within the digestive tract of sloths of the *Bradypus* and *Choloepus* genus. Additionally, panfungal PCR, targeting the ITS-2 gene, was performed in all cases to determine a specific etiology. We retrieved 11 cases of alimentary mycosis in three sloth species: *Bradypus variegatus* (n=1)*, Choloepus hoffmanni* (n=7), and *Choloepus didactylus* (n=3). Eight were free ranging, whereas 3 were held in captivity. Nine were females, and 7 were juveniles (ranged from 2 weeks to 13 months old). In 64% of the cases the lesions were gastric and at the muscular portion of the prepyloric stomach. In the rest of the animals, the lesions were located in the tongue and/or esophagus. Pustules, erosions, ulcers and hyperkeratosis within the keratoid layer with intralesional yeast, pseudohyphae and hyphae characterized alimentary mycotic infections. Panfungal PCR incriminated *Trichosporon asahii* infection in 45% (5/11) of the cases, from gastric and lingual lesions and *Penicillium* sp. and *Wallemia mellicola* in a gastric lesion in one case respectively. *Candida* sp. infection was not confirmed in any of the cases. *Trichosporon asahii* has overlapping histological features with *Candida* and poses a diagnostic challenge when conventional culture or molecular methods are unavailable. Trichosporonosis is a differential diagnosis in cases of fungal alimentary lesions in sloths. Predisposing factors for alimentary mycosis in sloths include age (younger animals), canine distemper virus co-infection, late pregnancy, and chronic antibiotic use.

DO DEER DIE OF OR WITH CHRONIC WASTING DISEASE? PATHOLOGICAL FINDINGS OF FREE-RANGING WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) IN PENNSYLVANIA'S CHRONIC WASTING DISEASE ENDEMIC ZONE

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Chronic wasting disease (CWD) is a fatal prion disease of Cervidae animals, including the economically and culturally important white-tailed deer (WTD; *Odocoileus virginianus*). Despite the investment in monitoring and studying this disease, there are gaps in our understanding of morbidity and mortality of individual animals as well as the population-level effects. This study describes the gross and microscopic lesions and overall causes of mortality of WTD in Pennsylvania's CWD endemic zone. Seventy-six GPS-collared, free-ranging WTD that died between 2023 and 2025 were examined. Forty-six of the 76 deer (65%) were CWD positive. Of the 31 (41% of total) deer that died from natural, non-human associated causes (predation, end stage CWD, and non-CWD infections), 29 (94%) were CWD positive. Ten of 76 (13%) deer died from end-stage CWD, with eight (80%) also having aspiration pneumonia. Seven of 76 deer (9%), all being CWD positive, died from non-CWD infections (primarily bacteria). Twenty-three of 76 deer (30%) died from anthropogenic causes (hunter harvest and vehicle collisions among others), and 11(48%) of these were CWD positive. Despite being a hallmark of the disease, spongiform changes in the brain were recognized in only 19 of 36 (53%) of the CWD-positive cases where brain was available. These results suggest that WTD in Pennsylvania's CWD endemic zone die both directly from CWD as well as from other causes prior to end stage disease. Additionally, inclusion of collared deer may be a useful tool for surveillance, studying epidemiology, and defining the pathobiology of CWD in wild WTD.

USING GOLD STANDARD DIAGNOSTIC TESTS AND DIGITAL IMAGE ANALYSIS TO STAGE WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) FOR CHRONIC WASTING DISEASE INFECTION

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Chronic wasting disease (CWD) is a fatal prion disease of deer, elk, moose, and other cervids. It is one of the most important infectious diseases of cervids with significant wildlife management and economic implications. The duration from CWD prion infection to death can be up to 2-3 years, where an animal can shed prion via bodily fluids, and prion can remain infectious in the environment for a decade or more, making management of this disease challenging. Considering the chronicity of the disease, understanding the stage of infection can be useful for determining the duration of infection, the burden of prion in the tissues, and how long the deer has been shedding prion in the environment. We attempted to determine if digital image analysis could aid in staging white-tailed deer for CWD infection and subsequent disease. Retropharyngeal lymph nodes and obex from CWD positive deer were analyzed to determine if the intensity and/or total area of immunoreactivity for the CWD prion were associated with body condition score (BCS) and therefore indicative of disease stage. While not reaching statistical significance, the total area of immunoreactivity in the lymph node follicles showed the strongest correlation with BCS (Kendall's Tau-b = -0.39, $p = 0.1$). Intensity of staining in the lymph nodes and obex showed slightly lower correlation coefficients. These data suggest that, with continued optimization, digital

image analysis may be a useful tool to stage CWD in white-tailed deer and assess infection duration, prion burden, and environmental shedding.

TISSUE TROPISM OF HIGHLY PATHOGENIC AVIAN INFLUENZA ACROSS VARIOUS RAPTOR SPECIES IN THE STATE OF NEW YORK

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Highly pathogenic avian influenza (HPAI) has devastating effects on wild and domestic birds and mammalian populations. Since 2022, HPAI H5N1 clade 2.3.4.4b viruses have evolved into several genotypes exhibiting expanded host range and a broad tissue tropism. The objectives of this study are to 1) compare viral cell tropism and histologic lesions in raptor species with differing diets and 2) determine if viral lesions differ between years.

From 2022 to 2025, we performed necropsies on raptors submitted to the Animal Health Diagnostic Center. Avian influenza infection was confirmed via RT-PCR, and HPAI infection was confirmed via detection of the H5 sequence. Brain, heart, and spleen from 21 H5N1-positive birds from 2022-2023 have been evaluated for cell tropism and lesion severity via histologic examination and RNA-scope *in situ* hybridization (ISH). Necrosis, vasculitis, and severity of inflammation were scored via a semiquantitative method, and viral presence and cell tropism were confirmed via ISH. Currently, histologic lesions in different species are being compared utilizing a multivariate regression. Additionally, histologic data from birds dying in 2025 are currently being analyzed and will be compared to results from previous years using similar statistical analysis.

MORPHOLOGIC AND IMMUNOPHENOTYPIC CHARACTERIZATION OF NEUROBRUCELLOSIS IN STRANDED DOLPHINS IN COSTA RICA FROM 2010–2020

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Background: Neurobrucellosis is a recognized manifestation of chronic *Brucella ceti* infection in cetaceans, with important zoonotic, conservation, and comparative pathology implications.

Objective: To characterize neurobrucellosis in dolphins stranded along the Pacific coast of Costa Rica from 2010 to 2020 using histopathology, immunohistochemistry (IHC), and in situ hybridization (ISH).

Methods: Formalin-fixed, paraffin-embedded (FFPE) brain tissue from 25 dolphins with confirmed neurobrucellosis (by culture and serology) was evaluated histologically.

Select brain regions were assessed by immunohistochemistry (IHC) for CD3, CD20, IBA-1, and *Brucella* spp., and by RNAscope® probe-based in-situ hybridization (ISH) assay for *Brucella* spp.

Results: Most cases involved striped dolphins (24/25, 96%) and juveniles (21/25, 84%) in postmortem code 1 (freshly dead) (64%) or 2 (mild postmortem autolysis) (36%). All cases exhibited lymphoplasmacytic and histiocytic meningoencephalomyelitis, most severe in the spinal cord, brainstem, cerebellum, and periventricular regions, often with choroid plexitis, neuritis, and vasculitis. *Brucella ceti* was isolated from cerebrospinal fluid (CSF), and all evaluated dolphins were seropositive in serum and CSF with Rose Bengal test. Immunolabeling against *Brucella* antigen and hybridization signal were detected in meningeal macrophages in 21/25 (84%) cases. $CD3^+$ T cells were present in 15/25 (60%), $CD20^+$ B cells in 24/25 (96%), and IBA-1^+ histiocytes in 25/25 (100%).

Conclusion: Neurobrucellosis is a common and severe manifestation of *Brucella ceti* infection in juvenile striped dolphins. CSF is the most reliable sample for bacterial isolation and advised for serologic diagnosis. Histopathology is essential for correlating lesions with characteristic inflammatory patterns, while IHC and ISH serve as valuable complementary tools.

HERITABLE ANOPHTHALMIA AND MICROPHTHALMIA IN A LABORATORY MARSUPIAL MODEL

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Background: Anophthalmia and microphthalmia (A/M) are rare congenital ocular malformations in humans and animals. The genetic and developmental mechanisms underlying these anomalies remain incompletely understood, and spontaneous models are limited.

Objective: To characterize the clinical, macroscopic, histopathological, and heritable features of spontaneous A/M in *Monodelphis domestica*, a laboratory marsupial.

Methods: Affected and control animals underwent ophthalmic exams and full postmortem evaluation. Eleven animals, selected to represent the clinical spectrum of disease, were euthanized for pathologic examination. Serial histologic sections of the head and major organs were assessed. Phenotypes of the anomalies were annotated and modeled using pedigree-based genetic analysis.

Results: Eleven opossums with clinical ocular malformations were selected. Histology

confirmed true anophthalmia in seven animals, defined by the complete absence of ocular tissues. Microphthalmic cases had rudimentary, aphakic globes with disorganized neuroretina, sometimes with coloboma or retinal dysplasia. Even clinically normal eyes in some of the unilateral cases exhibited retinal degeneration and/or cataract. Pedigree analysis revealed that these conditions are predominantly influenced by one or more recessive X-linked genes, and that penetrance of the phenotype is considerably reduced in both sexes.

Conclusions: This is the first report of spontaneous, non-syndromic A/M in a laboratory marsupial, offering a novel model for ocular developmental disorders. The findings highlight the complexity of A/M pathogenesis, which involves disruptions at multiple stages of eye development and exhibits phenotypic variability, offering valuable insights into similar conditions in humans.

Clinical Pathology Focused Scientific Session II

Chairs: Samantha Evans, DACVP (CP); Lisa Schlein, DACVP (CP)

RED BLOOD CELL DESIALYLATION IN DOGS WITH IMMUNE-MEDIATED HEMOLYTIC ANEMIA

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Background: Sialic acid (SA) on red blood cell (RBC) glycocalyx maintains membrane integrity and prevents premature clearance. RBC desialylation contributes to non-antibody-mediated clearance of senescent RBCs by promoting phagocytic removal. We hypothesized that dogs with immune-mediated hemolytic anemia (IMHA) have reduced RBC surface SA compared to healthy dogs and anemic dogs without IMHA.

Objective: Develop and validate flow cytometry assay for detecting surface SA on canine RBCs and compare RBC surface SA expression between healthy dogs, anemic dogs without IMHA, and dogs with IMHA.

Methods: FITC-conjugated lectins with affinity for SA (SNA and WGA) were screened for SA binding on canine RBCs by flow cytometry. SA expression level was calculated from the lectin-FITC MFI of native RBCs normalized to MFI of RBCs treated with sialidase (NeuC). SA levels were compared among healthy dogs (n=21), anemic dogs without IMHA (n=21), and dogs with IMHA (n=4).

Results: WGA at 0.1 µg/mL showed optimal binding characteristics and separation of SA signal between native and sialidase treated RBCs. SA signal remained stable in whole blood stored at 25°C and 4°C for up to 48 hours and sample HCT (40%-10%) did not affect results. IMHA dogs had higher SA levels compared to healthy dogs *(p=0.045).*

Conclusions: Our preliminary findings show RBCs from dogs with IMHA have relatively high SA levels; higher levels could reflect either true increased density of RBC surface SA or be from autoantibodies affixed to RBCs. Desialylation may not contribute to pathologic destruction of RBCs in canine IMHA.

CANINE SERUM AMYLOID A CONCENTRATIONS IN NON-ASSOCIATIVE IMMUNE-MEDIATED POLYARTHRITIS

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Background. Serum C-reactive protein (CRP) concentrations are increased in canine immune-mediated polyarthritis (IMPA), however, serum amyloid A (SAA) concentrations have not been evaluated in canine IMPA.

Objectives. Investigate SAA concentrations in canine non-associative IMPA and undertake additional validation of the SAA assay in dogs.

Methods. Dogs were diagnosed with non-associative IMPA based on compatible cytological findings in multiple joints and screening for associative conditions. Bilirubin, hemoglobin and lipemia interference studies were performed, with interference inferred when recovery percentage was <80% or >120%. Data are presented as median [minimum–maximum]. Correlations were assessed using Spearman's rank correlation.

Results. At the time of diagnosis, SAA and serum CRP concentrations were 32.9 [0.9-285.6] mg/L and 34.9 [2.3-76.5] mg/L respectively and were strongly positively correlated (r_s=0.976, n=10, P<0.001). SAA and serum CRP concentrations were above reference intervals (<2.2 mg/L and <8.2 mg/L) in 7/10 and 8/10 dogs respectively. Circulating neutrophil concentration was strongly positively correlated with SAA concentration (r_s=0.738, n=8, P=0.046), however, the correlation with serum CRP concentration failed to reach statistical significance (r_s=0.714, n=8, P=0.058). Post treatment SAA concentrations were 2.0 [<0.3-160.4] mg/L and were above reference interval in 2/6 dogs. No bilirubin interference was detected (up to 0.15 g/L), although hemoglobin interference was detected at the highest spiked concentration (10 g/L). Lipemia interference was detected in 1/3 samples (recovery 79%).

Conclusions. SAA and CRP concentrations are highly correlated in IMPA. SAA may be an alternative biomarker for non-associative IMPA, but measurement is subject to interference by hemoglobin.

ARTIFICIAL INTELLIGENCE-ASSISTED AUTOMATED ELECTRONIC MEDICAL RECORD RETRIEVAL USING A LARGE LANGUAGE MODEL – A PILOT STUDY

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Background

Extracting data from electronic medical records (EHRs) is a common but time-consuming task in veterinary clinical research. Large language models (LLMs), or artificial intelligence (AI) chatbots, have demonstrated high sensitivity and specificity in identifying clinical signs from veterinary EHRs. No prior veterinary studies have proven using LLMs can save time in data extraction.

Objective

To compare the efficiency and accuracy of manual and AI-assisted data extraction methods.

Methods

We used ten canine acute pancreatitis cases, each containing five de-identified PDF files: discharge summary, CBC, chemistry, Spec cPLI, and abdominal ultrasound. We used Google-developed Gemini with tailored prompts to extract 61 parameters from all files into an Excel spreadsheet. Parallel Manual data extraction was performed for comparison. For each method, time spent in data extraction and verification is recorded.

Results

Gemini extraction was significantly faster than manual extraction, with a median (IQR)

of 7.17 seconds (7.06–7.38) and 297.38 seconds (281.51–314.18), respectively ($p < 0.001$). Time spent in verification was similar in both **Methods:** 177.18 seconds (152.97–195.44) for Gemini and 197.50 seconds (177.20–218.73) for the manual method ($p = 0.1$). Error counts per case were not significantly different between methods ($p = 0.76$): Gemini generated 16 errors (error rate 2.6%), mainly in narrative fields, while manual extraction had 11 errors (error rate 1.8%), primarily typos in numerical data.

Conclusion

Compared to manual extraction, the AI-assisted approach significantly reduced time without increasing the errors, demonstrating the potential of AI to accelerate routine data extraction for veterinary clinical researchers.

A COMPARISON OF ORGAN-BASED AND SIMULATION-BASED APPROACHES TO TEACHING CYTOLOGY SAMPLING AND CELL IDENTIFICATION

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Background. Cytological sampling and basic cell recognition are key day-one competencies for veterinarians. Various teaching methods within the veterinary curriculum may support the development of these skills. While organ-based practicals have been used for years, a simulator-based version was recently introduced at the University of Edinburgh.

Objectives. Organ-based and simulator-based cytology practicals were compared in two consecutive 4th-year student cohorts using anonymised, paired pre- and post-practical questionnaires. Confidence in practical skills, accuracy in cell type recognition, and class enjoyment were compared.

Material and Methods. Students completed a pre-activity questionnaire evaluating confidence and cell recognition ability. Both cohorts received an introductory presentation covering sampling techniques and differences in cell types. One cohort then practised on organs; the other on simulators. After the session, students completed the post-activity questionnaire. Data was analysed using Mann-Whitney, Wilcoxon Signed-Rank and Kruskal-Wallis tests.

Results. 137/138 (organ) and 160/171 (simulator) students participated. The simulator group had less prior experience, but both cohorts had similar pre-activity confidence and perceived difficulty. Both practicals improved confidence, reduced perceived difficulty, and significantly enhanced cell recognition ability. Impression smears were considered the easiest method, followed by needle-only and fine-needle aspiration. Enjoyment levels were comparable, although in the simulator cohort higher prior experience negatively correlated with class enjoyment.

Conclusion. Both practicals effectively improved students' confidence and knowledge in cytology. Being a first-time session, the simulator practical lacked refinement, likely

impacting enjoyment. More experienced students found simulators less beneficial. Introducing simulation-based training earlier in the curriculum may enhance its perceived value.

WHOLE SLIDE IMAGING AS A TEACHING TOOL FOR CYTOLOGY AND HEMATOLOGY SUBMISSION PRACTICES

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In this study, 251 third- and fourth-year clinical veterinary students participating in a three-week pathology rotation between November 2023 and July 2025 were provided the opportunity to use whole slide imaging (WSI) as a learning tool for cytology and hematology specimen submission. Preliminary knowledge and opinions about using WSI to submit cases to remote pathologists were collected through a pre-test survey. Students worked in small teams to select a cytology or hematology specimen that had been previously submitted to a clinical pathology laboratory at an academic institution. They scanned the glass slide(s) using a commercially available digital slide scanner, uploaded the case information they deemed clinically relevant, and submitted the case for interpretation by a remote pathologist unaffiliated with their own institution. After the report was finalized, students then debriefed within and between teams to explore their experiences and answer any lingering questions. A post-test survey collected knowledge and opinions about using WSI after the hands-on experience was complete. In the post-scanning survey, students indicated they were now more familiar with using WSI, and they were more confident in the following: providing clinically relevant information to offsite pathologists, understanding cytology reports, the utility and limitations of WSI, and submitting cytology specimens using WSI. They were also more motivated to provide relevant information to offsite pathologists than in the pre-scanning survey. In conclusion, clinical veterinary students and their instructors had a positive experience using WSI to submit cytology and hematology specimens.

INVESTIGATING THE DIAGNOSTIC UTILITY OF CELL BLOCKS PREPARED FROM CANINE TUMORS

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For the diagnosis of canine tumors, fine needle aspirates are typically spread onto slides for cytologic evaluation, with limited options to assess molecular phenotype. Cells blocks, which are prepared by embedding aspirated material into some type of matrix, can be formalin-fixed, sectioned, and stained similarly to a tissue biopsy. We hypothesized that immunohistochemical (IHC) results would be similar between agarose cell block and tissue sections from canine tumors. To investigate this hypothesis, we collected aspirates and tissues from canine tumors post-mortem. Mesenchymal, epithelial, and round cell tumors were included in the study. Cell blocks were prepared from aspirated tumor material using 3% agarose in Hanks' buffered salt solution. Once solidified, cell blocks were fixed in 10% neutral buffered formalin for 24 hours. Tissues were collected and formalin-fixed. Both cell blocks and tissues were then

paraffin-embedded and sectioned for hematoxylin and eosin (H&E) staining and IHC. Antibodies for IHC (e.g. vimentin, cytokeratin, CD3, Pax5) were chosen based on morphologic assessment of the tissue by H&E. Cell blocks were evaluated for cellularity, presence of architectural structure, quality of H&E staining, background artifacts, and localization of immunoreactivity on IHC. Case recruitment is ongoing, and preliminary results indicate cells blocks have good cellularity with no background artifact on H&E or IHC. Immunohistochemical reactivity was appropriate for each antibody and tumor tested. These results suggest that cell blocks prepared from canine tumor aspirates could be a valuable minimally invasive diagnostic technique.

DIAGNOSTIC QUALITY AND CELLULARITY OF SEQUENTIAL FINE-NEEDLE ASPIRATES IN CLINICAL PRACTICE

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Background: Veterinarians routinely obtain more than one fine-needle aspirate (FNA) from the same lesion, assuming additional samples improve diagnostic yield. Yet, objective evidence for this practice is scarce, and it remains unknown whether sequential FNAs differ in cellularity, diagnostic adequacy, or cytologic interpretation.

Objective: Evaluate agreement in diagnostic quality of serial FNAs obtained from the same lesion assessed by clinical pathologists.

Methods: This was a cross-sectional study. Two serial FNAs of lesions from 195 dogs and 5 cats were collected at Purdue University and digitized by Motic scanner. Digitized images from paired aspirates were randomly assigned to IDEXX pathologists for blinded review after a 2-week washout. Pathologists recorded sample quality attributes using predefined structured inputs. Paired response frequencies for both aspirates were compiled in contingency tables. Log-linear models were used to assess joint distributions of responses. Reliability was quantified using Krippendorff's alpha with 95% confidence intervals.

Results: Slide diagnostic quality showed strong evidence of symmetry (G^2=0.136, df=3) and moderate reliability (α=0.500 [0.394, 0.606]). Overall cellularity showed strong evidence of symmetry (G^2=8.469, df=9) and substantial reliability (α=0.633 [0.555, 0.711]). Bias towards higher values of blood contamination was seen on the second aspirate. This was best described by an ordinal quasi-symmetry model (G^2=2.160, df=5) and had fair reliability (α=0.404 [0.282, 0.525]).

Conclusion: Sequential FNAs from the same lesion showed moderate to substantial agreement between reads in diagnostic quality and cellularity, supporting the clinical practice of multiple samplings. Variability in blood contamination was observed, but its overall impact on diagnostic quality is limited.

DOUBLE IMMUNOCYTOCHEMISTRY ASSAY ON ROMANOWSKY-STAINED SMEARS FOR B AND T-CELL MARKERS ON CANINE SAMPLES

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Background: Romanowsky-stained smears can be used for immunocytochemical (ICC) immunophenotyping of lymphoma and leukemia. This technique typically involves incubating the antibodies separately; however, the description of a manual double ICC to characterize lymphocytes in canine samples is scarce.

Objective: To develop and optimize a double ICC assay for identifying B- and T-cells on Romanowsky-stained smears from dogs and to compare findings with confirmatory ancillary tests or previous diagnoses of lymphoma/leukemia cases.

Methods: Unstained and methanolic Romanosky-stained bone marrow, spleen, and lymph node aspirates and peripheral blood smears (2020-2024) were included (n=85). Of six candidate markers (CD3ε, CD5, CD20, CD21, CD79a, and PAX-5), CD20 and CD3ε showed the strongest signal and minimal background reaction on stained smears and were further used for double ICC assay development (n=35). Assay optimization was performed in additional 19 samples. Alkaline phosphatase (ALP) and horseradish peroxidase (HRP), and two substrates were used for chromogenic detection (blue and red).

Results: Normal/hyperplastic lymph node samples showed a heterogeneous lymphoid population of B and T-cells with moderate to strong labeling. Lymphoma and leukemia cases also displayed moderate to marked immunolabeling in most cases and were correctly immunophenotyped based on ancillary tests and/or prior diagnoses.

Conclusion: A double ICC protocol for B- and T-cell immunophenotyping on Romanowsky-stained smears was successfully developed. This technique is applicable to prospective and retrospective studies and can be adapted for different antibodies. Additionally, it enables diagnosis and immunophenotyping from a single smear and is suitable for future development of automated double ICC protocols.

ASSESSMENT OF THE FLUROESCENT DISTRIBUTION AND MORPHOLOGICAL FEATURES IN CANINE LYMPHOMA USING IMAGING FLOW CYTOMETRY: A PILOT STUDY

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Background: Imaging flow cytometry (IFC) combines elements of light microscopy and flow cytometry (FC), allowing assessment of cell morphology and fluorescence patterns at the single-cell level.

Objective: To characterize fluorescent patterns and cellular features in canine lymphoma and correlate these findings with cytology and FC.

Methods**:** Eleven canine lymphoma cases were prospectively enrolled between January 2023 and December 2024. Inclusion required accompanying cytological smears and sufficient residual volume post-traditional FC for analysis with ImageStreamX (ISX) IFC. All cases were positive for either CD5 (T-cell) or CD21 (B-cell) on FC and based on initial IFC observations, two distinct fluorescence patterns (punctate and uniform) were assessed for these antigens. Cell diameter and additional morphological characteristics were evaluated and correlated with cytology findings.

Results: The average cell diameter measured by the IFC was 10.97 µm for large B-cell lymphomas and 11.15 µm for large T-cell lymphomas. On average uniform fluorescence was predominant, though T-cell lymphomas reported a greater proportion of punctate fluorescence. Punctate expression correlated with higher maximum pixel intensity and modulation values, indicating differences in fluorescence signal and texture. IFC was able to detect nucleoli, although its sensitivity was limited when compared to traditional microscopic analysis.

Conclusion: IFC provides morphological insights that partially correlate with conventional cytology, while uniquely distinguishing fluorescence distribution patterns. These features may have clinical implications and warrant further investigation.

Natural Disease Focused Scientific Session II

Chair: Mason Jager DVM PhD DACVP (AP)

LISTERIA MONOCYTOGENES OUTBREAK IN A CLUTCH OF TEXAS HORNED LIZARDS (PHRYNOSOMA CORNUTUM)

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Background: Texas horned lizards (*Phrynosoma cornutum*) have been in steady decline and even extirpated in certain areas of Texas. To increase populations, the Texas Parks and Wildlife Department in collaboration with several zoos have successfully reintroduced zoo-hatched *P. cornutum* back into the wild since 2021. In 2024, a clutch of hatchling *P. cornutum* at Caldwell Zoo experienced an outbreak of sudden mortality secondary to *Listeria monocytogenes*.

Objective: This study describes the gross, cytologic, histologic, and microbiologic results associated with the mortality event due to *L. monocytogenes*

Material and Methods: Complete gross and histologic examination was performed on 24 hatchling *P. cornutum*. Immunohistochemical assays directed toward *Listeria monocytogenes* were completed on 10/24 animals. Cytologic examination of hepatic impression smears was performed in 13/24 animals. A combination of aerobic and Listeria-specific bacterial cultures were conducted on 4/24 animals, all dams, and several environmental samples.

Results: 22/24 (91.67%) of animals had significant histologic changes. Notable changes included necrotizing heterophilic gastroenteritis, hepatitis, and myocarditis. In 18/24 (75%) animals, Gram-positive bacilli were detected in the gastrointestinal tract, liver, bone, and heart. In 10/10 (100%) animals, immunohistochemistry detected *L. monocytogenes* in the liver, gastrointestinal tract, bone, heart, coelom, kidney, and lung. Intracellular bacilli were found on all impression smears of liver (13/13; 100%). Aerobic and Listeria culture isolated *L. monocytogenes* in coelomic swabs, liver, incubator, and hatchling tanks.

Conclusions: *L. monocytogenes* is a rare cause of mortality in the class *Reptilia,* and this study is the first description of an outbreak in hatchling reptiles.

PREVALANCE AND PATHOLOGY OF LIVER DISEASE IN DONKEYS

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Background: Donkeys play a critical role in global livelihoods, with an estimated 51 million animals supporting over 600 million people through agricultural labor and goods transport. Despite their importance, donkey health remains understudied, particularly liver disease—a leading cause of morbidity and mortality in the United Kingdom.

Currently, no species-specific studies detail the pathology or prevalence of liver disease in donkeys.

Objective: To characterize the prevalence and histopathologic features of liver disease in donkeys using diagnostic data and archived tissue samples.

Methods: Serum biochemistry results from 1,200 donkeys submitted to the Cornell Animal Health Diagnostic Center (AHDC) were analyzed to estimate liver disease prevalence, defined as elevations in ≥2 liver-associated markers. Archived liver samples (n=28; 2010–2022) were evaluated histologically for 15 features on H&E-stained sections. Special stains (Masson's trichrome, reticulin, rhodanine, and Prussian blue) were used to assess fibrosis, architecture, copper, and iron.

Results: Liver disease was identified in 20% of serum submissions. Bridging fibrosis was present in 16/28 cases (57%) and associated with hepatic encephalopathy, indicating poor clinical outcomes. Portal infiltrates (17/28; 61%) and periductal fibrosis (13/28; 46%) were also common. Hepatic lipidosis, previously assumed to be frequent, was detected in only 3/28 cases (11%).

Conclusions: Liver disease is common and often severe in donkeys. These findings offer critical insights to improve diagnosis, prognosis, and clinical management in this under-recognized species.

CHRONIC ENTEROPATHY IN DOGS AND CATS: UPDATES AND INSIGHTS FOR 2026

Mark Ackermann
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Chronic enteropathy (CE) (also called inflammatory bowel disease (IBD)) can affect many breeds of dogs and cats but some appear to be predisposed (DOG: Weimaraner, Rottweiler, German Shepherd Dog, Border collie, Boxer, Yorkshire terrier, and French Bulldog, CAT: Siamese/Asian). CE can be classified clinically into four types most common of which is Food Responsive Enteropathy (FRE) followed by Antibiotic Responsive Enteropathy (ARE) (e.g., Microbiota-Related Modulation – Responsive Enteropathy (MrMRE)), then Immunosuppressant Responsive Enteropathy (IRE) and Nonresponsive enteropathy (NRE). Protein Losing Enteropathies (PLE) parallel the above and are Food Responsive-PLE (FR-PLE), Immunosuppressant Responsive-PLE (IR-PLE) and Non-Responsive-PLE (NR-PLE). Many chronic enteropathies of dogs and cats can be managed with dietary modifications antibiotics, corticosteroids, immunosuppressants, monitoring and alternative treatments and some dogs can change from immune-responsive enteropathy (IRE) to food-responsive enteropathy (FRE). Other diagnostic markers are useful adjuncts and there are emerging diagnostics. Clinically, intestinal full-thickness needle core biopsy via laparotomy can be effective and less invasive than standard incision biopsy. Differentiating CE from low-grade intestinal T cell lymphoma (LGITL) in cats and lymphoma in dogs can be difficult even with histopathology and ancillary tests/data. Diagnostic trouble spots I encounter and will discuss handling include: insufficient sampling (e.g., sampling only stomach and

duodenum), including villi but missing crypts, not submitting both fundic and antral areas of stomach, compressed/smashed-appearing villi, limited or hard to read history, lack of information on therapies used, samples from very young animals and classifying villous shortening/widening, crypt hyperplasia, lacteal dilation.

UNDERSTANDING THE METASTATIC OSTEOSARCOMA TUMOR MICROENVIRONMENT USING CROSS-SPECIES SPATIAL TRANSCRIPTOMICS

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Background: Osteosarcoma (OS) is the most common primary malignant bone tumor in both humans and dogs. Despite aggressive treatment, up to 40% of children and over 90% of dogs die due to multi-drug-resistant metastatic disease. Canine and human OS share significant similarities in clinical presentation, histopathology, and molecular aberrations, establishing the dog as a translationally relevant model to accelerate treatment discovery in human OS. Advancements in spatially resolved multi-omic techniques have enabled profiling of the tumor microenvironment at unprecedented resolution, offering greater insight to disease susceptibilities. However, few established frameworks exist for meaningful data integration, particularly in comparative oncology.

Objective: Identify conserved cellular signals in metastatic OS through independent and integrated analyses of canine and human GeoMx datasets.

Method: Archived FFPE lung metastases from canine (n=7) and human (n=5) OS patients were spatially profiled via GeoMx. Macrophage (CD163+ or CD68+), T cell (CD3+), and negative (CD163-/CD3- or CD68-/CD3-) tumor/stroma compartments were captured from intratumoral and extratumoral regions. Data were analyzed independently using GeomxWorkflows and integratively with reciprocal PCA within Seurat, focusing on orthologous genes.

Results: Both independent and integrated analyses demonstrate intratumoral enrichment of immunosuppressive transcripts in metastatic OS across species. Integrated clustering showed strong cell-type segregation independent of species. Intratumoral macrophages were enriched for JAG1, an emerging mediator of immune evasion and treatment resistance, while intratumoral T cells were enriched for Galectin-1 (LGALS1), also linked to immunosuppression in other cancers.

Conclusion: We demonstrate conserved, targetable genes conferring immunosuppression in metastatic OS using a novel, integrated, cross-species spatial transcriptomics approach.

EARLY DIAGNOSIS OF SPLENIC HEMANGIOSARCOMA IN GOLDEN RETRIEVER DOGS USING MULTIPLE SERUM MICRORNA MODELS

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Splenic hemangiosarcoma (sHSA) is one of the most common visceral tumors in Golden Retriever dogs. Diagnosis commonly occurs late in the course of disease. Identification and surgical removal of solitary, non-ruptured, sHSAs provide a longer survival time and improved quality of life as it avoids emergency treatment for hemorrhage. No reliable, easily accessible biomarkers exist for diagnosis of this tumor. MicroRNAs are small, non-coding, RNAs present in circulation and are dysregulated in a variety of tumors at diagnosis. There has been limited investigation in humans and no investigation in dogs to determine if microRNAs can serve as an early diagnostic biomarker of cancer. We quantified microRNAs by RT-qPCR in serum samples from the Golden Retriever Lifetime Study taken at and up to 3 years prior to diagnosis of sHSA (n = 42) and compared them to healthy Golden Retrievers (n = 33). Logistic regression (single and multi-microRNAs) and decision-tree models were investigated. Our multiple microRNA logistic regression model provided an accuracy of 94.8% when trained in samples obtained between 3 weeks-12 months prior to diagnosis. Using this model in the training set, only three dogs were misclassified. When a sample was above the model's threshold in a mixed sample time model, 34/42 (81%) of dogs with sHSA were appropriately classified. Multiple microRNA models using serum samples represent a promising, highly sensitive method of detecting sHSA, but ancillary diagnostics, such as ultrasound, would be required to confirm a diagnosis given the low specificity of the models.

HISTOLOGIC AND IMMUNOHISTOCHEMICAL FEATURES OF CANINE OCULAR GLIOMA

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Background: Canine ocular glioma, primarily oligodendroglioma and astrocytoma, is an uncommon intraocular neoplasm with poorly-described histopathologic features and immunohistochemical profile.

Objective: To characterize the histologic and immunohistochemical features of canine ocular glioma.

Methods: A retrospective search of the COPLOW database for canine ocular glioma retrieved 18 cases that were reviewed by two board-certified pathologists (RT and ADM) for histologic features and diagnosis corresponding to the canine intracranial glioma diagnostic criteria. Immunohistochemistry for OLIG2, CNPase, and GFAP was performed on all cases.

Results: 18 cases were included. Oligodendrogliomas (7/18) were characterized by round nuclei, nuclear rowing, myxoid/mucinous matrix, and nuclear molding. Astrocytomas (8/18) were characterized by oval to elongate nuclei, pleomorphic cells, abundant eosinophilic cytoplasm, disorganized pattern, spindle cell morphology and fibrillar eosinophilic stroma. Undefined glioma (3/18) were characterized by undifferentiated cellular morphology and biphasic phenotype. High-grade features included microvascular proliferation (50%), necrosis (93%) and mitotic activity (88%).

Retinal spread was noted in 2 cases and intraocular seeding in 4 cases. OLIG2 labeling was noted in 7/7 (100%) oligodendrogliomas, 3/8 (37%) astrocytomas, and 2/3 (66%) undefined gliomas. CNPase labeling was noted in 4/7 (57%) oligodendrogliomas, 0/8 (0%) astrocytomas, and 2/3 (66%) undefined gliomas. GFAP labeling was noted in 3/7 (42%) oligodendrogliomas, 8/8 (100%) astrocytomas, and 2/3 (66%) undefined gliomas.

Conclusions: Canine ocular gliomas share histologic features with intracranial gliomas. Novel findings include extensive retinal spread, intraocular seeding, and the presence of OLIG2 labeled cells in the normal canine retina. Immunohistochemistry for OLIG2, CNPase, and GFAP often compliments the histologic diagnosis.

Experimental Disease Focused Scientific Session

Chair: Natalie Fowlkes, DACVP (AP)

ELUCIDATING THE IMMUNOPATHOLOGY OF EPIDERMAL STAPHYLOCOCCUS AUREUS INFECTION IN A NOVEL MURINE MODEL

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Background: Atopic dermatitis (AD) is a common skin disorder in developed countries, affecting 15%–20% of individuals over their lifetime. AD patients frequently experience recurrent skin infections, often associated with colonization by *Staphylococcus aureus* on both lesional (>80%) and non-lesional (~40%) skin. Although several invasive mouse models have been used to study *S. aureus*-induced skin pathology, these models are relatively artificial and fail to fully recapitulate the natural disease process. To address this limitation, we developed a novel topical infection model that mimics AD onset.

Objective: To determine the role of *S. aureus* in promoting skin inflammation and to uncover the underlying immune mechanisms.

Methods: Mice received daily topical application of *S. aureus* USA300 strain to the ear skin for 10 consecutive days.

Results: Hematoxylin and eosin (H&E) staining of ear tissue revealed epidermal hyperplasia and leukocyte infiltration, consistent with inflammatory response. Flow cytometry showed a significant increase in the frequencies of T cells. Notably, $CD4^+$ T cells and γδ T cells exhibited elevated IL-17A production. However, IL-17A-deficient mice displayed a same phenotype to wild-type mice, suggesting that IL-17A is not essential in this context. In contrast, RAG1-deficient mice, which lack lymphocytes, showed reduced ear thickness, implicating a key role for T cells in disease development.

Conclusion: These results suggest that T cells are critical mediators of *S. aureus*-induced skin inflammation, while IL-17A may be dispensable for disease progression. Ongoing studies aim to identify the specific immune cell subsets, cytokines and molecular pathways driving this response.

ANTIBODY SUBCLASS DEFICIENCY ACCELERATES TUMORIGENESIS IN GENETICALLY ENGINEERED MOUSE MODELS OF PANCREATIC CANCER

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Background: Pancreatic cancer is projected to be the 2nd leading cause of cancer death in the United States by 2030, with 5-year overall survival (OS) of 13% . Poor overall prognoses is attributed to reduced vascularization, a dense extracellular matrix (ECM), abundant immunosuppressive cancer-associated fibroblast (CAF), myeloid, regulatory T and B cell subsets within the tumor microenvironment (TME). However, the

presence of tertiary lymphoid structures (TLS) and *in situ* anti-tumor antibody generation correlates with favorable prognosis and increased OS implying an importance of antibodies in anti-tumor immunity.

Objective: Evaluate the impact of antibody deficiency during $Kras^{G12D}$ oncogene-driven development of pancreatic ductal adenocarcinoma (PDAC) using Pdx1-Cre;LSL-$Kras^{G12D}$;$Tp53^{FL/+}$ (KPC) mice.

Methods: KPC mice were bred mice lacking all circulating antibody, or all class-switched immunoglobulin (Ig) (KPC-$\mu S^{-/-}AID^{-/-}$ or KPC-$AID^{-/-}$ mice, respectively) and formalin fixed patient tumor biopsies were evaluated for antibody localization. In mice, changes in stromal, tumor, and immune biology were assessed using gross assessment of tumor development, histology, IHC, IF, ELISA, and FACS.

Results: Antibody subclass deficiency accelerated tumorigenesis (local and metastatic growth) and significantly reduced median survival, which was associated with reductions in ECM density and podoplanin^{+} cancer-associated fibroblast (CAF) abundance. Intratumoral Gr-1^{hi} MDSC and F4/80^{+} macrophage were also increased with antibody subclass deficiency. IF analysis of pancreatic tissues from tumor-bearing mice and patients showed that IgG predominantly localized in the ECM adjacent to podoplanin expressing CAFs.

Conclusions: Genetic ablation of IgG subclass antibodies demonstrates an important anti-tumor role for antibody in regulating pancreatic tumorigenesis and tissue fibrosis.

THE RIP1TAG2 MOUSE MODEL: INVESTIGATING LIPID AND GLUCOSE METABOLISM IN PANCREATIC NEUROENDOCRINE TUMORS

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This study investigated FASN and GLUT-1 expression in the Rip1Tag2 mouse model and human PanNETs. Pancreatic neuroendocrine tumors (PanNETs) are rare, heterogeneous neoplasms characterized by deregulated lipid and glucose metabolism, driven by fatty acid synthase (FASN) and glucose transporter 1 (GLUT-1).

In Rip1Tag2 mice, PanNETs exhibited intense FASN and GLUT-1 expression, which was reduced or absent in normal pancreatic islets. Both markers were more pronounced at the tumor periphery and co-localized with Ki-67, suggesting a link between metabolic activity and proliferation.

Similar patterns were observed in human PanNETs: GLUT-1 was minimally expressed in normal islets but diffusely present in tumor cells. Grade 3 tumors showed significantly lower GLUT-1 expression than Grades 1 and 2 ($p < 0.05$). FASN displayed strong cytoplasmic staining in both normal and neoplastic pancreatic tissue, with consistently higher expression in tumors. FASN positivity exceeded 93% in Grades 1, 2, and

metastatic cases, but decreased to 65.6% in Grade 3 tumors. Staining intensity was significantly higher in Grades 1 and 2 compared to Grade 3 ($p < 0.05$).

The Rip1Tag2 model effectively mirrors the metabolic characteristics of human PanNETs. GLUT-1 overexpression appears to be tumor-specific, whereas FASN upregulation reflects an amplification of pre-existing lipid metabolic activity within pancreatic tissue. These findings validate the Rip1Tag2 model for studying metabolic reprogramming in PanNET tumorigenesis and may be used to identifying targeted therapies.

ESTABLISHING A MURINE MODEL OF RIFT VALLEY FEVER VIRUS (RVFV) ENCEPHALITIS USING THE MP12 STRAIN IN A BIOSAFETY LEVEL-2 SETTING

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Rift Valley fever virus (RVFV) causes recurring outbreaks in Africa and the Middle East. While most human infections are asymptomatic, 8–10% progress to severe disease, including ocular and neurological complications that can result in long-term deficits or death. Due to limited clinical data, a well-characterized animal model is essential to understanding RVFV neuropathogenesis. This study establishes a biosafety level-2-compatible mouse model of RVFV encephalitis using the attenuated MP12 strain. BALB/c mice were infected intranasally and monitored for clinical signs, weight loss, and survival. Samples from mice euthanized at 2, 4, 6, 8, 9, and 10 days post-infection (dpi) were analyzed for viral titers, cytokine expression, and/or histopathology. Symptoms emerged at 7dpi, peaking at 8–10dpi with ~20% weight loss. Histopathology showed lesions in the olfactory bulb, cortex, and hippocampus, peaking at 8–10dpi. Infectious virus was detected in the brain (but not serum or liver) via plaque assay at 8dpi and in moribund mice at 9–10dpi. Viral titers in the olfactory bulb, cortex, midbrain, cerebellum, and brainstem—especially at 8 and 10dpi—revealed broader caudal spread of virus than previously suggested by histopathology, indicating more extensive CNS involvement. Brain cytokine gene expression showed increased MMP9 (9–10 dpi), IL-1β, IFN-β, and CXCL10 (8–10dpi), indicating robust neuroinflammation. Liver cytokines remained unchanged. In conclusion, intranasal infection with the MP12 strain reliably induces encephalitis in BALB/c mice, recapitulating neuropathological features of severe human disease. This model provides a platform for studying RVFV neuropathogenesis and evaluating antiviral therapies under BSL-2 conditions.

SKIN DEEP: CUTANEOUS MANIFESTATION OF GVHD IN HUMANIZED NSG AND NBSGW MICE

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Immunodeficient mice such as NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) and NOD.Cg-*Kit*$^{W-41J}$ *Tyr*$^{+}$ *Prkdc*^{scid} *Il2rg*^{tm1Wjl}/ThomJ (NBSGW) strains are widely used in biomedical research when humanized models are needed. A cohort of 9-month-old, female, CD34+ humanized mice (33/38 NSG and 3/93 NBSGW) presented with severe hair loss, poor body condition score (2/5), and dry, thickened skin with crusting lesions. Mice were housed adjacent to, and shared an anteroom with, a cohort of immunodeficient mice that had recently tested positive for *Corynebacterium bovis.* Affected NSG and NBSGW mice were humanely euthanized and submitted for diagnostic pathology evaluation. Gross examination revealed poor body condition, extensive scaling, and severe hypotrichosis. Microscopically, the mice had variable epidermal hyperplasia and hyperkeratosis accompanied by interface dermatitis and replacement of hair follicles by fibrosis. Multifocal epidermal erosions and ulcerations with crust formation were also present. Intralesional bacteria were not observed in any of the mice. NSG mice had mild, multifocal perivascular mononuclear inflammatory cell infiltrates in the lung. The other examined tissues were grossly and microscopically unremarkable. Infectious disease PCR testing performed at Charles River Laboratories was negative for *C. bovis* and all other agents included in the panel. Given the constellation of tissue changes and absence of an infectious cause, the diagnosis of chronic graft-vs-host disease (cGvHD) was made. Of note in this case, while NSG mice are reportedly stable for one-year post-engraftment, these mice developed disease at 9 months old. GvHD should therefore be considered in humanized immunodeficient mice that develop skin lesions less than one-year post-engraftment.

STAPHYLOCOCCUS AUREUS SUPERANTIGENS TARGET SEBACEOUS GLAND MATURATION TO PROMOTE SKIN PATHOGENESIS AND COLONIZATION

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Atopic dermatitis (AD) is a chronic, itchy, and inflammatory skin disorder that affects children and adults with increasing prevalence. AD is associated with cutaneous *S. aureus* dysbiosis, with *S. aureus* abundance correlating with disease severity and increased barrier disruption via virulence factor production (e.g., proteases, superantigens). Although expansion on the skin precedes disease flares, the mechanisms S. aureus employs to disarm skin defenses and enable pathogenic colonization are poorly understood. Using a mouse model of AD-like skin inflammation induced by epicutaneous application of *S. aureus* to the shaved and depilated dorsal skin of C57BL/6J mice for 7 days, we found that mice exposed to toxic shock syndrome toxin (TSST) superantigen developed increased epidermal thickening, cutaneous immune cell recruitment, and serum IgE production than mice exposed to TSST-deficient *S. aureus*. RNAseq analysis of infected skin revealed TSST-mediated suppression of sebocyte maturation and lipid synthesis genes, which functionally corresponded to a three-fold reduction in mature sebaceous glands in the skin and increased *S. aureus* colonization. Excitingly, we found that multiple S. aureus superantigens including TSST, staphylococcal enterotoxin B, and staphylococcal enterotoxin C suppressed sebaceous gland numbers in the skin, suggesting a

conserved interaction between superantigens and sebaceous glands. Collectively, our data demonstrates that S. aureus superantigens disrupt sebaceous gland maturation leading to reduced numbers of sebaceous glands in the skin and increased S. aureus colonization. These findings provide the foundation for new therapies that target S. aureus skin colonization to treat patients with AD and other skin disorders with S. aureus involvement.

IDENTIFICATION OF DISEASE-ASSOCIATED POLYOMAVIRUSES FROM CYNOMOLGUS MACAQUES UNDERGOING STEM CELL TRANSPLANTATION

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BK polyomavirus (BKPyV) contributes to major complications after hematopoietic stem cell transplantation (HSCT) in humans, with limited therapeutic options. Similar complications occur in Mauritian cynomolgus macaques (MCM, *Macaca fascicularis*) after HSCT, including hemorrhagic cystitis and nephropathy, and an associated polyomavirus was previously identified by PCR without full characterization.

One spleen and 80 urine samples were analyzed from 17 MCM post-HSCT and 42 control cynomolgus macaques. Five MCM post-HSCT had urinary clinical signs +/- nephritis/cystitis post-mortem. Short-read sequencing results of rolling circle amplified urine DNA were de novo assembled and annotated for viral sequences, which identified three polyomaviruses.

MafaPyV2 was most frequently identified and is the closest relative to BKPyV known to date. SV40-type-IIB is a new, distinct strain of SV40-type-II, a rare SV40 type identified in a few rhesus macaques. MafaPyV3 is most closely related to human WU polyomavirus and is shed overall at much lower relative loads. MafaPyV2 and MafaPyV3 were found 1.5-times as frequently and shed at higher relative loads in post-HSCT animals compared to controls, while SV40-Type-IIB was found at similar prevalence between groups but shed at higher relative loads in post-HSCT animals.

All three viruses were identified in post-HSCT animals that presented with urologic disease and multiple polyomaviruses were frequently identified in a single urine sample. Additionally, immunoreactivity for T antigen was identified in the kidney and spleen of one overtly clinical animal with SV40-type-IIB. This MCM model offers opportunities to study analogs of polyomavirus-associated disease in humans and evaluate interventions, such as vaccines and antivirals.

PATHOLOGY OF INTRADERMALLY INOCULATED LA CROSSE VIRUS IN A MOUSE MODEL

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La Crosse Virus (LACV), an arbovirus within the California serogroup of Peribunyaviruses, is a common cause of childhood encephalitis in the United States. LACV is primarily transmitted by the eastern treehole mosquito, *Aedes triseriatus*, although other species of *Aedes* mosquitos may also be capable of transmission. Until recently the role of mosquito saliva in LACV pathogenesis has not been extensively studied. In previous studies, LACV was found to infect the panniculus carnosus (PC), a subcutaneous muscle underlying regions of the skin. To investigate a potential role of the vector in virus infection of the PC, LACV was inoculated with and without mosquito saliva proteins and tissues were collected at 2 days post-infection (dpi) or when mice reached humane euthanasia criteria. Tissues were processed for histopathologic evaluation or plaque assays to assess viral load. Lesions associated with viral encephalitis were found throughout the brain, in conjunction with viral distribution to the brain at the time of humane euthanasia but not 2 dpi. LACV was isolated from the eyes of mice at the time of humane euthanasia but was not associated with lesions. Mosquito saliva did not significantly affect the viral load or lesion development in these tissues of animals at humane euthanasia endpoints. Despite previous evidence of LACV infecting the panniculus carnosus at the site of inoculation and the presence of virus at humane euthanasia, there was no evidence of muscular degeneration or myositis associated with LACV.

EARLY-LIFE LUNG DISEASE IN A NOVEL PIG MODEL OF PRIMARY CILIARY DYSKINESIA

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Primary ciliary dyskinesia (PCD) is a condition that can be caused by mutations in over 50 different genes and has an incidence of 1:15.000 – 1:30,000 human births. People with PCD have impaired airway cilia motility that disables effective mucociliary transport. This loss in host defense is assumed to contribute to respiratory distress seen as early as the first day of postnatal life, but the initial appearance of lung lesions as not been well-defined due to PCD diagnosis often occurring later in life. To better understand early PCD pathogenesis and lesions, we recently produced a DNAI1 deficient pig as a novel model of PCD. Newborn pigs were euthanized at birth to postnatal day two and the lungs collected for pathology studies using HE and dPAS stains. Wildtype lungs had many bronchi that lacked luminal mucus, but a few had minor rims of wispy mucus along the bronchial walls. PCD bronchi had more detectable mucus than wildtype including airway accumulation causing partial obstruction of airways. Regions of lung with several affected airways had retrograde mucus accumulated in scattered pockets of alveoli. Rims of dPAS+ mucus were sometimes accompanied by a layer of pale eosinophilic liquid and sloughed cellular debris in PCD airways. By the second postnatal day, evidence of airway mucus, localized bronchopneumonia, and air-trapping were increased in the PCD groups. Our studies suggest that defective mucociliary transport and its lesions are present by birth, and that subsequent postnatal airway infection and air trapping likely contribute to early clinical disease.

Poster Presentation: Clinical Pathology

1: OOMYCETE INFECTIONS IN TWO DOGS: CYTOLOGY, HISTOPATHOLOGY, AND HISTOCHEMICAL STAINING WITH STRIKINGLY DIFFERENT CLINICAL PROGRESSION AND PROGNOSES

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Oomycetes, including *Lagenidium* and *Pythium* species, are emerging, aquatic, dimorphic water molds found in tropical and subtropical regions. We report two dogs presented to The Ohio State University College of Veterinary Medicine with chronic non-healing cutaneous wounds. Case 1 (ASVCP-2024) was a 1-year-old female German Shepherd with a history of swimming in Georgia. Case 2 was a 2-year-old female miniature poodle with a travel history to Oklahoma. Fine-needle aspirates (FNA) of the cutaneous lesions revealed broad (7-10 µm, Case 1 and 5–7 µm, Case 2), poorly septate, hyphal-like structures with non-parallel sides, most consistent with oomycetes. Histopathology showed marked eosinophilic and pyogranulomatous deep dermatitis with numerous bulbous, poorly septate fungal hyphae (6–15 µm, Case 1, and 2-8 µm, Case 2) that were visualized with Grocott-Methenamine Silver staining. Anti-*Pythium* MiraVista Sandwich Enzyme-Linked Immunoassay was negative in Case 1 and positive in Case 2. Polymerase chain reaction with sequencing on formalin fixed paraffin embedded tissue from Case 1 identified *Lagenidium giganteum* forma *caninum*. Case 1 developed multifocal lymphadenopathy within 3 months, and FNA cytology showed hyphae-like structures in multiple lymph nodes. A computed axial tomography (CT) scan showed further spread of disease throughout the abdomen and lymph nodes. Surgical control was not feasible, and palliative care was recommended. Case 2 underwent hindlimb amputation and antifungal treatment. Initial and recheck abdominal and chest CT were unremarkable for disease outside the visible lesions. This report highlights cytological and histopathological characterization and variable prognosis of oomycete infections.

2: MORPHOLOGICAL AND CYTOCHEMICAL FEATURES OF BLOOD CELLS FROM THREE SPECIES OF PALM PITVIPER SNAKES (GENUS: BOTHRIECHIS)

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Background- *Bothriechis* (*B.*) is a genus of venomous snakes widely distributed and responsible for 7% of envenomation cases in Costa Rica. Certain species are kept in captivity for venom research. Hematologic evaluation of captive reptiles is important for health assessment, but there is a lack of knowledge on basic hematology of this genus.

Objective- To describe blood cell features in *B. lateralis*, *supraciliaris* and *nigroadsperus* with routine hematologic and cytochemical stains.

Methods- We obtained blood samples from 16 captive snakes in Costa Rica. We described cell morphology and leukocyte proportions in modified Wright-stained smears and leukocyte staining characteristics with eight cytochemical stains: alkaline phosphatase (ALP), alpha-naphthyl butyrate esterase (ANBE), Luna, Luxol Fast Blue (LFB), myeloperoxidase (MPx), periodic acid-Schiff (PAS), Sudan black B (SBB), and toluidine blue (TB).

Results- Cell morphologic features, leukocyte proportions and cytochemical reactions were similar between the species. Lymphocytes were the most abundant leukocyte, followed by azurophils. Lymphocytes were variably positive for LFB but negative with the other stains. Azurophils were positive for ANBE, MPx, LFB, SBB, and PAS. Eosinophils and heterophils stained with Luna, LFB, and PAS, although staining pattern (granule versus cytoplasm) differed. Basophils were positive for ANBE, LFB and PAS, with variable TB positivity in *B. lateralis* while being negative in the other species.

Conclusions- Our results will aid in assessing health and disease status in these three snake species, by providing guidance on stain use for leukocyte detection in cytologic and histologic specimens and helping with determination of cell lineage in hematopoietic neoplasia.

3: CYTOLOGIC DIAGNOSIS OF TWO FELINE COLONIC DUPLICATION CYSTS

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Two feline colonic duplication cyst cases were recently diagnosed using fine needle aspiration cytology at the Mizzou VHC. Colonic duplication cysts are a type of enteric duplication, which refers to a segment of the GI tract that is duplicated either partially or completely. These rare congenital anomalies can arise anywhere in the gastrointestinal tract, share a common blood supply with the surrounding alimentary tissue, and most often do not communicate with the intestinal lumen. In small animals, enteric duplication cysts (EDCs) may be subclinical or associated with anorexia, depression, vomiting, melena, a palpable abdominal mass, and gastrointestinal obstruction. Ultrasound evaluation may prove challenging due to variations in shape and wall thickness and potential for ingesta associated artifact, and may require supplemental diagnostics. Cytology may prove a useful tool for initial diagnosis. Cytologic evaluation in both cases reported revealed slides of low to moderate nucleated cellularity and mucinous fluid backgrounds. Intact cells were predominantly macrophages, including rare multinucleated forms. Enteric duplication cyst was considered as the top differential based on cytologic findings and provided history in both cases. Follow up histopathology on one case confirmed a diagnosis of colonic duplication cyst. The other case was lost to follow-up, however imaging and cytologic findings were considered diagnostic. The prognosis of intestinal duplication cysts is good, but recurrence can

occur if the cystic lesion is incompletely excised and rare cases of malignant transformation have been reported. Greater knowledge of the cytologic findings of EDCs can help clinical pathologists recognize this rare condition.

4: PULMONARY PARAGONIMIASIS IN A DOMESTIC CAT

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An 8.5-year-old spayed female domestic shorthair cat presented for evaluation of a chronic, non-productive cough of at least four years' duration and a lung mass. Thoracic CT revealed a fluid-filled lesion in the caudodorsal aspect of the left caudal lung lobe with diffuse bronchial thickening. Differential diagnoses included feline asthma, bronchitis, neoplasia, and infectious disease. Bronchoalveolar lavage (BAL) was performed for cytologic evaluation. Cytology revealed moderate mucus, marked necrosis and cellular degeneration, marked inflammation composed predominantly of neutrophils with fewer macrophages, and scattered degenerate respiratory epithelial cells. Notably, several large (~98 × 54 µm), oval, golden-yellow operculated structures were observed, morphologically consistent with trematode ova. Although pollen contamination could not be excluded cytologically, *Paragonimus kellicotti* was suspected. Diagnosis was confirmed via centrifugal fecal flotation using Sheather's sugar, which revealed many *P. kellicotti* eggs. Additional testing, including Mycoplasma PCR, bacterial culture, heartworm antigen/antibody testing, and urine Blastomyces antigen testing, was negative. The life cycle of *P. kellicotti* involves aquatic snails and crayfish or crabs as intermediate hosts. Cats become infected by ingesting raw crayfish/crabs containing encysted metacercariae. Juvenile flukes migrate through the peritoneal and pleural cavities to reach the lungs. Here, adult flukes release operculated eggs, completing the cycle. Clinical signs are often nonspecific and include chronic cough; radiographic findings may mimic neoplasia (cystic masses) or inflammatory pulmonary disease. This case highlights the diagnostic utility of BAL cytology in identifying parasite eggs and directing confirmatory testing. *P. kellicotti* infection should be considered in cats with chronic cough and pulmonary masses.

5: VALIDATION OF THE AUTOMATED HEMATOLOGY ANALYZER SYSMEX XN-1000V™ FOR EDTA WHOLE BLOOD EVALUATION IN HAMSTERS

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Introduction: The Sysmex XN-1000V™ hematology analyzer provides multispecies complete blood counts (CBC) with differential. Standard operation mode requires 88ul of whole blood, low aspiration mode (50ul), low white blood cell mode (88ul), and predilution mode (20uL of blood diluted 1:7). This instrument provides a useful alternative for species with small whole blood volumes.

Objective: Validation of the Sysmex XN-1000V™ for laboratory hamsters using the different modes, including a comparison with manual leukocyte differentials.

Design: Whole blood samples were collected from twenty clinically healthy Golden Syrian male and female hamsters. Blood and reticulocyte smears were prepared for microscopic leukocyte and reticulocyte assessments.

Results: The method showed acceptable precision in whole blood mode for all CBC parameters: Intra-assay (CV ≤6.2%), inter-assay and accuracy (CV ≤4.5%), linearity of dilution (r= 0.99-1.00, slope =0.99-1.02). Absolute bias of Sysmex leukocyte differential (whole blood, low white blood cell, low aspiration and pre-dilution modes) versus manual correlation was: 4.9-7.2 % neutrophils, 5.0-6.0 % lymphocytes, 4.7-6.1 % monocytes, 0.7-0.8 % eosinophils, 0.5-0.8 % basophils, and 1.0-1.4 % reticulocytes. Whole blood stability was 8 hours at room temperature and 3 days at 4°C. Low aspiration and low white blood cell modes provided similar results. Monocytes did not correlate well to microscopic evaluation and a 50/50 monocyte/lymphocyte gating adjustment was made to the hamster analyzer setting for better correlation. Reference values were generated.

Conclusion: The Sysmex XN-1000V™ provides reliable data for assessment of hamster whole blood. A gating adjustment is needed to the hamster analyzer setting for leucocyte differential.

6: LYMPHOCYTE CLONALITY TESTING IN FELINE INTESTINAL LYMPHO-PLASMACELLULAR INFILTRATION: FRIEND OR FOE?

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Background. Lymphoplasmacytic enteropathy and alimentary small cell lymphoma are most common gastrointestinal diseases in cats. Clonality testing (PARR) detects clonal B- or T-lymphocyte populations.

Objective. In this retrospective study on feline chronic enteropathy, PARR was added to the diagnostic work-up. We asked if analyzing the CDR3 repertoire of the B- and T-cell receptors can be seen as a suitable stand-alone method.

Methods. Based on histopathological findings of mild or moderate lymphoplasmacytic intestinal infiltration, 34 archived small intestine feline patient samples were analyzed. Samples were assayed by clonality testing, together with immunohistochemistry (CD20, CD3), and histopathological examination.

Results. None of the 34 samples subjected to clonality testing showed B-cell clonality, whereas in 23 cat patients, T-cell clonality was detected. In a lymphocytic-plasmacytic inflammation, the infiltration is usually confined to the *Lamina propria mucosae* and whereas in lymphoma, cells often infiltrate beyond the mucosa. Mild to moderate lymphoplasmacytic infiltration of the *Tela submucosa* was considered as potential indication for early lymphoma, hence explaining the high number of monoclonal PARR results. In 15 out of 34 cases, mild lymphoplasmacytic infiltration was confined to the

Lamina propria mucosae and 80 % of these samples showed a 50:50 ratio of T- and B-cells.

Conclusion. This study underlines that PARR can be used as adjunct diagnostic approach in feline patients with chronic enteropathy. However, this technique is far from being a stand-alone tool and the obtained results must be interpreted in the context with the patient's history, clinical status and histopathological examination.

7: SAMPLING SCHEME AND CORRECTION FORMULA DETERMINATION FOR IOHEXOL-DETERMINED GLOMERULAR FILTRATION RATE IN PUPPIES WITH AND WITHOUT X-LINKED HEREDITARY NEPHROPATHY

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Background: Iohexol plasma clearance is a common method for estimating glomerular filtration rate (GFR) to assess kidney function in dogs. To most accurately determine GFR, an 8-point sampling method is required. Sampling schemes that require fewer post-iohexol blood draws are possible, but this requires application of a correction formula. Sampling schemes with associated correction formulas have been previously developed for adult dogs but not for puppies.

Objective: To determine the best sampling schemes and correction formulas in a population of puppies with and without X-linked hereditary nephropathy (XLHN).

Methods: GFR was determined post-iohexol administration based on 8-point sampling measurements (5, 15, 30, 60, 120, 180, 240, and 360 minutes) using WinNonlin and a non-compartmental analysis, wherein the trapezoidal rule was applied to determine the area under the curve. GFRs were normalized to dog (mL/min/dog). Using the 8-point GFR as the response variable, multiple potential correction formulas were generated via linear regression models, where uncorrected GFR determinations were the explanatory variable (1-, 2-, 6-hour; 1-, 4-, 6-hour; 1- and 6-hour; and 2-, 3-, 4-hour).

Results: For GFR determination in these puppies, all population-specific correction formulas out-performed previously generated correction formulas for adult dogs. Sampling schemes varied by age; however, the widely accepted 2-, 3-, and 4-hour sampling scheme consistently produced GFRs that corresponded the least well with the 8-point WinNonlin-generated GFRs.

Conclusions: This study highlights the importance of using population-specific correction formulas to attain the most accurate GFR and that the standard sampling scheme might not be ideal for every population.

8: DETECTION OF PUTATIVE B REG CELLS ASSOCIATED WITH PERSISTENT LYMPHOCYTOSIS IN BLV INFECTED COWS: PRELIMINARY RESULTS

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Persistent lymphocytosis (PL) is the hallmark of bovine leukemia virus (BLV) infection which is considered a susceptibility trait, and related to BLV-induced immune suppression in dairy cows. Previously, significantly lower levels of IL12, IFNg, and IL4 were associated with high expression of TGFb and IL10 in BLV^+PL^+ cows compared to BLV^+PL^- and BLV^- animals. Hypothetically, polyclonal IgM^+, $CD5^+$ B target cell proliferation may enrich a subpopulation of TGFb- and IL-10-secreting B regulatory cells (Bregs) whose phenotype is similar to that of the BLV target cells, promoting immune suppression. To identify cells with Breg phenotype, 38 cows were grouped as follows: BLV^-PL^- (n= 10), BLV^+PL^- (n= 14) BLV^+PL^+ (n=14). Then, six monthly blood samplings were completed to perform routine lymphocyte counts, and PBMC phenotyping (IgM, CD5 and IL10 or TGFb) by flow cytometry. IL10-, and TGFb-secreting B cells were detected throughout the experiment, being predominantly $CD5^+$, but $CD5^-$ were identified as well. These populations were significantly higher in the BLV^+PL^+ group than in the BLV^+PL^- and BLV^-PL^- groups. In addition, the TGFb-secreting B cells were considerably higher compared to the IL10-secreting B cell, in both CD5+ and CD5- populations, being more remarkable in the former. Our data suggest that IL10-, and TGFb-secreting B cells might have a key role in the BLV-induced immune suppression described in cows with the outcome of PL, and that TGFb secretion might have a distinct effect in the BLV infection. Further studies must be performed to thoroughly characterize the putative Breg population.

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9: REFERENCE INTERVALS FOR RENAL BIOMARKERS IN FREE-RANGING CALIFORNIA SEA LIONS (ZALOPHUS CALIFORNIANUS)

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Background: Renal disease is common in stranded California sea lions (*Zalophus californianus;* CSL) and is typically assessed using serum creatinine (SCR) and blood urea nitrogen (BUN). Use of other renal biomarkers such as symmetric dimethylarginine (SDMA) and urine protein: creatinine ratio (UPC) is rare, and reference intervals (RIs) were not available.

Objective: Establish RIs for SCR, BUN, and SDMA, UPC, and urine microalbumin (uALB) in healthy free ranging CSLs, and evaluate effects of long-term freezing.

Methods: SCR, BUN, and SDMA, UPC, and uALB were obtained from 113 apparently healthy CSLs. RIs were computed following ASCVP guidelines. Analyte stability was determined by comparing results from fresh vs frozen samples for 13 additional CSLs.

Results: RIs were established as follows: BUN: 12.49-50.22 mg/dL; SCR: 0.50-1.44 mg/dL; serum SDMA: 3.67-17.83 µg/dL; UPC 0.1-1.8; uALB: 0.35-26.51 mg/dL. Sample handling (fresh vs freeze/thaw) did not significantly affect SCR (p=.65) or SDMA (p=.18). Although BUN, uALB, and UPC showed slight decreases in frozen samples (mean differences: 0.83 (p=.005), 0.44 (p<.005), and 0.031 (p=.04), respectively), values remained highly correlated (.997, .997, .947, respectively).

Conclusion: CSLs exhibited higher uALB and UPC than domestic animals, independent of sex or age, suggesting these findings were not attributable to seminal fluid. Freezing affected several analytes, most notably in uALB. Differences between fresh and frozen analyte measurements were generally small and showed strong correlation. Additional studies with more robust sample sizes are needed to determine analyte stability and adjustments for using the frozen sampled derived RIs from the current study.

10: DIRECT ANTIGLOBULIN TESTING FOLLOWING ALLOGENIC RED BLOOD CELL TRANSFUSION OF DOGS

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Background: Immune-mediated hemolytic anemia (IMHA) has been associated with high mortality and morbidity in dogs, and distinguishing IMHA from other causes of anemia is critical for prompt treatment. The Direct Antiglobulin Test (DAT) plays an important role in IMHA diagnosis by detecting autoantibodies and complement attached to RBCs. Anemic patients often receive blood transfusions (potential sensitizing event) before a cause of anemia is diagnosed. The DAT has therefore been deemed unreliable post-transfusion, and often eschewed by clinicians, however, no cited cases or studies support this assertion.

Objectives: To characterize the frequency that transfusion naïve, DAT negative dogs convert to DAT positive, post-allogenic blood transfusion and to characterize the time interval of conversion, to inform clinicians regarding the reliability of DATs for diagnosing IMHA in previously transfused dogs.

Methods: Transfusion naïve dogs requiring blood transfusion have pre-transfusion DAT and major crossmatching performed using polyspecific canine anti-globulin/anti-

complement gel column technology (Alvedia). Patients with a positive pre-transfusion DAT, history of steroids and blood product transfusion, and suspected autoimmune disease are excluded. Samples are collected 24 hourly up to 72 hours post-transfusion, at 1 week, and at 4 weeks post-transfusion for DAT testing, depending on patient availability.

Preliminary Results: In 22 patients, one positive DAT has been detected (T24), which was followed by further negative results (T36, T48). Time points of post-transfusion DATs range from T24 to T792 (33 days).

11: FLOW CYTOMETRIC CHARACTERIZATION OF A NOVEL POPULATION OF T-LYMPHOCYTES EXPRESSING B-CELL ANTIGEN (TEBA)

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Background

Co-expression of T- and B-lineage markers in dogs' lymphoid cells suggests neoplasia. T-zone lymphoma (TZL) typically features CD5+ T cells with low CD21 and absent CD45 expression. However, we previously described non-neoplastic CD5+CD21+dim lymphocytes (TEBA) in reactive lymph nodes (rLNs).

Objective and Methods

This study characterizes TEBA in rLNs and compares their immunophenotypic profile in lymphoma-affected nodes (LSAs) and nodes assessed for minimal residual disease (MRD) post-chemotherapy. Flow cytometric panel included CD5, CD21, CD4, CD8, CD45, and CD25.

Results

Eighteen rLNs, 17 LSAs and 10 MRDs were included. TEBA accounted for about 20 percent of the nonneoplastic population in all groups. In rLNs, TEBA were mainly CD4+CD8- (median 83.9%, range 55.1%-91.2%). In contrast, CD5+CD21- lymphocytes comprised a mixture of CD4 and CD8 subsets. TEBA profiles in MRDs mirrored rLNs, but LSAs showed increased CD4-CD8+ ($p = 0.034$) and CD4+CD8+ subsets ($p < 0.001$). CD25 was more expressed in TEBA (32.4%) than in CD5+CD21- cells (12.3%). CD45-CD5+ cells were found in 50% of rLNs, 67% of MRDs, and 31% of LSAs. Except in one MRD, CD45- cells were primarily TEBA.

Conclusion

Non-neoplastic CD5+CD21+dim lymphocytes exist in dogs and differ from CD5+CD21- counterpart for increased CD4+CD8- subset predominance and CD25 expression. Their altered composition in some LSAs warrants further study for potential prognostic significance. Finally, it is noteworthy that CD45-CD5+ cells are present in individuals

without TZL. This suggests that detecting low percentages of such elements in nodal aspirates is not enough for diagnosing a T-zone lymphoma.

12: OBSERVATIONS ON HEMATOLOGIC AND BIOCHEMICAL DATA IN A COHORT OF CATS WITH GASTROINTESTINAL LYMPHOMAS

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Background. Gastrointestinal lymphoma is the most common hematopoietic malignancy in cats. However, there is no data available describing results of routine hematologic and biochemical data regarding immunophenotypic differences or survival.
Objective. Aim of this retrospective study was to describe differences of routine hematology and biochemical data and their course during follow up between flow cytometric (FCM) immunophenotype, WHO entity, and survival time groups.
Methods. CBCs, Glucose, urea, creatinine, uric acid, total protein, albumin, AP, AST, ALT, GLDH, GGT, bilirubin, bile acid, triglycerides, cholesterol, lipase, LDH, Na, K, Cl, Ca, P, Mg, Fe, Vit B12, USG, UPC were measured in 33 cats suffering from gastrointestinal lymphoma. Differences between FCM immunophenotypes regarding B-cell and T-cell type, WHO entity (Diffuse large B-cell lymphoma, DLBCL; Peripheral T-cell lymphoma, PTCL; Enteropathy associated T-cell lymphoma EATL-I and EATLII), and survival time (ultrashort(1-10days), short(10-100days), intermediate(100-180 days), long(180-356 days), ultralong(>365days)) were investigated.
Results. Regarding FCM and WHO entity no clinically significant differences were found. Short time survivors showed anemia, an inflammatory leukogram and thrombocytosis more frequently than long time survivors. Left shift and an increased myeloperoxidase-index was observed in patients with ultrashort survival time. Hypoproteinemia, hypalbuminemia, and increased LDH-activities was seen in patients with short survival time. Low calcium concentration was seen with ultrashort survival.
Conclusion. The observed changes as the inflammatory leukogram and the anemia can be explained by paraneoplastic inflammation and gastrointestinal disease. The left shift, increased myeloperoxidase index, the pan-hypoproteinemia, hypalbuminemia and decrease of total calcium concentration may indicate a negative prognostic factor.

13: CAN EPCAM DISTINGUISH MESOTHELIAL FROM EPITHELIAL CELLS IN DOGS AS IN HUMANS?

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BACKGROUND

Epithelial cell adhesion molecule (EpCAM) is widely used in human medicine to distinguish epithelial (positive) from mesothelial cells (typically negative) in tissues and

effusions, aiding diagnosis of carcinomas versus mesotheliomas, except for rare epithelioid variants.
In veterinary medicine, distinguishing non-hematopoietic (NH) cells in canine effusions remains challenging due to overlapping morphology and limited markers. Despite its relevance in humans, EpCAM has not been investigated in these canine cell populations.

METHODS
Samples were collected for diagnostic purposes with owner consent; ethical approval was not required (Turin University, protocol 1965–2017). NH cells in cell blocks from 20 canine effusions were classified as epithelial or mesothelial based on cytokeratin (CK), vimentin (VIM), and desmin (DES) detection by immunohistochemistry (IHC). EpCAM expression was then assessed in these samples and in 10 formalin-fixed paraffin-embedded (FFPE) tissues (2 carcinomas, 6 mesotheliomas, 2 reactive hyperplasias) by IHC.

RESULTS
NH cells were classified as mesothelial in 10 samples (9 CK+VIM+DES+; 1 CK+VIM−DES+) and epithelial in 10 samples (8 CK+VIM−DES−; 2 CK+VIM+DES−). EpCAM was strongly expressed in both groups (80–100% positive target cells), with staining intensity of 2–4 in epithelial and 3–4 in mesothelial cells. Localization was predominantly membranous in epithelial cells and mixed membranous-cytoplasmic in mesothelial cells. All FFPE tissues were EpCAM-positive (median intensity: 3.5; range: 2–4).

CONCLUSIONS

Contrary to human data, EpCAM is consistently expressed in canine mesothelial cells, limiting its utility for differentiating carcinomas, mesotheliomas, and reactive proliferations. Further studies, including mRNA-level validation, are needed to clarify its diagnostic and biological role in canine pathology.

14: MRI, CYTOLOGIC, AND HISTOLOGIC FINDINGS FROM A DOG WITH DISSEMINATED PROTOTHECOSIS

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A 6-year-old, female spayed American Cocker Spaniel presented to Mississippi State University College of Veterinary Medicine for progressive vestibular signs. Three weeks prior to presentation, the patient developed a head tilt, nystagmus, and ataxia. On neurological examination, the patient was moderately obtunded, non-ambulatory tetraparetic, and lacked postural reactions in all limbs. Numerous cranial nerve deficits were appreciated. Severe scleritis was noted in both eyes. The right eye revealed white precipitate in the ventral anterior chamber with corneal opacity. MRI findings included moderate hydrocephalus, cerebellar herniation, bilateral chorioretinitis, and retinal detachment. The cranial cervical spine contained a large T2/T2 FLAIR and FS

hyperintense ovoid, central region occupying approximately 75% of the cross-sectional area of the spinal cord. CSF analysis showed moderate eosinophilic pleocytosis with evidence of acute hemorrhage. Rare 5-10 x 3-5 µm, round to ovoid shaped organisms were detected within macrophages and extracellularly. The cytoplasm was extremely basophilic and granular, with a thin, non-staining cell wall. Due to the poor prognosis, the patient was euthanized and presented for necropsy. Gross examination revealed an amorphous, white, free-floating exudates present in both eyes. The meninges were subjectively thickened. Histological evaluation of the brain, pituitary, eyes, kidney, heart, and spleen revealed a marked number of variably sized, unicellular, circular to ovoid organisms measuring approximately 15 μm in diameter. They had a thick, semi-refractile cell wall containing abundant intracellular endospores. The organisms were PAS and GMS positive. PCR with sequencing identified these organisms as *Prototheca zopfii.* Disseminated Protothecosis was diagnosed.

15: DEVELOPMENT OF A DEEP LEARNING ALGORITHM FOR MITOTIC FIGURE DETECTION IN CANINE LYMPHOMA CYTOLOGY WHOLE-SLIDE IMAGES

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Background

Enumeration of mitotic figures (MFs) is routinely performed in cytological samples from canine lymphomas for tumor grading. Since MFs are assumed to be unevenly distributed throughout the specimen, evaluation of a relatively small area (5 fields at 500x) might result in non-representative, non-reproducible counts. Despite this challenge, automated MF detection in cytology whole slide images (WSIs) has not yet been explored.

Objective

We present a deep learning algorithm for MF quantification in canine lymphoma cytology WSIs using a fully convolutional one-stage (FCOS) object detection model with a ResNet-18 backbone.

Methods

Thirteen WSIs (11,276 MF annotations) were used: eight for model development via three-fold cross-validation, five as an independent test set. To evaluate different training strategies, we trained two FCOS models: one using ImageNet-pretrained weights and one using weights from a MF detector trained on hematoxylin-eosin-stained

histopathology images. Training of each model on each fold was repeated three times (18 sessions).

Results

The FCOS-ResNet18 model with ImageNet initialization achieved a mean F1 score of 0.72 (±0.09), precision of 0.61 (±0.13) and recall of 0.88 (±0.02). In comparison, the model initialized with histopathology-derived weights showed a higher F1 score of 0.78 (±0.05), higher precision (0.75 ± 0.1) and slightly lower recall (0.82 ± 0.03).

Conclusions

These findings demonstrate the feasibility of deep learning for MF detection in cytology WSIs, suggesting that transfer learning from histopathology images can improve overall performance. Our approach represents an initial step toward automated MF quantification in veterinary cytology samples, with potential research and diagnostic applications.

16: CYTAUXZOONOSIS IN PARIS: CYTAUXZOON SP. IN AN URBAN CAT

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Background: Cytauxzoonosis, caused by *Cytauxzoon felis*, is a well-known tick-borne disease in cats in America. However, its presence in France is uncommon and the European strain of *Cytauxzoon* sp. has only been described recently. Clinical reports are scarce, but the disease outcome appears to be less severe than *C. felis* infection with some asymptomatic or subclinical forms. The arthropod vector responsible for the transmission remains unidentified in Europe and the parasite appears to circulate among both wild and domestic felids.

Case presentation: A sixteen-year-old FIV-positive cat, followed for a low-grade intestinal T-cell lymphoma and treated with chlorambucil and prednisolone, was evaluated for pale mucous membranes and a heart murmur. A complete blood count showed a marked non-regenerative anemia and a lymphopenia while a blood smear revealed many small forms of piroplasm. PCR testing identified a *Cytauxzoon sp*. and '*Candidatus* Mycoplasma haemominutum' co-infection. Sequencing confirmed a European strain of *Cytauxzoon sp.* The cat was treated with imidocarb and doxycycline, followed by atovaquone two days later. After two additional days, no improvement in anemia and parasitemia was observed, yet the cat was clinically stable and was therefore discharged. One month later, his clinical condition worsened and he died.

Conclusion: This is the second clinical report of cytauxzoonosis in France and the first in an urban area. It involved an indoor cat in the city and the source of infection remains unclear. The cat's immunocompromised status was likely a contributing factor.

Poster Presentation: Experimental Pathology

17: TRANSCRIPTOMIC PROFILING OF DH82 CELLS REVEALS DIFFERENTIAL GENE EXPRESSION IN RESPONSE TO CANINE DISTEMPER VIRUS INFECTION DEPENDENT ON THE MICROENVIRONMENT

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Background: Understanding of the cellular response to therapeutic agents is essential for the development of effective interventions against neoplasms.

Objective: This study describes a comparative transcriptome analysis of canine histiocytic sarcoma cells (DH82 cells) to investigate gene expression changes induced by a persistent canine distemper virus (strain Onderstepoort; CDVOnd) infection under various culture conditions simulating different zones within a solid tumor.

Methods: Persistently CDVOnd-infected DH82 cells and noninfected controls were cultured under control conditions, 1% hypoxia, and starvation and harvested on day 1 (short-term) and day 3 (prolonged exposure). RNA sequencing was performed, and data were processed using standard bioinformatic pipelines. Furthermore, functional enrichment analysis was performed.

Results: Analysis revealed upregulated genes involved in immune and stimulus response in all conditions. Genes associated with cytokine production were upregulated during short-term hypoxia and starvation, those associated with metabolism under short-term hypoxia and those associated with cell cycle under prolonged starvation. Conversely, there was a downregulation of genes associated with tissue development across all conditions. Genes associated with vessel formation were downregulated under short-term hypoxia and starvation, prolonged starvation, and prolonged cultivation of controls. Genes associated with cell adhesion were downregulated in controls and hypoxic cells at both time points and in starved cells after prolonged exposure.

Conclusions: CDVOnd treatment leads to transcriptomic alterations involved in immune responses in all zones of a tumor, inflammation and cell metabolism in the intermediate zone while suppressing angiogenesis and metastasis in all zones of the neoplasm, offering a starting point for future investigations.

18: IMMATURE GLOMERULI IN DOGS WITH GLYCOGEN STORAGE DISEASE TYPE IA NEPHROPATHY

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Dogs with glycogen storage disease type Ia (GSD Ia) are a model for the human disease, in which a similar renal pathology is observed, including progressive focal segmental glomerulosclerosis (FSGS) and tubular glycogen accumulation. Recently, animals from the GSD Ia dog colony at our institution enrolled in adeno-associated virus (AAV) vector therapy studies succumbed to renal disease earlier than historically expected. In addition to previously described microscopic findings, frequent immature glomeruli without additional features of renal maldevelopment were detected. To investigate the association of immature glomeruli with GSD Ia nephropathy, AAV vector therapy, or an unrelated predisposition, renal sections of GSD Ia affected (n = 14: 5 females, 9 males, 0.75-8 years old) and heterozygote (carrier) dogs (n = 3: 1 female, 2 males, 1-3 years old) were retrospectively assessed. Microscopic features evaluated included the frequency of immature glomeruli, features of FSGS, tubular glycogen, and interstitial disease. One of three carriers displayed immature glomeruli at an average rate of 0.4 per 10x field. Three affected dogs displayed no immature glomeruli, six displayed a similar rate to the carrier, while five affected dogs displayed a rate of immature glomeruli at 0.8-1.4 per 10x field. The frequency of immature glomeruli positively correlated to the severity of tubular glycogen accumulation. Though present in a carrier animal, the association of tubular disease with increased immature glomeruli suggests GSD Ia nephropathy might affect glomerular maturation. Wilms tumor protein 1 (WT-1) immunohistochemistry and transmission electron microscopy (TEM) are being conducted to further characterize these initial findings.

19: LAYER-SPECIFIC TRANSCRIPTOMIC CHANGES IN RAT VOCAL FOLDS UNDERGOING ESTROGEN MANIPULATION AND SYSTEMIC DEHYDRATION

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Human and rat vocal folds (VFs) have a comparable three-layer anatomical structure comprising a stratified squamous epithelium, lamina propria (LP), and muscle, and similar extracellular matrix composition of fibrous proteins. VFs are sensitive to hydration and hormonal changes such as estrogen. While systemic dehydration alters gene and protein expression in VF tissue, the interaction between estrogen and dehydration across distinct VF layers remains unexplored. This study utilized 5-month-old female Sprague-Dawley rats as a model for estrogen manipulation and systemic dehydration to investigate the layer-specific transcriptomic responses of VF tissue. Forty-eight rats were assigned to six groups based on hormonal status (intact: control; ovariectomized (OVX): estrogen loss; OVX+estradiol: replacement) and hydration status: intact-euhydrated, intact-dehydrated, OVX-euhydrated, OVX-dehydrated, OVX+estradiol euhydrated, and OVX+estradiol dehydrated. Systemic dehydration was induced by water restriction, confirmed by weight loss and increased renin expression. Spatial transcriptomic analysis revealed that estrogen’s regulatory effects were more noticeable under dehydration, especially in the epithelium and LP. In dehydrated epithelium, estrogen loss upregulated genes related to stress, remodeling, and inflammatory responses, while estrogen replacement upregulated genes related to anti-inflammatory pathways. In dehydrated LP, estrogen loss altered gene expression related to extracellular matrix, whereas replacement downregulated genes related to mesenchymal proliferation. The muscle layer exhibited minimal gene changes

with estrogen manipulation but showed gene downregulation related to metabolic suppression during dehydration, suggesting energy conservation and protection against oxidative stress. These findings suggest a protective role of estrogen in maintaining VF homeostasis during dehydration and demonstrate the value of analyzing molecular responses by VF layer.

20: ANTI-VEGFA, ANTI-CTLA4, AND ANTI-PDL1 TRIPLE THERAPY RESHAPES THE IMMUNE RESPONSE IN PATIENTS WITH PRIMARY LIVER CANCER

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Background

Recent advances in immunotherapy, including anti-VEFGA, anti-PDL1, and anti-CTLA4 antibodies, have emerged as first line options for primary liver cancer patients. Although VEGFA is known to mediate angiogenesis, its effects on anti-tumor immunity are poorly understood. We initiated an open label phase I trial combining all three antibodies in patients with hepatocellular carcinoma (HCC, n=1) and cholangiocarcinoma (CCA, n=6). Although strong clinical responses were observed, most patients exhibited immune-related adverse events (irAEs).

Objective

We aimed to characterize the immunologic landscape induced by triple therapy in both humans and murine models to highlight immunologic changes driving treatment efficacy and irAEs.

Methods

Paired patient tumor biopsies pre- and post- therapy were examined and scored based on severity of tumor-associated inflammation, portal hepatitis, and interface hepatitis. Immune infiltrates and other histologic alterations were evaluated in triple-therapy treated mice with or without subcutaneous CCA tumors. Quantification of major immune subsets in both human and mice tissues was performed using spectral flow cytometry.

Results

Patient tumor biopsies revealed increased mononuclear inflammation post-triple therapy compared with pre-treatment. Flow analysis demonstrated significant increases in non-classical monocytes and Tregs in triple-therapy patients compared with double-therapy (no anti-VEGFA) patients. Naïve mice exhibited increased lymphocytic infiltrates in the liver and kidneys upon triple therapy treatment. With subcutaneous CCA tumors, triple-therapy treated mice expanded T and B cell compartments and decreased suppressive macrophages.

Conclusion

Triple therapy with anti-VEGFA increased inflammatory infiltrates in both human patients and murine models and reshaped the immune landscape by expanding and reinvigorating lymphocytes and macrophages.

21: CANINE TUMOR–BRAIN CROSSTALK: OPTIMIZATION OF A PROTOCOL TO CO-CULTURE TUMOR CELLS WITH BRAIN SLICES

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In dogs, brain tumors are associated with poor prognosis following conventional treatment, making them interesting research topics. Organotypic brain slices (OSCs) provide a highly organized tissue culture system composed of neuroparenchyma, neurons and supporting glial cells, closely mimicking the *in vivo* environment. They offer an excellent platform to study the behavior of tumor cells and immortalized cell lines known to either cause brain tumors or metastasize to the brain.

A Krumdiek tissue slicer was used to obtain OSCs from recently deceased dogs within 1-4 hours postmortem. Subsequently, slices were injected with 40,000 cells of DH82 (canine histiocytic sarcoma), CLBL-1 (canine lymphoma) or D-GBM (canine glioblastoma) cells, using a stereotactic injector. OSCs were fixed after 1, 3 and 7 days of co-culture in 10% buffered formalin. Multiple embedding strategies were tested to visualize tumor cell migration in multiple planes. Serial sections were stained with Hematoxylin and Eosin (H&E) and used for immunohistochemistry.

Expanding tumor cell populations were observed in HE-stained sections after 3 and 7 days of co-culture. Trimming of OSCs after formalin fixation and prior to paraffin embedment preserved structure better than post-embedding trimming and allows better visualization of the invading tumor cells in tissue sections.

OSCs of recently deceased dogs co-cultured with canine tumor cells were successfully used for the investigation of tumor cell migration in a 3D environment that closely resembles *in vivo* conditions. Such setups provide a robust and versatile environment for a variety of oncological experiments without the need of additional *in vivo* transplantation studies.

22: HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL ANALYSIS OF CEREBRAL MICROVASCULOPATHY IN A NEONATAL RAT MODEL OF HYPOXIC-ISCHEMIC ENCEPHALOPATHY

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Background: Hypoxic-ischemic encephalopathy (HIE) is a leading cause of neonatal brain injury, contributing to infant mortality and chronic neurological disabilities in term newborns. Currently, no effective cure exists. Microvessels play a crucial role in forming the blood-brain barrier (BBB), and alterations in cellular and non-cellular components of the BBB may contribute to HIE. Despite their importance, the response of the

developing microvasculature to HIE remains underexplored. **Objective:** Here, we perform a histopathological and immunohistochemical assessment of the microvasculature and surrounding regions in a rodent model of neonatal hypoxic-ischemic (HI) brain injury. **Methods:** The Vannucci model of unilateral hypoxic-ischemic brain injury was performed in postnatal day (P) 10 rats. Brains were harvested at P13 and P17 for histopathology and immunohistochemistry (CD31 and Ki-67). A modified version of a previously reported nine-step scoring system for HIE was used to grade the cerebral cortex, striatum, thalamus, hippocampus, and microvessels. **Results:** Histopathology revealed extensive unilateral neuronal necrosis, neuronal loss, and neuropil rarefaction. Affected areas showed marked microvascular proliferation, with tortuous, small-caliber vessels, endothelial hypertrophy, and increased perivascular gitter cells. Vascular lesions were more severe in animals with higher histopathology scores, and at P13 compared to P17. Immunohistochemistry suggested co-localization of CD31 and elevated Ki-67 expression in infarcted hemispheres showing microvasculopathy. Ki-67 staining was also increased in ischemic regions compared to contralateral, non-ischemic brain tissue. **Conclusions:** These findings highlight the dynamic microvascular response to HI injury in the developing brain and support further investigation into vascular-targeted therapies for neonatal HIE.

23: NOT JUST LIVER CANCER: EXTRAHEPATIC SEQUELAE OF CHRONIC WOODCHUCK HEPATITIS VIRUS INFECTION IN THE LABORATORY WOODCHUCK

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Background: The Eastern woodchuck (*Marmota monax*) is a well-established model for studying hepatitis B virus (HBV) pathogenesis and hepatocellular carcinoma (HCC), due to its susceptibility to woodchuck hepatitis virus (WHV). While hepatic outcomes in this model are well characterized, extrahepatic immune-mediated manifestations are less frequently reported.

Objective: This case highlights extrahepatic immune pathology in a WHV-infected woodchuck, including glomerulonephritis and systemic arteritis, to expand awareness of the broader disease spectrum in this model.

Methods: A 354-day-old female woodchuck experimentally infected with WHV as a neonate was euthanized following clinical signs of abdominal distension. Complete necropsy, histopathology, clinical chemistry and hematology were performed.

Results: Gross findings included prominent ventral subcutaneous edema and tricavitary effusion, consistent with a transudate. Clinical pathology revealed marked hypoalbuminemia. Histologic examination confirmed chronic lymphoplasmacytic hepatitis with hepatocyte dissociation and vacuolar degeneration, consistent with chronic WHV infection. Notably, there was diffuse chronic glomerulonephritis and multifocal proliferative and necrotizing arteritis in the kidneys, pancreas, and bladder. These lesions were consistent with immune complex-mediated disease.

Conclusion: This case illustrates that chronic WHV infection in woodchucks can produce extrahepatic lesions analogous to HBV-associated glomerulonephritis and polyarteritis nodosa in humans. These findings highlight the broader utility of the woodchuck as a model not only for HCC but also for studying less well-characterized extrahepatic immune-mediated complications of chronic hepadnavirus infection.

24: GENETIC AND PATHOGENIC CHARACTERISTICS OF AN EMERGING HIGHLY VIRULENT RECOMBINANT LINEAGE KOREAN CLADE C PRRSV STRAIN (LINEAGE 1J)

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A strain of porcine reproductive and respiratory syndrome virus (PRRSV) was isolated from lung tissue of a pig showing severe respiratory clinical signs from a farm in Gyeongsang province of South Korea. This PRRSV strain, designated as SNUVR220803, was classified within the lineage Korean clade C (LKC, recently reclassified L1J) based on a phylogenetic analysis of the ORF5 gene. Whole-genome sequencing revealed it as a recombinant between Korean Lineage C (LKC) strain K07-2273 and Lineage 5 vaccine strain Ingelvac MLV. The Nsp2 amino acid sequence of this strain features a deletion of four additional amino acids, setting it apart from the typical Korean clades A, B, and C lineages. Inoculated pigs exhibited persisting hyperthermia for 5 days, palpebral edema, and cyanosis. Subsequently, these pigs suffered from severe respiratory distress and cachexia, leading to a mortality rate of 58.3% (7 out of 12 pigs) at 14 dpi. Gross pathology revealed noncollapsed lungs and serous effusion in the pericardial and peritoneal cavities. Microscopic analysis revealed severe interstitial pneumonia, while immunohistochemistry confirmed the presence of PRRSV antigen in the lungs, lymph nodes, thymus, kidneys, and the heart. Additionally, the levels of cytokines were significantly elevated in the plasma of infected pigs. These observations indicate that the LKC recombinant strain, combined with Lineage 5, possesses high virulence and infectivity as characterized by distinctive exudative lesions.

25: ROLE OF GLUCOSE IN EXPERIMENTAL URINARY TRACT INFECTION IN A DIABETIC MOUSE MODEL

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Background: Diabetic individuals are at higher risk of developing urinary tract infections (UTI) compared to healthy people; however, the pathogenesis is poorly understood. Whether diabetes increases UTI risk due to glucose fueled bacterial growth or changes in host-response to pathogen or combination of both remains to be addressed.

Methods: Dapagliflozin was used to induce hyperglucosuria in female adult diabetic, KK-Ay (n = 5-10/group) mice, and healthy, wild-type littermates (n = 5-10/group) prior to transurethral inoculation with uropathogenic *E. coli* (UPEC). Urinary glucose, bacterial

load, neutrophil infiltration, tissue inflammation and remodeling, myeloperoxidase enzyme (MPO), and pro-inflammatory cytokine levels were determined at 48-hour post-infection by colorimetric assay, bacterial plate count, flow cytometry, histopathology and enzyme-linked immunosorbent assay.

Results: Significantly increased in glucose concentration was observed in dapagliflozin-treated diabetic mice compared to vehicle-treated mice. UPEC load was significantly higher in the urinary tract of dapagliflozin-treated hyperglucosuric diabetic mice accompanied by bacteremia, higher degree of cystitis, pyelonephritis and neutrophil infiltrations compared to vehicle-treated mice. Additionally, our results revealed higher bacterial titer in urinary tract of diabetic mice compared to healthy mice. Myeloperoxidase enzyme, and proinflammatory cytokine TNF-α, IL1-β, and IL6 in urine and homogenates of urinary bladder, and kidneys were similar across treatment groups, and between genotypes.

Conclusion: In conclusion, both hyperglucosuria and diabetes-associated pathological changes exacerbate UPEC colonization and UTI severity in diabetic mice.

26: FOAM-BASED DEPOPULATION METHODS OF SWINE DIFFER FROM WATER SUBMERSION BASED ON POSTMORTEM LESIONS AND COMPUTED TOMOGRAPHY FINDINGS

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Objective:

Investigate the extent of fluid incursion throughout the respiratory tract of anesthetized pigs terminated using three different depopulation methods, compared to termination by water submersion.

Methods:

Inclusion criteria included pigs aged approximately 40d. Study occurred over two consecutive days during spring. Pigs were anesthetized and terminated using their assigned **Method:** water-based foam (WBF), high-expansion nitrogen foam (N_2F), carbon dioxide gas (CO_2), or water submersion (H_2O). Respiratory tracts were evaluated three ways: gross examination, histopathology, and computed tomography (CT). Immediately after death, pigs were CT scanned, and opacity changes were scored. After gross examination, representative respiratory tissues were scored microscopically.

Results:

Forty-eight pigs were assigned to four treatment groups of 12 pigs each. All groups had pulmonary petechiae to regional hemorrhages and subpleural hemorrhages. Emphysema aquosum was observed exclusively in H_2O pigs (33.3%, 4/12).

Histologically, CO_2 had 97.8% decreased odds of pulmonary hemorrhage compared to H_2O, after accounting for sex. Compared to all other methods, water submersion had higher odds of increased opacities on CT in several proximal structures and bronchi and pulmonary parenchyma of multiple lung lobes.

Conclusions:

The postmortem lesions and CT opacity patterns associated with WBF and N_2F are dissimilar to H_2O. Foam-based methods involve mechanistic differences from drowning through either environmental or occlusional anoxia, not overt fluid inundation of airways. Foam-based methods are valuable candidates for U.S. swine production and must be separated from the mislabel of drowning. Our findings provide newfound information regarding large-scale depopulation tools for emergency response efforts.

27: YOLK EMBOLISM AND OVARIAN HYPERSTIMULATION SYNDROME IN FEMALE LABORATORY HOUSED AFRICAN CLAWED FROGS (XENOPUS LAEVIS)

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Background

Female African clawed frogs (*Xenopus laevis)* were used for oocyte harvest following induction with human choriogonadotropin (hCG) or pregnant mare serum gonadotropin (PMSG). Increased morbidity (including poor ovulation, bloating, and/or coelomic distention) and mortality were reported over several months. Six deceased or euthanized frogs were submitted to necropsy.

Results

Frogs had varying degrees of subcutaneous edema and/or coelomic effusion (3/6 frogs), free floating oocytes within the coelomic cavity (2/6 frogs), grossly visible intrapericardial, subserosal and retroperitoneal oocytes (2/6 frogs) or luminal occlusion of the oviduct by oocytes and yolk protein (2/6 frogs).

Histologically, granulomatous serositis was present in multiple organs and was characterized by both intact and degenerated oocytes embedded in tissues. Strikingly, intact oocytes and yolk platelets (Fite's and Luxol Fast Blue positive) were present in the cardiovascular system of four frogs, including intramural and coronary vessels.

Discussion

Increased morbidity and mortality were attributed to oviduct impaction, yolk embolism, and ovarian hyperstimulation syndrome (OHSS), a complication of induced ovulation characterized by peritonitis and effusions. In human medicine, OHSS induces capillary leakage by secretion of vasoactive factors. Increased vascular permeability in the affected frogs may have contributed to the dislocation of oocytes into the vascular system. No traumatic events (e.g. dropping, massaging) that may have caused

embolism were reported in these frogs. Further investigations are on-going to determine contributing factors such as underlying disease, handling, or dosage of hCG and PMSG.

28: IN VITRO REPLICATION AND ASSEMBLY OF CANINE AND FELINE CORONAVIRUS AS SEEN BY CONVENTIONAL TRANSMISSION ELECTRON MICROSCOPY

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Background:

Coronaviridae morphogenesis is still not well understood. Significant reorganization of the endoplasmic reticulum (ER) by the virus generates sites for viral replication, followed by assembly within the ER-Golgi intermediate complex (ERGIC). Generally, inoculation of different species-associated coronaviruses within different cell cultures can exhibit varying susceptibility and responsiveness across species. Comprehensive studies regarding feline coronavirus (FeCoV) and canine coronavirus (CaCoV) morphogenesis are scarce and do not detail replication and assembly in full.

Objective:

Using a cell model to explore similarities and differences between the replication and assembly processes of FeCoV and CaCoV.

Methods:

Conventional transmission electron microscopy (CTEM) was performed on an A-72 cell line infected with FeCoV and CaCoV separately.

Results:

Ultrastructurally, both FeCoV and CaCoV replication and assembly compartments are characterized by 1) double membraned vesicles (DMVs) 2) convoluted membranes (CMs) 3) large virion-containing vacuoles (LVCV) 4) presence of mature virions in the ERGIC and 5) extracellular virions.

Conclusion:

A-72 cell models are a successful method for studying FeCoV and CaCoV replication and assembly to demonstrate similar characteristics between alphacoronaviruses in vitro. Findings were consistent with that of SARS-CoV-2 (COVID-19) of the betacoronavirus in vivo, suggesting that in vitro results should be further investigated as

a means to understanding pathogenesis in vivo and advancing therapeutic and detective methods.

29: SCAAV-MEDIATED IL-1RA/IGF-1 THERAPY IN EQUINE POST-TRAUMATIC OSTEOARTHRITIS

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Gene therapy approaches using self-complementary adeno-associated viruses (scAAVs) have been successfully tested in the equine post-traumatic osteoarthritis (PTOA) model. This study aimed to develop a scAAV- based approach to diminish the progression of PTOA in a highly translational equine preclinical model. A 10-week dosing trial identified 5×10^{11} vg as a safe therapeutic dose of scAAVIL-1ra. This dose was further used in this 4-month preclinical trial evaluating the efficacy of intra-articular injections of scAAVIL-1ra or scAAVIGF-I alone or in combination. Thirty-one horses were divided into four groups and treated with scAAVIL-1ra, scAAVIGF-I, a combination of both, or saline (control). Horses were evaluated throughout and at the end of the study. Synovial IL-1ra levels were significantly higher in the scAAVIL-1ra and the combination groups compared to the control. The peak synovial fluid IL-1ra levels in the scAAVIL-1ra and the combination groups were observed at 14 days and 7 days post-treatment, respectively. Correlating with IL-1ra expression levels, the cartilage summation scores were lower (improvement) in the scAAVIL-1ra and the combination group compared to the scAAVIGF-I group. No differences in synovial membrane summation scores were observed across the different treatment groups. These preliminary results suggest that suppression of IL-1β via IL-1ra alone or in combination has a positive treatment effect for PTOA. IGF-I expression alone did not improve cartilage healing. A combinatorial gene therapy approach aimed at reducing inflammation while enhancing the joint anabolic environment may offer a promising gene therapy strategy for PTOA.

Poster Presentation: Industrial and Toxicologic Pathology

30: DEVELOPMENT OF A DEEP LEARNING MODEL FOR SEGMENTATION OF HEPATIC VASCULAR AND BILIARY STRUCTURES FOR QUANTITATIVE ANALYSIS IN VIRAL HEMORRHAGIC FEVERS

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Background: Viral hemorrhagic fevers (VHFs), such as Ebola virus and Lassa virus infections, often cause hepatocellular degeneration, necrosis, and inflammation. However, reports on the zonal distribution of these lesions vary. Mapping lesions relative to bile ducts, arteries, and veins provides a reproducible analysis framework, yet quantifying these structures remains challenging due to morphological complexity and interobserver variability. **Objective:** To develop a deep learning model that accurately segments hepatic vascular and biliary structures in VHF-infected liver tissue, supporting objective spatial lesion mapping. **Methods**: A U-Net–based model was trained to segment bile ducts, arteries, and veins in H&E-stained liver whole slide images (n=86) from VHF-infected non-human primates. Expert annotations served as ground truth. Data were split into training (70%), validation (20%), and test (10%) sets with class balancing by oversampling and random spatial augmentations to increase the diversity of the training data. Loss metrics were tracked across 100 epochs. **Results:** The model showed effective convergence with validation performance stabilizing after approximately 40 epochs. The specificity exceeded **99.5%**, and negative predictive value surpassed **99%** for all structures. The positive predictive value ranged from **81–93%**, with a pixel-wise sensitivity near **70%**. **Conclusion:** This deep learning model reliably segments hepatic structures in infected liver tissue, providing an automated and reproducible method to map lesion distribution in VHFs. Future work will integrate lesion detection (e.g., necrosis), immunohistochemistry, and cross-species applications to refine spatial pathology insights.

31: DEVELOPMENTAL EXPOSURE TO TRICHLOROETHYLENE DISRUPTS DNA METHYLATION AND INDUCES MORPHOLOGIC ALTERATIONS IN ZEBRAFISH (DANIO RERIO)

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Background: Trichloroethylene (TCE), a volatile organic solvent and legacy environmental toxicant, may induce epigenetic toxicity in developmental stages by altering DNA methylation.

Objective: This study assessed whether TCE exposure during zebrafish development affects DNA methyltransferase expression, transgenerational DNA methylation and morphologic traits.

Methods: Zebrafish embryos were exposed to 0, 5, 50, or 500 ppb TCE from 1-120 hours post fertilization (hpf). Larvae were evaluated for morphologic alterations at 120

hpf and then rinsed, pooled, and processed for RNA extraction and cDNA synthesis or genomic DNA isolation. Expression of seven DNA methyltransferase genes was quantified via qPCR and DNA methylation (5-hmC) levels were measured in F0, F1, and F2 generations using ELISA.

Results: Significant changes in gene expression were demonstrated with increased relative expression of *dnmt1* in the 5 and 50 ppb groups and decreased relative expression of *dnmt3bb.2* in the 500 ppb group. 5-hmC DNA quantification indicated significantly lower levels of global DNA methylation in the F0 generation for all exposure concentrations, while the F2 500 ppb had significantly higher 5-hmC than the F0 500 ppb. Significant morphology alterations were observed in the F0 generation with morphology alterations persisting in the F1 and F2 generations, especially in the 50ppb and 500 ppb groups.

Conclusion: Developmental exposure to TCE does change gene expression involved in DNA methylation, with significantly reduced 5-hmC levels at the exposed F0 generation and tendency to transgenerational epigenetic compensation, and morphologic changes at higher concentrations.

32: VALIDATION OF THE AUTOMATED HEMATOLOGY ANALYZER SYSMEX XN-1000V™ FOR EDTA WHOLE BLOOD EVALUATION IN GUINEA PIGS

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Introduction: The Sysmex XN-1000V™ hematology analyzer provides complete blood counts for animal species. Different operation modes are available: whole blood mode necessitating 88ul of blood, low aspiration mode (50ul), low white blood cell mode (88ul), and predilution mode (20ul of blood diluted 1:7). This instrument provides a useful alternative for species with small whole blood volumes.

Objective: Validation of the Sysmex XN-1000V™ for laboratory guinea pigs using the different modes, including a comparison with manual leukocyte differentials.

Design: Whole blood samples were collected from twenty-four clinically healthy Hartley male and female guinea pigs. Blood and reticulocyte smears were prepared for manual leukocyte and reticulocyte assessment.

Results: The method showed acceptable precision in whole blood mode for all parameters: Intra-assay (CV ≤2.2%), inter-assay and accuracy (CV ≤4.5%), linearity of dilution (r= 0.99-1.00, slope =0.98-1.03). Absolute bias of Sysmex leukocyte differential (whole blood, low white blood cell, low aspiration and pre-dilution modes) versus manual correlation was: 3.9-4.5% neutrophils, 3.9-4.7% lymphocytes, 1.1-1.5% monocytes; 0.5-0.8% eosinophils, 0.8-1.6% basophils, and 0.9-1.5% reticulocytes. Whole blood stability was 8 hours at room temperature and 3 days at 4°C. Low aspiration and low white blood cell modes provided similar results. Few discrepancies

were observed in predilution mode: Intra-assay was higher for red blood cell parameters, CV ≤ 7.8%, white blood cell counts, CV= 4.4%, and platelet counts, CV=16.2% (≤10%). Reference values were generated.
Conclusion: The Sysmex XN-1000V™ provides reliable data for assessment of guinea pig whole blood. Intra-assay precision of platelet counts was not acceptable under predilution mode.

33: ENHANCING TOXICOLOGIC PATHOLOGY WITH AI: EFFICIENCY GAINS FROM PATHOLYTIX FORESIGHT V1 IN PRECLINICAL STUDY EVALUATIONS AND THE DEVELOPMENT OF FORESIGHT V2

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Background
Pathology workflows in preclinical toxicology require significant time investment from board-certified pathologists, creating bottlenecks in drug development timelines.

Objective
To evaluate the efficiency gains and diagnostic concordance of AI-assisted pathology evaluation using Patholytix Foresight V1, a suite of pre-trained deep learning models for toxicologic pathology.

Methods
To assess workflow efficiency, six board-certified veterinary pathologists reviewed 105 rat slides from seven organs using Patholytix Study Browser. After a two-month washout period, the same slides were re-evaluated with AI decision support. Review times, findings, and gradings were recorded for both conditions, for the V1 classifiers.

Results
AI-assisted reviews demonstrated a 21% reduction in average review time compared to manual assessment. Diagnostic concordance between AI-assisted and manual reviews remained high, with no significant differences in lesion detection or grading accuracy.

Conclusion
Patholytix Foresight V1 significantly improves non-clinical pathology workflow efficiency while maintaining diagnostic accuracy. The time reduction translates to accelerated evaluation. The qualified classifier suite successfully integrated into the workflow without disrupting existing processes.

As a result of V1's success, additional models have been developed using three AI classifier types: pixel segmentation for area-based lesions, object detection for single-cell findings (mitosis, apoptosis), and density-based approaches for lesions like hepatocellular hypertrophy. The Foresight V2 classifier suite now covers 14 commonly

reviewed organs and 40 frequent lesions, representing 80% of the findings observed in rat toxicity studies.

34: PATHOLOGY FINDINGS IN NONCLINICAL STUDIES EVALUATING A HELIOS CELMOD DEGRADER FOR SOLID TUMOR INDICATIONS

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Background: The discovery of T cell checkpoint inhibitor therapy has transformed the treatment of multiple cancers; however, many patients do not respond or develop resistance to current therapies, underscoring the need for next generation of immune-modulating approaches. Clinically, increased regulatory T cells (Tregs) in the tumor microenvironment (TME) are correlated with worse outcomes in multiple solid tumor indications.

BMS-986449 is a cereblon E3 ligase modulator (CELMoD) degrader of the transcription factors Helios and Eos which are abundantly expressed in Tregs; preclinical models have demonstrated potential for intense BMS-986449-driven Helios degradation in the TME. Additionally, degradation of Helios and Eos can intrinsically amplify cytokine production by a subset of CD8+ T cells. By exerting these effects on T lymphocytes in the TME, BMS-986449 is expected to promote effector T cell activation and increase antitumor immune responses.

Objective and Methods: The safety profile of BMS-986449 was assessed in single- and repeat-dose general toxicology studies in rats and monkeys and included pathology (clinical/anatomic), immunotoxicology, pharmacodynamic, toxicokinetic, and in-life evaluations.

Results: Hematology findings denoted dyserythropoiesis. Ovarian, testicular, and bone marrow toxicity and also pathology findings reflecting immune system activation with no associated T-cell count increases were identified and displayed varying degrees of reversibility.

Conclusion: Nonclinical studies provided adequate safety margins for the initiation of clinical dosing in humans. BMS-986449 administration yielded species differences in toxicity profiles and Helios protein degradation correlated poorly with pathology findings in associated tissues overall. Pathology findings also illustrate the challenge of attributing cereblon-mediated, off- and on-target-mediated effects.

35: CHARACTERIZATION OF THE PORTOSYSTEMIC SHUNT PHENOTYPE AND ITS AGE-ASSOCIATED EFFECTS IN C57BL/6J MICE

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Portosystemic shunts (PSS) in C57BL/6J mice are associated with altered hepatic and neurologic physiology and morphology. This study defines the clinicopathologic features of PSS and assesses the impact of PSS on age-related changes in the liver of C57BL/6J mice. Gross images and HE-stained sections of liver and brain from 45 C57BL/6J mice with PSS (1 – 21 months, median 5 months) and 48 controls (1 – 20 months, median 5 months) were reviewed, and brains were subjected to anti-GFAP and Iba-1 immunohistochemistry. To determine whether PSS increased age-related changes, livers from aged C57BL/6J mice with PSS were histologically scored and compared to controls. Presenting complaints included lethargic, female breeders or aged males exhibiting seizure-like activity. Grossly, livers were small and light brown with pitted capsular surfaces. Histologically, portal areas had portal vein hypoperfusion, hepatic arteriolar reduplication, bile duct hyperplasia, lymphangiectasia, and lobular atrophy. In the brain, Alzheimer type II cells, defined as glial cells with nuclei at least twice the size of adjacent glial cells, greater than 75% chromatin clearing, and chromatin peripheralization, occurred with greater prevalence in PSS mice ($p < 0.0001$) and were more likely to be in the hippocampus than the cerebral cortex ($p = 0.0015$) or basal ganglia ($p = 0.0121$). Iba-1 immunoreactivity was equivalent between groups. GFAP immunoreactivity was significantly reduced ($p = 0.006$) in the brains of PSS mice. In aged C57BL/6J mice, PSS mice had significantly higher hepatic geropathology scores ($p <0.0001$), regardless of sex (females $p = 0.0001$; males $p = 0.006$).

36: UPDATED HISTORICAL CONTROL INCIDENCE OF MICROSCOPIC BACKGROUND LESIONS IN GÖTTINGEN MINIPIGS (2014-2024) FOR TOXICOLOGICAL SAFETY ASSESSMENT

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The Göttingen minipig remains a widely used nonrodent species in toxicological safety assessments. To support accurate interpretation of study findings, updated historical control data are essential for distinguishing background lesions from potential test article-related effects. Microscopic pathology data were compiled from 1,199 untreated Göttingen minipigs (597 males, 602 females) across 144 nonclinical studies conducted between 2014 and 2024 at three Charles River laboratory sites in North America. All animals were sourced from Marshall Farms, Inc., and data were captured using Provantis©. Histopathology terminology was standardized and harmonized using INHAND guidelines, relevant literature, and the global open Registry Nomenclature Information System (goRENI). Background findings are summarized across 13 organ systems. The most frequent microscopic observation included intrasinusoidal erythrocytes in lymph nodes (47.8% males; 53.4% females), inflammatory cell infiltration

in kidneys (22.7%; 22.9%), and pigment-laden macrophages in lymph nodes (20.9%; 22.7%). Additional findings with incidence rates exceeding 2% are also reported. No comprehensive historical control database for Göttingen minipigs has been published in nearly a decade. Given their continued prominence in regulatory toxicology, current reference data are critical for evaluating background pathology. This dataset represents the most extensive recent compilation of spontaneous historical changes in control Göttingen minipigs and provides an essential resource for pathologists and toxicologists involved in nonclinical safety studies.

37: THE LANDSCAPE OF MULTIMODAL LARGE LANGUAGE MODELS (MLLMS) APPLICATIONS IN VETERINARY PATHOLOGY

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Thanks to their adaptability and versatile functionality, large language models (LLMs) are increasingly applied in every discipline, and their integration is transforming the way human users are performing their job responsibilities. Given its reliance on both textual and visual data, the veterinary pathology field is no exception and is well-positioned to benefit from the integration of multimodal LLMs (MLLMs) to streamline workflow and enhance productivity. On the textual aspect of pathology work, MLLMs can assist with supervised data summarization, customized report writing, and interactive knowledge retrieval. The potential benefits of these MLLMs-based tools are user-friendly searchable archiving, standardized report generation with consistent use and automated updating of nomenclature, avoiding jargons, and inclusion of accurate, comprehensive, multidimensional information enabling pathologists to have effective cross-disciplinary communication with other scientists. On the visual front, MLLMs can assist pathologists in high-throughput lesion screening, accurate diagnosis, and consistent grading, while their integrated text-image capabilities offer insights into disease pathogenesis and recommendation for ancillary testing strategies. Overall, it can be concluded that the pattern recognition and report writing aspect of pathology work will be affected the most by the MLLM-based tools. In this regard, we evaluated current capabilities of MLLMs and outline an optimized framework for their application in veterinary pathology.

38: AI-DRIVEN HISTOLOGICAL ANALYSIS OF RAT KIDNEYS: INVESTIGATING THE IMPACT OF REPEATED GLYPHOSATE AND HEAT EXPOSURE IN THE DEVELOPMENT OF CHRONIC KIDNEY DISEASE OF UKNOWN ETIOLOGY (CKDU)

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Advancements in digital pathology have led to significant developments in Whole Slide Imaging (WSI) analysis, particularly through the integration of Artificial Intelligence (AI)

and deep learning algorithms. These innovations offer new opportunities to investigate complex diseases such as Chronic Kidney Disease of unknown etiology (CKDu), an urgent health issue with increased mortality among farmworkers across the globe, for which the underlying cause(s) remain unclear. Here, we established a controlled exposure system in male Sprague-Dawley (SD) rats to model the effects of glyphosate and heat stress exposure on the development of CKDu. To improve the objectivity and precision of histopathological analyses, we employed an AI-based analysis platform (HALO AI) that used customized algorithms to analyze rat kidneys. Briefly, an initial phase involved manual annotations supervised by a trained anatomic pathologist to guide AI in identifying key structures. A second algorithm was designed to combine structural analysis with special stains and immunohistochemistry markers, including PAS and IBA-1, allowing for precise assessment within the different regions of the kidney. Our analysis suggested increased PAS-positive material in glomeruli and decreased cortex area size in animals exposed to glyphosate and/or heat. Moreover, these animals had significantly higher macrophage count and intratubular protein area compared to the age-matched control group.

Together, these AI-driven algorithms enable the objective quantification of subtle histopathological changes that may be difficult to discern through traditional pathology. Future work will focus on expanding the application of these tools to provide reproducible and accurate assessments of kidney disease in clinical and research settings.

Poster Presentation: Natural Disease

39: DISSEMINATED CRYTOCOCCUS NEOFORMANS INFECTION IN A CAPTIVE CHREVROTAIN (TRAGULUS SPP.): A CASE CONFIRMED BY CULTURE, HISTOPATHOLOGY, AND MOLECULAR ANALYSIS

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Cryptococcus neoformans (*C. neoformans*) is an encapsulated, invasive yeast that causes opportunistic infections in humans and animals, primarily acquired via inhalation of environmental propagules. Disseminated infections in wild ungulates, especially chevrotains (Tragulus napu) are exceptionally rare.

This report describes a disseminated *C. neoformans* infection in an 11-year-old male chevrotain housed at the Topeka Zoo, Kansas. The animal showed intermittent respiratory distress over seven days before it was found dead. Necropsy revealed consolidated, multinodular lungs; a globoid heart; moderate serosanguineous abdominal effusion; and mild epaxial muscle atrophy.

Histologic examination identified severe granulomatous, minimally exuding pneumonia with scarring fibrosis and pleuritis containing numerous yeast cells (4–8 µm diameter) encased within 1–30µm clear capsules, often exhibiting narrow-neck budding. Mucicarmine, periodic acid–Schiff (PAS), and Grocott's methenamine silver (GMS) confirmed the presence of encapsulated yeasts. Biventricular dilation with interstitial fibrosis, hepatocellular necrosis, and acute suppurative diaphragmitis were also evident.

Aseptic cultures from lung, brain, spleen, and ocular swabs plated on Sabouraud dextrose agar and tryptic soy agar with 5% sheep blood grew smooth, mucoid, white-to-cream colonies at room temperature. MALDI-TOF mass spectrometry confirmed the causative organism as *Cryptococcus neoformans*.

To our knowledge, this is the first published case of disseminated cryptococcosis in a chevrotain. Although infections in small ruminants and exotic cervids are rare, their occurrence should prompt evaluation of environmental exposure or underlying immunosuppression in zoological collections. Recognizing fungal pathogens in zoo and exotic species is critical for both animal welfare and public health, particularly given shared habitats and the emergence of fungal threats.

40: INTESTINAL LEIOMYOSARCOMA IN A GRANT'S ZEBRA

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An 8-year-old female Grant's zebra with a history of sudden limping on the right front leg was euthanized. On postmortem examination, there were multifocal to coalescing, raised, grey, firm, ~3 cm in diameter, round plaques along the serosal surface of the ileum and colon. There was transmural thickening of the intestinal wall with an irregular mucosa at the level of the ileocecal junction. On cut section, the plaques had a central area of mineralization. Microscopically, the plaques consisted of spindle cells arranged in bundles and streams. Neoplastic cells had strong cytoplasmic immunolabeling for smooth muscle actin and vimentin immunohistochemistry (IHC). IHCs for desmin and C-KIT were negative. On transmission electron microscopy (TEM) the neoplastic cells were elongated and had abundant rough endoplasmic reticulum, intermediary filaments, cytoplasmic and plasmalemmal density with small intercellular anchoring attachments and a discontinue basal lamina, in which no caveolar vesicles were seen. Gross, microscopic, immunohistochemical and TEM results confirmed a diagnosis of intestinal leiomyosarcoma. In horses, intestinal leiomyosarcomas are uncommon and constitute ~9% of all intestinal neoplasms. Clinical signs include chronic abdominal pain, weight loss, fever, depression and anorexia and the mass(es) can result in luminal obstruction. Reports of intestinal tumors in zebras are uncommon and, to the best of our knowledge, this is the first report of an intestinal leiomyosarcoma in a zebra.

41: ASSESSMENT OF INTESTINAL IMMUNE CELLS, ENTERIC NEURONS AND INDOLEAMINE DEOXYGENASE-1 EXPRESSION IN TYPE 2 DIABETIC AFRICAN GREEN MONKEYS (CHOLOROCEBUS AETHIOPS SABAEUS) USING VISIOPHARM ALGORITHMS

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Diabetic enteropathy (DE) is an under-recognized complication of type 2 diabetes mellitus (T2D) that is linked to enteric nervous system dysfunction. T2D is a chronic inflammatory disease, studies have shown that chronic inflammation contributes to enteric neuropathies. In T2D patients, tryptophan metabolism is increased via indoleamine 2, 3 dioxygenase-1 (IDO1). It is regulated by inflammatory cytokines. The role of tryptophan metabolism in immune signaling of T2D patients and its effect on the enteric nervous system have not been very well studied. African green monkeys (AGMs) spontaneously develop T2D with age. We hypothesized that IDO1-driven chronic intestinal inflammation contributes to enteric neuronal loss in diabetic AGMs. To study this, we used small and large intestinal samples from five female AGMs withT2D and age-matched nondiabetic controls using immunohistochemistry to detect immune cells (CD3, CD4, CD8, CD20, CD68, and FoxP3), enteric neurons (NSE and GFAP) and IDO1 expression. Immunostains were quantified using basic and deep learning Visiopharm algorithms. The results demonstrated that there were no significant differences in the numbers of immune cells, enteric neurons or IDO1 expression in diabetic AGMs compared to controls. However, the basic algorithm demonstrated a higher trend in the immune cell population, NSE and IDO1 expression in the mucosa of small and large intestine. This was not observed in deep learning algorithm data, which demonstrated the spatial distribution of the cells and IDO1. Although nonhuman

primates are great translational models of T2D, establishment of AGMs as models of DE, would require additional studies with larger sample sizes.

42: THEILERIA CERVI DETECTIONS AND ASSOCIATED PATHOLOGY IN WHITE-TAILED DEER, ELK, AND MULE DEER IN THE EASTERN AND MIDWESTERN UNITED STATES

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Background: *Theileria* spp. are vector-borne, protozoal parasites. *Theileria cervi* is transmitted by the lone star tick (*Amblyomma americanum*) and circulates in wild deer throughout much of North America. *T. cervi* prevalence is poorly understood and reports of associated disease in deer are rare.

Objective: To document clinical and subclinical cases of *Theileria* spp. infection in cervids.

Methods: A retrospective review of diagnostic data from the Southeastern Cooperative Wildlife Disease Study (SCWDS) was conducted to identify cervid cases with *Theileria* spp. detection from 2014-2024 (n=10). Inclusion criteria were those with *Theileria* sp. detected via PCR, with or without morphologically consistent protozoa on histopathology. Species included white-tailed deer (*Odocoileus virginianus*), elk (*Cervus canadensis*), and mule deer (*Odocoileus hemionus*) from Georgia, West Virginia, Missouri, and Nebraska; all were wild except for one captive white-tailed deer.

Results: *Theileria* spp. were detected via PCR in spleen and/or liver (7/10) and brain (3/10). Detections occurred in adults (5/10), fawns (4/10), and a juvenile (1/10). Histologically identified piroplasms were limited to fawns (2/4). Meronts, merozoites, and zoites were visualized mostly in the liver (3/5) and spleen (2/5) and also in brain, skin, lymph node, and bone marrow of juveniles and fawns (4/5). Lesions attributed to *Theileria* spp. infection included hepatitis/hepatocellular necrosis (4/10) and icterus (1/10); comorbidities included pneumonia (5/10), and 4/10 were in poor to emaciated nutritional condition.

Conclusion: *Theileria* spp. infection in cervids manifests both clinically and subclinically. Tracking vector-borne diseases in wildlife and domestic animals is imperative, particularly in light of changing environmental conditions.

43: A SURVEY OF NATURAL DISEASE IN WILD-CAUGHT HOUSE SPARROWS (PASSER DOMESTICUS) FROM THE NORTHERN COLORADO FRONT RANGE

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House Sparrows (Passer domesticus, HSp) are invasive passerine birds in the United States that contribute significantly to the sylvatic cycling of avian and zoonotic pathogens making them useful sentinels for disease surveillance. In this study, we surveyed naturally acquired infections in HSp (n = 52) captured in the Northern Colorado Front Range using mist nets. Birds were tested for West Nile virus (WNV) seropositivity via plaque reduction neutralization test (PRNT) and underwent full postmortem examinations. Blood films from 15 birds were also screened for haemoparasites. Of the birds sampled, 32.7% (17/52) were adults and 67.3% (35/52) were fledglings; 32.7% were male, 32.7% female, and 34.6% were undetermined due to immaturity of gonads. WNV seroprevalence was 13.5% across all birds. All 15 blood films were negative for haemosporidians, though one film demonstrated rare microfilaria. On postmortem examination, 88% of birds had intestinal coccidia and 22.0% harbored intestinal helminths (54.6% trematodes; 18.2% cestodes). Among birds with coccidiosis, 13.6% exhibited associated lymphoproliferative disease most commonly affecting the duodenum. Overall, HSp appear to be important reservoir hosts for WNV and intestinal parasites in this region. The lack of haemoparasitism or overt chronic disease may reflect the overrepresentation of fledglings (immunologically naive hosts) and the timing of blood collection which likely occurred after peak parasitemia. Future studies that control for age-related bias and screen blood upon initial capture are warranted to better characterize true infection prevalence and disease burden in HSp populations of Northern Colorado.

44: SCENT (APOCRINE) GLAND ADENOCARCINOMA IN A COMMON SQUIRREL MONKEY (SAIMIRI SCIUREUS): MACROSCOPIC, HISTOLOGICAL AND IMMUNOHISTOCHEMICAL FEATURES

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Saimiri sciureus, commonly known as the common squirrel monkey, is listed as **Least Concern** on the IUCN Red List. Despite facing habitat loss and pet trade pressures, it maintains stable populations across its range in South America due to adaptability and widespread distribution. We report the gross, histopathological and immunohistochemical features of a sweat (scent) gland apocrine adenocarcinoma in a captive specimen. Grossly the neoplasm arised in the chest region, as a 1x1x0.9cm, moderately demarcated, mobile, green-purple, cystic, alopecic, nodule. The neoplasm was removed with narrow surgeon-cut margins under general anaesthesia. Histopathological examination revealed a dermal, well-demarcated, multilobular, cystic, benign, glandular neoplasm. Cells formed papillae, lined by a bilayer of luminal epithelial and myoepithelial cells. Luminal cells had well defined borders, a moderate amount of amphophilic cytoplasm and one round nucleus with coarsely stippled chromatin. The apical border depicted cytoplasmic decapitation, a classical feature of sweat apocrine glands. Anisokaryosis and anisocytosis were minimal and there were 6 mitoses in 2.37mm^2. Myoepithelial cells exhibited minimal atypia and no mitotic activity. Immunohistochemical examination using AE1AE3, CK7, CK8/18, vimentin, P63, 14-3-3 sigma, COX2 and Ki67 antibodies revealed a glandular immuneprofile, low Ki67 index

and a discontinuous myoepithelial cells layer, confirming an invasive growth. These results are in accordance with a previous published case in a capuchin monkey and with cases reported in human medicine, in which the chest region, alongside axillary and pudendal areas, are classical anatomical regions for this cutaneous neoplasm.

45: MYOFIBROBLAST DIFFERENTIATION IN FELINE EOSINOPHILIC SCLEROSING FIBROPLASIA

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Background: Feline eosinophilic sclerosing fibroplasia (FESF) is a fibroproliferative disease of cats marked by eosinophilic inflammation and extensive tissue fibroplasia predominantly affecting the gastrointestinal tract. Its pathogenesis remains poorly understood. A recent study suggests that myofibroblast differentiation and macrophage-to-myofibroblast transition (MMT) may play pivotal roles in tissue fibrosis in FESF, similar to scleroderma and systemic sclerosis in humans.

Objective: The objective of this study was to characterize the distribution of macrophages and myofibroblasts in FESF and investigate their roles in lesion development.

Methods: Tissue blocks of fifteen domestic cats diagnosed with FESF were retrieved from the database. Histochemical stains were performed to highlight the collagenous extracellular matrix (ECM). Different areas of FESF lesions were categorized as follows: (1) Inflammatory Zone (inflammatory infiltrates predominate with <10% ECM), (2) Profibrotic Zone (mixture of mesenchymal and inflammatory cells with 10%-50% ECM), (3) Fibrotic/ Sclerotic Zone (mature fibrous tissue and collagen predominate with >50% ECM). Dual immunohistochemistry (IHC) of smooth muscle actin (SMA) and ionized calcium–binding adapter molecule 1 (IBA1) were performed. Myofibroblasts, macrophages, and SMA/IBA1 double positive mesenchymal cells, interpreted as MMT cells, were counted in 2.37 mm^2.

Results: Macrophages were found scattered throughout FESF lesions, with a noticeable concentration in Inflammatory and Profibrotic zones. Myofibroblasts were mainly seen in Profibrotic and Fibrotic/Sclerotic zones. The double positive MMT cells were sparse and most frequently noted in Profibrotic Zone.

Conclusions: Our findings underscore the marked myofibroblast differentiation and the potential role of macrophages in tissue fibrosis, supporting the hypothesis of MMT in FESF.

46: REPLICATION AND ASSEMBLY OF RABIES VIRUS AS SEEN BY CONVENTIONAL TRANSMISSION ELECTRON MICROSCOPY IN NEURONS OF TWO GREY FOXES (UROCYON CINEREOARGENTEUS): DYNAMIC OF THE NEGRI BODY FORMATION

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Background:

Rabies virus (RV) is a fatal neurotropic Lyssavirus causing encephalitis. Globally, 59,000 human deaths per year are attributed to this neglected zoonotic disease; rapid diagnosis is critical for effective intervention and patient survival. Histopathologically, identification of eosinophilic intracytoplasmic inclusions, known as Negri Bodies (NB), are characteristic of rabies infection and are the replication and assembly organelle (RAO) of RV. Deeper analysis of RAO formation provides insight for understanding disease pathogenesis, developing antiviral therapies, and vaccines.

Objective:

To assess the formation of RV RAO in neurons using conventional transmission electron microscopy (CTEM).

Methods:

Two grey foxes presenting abnormal behavior were necropsied and tested rabies-positive (CDPH). One case involved human exposure. Brain cortex and hippocampus samples presenting numerous NBs were examined using CTEM.

Results:

Replication and assembly organelles or NBs were located in axons, dendrites, and soma of neurons. RAOs were round to oval, and composed of a granular fibrillar matrix scattered with electron-dense supercoiled fibrils. While oval-shaped RAO ranged from 0.41 µm to 10.26 µm in its larged lengths, round-shaped were up to 4.24 µm in diameter. Frequently, RAOs exhibited vacuole-like clearings containing virion free or within vesicles. Virions showed brick-to bullet-shaped morphology measuring up to 290 nm in length by 80 nm in diameter.

Conclusion:

Negri Bodies are a membraneless organelle in which RV replicate and virions assemble. This process is highly dynamic and progressive. Virions can be found in transport vesicles through the cell body, axon and dendrites.

47: REPLICATION AND ASSEMBLY OF AVIAN POXVIRUS AS SEEN BY CONVENTIONAL TRANSMISSION ELECTRON MICROSCOPY IN TWO CHICKENS (GALLUS GALLUS DOMESTICUS): DYNAMIC OF THE BOLLINGER BODY FORMATION

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Background:

Bollinger Bodies (BB), eosinophilic intracytoplasmic inclusions in chicken epidermal epithelial cells (CEEC), are a diagnostic feature of fowlpox virus (FPV, Avipox) infection. Studies using conventional transmission electron microscopy (CTEM), characterized BBs as large aggregations of mature virions and further identified another inclusion composed of viroplasm and membranes. These inclusions constitute the FPV replication and assembly organelle (RAO). Although poxviruses exhibit extensive taxonomic diversity and broad vertebrate and invertebrate host ranges, studies remain focused on prototypic strains in limited in vitro systems.

Objective:

To assess the formation of FP-RAO in CEECs using CTEM.

Methods:

Two backyard-flock chickens presenting multifocal proliferative dermatitis were necropsied and confirmed FPV infection. CTEM was used on glutaraldehyde-fixed samples.

Results:

In CEEC, FPV-RAO consists of: 1) Electron-dense, granular fibrillar matrix (viroplasm); 2) Crescent-shaped membranes (CSM); 3) Immature virions (IV), spheres derived from CSM with viroplasm developing into IV nucleoids; 4) Mature virions (MV); 5) MVs internalized within lipid droplets (LD) forming wrapped mature virions (WMV), averaging 354.56 ± 32.2 x 235.04 ± 22.1 nm. WMV, electron-dense tubules, and rodlets progressively replace LD lipid components forming the so-called, BBs. CEEC containing FPV-RAO presented rarefaction of organelles and cytoskeleton.

Conclusion:

FPV replication and assembly is a complex process occurring in compartmentalized, membraneless RAOs in the stratum spinosum and granulosum. While the wrapping of the prototypic poxvirus MVs occur in the Golgi-vesicular system, wrapping of FPV-MV appeared to occur in membrane-bound LDs in a multicentric manner. BBs are WMV aggregates evolving from coalescing LDs containing WMV.

48: MYCOTOXICOSIS IN TWO HORSES IN NEW JERSEY

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In the spring of 2025, two horses from different premises were submitted to the New Jersey Animal Health Diagnostic Laboratory for necropsy. The first horse was a yearling Standardbred filly found dead without known pre-existing disease, and the second was a 16-year-old Standardbred mare with a 1-day history of blindness who escaped from the pasture and was found trapped in a small ditch. A pasture-mate of the filly was found dead one week prior, but an investigation was not performed. Gross necropsy did not reveal a definitive cause of death for either horse though cerebral softening was noted in both cases (more pronounced in the older horse). Histopathology of the filly revealed areas of chronic lymphoplasmacytic perivascular cuffing multifocally in the cerebral cortex, and the older horse had leukoencephalomalacia with vascular necrosis, gliosis, hemorrhage and edema. The stomach contents and/or grain from each horse was submitted for mycotoxin testing for both cases which revealed an elevated level of total fumonisins (B1,2,3) with highest elevations in B1 and B2 for the filly and elevated fumonisin B1 for the older horse. A single feed source was suspected and under investigation. A similar occurrence associated with the death of 16 horses was reported in nearby Delaware around the time of our investigations. This small case series illustrates an ongoing need for food safety vigilance, as losses can be significant.

49: PROLIFERATIVE TENOSYNOVITIS IN FREE-RANGING IN FREE-RANGING EGYPTIAN ROUSETTE BATS (ROUSETTUS AEGYPTIACUS) FROM ISRAEL

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The Egyptian rousette bat (ERB; *Rousettus aegyptiacus*) is a frugivorous, cave-dwelling pteropodid bat widely distributed across Africa, the Middle East, and parts of South Asia, and is recognized as a natural reservoir for multiple high-consequence zoonotic viruses. In Israel, approximately 3% of free-ranging ERBs are afflicted by a chronic, progressive syndrome characterized by bilateral enlargement of the interphalangeal and metatarsal joints, along with elongation and deformation of the claws and progressive loss of digital flexion and grasping function. Between 2023 and 2025, five ERBs were euthanized after failing to respond to clinical intervention. On histopathologic examination, proliferative tenosynovitis was characterized by expansion of synovial and periarticular soft tissues, reactive periosteal proliferation, inflammation of the deep digital flexor tendons, and frequent neutrophilic infiltrates. In two ERBS, aerobic bacterial culture followed by Illumina sequencing identified *Staphylococcus aureus* strain 15, a bat-adapted lineage previously reported in ERBs in Israel. PCR and Sanger sequencing additionally confirmed the presence of *Mycoplasma/Ureaplasma* spp. within affected tissues. Comprehensive toxicologic evaluation, including assessment of micronutrient levels and heavy metal exposure, failed to identify an exogenous toxicant. We hypothesize that *Mycoplasma* spp. is the primary driver of the proliferative tenosynovitis, with *S. aureus* strain 15 acting as an opportunistic secondary invader—potentially facilitated by *Mycoplasma spp.*-associated immunomodulation. While *Mycoplasma spp.*-associated arthritis and tenosynovitis has been documented in other species, including big brown bats (*Eptesicus fuscus*), this is the first report in ERBs.

Characterization of this emerging syndrome represents a critical component to understanding host-pathogen interactions in natural reservoir species.

50: PANULIRUS ARGUS VIRUS 1 VIRURIA ASSOCIATED WITH A PODOCYTOPATHY IN THE CARIBBEAN SPINY LOBSTER, PANULIRUS ARGUS (LATREILLE, 1804)

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Background: *Panulirus argus* virus 1 (PaV1) infects the Caribbean spiny lobster, *Panulirus argus* (Latreille, 1804), and causes metabolic wasting and mortality mainly in juveniles. The mechanism of environmental shedding is poorly understood for this and many other crustacean viral diseases. The excretory system is an important route of viral entry and potentially exit in decapods; however, the mechanisms of urine shedding (viruria) have not been explored.

Objective: The goal of this study was to determine if PaV1 is shed in the urine and to elucidate potential mechanisms of viruria.

Methods: Four wild, naturally infected adult *P. argus* were caught and euthanized. Hemolymph and urine were collected for PaV1 quantitative PCR, and tissues were fixed and processed routinely for histologic examination and PaV1 *in situ* hybridization (ISH).

Results: PaV1 was detected in the urine of all lobsters with viremia (3 of 4), with the quantity being proportional but less than that circulating in the hemolymph of two subclinically and moderately infected lobsters. However, in the severely infected lobster, the viral quantity exceeded that in circulation. Histologically, hemocytes in moderate and severely infected lobsters exhibited classic intranuclear inclusions. Similar inclusions were also evident in the urinary podocytes (coelomocytes) of the excretory organ (antennal gland) in the severely infected lobster, with coelomocyte sloughing, degeneration, and apoptosis. Both cell types were intensely labeled with ISH.

Conclusions: Although previously only known to infect hemocytes, PaV1 also infects coelomocytes within the antennal gland, resulting in a podocytopathy. Viruria occurred in PaV1 infected lobsters with detectable viremia.

51: ENDOLIMAX AMOEBIASIS IN GOLDFISH

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Amoebae from the genus *Endolimax* are best characterized as components of the human gastrointestinal microbiota. However, *Endolimax* spp. have also been identified in feces from non-mammalian hosts, including amphibians, birds, reptiles, insects, and fish. Recently, two *Endolimax* species, *E. carassius* and *E. piscium*, have been reported in association with systemic granulomatous disease in goldfish (*Carassius auratus*) and

farm-raised sole (*Solea senegalensis*), respectively, in Europe. Multiple goldfish (*C. auratus*) from a backyard pond in the southeastern United States presented with a history of circling behavior and lying on the bottom of the pond. Two fish were submitted for postmortem examination, and microscopically, both fish exhibited disseminated granulomatous inflammation characterized by variably sized granulomas containing necrotic cores and large numbers of 3-4 µm diameter protozoa with a single, central to paracentral nucleus. Polymerase chain reaction designed to amplify a short segment of the 18S rRNA gene of *Endolimax* spp. and other closely related archamoebae produced an amplicon of the expected size, exhibiting 99.1% nucleotide identity over a 330 bp region to *E. carassius* previously reported in Europe. The identification of this organism in a nondescript backyard pond suggests that *Endolimax* amoebae may be an underdiagnosed cause of disease in at least goldfish in North America, and pathologists should be aware of this organism as a differential for granulomatous disease in goldfish.

52: MORPHOLOGIC AND IMMUNOPHENOTYPIC CHARACTERIZATION OF NEUROBRUCELLOSIS IN STRANDED DOLPHINS IN COSTA RICA FROM 2010–2020

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Background: Neurobrucellosis is a recognized manifestation of chronic *Brucella ceti* infection in cetaceans, with important zoonotic, conservation, and comparative pathology implications.

Objective: To characterize neurobrucellosis in dolphins stranded along the Pacific coast of Costa Rica from 2010 to 2020 using histopathology, immunohistochemistry (IHC), and in situ hybridization (ISH).

Methods: Formalin-fixed, paraffin-embedded (FFPE) brain tissue from 25 dolphins with confirmed neurobrucellosis (by culture and serology) was evaluated histologically. Select brain regions were assessed by immunohistochemistry (IHC) for CD3, CD20, IBA-1, and *Brucella* spp., and by RNAscope® probe-based in-situ hybridization (ISH) assay for *Brucella* spp.

Results: Most cases involved striped dolphins (24/25, 96%) and juveniles (21/25, 84%) in postmortem code 1 (freshly dead) (64%) or 2 (mild postmortem autolysis) (36%). All cases exhibited lymphoplasmacytic and histiocytic meningoencephalomyelitis, most severe in the spinal cord, brainstem, cerebellum, and periventricular regions, often with choroid plexitis, neuritis, and vasculitis. *Brucella ceti* was isolated from cerebrospinal fluid (CSF), and all evaluated dolphins were seropositive in serum and CSF with Rose Bengal test. Immunolabeling against *Brucella* antigen and hybridization signal were detected in meningeal macrophages in 21/25 (84%) cases. $CD3^+$ T cells were present in 15/25 (60%), $CD20^+$ B cells in 24/25 (96%), and IBA-1^+ histiocytes in 25/25 (100%).

Conclusion: Neurobrucellosis is a common and severe manifestation of *Brucella ceti* infection in juvenile striped dolphins. CSF is the most reliable sample for bacterial isolation and advised for serologic diagnosis. Histopathology is essential for correlating lesions with characteristic inflammatory patterns, while IHC and ISH serve as valuable complementary tools.

53: PATHOLOGY, TISSUE DISTRIBUTION, AND PHYLOGENOMIC CHARACTERIZATION OF LARGEMOUTH BASS VIRUS ISOLATED FROM A WILD SMALLMOUTH BASS (MICROPTERUS DOLOMIEU)

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We performed a diagnostic disease investigation on a wild smallmouth bass (*Micropterus dolomieu*) with skin ulcers that was collected from Lake Oahe, South Dakota. Gross and histologic lesions of ulcerative dermatitis, myositis, and lymphocytolysis within the spleen and kidneys were consistent with largemouth bass virus (LMBV) infection. LMBV was detected by conventional PCR in samples of a skin ulcer, and the complete genome sequence of the LMBV (99,184 bp) was determined from a virus isolate obtained from a homogenized skin sample. A maximum likelihood (ML) phylogenetic analysis based on the concatenated sequence of 21 core iridovirus proteins supported the LMBV isolate (LMBV-SD-2023) as a member of the species *micropterus1*. The ultrastructure of the LMBV isolate exhibited the expected icosahedral shape of virions budding from cellular membranes. Viral RNA in infected cells was visualized via *in situ* hybridization (ISH) within dermal granulomas, localized predominantly at the margin of epithelioid macrophages and central necrosis.This is the first report of the use of ISH to examine the tissue distribution of active LMBV infection in a case of naturally acquired infection.

54: RANAVIRUS RANA1 (FROG VIRUS 3)-ASSOCIATED LESIONS, VIRAL TROPISM, AND GENOMIC ANALYSIS DURING A NATURAL OUTBREAK IN EASTERN BOX TURTLES (TERRAPENE CAROLINA CAROLINA)

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Rationale: Ranavirus rana1 (formerly Frog virus 3 [FV3]) is an emerging pathogen causing mass mortality in ectothermic vertebrates. Viral pathogenesis and lesion distribution have not been extensively investigated in reptiles compared to amphibians.

This study aimed to perform detailed histological evaluations, correlate lesion distribution with viral tropism, and perform genomic characterization of a Ranavirus rana1 outbreak in eastern box turtles (Terrapene carolina carolina) in Louisiana.

Methods: Five eastern box turtles with lethargy, inappetence, nasal discharge, and periocular swelling were clinically examined, and three were submitted for postmortem examination (Turtles #1-3). Pan-ranavirus qPCR and ISH targeting the major capsid protein (Orf90R) were performed in addition to virus isolation, transmission electron microscopy, and whole genome sequencing.

Results: Turtles #1 and #2 had severe multisystemic, necrotizing inflammation, while in #3, mild to moderate necrotizing lesions restricted to the upper gastrointestinal tract (GIT). Lesions were associated with ranavirus DNA, within situ, DNA signal abundant in epithelial, endothelial, and histiocytic cells across organs of #1 and #2 (correlating with higher viral load), and in the upper GIT in #3. Phylogenetic analysis following whole genome sequencing determined this isolate was closely related to Frog Virus 3 isolate OP/2025/Netherlands, with a major and minor parent derived from isolates identified in Canada and China, respectively.

Conclusion: Ranavirus rana1 is a multisystemic viral disease with high mortality. In this outbreak, lesion severity corresponded to increased viral load with abundant in situ DNA signal. The Ranavirus rana1 isolate from this outbreak derives from Canadian and Chinese parents.

55: SINONASAL POXVIRUS INFECTION IN BLACK-BELLIED WHISTLING DUCKS

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Avipoxvirus infection is common in many species of birds, but less commonly described in ducks with most reports from captive domestic ducks. In most avian species, poxvirus lesions are cutaneous or oral with respiratory infections being less common and when they do occur are tracheal or pulmonary. We report an unusual presentation of poxvirus infection in two free-ranging Black-bellied Whistling Ducks (BBWDs) where viral infection caused large subcutaneous neoplastic-like masses, up to 10 cm in diameter, on the head. We suspect these arose from the frontal sinus. On section, the masses were cystic with a thick (1-3 cm) wall and filled with keratin. Microscopically, the wall was composed of proliferated squamous epithelium with many Bollinger bodies which contained typical avipoxvirus particles on electron microscopy. Interestingly, biologists have recognized these unusual masses in multiple BBWD from the same area which, in some cases, have disappeared over time. Biologists have observed small numbers of other BBWDs in this population with typical cutaneous poxvirus lesions on the feet and legs. Future genomic analysis is warranted to determine if the head masses in these BBWDs are caused by a novel avipoxvirus or if this is just an unusual distribution caused by the same virus associated with more typical cutaneous lesions.

56: PATHOLOGICAL AND GENOTYPICAL CHARACTERIZATION OF SEPTICEMIC AND ENCEPHALIC LISTERIOSIS IN GOATS - A CASE REPORT

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Listeriosis caused by *Listeria monocytogenes* manifests in three forms including encephalitis, septicemia, and intrauterine infections. On November 2024, a goat farm rearing 200 mixed breed goats located in Seosan-si reported death of 10 goats manifested with neurological signs and abortion. Two female adult dead goats were requested for autopsy. Grossly, there were no lesions except for uterus containing hemorrhagic purulent exudate. Histopathologically, both goats revealed suppurative meningoencephalitis with microabscesses in brainstem, pyogranulomatous nodule with hepatocellular necrosis and thrombosis, and suppurative endometritis. *L. monocytogenes* antigens were identified in lesions of microabscess and necrotized endometrium by using the immunohistochemistry. Following the laboratory examination, *L. monocytogenes* was isolated from brainstem and uterine exudates. Finally, the case was diagnosed as listeriosis mixed with encephalitis and septicemia. *L. monocytogenes* isolates were genotypically characterized for whole-genome sequencing. All *L. monocytogenes* isolates were identified as sequence type 3354 and sublineage 8. According to the genomic comparisons for correlating the lesions with genomic profiles, *L. monocytogenes* isolates harbored *Listeria* pathogenicity island (LIPI) 1 and stress survival islet-1 (SSI-1). According to the previous study, LIPI 4 was known as a genomic island related with infections of central nervous system and placenta and SSI-1 help *L. monocytogenes* isolates to withstand the environmental stresses in the stomach. Although *L. monocytogenes* isolates in this study don’t harbor LIPI 4, SSI-1 might affect the pathogenicity in this case. Further study is required for the understanding the function of SSI-1 in animal hosts to elucidate the pathogenicity of *L. monocytogenes*.

57: USING GOLD STANDARD DIAGNOSTIC TESTS AND DIGITAL IMAGE ANALYSIS TO STAGE WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) FOR CHRONIC WASTING DISEASE INFECTION

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Chronic wasting disease (CWD) is a fatal prion disease of deer, elk, moose, and other cervids. It is one of the most important infectious diseases of cervids with significant wildlife management and economic implications. The duration from CWD prion infection to death can be up to 2-3 years, where an animal can shed prion via bodily fluids, and prion can remain infectious in the environment for a decade or more, making management of this disease challenging. Considering the chronicity of the disease, understanding the stage of infection can be useful for determining the duration of infection, the burden of prion in the tissues, and how long the deer has been shedding prion in the environment. We attempted to determine if digital image analysis could aid

in staging white-tailed deer for CWD infection and subsequent disease. Retropharyngeal lymph nodes and obex from CWD positive deer were analyzed to determine if the intensity and/or total area of immunoreactivity for the CWD prion were associated with body condition score (BCS) and therefore indicative of disease stage. While not reaching statistical significance, the total area of immunoreactivity in the lymph node follicles showed the strongest correlation with BCS (Kendall's Tau-b = -0.39, p = 0.1). Intensity of staining in the lymph nodes and obex showed slightly lower correlation coefficients. These data suggest that, with continued optimization, digital image analysis may be a useful tool to stage CWD in white-tailed deer and assess infection duration, prion burden, and environmental shedding.

58: PATHOLOGY OF THE ESOPHAGUS IN SOUTH AMERICAN CAMELIDS SUBMITTED TO TWO LABORATORIES IN NORTH AMERICA BETWEEN 1987 TO 2023

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Objective: To determine prevalence and underlying causes of esophageal lesions (EL) and demographic characteristics of affected South American camelids (SAC) submitted to two veterinary diagnostic laboratories between January 1st, 1987, and December 31st, 2023

Animals: 156 llamas and alpacas (84 and 70, respectively) with EL, including choke, esophagitis, hyperkeratosis, megaesophagus, myopathy, squamous cell carcinoma, squamous metaplasia and esophageal ulcers; submitted to the Oregon Veterinary Diagnostic Laboratory and any of the California Animal Health and Food Safety Laboratory facilities (64 and 92, respectively)

Procedures: The records were reviewed, and data collected included animal species, sex, geographic origin, and pathology. The categories of lesions, processes and causes were unified across the two laboratories. Descriptive statistics were compiled and reported as numbers and percentages.

Results: Ulcers in the esophagus (40/156), megaesophagus (56/156), esophagitis (32/156) and choke (17/156) were the most common ELs. The underlying cause of most EL was categorized as undetermined (both laboratories); followed by infectious and inflammatory non-infectious (CAHFS) or poor dentition and vascular ring anomalies (OVDL).

Conclusions and Clinical Relevance: For many ELs in SAC the underlying cause was not identified. In cases with an identifiable cause, most ELs were linked to inflammation or mechanical obstruction/impaction. The findings underscore the need for enhanced diagnostic procedures and strategies to discern the causes underlying the development

of ELs in SAC. Knowledge of the most common EL may aid in diagnosis and identification of associated risk factors.

59: UNDERSTANDING THE METASTATIC OSTEOSARCOMA TUMOR MICROENVIRONMENT USING CROSS-SPECIES SPATIAL TRANSCRIPTOMICS

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Background: Osteosarcoma (OS) is the most common primary malignant bone tumor in both humans and dogs. Despite aggressive treatment, up to 40% of children and over 90% of dogs die due to multi-drug-resistant metastatic disease. Canine and human OS share significant similarities in clinical presentation, histopathology, and molecular aberrations, establishing the dog as a translationally relevant model to accelerate treatment discovery in human OS. Advancements in spatially resolved multi-omic techniques have enabled profiling of the tumor microenvironment at unprecedented resolution, offering greater insight to disease susceptibilities. However, few established frameworks exist for meaningful data integration, particularly in comparative oncology.

Objective: Identify conserved cellular signals in metastatic OS through independent and integrated analyses of canine and human GeoMx datasets.

Method: Archived FFPE lung metastases from canine (n=7) and human (n=5) OS patients were spatially profiled via GeoMx. Macrophage (CD163+ or CD68+), T cell (CD3+), and negative (CD163-/CD3- or CD68-/CD3-) tumor/stroma compartments were captured from intratumoral and extratumoral regions. Data were analyzed independently using GeomxWorkflows and integratively with reciprocal PCA within Seurat, focusing on orthologous genes.

Results: Both independent and integrated analyses demonstrate intratumoral enrichment of immunosuppressive transcripts in metastatic OS across species. Integrated clustering showed strong cell-type segregation independent of species. Intratumoral macrophages were enriched for JAG1, an emerging mediator of immune evasion and treatment resistance, while intratumoral T cells were enriched for Galectin-1 (LGALS1), also linked to immunosuppression in other cancers.

Conclusion: We demonstrate conserved, targetable genes conferring immunosuppression in metastatic OS using a novel, integrated, cross-species spatial transcriptomics approach.

60: RENAL HISTOLOGIC FINDINGS IN AGED AFRICAN GREEN MONKEYS (CHLOROCEBUS AETHIOPS SABAEUS) IN ST KITTS

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INTRODUCTION

African Green monkeys (AGM) are commonly used as animal models in research, such as in hypertension and SARS-Covid 2019. There is scant information describing background and spontaneous histologic changes in aged AGMs.

MATERIALS AND METHODS

Thirty two AGMs from Behavioral science foundation that were euthanized at an endpoint, IACUC approved protocol which covers the entire colony were submitted for autopsy at Ross University School of Veterinary Medicine (RUSVM), Pathology department. None of the AGMs in this study showed clinical signs of renal disease. A standardized autopsy protocol was used, and all organs were sampled and fixed in 10% buffered formalin. Tissues were routinely processed and stained with Hematoxylin-eosin (HE) stain. For kidney sections, periodic acid Schiff (PAS), Jones Methenamine Silver stain and Masson's trichrome special stains were routinely performed. Perl's Prussian, Congo red and Von Kossa special stains were used as required.

RESULTS

Renal histological changes were identified in 21/32 (65.6%) of the monkeys comprised multiple cortical cysts (7), interstitial nephritis (13), lipofuscin (1) or hemosiderin (4) within the cytoplasm of the tubular epithelium, tubular degeneration and necrosis (5), intratubular crystals (4), glomerulonephritis (2), periglomerular fibrosis (1), glomerular sclerosis (9) and atrophy (6), and arterial hyalinosis(1).

CONCLUSIONS

In this group of AGMs, 65.6% were presented with renal histologic changes. The most common changes encountered were interstitial nephritis, glomerular sclerosis and fibrosis and multiple cortical cysts. We believe that identifying histologic changes in aged AGMs will assist researchers in better understanding and characterizing spontaneous and disease associated renal lesions.

61: COMMON FEATURES OF COASTAL BIRDS DIAGNOSED WITH HIGHLY PATHOGENIC H5 INFLUENZA A VIRUS IN THE EASTERN USA

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Background: Since its North American introduction in late 2021, highly pathogenic (HP) H5 influenza A virus (IAV) clade 2.3.4.4b caused countless mortalities in wild birds. Coastal species (Charadriiformes and marine Anseriformes) are particularly vulnerable to exposure through densely populated habitats shared with known IAV reservoirs.

Objective: Describe taxonomic patterns of gross and histopathologic lesions associated with HP H5 IAV in wild coastal birds in the eastern USA.

Methods: Pathology findings were reviewed for 34 coastal birds (11 species) from the eastern USA collected from 2022 to 2025 from which H5 IAV clade 2.3.4.4b RNA was detected by real-time RT-PCR of pooled oropharyngeal and cloacal swabs.

Results: Gross lesions included pulmonary and meningeal hemorrhage and/or congestion. Gulls, sanderlings, and terns were often in poor nutritional condition. Histopathologic lesions were common in brain (26; 76%), spleen (20; 59%), and liver (18; 53%). Necrosis was the predominant finding among affected tissues, occasionally with lymphoplasmacytic inflammation, hemorrhage, and/or lymphocytolysis. Pelicans, terns, and sanderlings exhibited acute, widely disseminated viral disease; while eiders had concurrent trauma and visceral gout. Gulls had concurrent amyloidosis and/or aspergillosis, suggesting a chronic disease course.

Conclusions: Pathology attributed to HP H5 IAV in coastal birds was most frequent and severe in the brain, consistent with viral neurotropism. Additionally, lymphoid destruction and multi-organ necrosis underscore the severity of infections in some species. Varied pathogenesis across species, however, is suggested by more chronic, secondary disease processes. Understanding HP H5 IAV pathogenesis among coastal birds is important toward recognizing associated impacts in at-risk species.

62: THE WAXING AND WANING OF "PIX": STUDYING THE SEQUENCE OF LYMPHOID NEOGENESIS IN CROCODILIAN SKIN

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Background

Tertiary lymphatic follicles (TLFs) are highly plastic lymphoid tissues that can form in any organ in response to inflammatory stimuli, a process known as lymphoid neogenesis. In crocodilian skin, lymphohistiocytic proliferative cutaneous lesions (LPCL) represent a form of lymphoid neogenesis within the dermis and are associated with “pix,” a lesion that reduces crocodilian leather quality. LPCL have previously been linked to West Nile virus (WNV) infection in American alligators (*Alligator mississippiensis*) and saltwater crocodiles (*Crocodylus porosus*).

Objective

To characterize the architecture of TLF developmental stages in crocodilians using naturally occurring LPCL in Nile crocodiles (*Crocodylus niloticus*) and to compare it to mammals and birds.

Methods

Immunohistochemistry on serial sections of LPCL was used to characterize the cell populations during different stages of formation and resolution in the crocodilian skin.

Results

LPCL formation began as small perivascular clusters of $CD3^+$ T cells and $MHCII^+$ antigen-presenting cells. These progressed into mature lymphoid structures with organized lymphocyte populations, including distinct $CD3^+$ T cell zones and $PAX5^+$ B cell follicles. Resolving LPCL presented with focus of dermal collagen loss and disorganization, replaced by an increased mucin matrix embedding sparse groups of histiocytes and lymphocytes mixed with heterophils, granular monocytes, and mast cells.

Conclusions

LPCL progression and resolution follows a similar sequence of TLF formation as seen in mammals and birds, suggesting a conserved (or convergent) molecular pathway for crocodilian lymphoid neogenesis. These results enhance our understanding of crocodilian adaptive immunity and can support future studies on comparative immunology.

63: A RETROSPECTIVE EVALUATION OF URINARY TRACT DISEASE IN THE SPADEFOOT TOAD

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Background:

Spadefoot toads (Spea spp.) are used to study various aspects of evolution, including the environmental impact on development, phenotypic plasticity, and mate selection. Despite growing knowledge of spadefoot ecology, little is known about the common diseases affecting these toads.

Objective:

To identify and characterize common spontaneous diseases, infectious agents, and background lesions in the spadefoot toad.

Methods:

17 years of spadefoot toad necropsy records from a wild-caught, captive colony were evaluated. Records from 160 spadefoot toads were further divided by organ system and specific conditions to determine overall trends in disease.

Results:

The urinary system was a commonly affected system in spadefoot toads (33/160, 20.6%), so it was the focus of this abstract. Of the 33 cases, 8 (24.2%) had multiple lesions within the urinary system. 12 of the 33 cases (36.3%) had glomerulonephritis, while another 12 (36.3%) had primarily tubulocentric changes. Occasionally, both were affected (4/12, 33.3%). Most cases involving the urinary bladder (8/33, 24.2%) were attributed to parasitic infection (7/8, 87.5%). Rarely, the ureters were also affected by parasites (2/33, 6.1%). Finally, extramedullary hematopoiesis was a common feature observed in the kidney (7/33, 21.2%).

Conclusions:

Urinary tract disease is a common problem contributing to morbidity or mortality in spadefoot toads. The retrospective evaluation demonstrates the value of examining this data across all body systems to gain a deeper understanding of diseases and background lesions associated with the spadefoot toad.

64: FINE STRUCTURE OF YELLOW FEVER VIRUS-INDUCED HEPATITIS IN A WILD OWL MONKEY (AOTUS SP.) IN THE EASTERN BRAZILIAN AMAZONIA

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Background:

Non-human primates (NHPs) are susceptible to yellow fever virus (YFV) infection, however, owl monkeys are considered a less sensitive species. Published studies focusing on the fine structure of YFV infection in NHPs are infrequent and morphogenesis of this Flavivirus remains in part elusive.

Objective:

To assess the ultrastructure of the lesion in the liver, the target organ of YFV infection, in a comparatively less susceptible NHP, with emphasis in viral replication and assembly.

Methods:

Conventional TEM (CTEM) performed on an adult female owl monkey from the Salgado micro-region of Para, Brazil, diagnosed with YFV-induced hepatitis.

Results:

Ultrastructurally there was loss of the stroma scaffold, vascular disruption, extravasation of erythrocytes, and individualization of hepatocytes. Hepatocytes displayed

consumption of glycogen and mitochondria swollen with floccular electron dense matrix. Endoplasmic reticulum (ER), Golgi and vesicles system were rearranged or effaced. Consistently, small to large sites composed of cisternae containing vesicles ranging 53-73 nm in diameter of botryoidal arrangement, and tubular structures ranging 315-573 nm by 69-103 nm were associated with lipid droplets and convoluted membranes through structural changes of the ER. Virion identification was equivocal. The nucleus displayed degeneration with clumping of the chromatin to fragmentation.

Conclusion:

Hepatocellular degeneration, membrane associated replication and assembly organelles, and cell death were confirmed in YFV infection. Although YFV continues to demonstrate a highly complex mechanism of viral assembly, which should be further investigated through advanced TEM, CTEM findings have diagnostic value through identification of the replication organelle even in the absence of viral particles.

65: NOVEL CHARACTERIZATION AND LOCALIZATION OF TBK-1 IN PRIMARY CANINE ORAL MELANOMAS: IMPLICATIONS FOR PD-AXIS MEDIATED IMMUNOTOLERANCE

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Background: Canine Oral Melanoma (COM) is an aggressive neoplasm with poor prognosis. Despite being highly immunogenic, its PD-axis overexpression suppresses T-cell function. This research investigates potential immune modulation in primary COM by TANK-binding kinase 1 (TBK-1), a potential novel regulator of the PD-axis that contributes to immunosuppression, in addition to cell proliferation.

Objective: To determine the gene expression and localization of TBK1 and the PD-axis in primary tumors based on their metastatic status.

Methods: Forty-four primary COM cases were analyzed by RT-qPCR for expression of PD-axis and TBK-1, classified by metastatic status at diagnosis, and clinical data was recorded for each case. Separately, selected COM samples from each group underwent RNAscope duplex in situ hybridization to assess distribution of these markers. Slides were digitalized, and Regions of Interest (ROI) were annotated from tumor core and interface regions. HALO AI was utilized for segregation of the cells and to quantify expression of these markers with the ISH module via H scores.

Results: On RT-qPCR, significantly higher expressions of PD-1, PD-L1, and TBK-1 were observed in COM with metastasis. On RNA scope, PD-axis and TBK-1 were consistently detected, with distinct distribution patterns. Notably, TBK-1 expression was significantly higher in tumor ROIs, and co-localized with PD-L1 in melanoma cells.

Conclusion: This is the first report of TBK-1 expression in COM. Given its ability to influence cell proliferation and PD-L1 expression, and due to its higher expression in

primary COM with metastatic disease, TBK-1 emerges as a promising therapeutic target and merits further investigation.

66: HIGHLY PATHOGENIC AVIAN INFLUENZA A (H5N1) CLADE 2.3.4.4B INFECTION IN WILD MAMMALS IN THE UNITED STATES

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Background: Highly pathogenic avian influenza A (H5N1) virus clade 2.3.4.4b (HPAIV) emerged in wild birds in 2020 and is responsible for an ongoing panzootic. Since 2022, it has had devastating impacts on U.S. backyard and commercial poultry with spillover into wild mammals, dairy cattle, cats, and humans.
Objective: To characterize HPAIV-associated pathology, genotypes, and seroprevalence in wild mammals.
Methods: HPAIV was diagnosed via histopathology and rRT-PCR in wild mammal carcasses that underwent postmortem evaluation from 2023-2025. Genotyping was performed at the National Veterinary Services Laboratory. Concurrently, wild raccoons were bled, and sera were screened for antibodies to influenza A virus (IAV), H5, and N1 via bELISA, virus microneutralization, and ELLA, respectively.
Results: HPAIV was detected in red foxes (n=4), raccoons (n=3), striped skunks (n=3), mountain lions (n=2), bobcats (n=2), an American black bear, and an American mink (n=1 each). HPAIV-associated lesions were observed in 10 cases and included lymphoplasmacytic meningoencephalitis (n=9) with neuronal necrosis (n=5) and/or gliosis (n=3). Genotyping on a subset of cases revealed strains B3.6 (n=2), D1.1 (n=1), and Minor60 (n=1). Among 83 live-sampled raccoons in Ohio from 2024-2025, IAV antibodies were detected in 58 (70%) and, of these, 46 (80%) had detectable H5 and N1 antibodies.
Conclusions: North American wild mammals are at risk for fatal neurologic disease from HPAIV infection. Viral detection without concurrent disease and serological data suggest that subclinical infection is common. Thus, wild mammals represent a potential reservoir for HPAIV and may pose a risk for future spillover to domestic animals and humans.

67: DNA POLYMERASE THETA AS A PROGNOSTIC MARKER IN CANINE CUTANEOUS MAST CELL TUMORS

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DNA Polymerase Theta (POLQ)-Mediated End Joining is one of the pathways responsible for DNA repair and maintenance. Altered POLQ expression has been reported in several cancers in humans and linked to genomic instability, poor prognosis, and resistance to therapies. The objective of this study was to evaluate the expression of POLQ as a prognostic marker for canine cutaneous mast cell tumors. Twenty-five cases of mast cell tumors, treated by wide surgical resection with curative intent, were subjected to immunohistochemistry using a rabbit polyclonal anti-POLQ antibody

(Invitrogen). POLQ expression was quantified in five high-power field images (total area = 0.4 mm^2). All positive and negative neoplastic mast cells were counted to calculate the percentage of positive cells. Dogs that were alive at the end of the study or had died due to unrelated causes were censored in the survival analysis. Cases were only censored if a minimum of 180 days of post-surgical follow-up was available. POLQ positivity varied from zero to 99.7%, and was significantly lower in dogs that died due to the disease (P=0.0191). Using a cut-off value determined by ROC curve analysis (AUC=0.8013; P=0.0106), we observed that dogs with tumors showing less than 75% of POLQ-positive cells had shorter post-surgical survival times (P=0.0069; median survival = 154 days; hazard ratio = 9.667). Lower POLQ expression in canine cutaneous mast cell tumors is a potential indicator of shorter post-surgical survival. The clinical significance of this observation, as well as potential new treatment strategies, warrants further investigation.

68: INTRATUMORAL T REGULATORY CELLS IN CANINE CUTANEOUS MAST CELL TUMORS

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Immune cells are commonly found in the tumor microenvironment. Lymphocytes may either stimulate or suppress neoplastic growth through their interactions with cancer cells. Regulatory T cells (Treg) are known to suppress the activity of effector lymphocytes, macrophages, and mast cells, thereby influencing cancer progression. This study aimed to characterize the intratumoral Treg population in canine cutaneous mast cell tumors and verify its association with post-surgical survival. Twenty-three cases of mast cell tumors (11 high-grade and 12 low-grade), treated by wide surgical resection with curative intent, were subjected to immunohistochemical analysis using an anti-FOXP3 antibody (clone FJK-16s, Invitrogen). The entire histological sections were examined using the 40x objective and classified as positive or negative for the presence of intratumoral Tregs. Dogs that were alive at the end of the study or had died due to unrelated causes were censored in the survival analysis. A minimum post-surgical follow-up of 180 days was required for censored cases. Nine tumors were Treg-negative (five low-grade and four high-grade), and 14 were Treg-positive (seven low-grade and seven high-grade). Dogs with Treg-negative mast cell tumors had significantly shorter survival (P=0.0369, median survival = 147 days) compared with Treg-positive cases (median survival = 433 days). Our preliminary results suggest that the presence of intratumoral Tregs may serve as a potential prognostic marker in canine cutaneous mast cell tumors. A larger number of cases and additional sample subsets are required to confirm these findings.

69: EVALUATION OF NEUTROPHIL SIZE AND GRANULARITY AS INDICATORS OF DYSFUNCTIONAL INFLAMMATORY RESPONSE IN CALVES AT HIGH RISK OF BOVINE RESPIRATORY DISEASE

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Neutrophil recruitment is an early response to bacterial infection; however, excessive activation can lead to tissue damage through the release of uncontrolled DAMPs, ROS, and NETs, thereby perpetuating inflammation. This may underlie the dysfunctional responses in bovine respiratory disease, often marked by suppurative bronchopneumonia. Neutrophil size and granularity, as measured by flow cytometry, are markers of neutrophil activation. We analyzed forward (FSC) and side scatter (SSC) of BALF and blood neutrophils in auction beef calves during the BRD risk period (days 1, 4, and 5 after arrival) and after adaptation (days 32 and 33) vs. low-risk non-comingled calves. Blood neutrophils of auction calves had higher FSC values only on days 1 and 4, reflecting their larger size compared to low-risk calves. Auction calves had more uniform neutrophil size (lower FSC variability) than low-risk calves. On Day 32, auction calves had lower SSC, reflecting decreased neutrophil granularity in blood during recovery. An aerosol lysate challenge on Days 5 and 33 did not affect blood neutrophil size or granularity of auction calves relative to low-risk calves. BALF neutrophils of auction calves consistently showed significantly lower SSC (granularity) on all days, compared to low-risk calves. Overall, although neutrophils appeared activated in blood during the high-risk early arrival phase, there was no evidence of activation in the lung using size and granularity markers. Their reduced granularity may instead suggest functional inferiority of neutrophils in the lungs of animals at high risk of BRD.

70: COLITIS ASSOCIATED WITH ALIARCOBACTER BUTZLERI IN RHESUS MACAQUES (MACACA MULATTA)

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Background: *Aliarcobacter butzleri* is a food and water-borne pathogen associated with watery diarrhea in humans. Reports of *A. butzleri* in nonhuman primates (NHP) are uncommon.

Objective: The objective of this abstract is to characterize the clinical, gross, and microscopic features of enteric disease associated with *A. butzleri* in rhesus macaques.

Methods: Using a retrospective approach, ten cases of *A. butzleri* infection were identified between 2023-2025, five of which progressed to clinical end point and were euthanized. Necropsy and histopathology were used to characterize colitis. Brown-Brenn gram and Steiner silver stains, and culture with MALDI-TOF were used for bacterial identification.

Results: Clinical features included persistent watery diarrhea, hematochezia, dehydration, and weight loss. In the five cases submitted for necropsy, gross lesions included emaciation (4/5), watery diarrhea (4/5), mesenteric and/or colonic lymphadenomegaly (3/5), and ulcerative colitis (2/5). Microscopic lesions included lymphoplasmacytic colitis (5/5) and severe ulcerative colitis with gram negative, argyrophilic bacterial rods (2/5). *A. butzleri* was cultured from all five cases, four of which were mixed populations. *A. butzleri* was the sole pathogen isolated from the colon in one case of ulcerative colitis; *Campylobacter coli* was co-isolated in the second case.

Conclusions: This is the first report of *A. butzleri* associated with ulcerative colitis in NHPs. Further investigation is needed to determine the cytopathologic potential and prevalence of *A. butzleri* in NHPs. However, *A. butzleri* should be considered a differential diagnosis in cases of diarrhea and colitis in rhesus macaques.

71: MITOCHONDRIAL DYSFUNCTION OF MESENTERIC LYMPH NODE LYMPHOCYTES IN CATS WITH FIP

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Mitochondrial dysfunction is increasingly recognized as a central driver of immune cell exhaustion in chronic inflammatory diseases. In a veterinary clinical trial, we investigated mitochondrial alterations in mesenteric lymph node (MLN) lymphocytes of 10 cats diagnosed with effusive feline infectious peritonitis (FIP). Cats were treated with the antiviral GS-441524 alone (n=5) or combined with two intravenous infusions of allogeneic adipose-derived mesenchymal stromal cells (MSCs; n=5). All cats recovered and completed the 12-week study.

MLN aspirates were collected at the time of presentation, pre-treatment, and at study completion. Single-cell RNA sequencing revealed pronounced mitochondrial perturbations in T and NK cells from untreated FIP-affected cats. Pathway enrichment analyses showed increased activation of mitochondrial apoptosis and energy metabolism pathways (e.g., cytochrome c release, ATP hydrolysis, mitochondrial SARS-CoV-2 response), suggesting bioenergetic stress. Concurrent downregulation of mitotic and proliferative pathways implied an immunosuppressed phenotype.

Notably, cytotoxic T cells exhibited elevated expression of exhaustion (CTLA4, PDCD1) and stress-associated markers (MAP1LC3A, SOD2), coupled with decreased expression of mitochondrial structural proteins TOMM20 and COX4I1, indicating impaired oxidative phosphorylation.

These findings suggest that mitochondrial stress may contribute to lymphocyte dysfunction and immune dysregulation in FIP. Ongoing studies using archived formalin-fixed and paraffin-embedded MLN tissues and serum from this cohort aim to corroborate transcriptomic data at protein and systemic levels. Clarifying the role of mitochondrial metabolism in FIP pathogenesis may reveal novel targets for immunotherapeutic intervention.

Poster Presentation: Diagnostic Pathology

1: SYSTEMIC TOXOPLASMOSIS IN A BRUSH RABBIT IN LOS ANGELES COUNTY, CALIFORNIA

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An adult, male, brush rabbit found recumbent and lethargic was euthanized and submitted for necropsy. On postmortem examination, the lungs were red, had pinpoint white foci and oozed red watery fluid on cut section. The liver had a single ~1 cm diameter round, white nodule. In the skeletal muscle of the right hindlimb, there were many 1–2 cm in diameter, irregular, white, foci. On histopathology, there were numerous, randomly scattered ~15 µm diameter cysts containing numerous ~1 µm elongate basophilic zoites in the liver, lung, spleen, lymph nodes, and heart. The cysts were admixed with large amounts of necrotic debris, lymphocytes, macrophages, and plasma cells. Electron microscopy of the liver showed single cysts containing numerous individual zoites, which measured approximately 4.88 µm by 1.53 µm. The zoites had an apical complex consisting of long conidia, a nucleus, glycogen, mitochondria, lipid droplets, and vacuoles. *Toxoplasma* spp. was detected by immunohistochemistry in the lungs, liver, and spleen. *T. gondii* DNA was identified by conventional PCR at the ITS-1 locus. DNA sequencing of the B1 and GRA6 loci showed 100% sequence identity with *Toxoplasma gondii* Type X. *T. gondii* is a serious concern due to its potential to affect other intermediate hosts, including humans and other animals, and can cause abortions and be fatal in immunosuppressed hosts.

2: NECROTIZING ATROPHIC RHINITIS AND ESOPHAGITIS CAUSED BY GEOTRICHUM CANDIDUM IN A TAMMAR WALLABY (MACROPUS EUGENII)

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A 4.5-year-old tammar wallaby with a 2-month history of dental disease, facial abscesses, and atrophic rhinitis was submitted for necropsy. Grossly, there was bilateral atrophy of the nasal turbinates with abundant caseous, firm, yellow/tan material throughout the nasal cavity. The left lung was markedly swollen, non-collapsed, and had multifocal to coalescing white/yellow purulent material in the airways. There was also focally extensive fibrinous pleuritis and pericarditis. Multifocal to coalescing white to yellow plaques were also noted along the esophageal mucosa. Diffusely, the liver had white to yellow foci surrounding the blood vessels. Histologically, there were numerous fungal hyphae characterized by ~0.3 µm wide segmented and parallel walls, admixed

with large amounts of eosinophilic necrotic debris, bacterial colonies, and lining the surface of the necrotic mucosal epithelium, the nasal turbinates and esophagus. Additionally, there was evidence of necrotizing bronchopneumonia with intralesional bacteria, and perivascular foci of hepatocellular necrosis and degeneration within the liver. *Geotrichum candidum* was isolated and identified by MALDI-TOF in samples from nasal turbinates. This fungus has been associated with immunocompromised hosts, including human patients with HIV and diabetes mellitus, dermatomycosis in horses, tonsillitis in a pig, and nasal granuloma(s) in cattle. Reports of nasal lesions in wallabies are uncommon and, to the best of our knowledge, this is the first report of necrotizing atrophic rhinitis associated with *Geotrichum candidum* in this species.

3: CYTOLOGIC AND HISTOLOGIC ANALYSIS OF ESCHERICHIA COLI BACTERIAL MENINGITIS IN A JUVENILE DROMEDARY CAMEL

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A 3-month-old male dromedary camel presented for progressive neurologic signs including obtundation and stargazing. A complete blood count (CBC) and cerebrospinal fluid (CSF) sample were submitted for pathologist review. Significant CBC changes included leukopenia (5.88 x 10^3/µL, RI: 7.35-18.36 x 10^3 /µL) characterized by a neutropenia (0.82 x 10^3/µL segmented neutrophils, RI: 4.59 - 8.41 x 10^3/µL) with a degenerative left shift (1.12 x 10^3/µL banded neutrophils, RI: 0.00/uL; 1.71 x 10^3/µL metamyelocytes, RI: 0.00/uL; 0.76 x 10^3/µL myelocytes, RI: 0.00/uL). Cytologic review of the CSF revealed marked, neutrophilic pleocytosis (2529 WBC/µL) with both intracellular and extracellular rod-shaped bacteria. Bacterial cultures of the CSF and peripheral blood yielded Escherichia coli, which was confirmed with 16S rRNA sequencing of the CSF. The camel declined clinically and was euthanized. Post-mortem examination revealed no evidence of bacterial translocation from the gastrointestinal tract. Histopathology confirmed severe, diffuse suppurative meningitis with intralesional bacilli. The source of the E. coli infection with subsequent bacterial meningitis was not identified.

E. coli is an economically important gastrointestinal pathogen of neonatal camels. Affected neonates present with watery diarrhea, which can result in inhibited development and high mortality. Gastrointestinal infection can occasionally lead to central nervous system infection and neurologic signs. To the authors' knowledge this is the first case report of extra-intestinal neurologic E. coli infection in a juvenile dromedary camel in the United States.

4: MAST CELL TUMOR IN A PET CARIO SPINY MOUSE (ACOMYS CAHIRINUS)

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Mast cell tumors (MCTs) are rare in rodents with only a few spontaneously occurring cutaneous MCTs reported in mice. A 3-year-old pet male Cairo spiny mouse (*Acomys*

cahirinus) that presented with a 1.5 x 1.0 cm, exophytic sessile dermal mass at the base of the left ear. The mass did not respond to antibiotic therapy and was surgically excised along with the entire left pinna and submitted to the University of Missouri Veterinary Medical Diagnostic Lab (VMDL) for histopathology. Microscopic examination demonstrated an unencapsulated, poorly demarcated densely cellular, neoplasm composed of variably dense sheets of round cells that expanded in the dermis and extended into the subcutis. Neoplastic cells have ample pale eosinophilic cytoplasm containing abundant fine basophilic granules with variably distinct cell borders. Nuclei are round with coarsely stippled chromatin and 1-2 distinct nucleoli. Anisocytosis is moderate with 2 mitotic figures were observed in 2.37 mm^2. Moderate numbers of eosinophils are scattered throughout the neoplasm, and excision was narrow (with <1mm margins). The intracytoplasmic granules of neoplastic cells exhibited metachromasia with toluidine blue and Giemsa. The neoplastic cells stained positively for c-kit (CD117) and tryptase by immunohistochemistry, consistent with mast cells. At two-month post-surgery, the mouse remained clinically well without evidence of tumor recurrence. To our knowledge, this is the first report of a MCT in the Cairo spiny mouse.

5: DEGENERATIVE SUSPENSORY LIGAMENT DESMITIS: COMPREHENSIVE HISTOCHEMICAL CHARACTERIZATION IN AN IRISH DRAFT HORSE

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An 8-year-old, neutered male, Irish Draft Horse was presented for chronic lameness, more pronounced in the pelvic limbs. Degenerative suspensory ligament desmitis (DSLD) was diagnosed, and the animal was euthanized. There was minimal variation in the gross diameter of the suspensory ligaments (SLs) among the limbs. Ill-defined white discolorations and tan striations were on cross sections of the formalin-fixed proximal SL. Histologically, the white discolorations mirrored deposits of faintly basophilic matrix (ground substance) in between collagen bundles with hematoxylin and eosin, with partial loss of normal birefringence under polarized light (PL). The tan striations reflected dense fibrous connective tissue in affected ligament septa, confirmed with Masson's Trichrome. To a higher extent in the pelvic limbs, ground substance deposits were often interspersed with foci of immature chondroid metaplasia. Proteoglycan accumulation was highlighted by Safranin O and Alcian blue stains. Picrosirius Red-stained sections suggested a gradual replacement of normal collagen (type I) with types III and IV based on tinctorial changes towards green hue under PL and loss of birefringence, respectively. DSLD is a progressive degenerative disease associated with behavioral issues, poor performance, and lameness in adult horses, driven by abnormal proteoglycan accumulation in the SLs. Though its systemic nature is debated, hereditary, nutritional, and exertional factors are likely contributors. Histochemistry was useful for distinguishing normal from affected collagen fibers and confirmed chronic SL degeneration due to proteoglycan accumulation, consistent with DSLD.

6: NASAL CLEAR CELL CHONDROSARCOMA IN A 12-YEAR-OLD MIXED BREED DOG

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Clear cell chondrosarcoma (CCC) is a rare subtype of undifferentiated chondrosarcoma that has previously been reported twice in the dog, both cases within the nasal cavity. A large, nasal mass from a 12 year old castrated male Husky mix was submitted for biopsy. This animal had a history of sneezing, stertor, unilateral serosanguinous nasal discharge, and worsening epistaxis for an unknown duration of time. Microscopically, the mass was composed of sheets to streams of cells with clear or vacuolated cytoplasm and a round to stellate nucleus. In few areas, neoplastic cells were individualized by small lakes of basophilic chondroid matrix. Neoplastic cells were positive for Vimentin and S100 immunohistochemical staining and negative for Melan A, PNL2, CD18, and Cytokeratin which is supportive of a diagnosis of CCC. CCCs are distinguished by abundantly vacuolated cytoplasm due to accumulation of glycogen. In humans, CCCs represent only 2-6% of chondrosarcomas and have a preference for long bones. This is a rare tumor in dogs which seems to exhibit a site predilection that differs from humans. Because of the low number of reported cases, further evaluation and research would be warranted.

7: INFLUENZA A-ASSOCIATED RHABDOMYOLYSIS IN A MYHM HETEROZYGOUS QUARTER HORSE: A CASE REPORT

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Rhabdomyolysis is a serious clinical syndrome with high morbidity and mortality and a wide range of reported triggers. While Influenza A-associated rhabdomyolysis is documented in humans, it is rarely reported in veterinary species. We describe a case in a 4-year-old male castrated Quarter Horse that presented with acute respiratory signs and fever, which rapidly progressed to pigmenturia and recumbency. Due to the grave prognosis, humane euthanasia was elected.

Postmortem examination revealed extensive monophasic rhabdomyolysis affecting all major skeletal muscle groups, accompanied by renal tubular necrosis, intratubular casts, and pigmenturia. There was also necrotizing bronchitis and bronchiolitis, and epithelial necrosis within the guttural pouches. The lungs were PCR positive for Influenza A. Accordingly, the widespread rhabdomyolysis was attributed, in part, to Influenza A infection.

Given the horse’s breed, we also explored the possibility that genetic factors contributed to its susceptibility to rhabdomyolysis. Periodic acid-Schiff (PAS) staining revealed no abnormal polysaccharide accumulation within the muscle, and genetic testing confirmed the absence of the Polysaccharide Storage Myopathy type 1 (PSSM1) allele. However, the horse was identified as a heterozygous carrier of Myosin-Heavy Chain Myopathy (MYHM), a disease associated with two distinct clinical presentations: immune-mediated myositis and non-extertional rhabdomyolysis. MYHM requires immune triggers, such as infection or vaccination, to manifest clinically. In this case, Influenza A likely served as the trigger, exacerbating disease severity.

This case highlights the importance of including Influenza A as a differential diagnosis or potential instigator for rhabdomyolysis in horses, particularly those with a known genetic predisposition.

8: MOLECULAR AND MORPHOLOGICAL CHARACTERIZATION OF EMERGING HEMOPROTOZOAL INFECTIONS IN IMPORTED REPTILES IN TAIWAN

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Hemoprotozoa are blood-parasitic microorganisms with complex life cycles. Despite their high prevalence, little is known about their diversity and interactions with reptilian hosts. This study investigated samples from 9 reptiles across 5 species imported to Taiwan as pets, employing molecular, clinical, and anatomical pathological techniques. In *Ctenosaura* spp., hemococcidian sporozoites were identified in both *C. quinquecarinata* and *C. similis*. Phylogenetic analysis of 18S rDNA and cytochrome c oxidase subunit 1 (*COI*) sequences revealed that all strains to belong to *Lankesterella* sp. In contrast, *Hepatozoon* sp. gametogenic stages in *C. quinquecarinata* were morphologically distinct from *H. gamezi* in *C. similis*. Phylogenetic analysis revealed that these sequences are closely related to *Hepatozoon* species from snakes. Further, novel *Hepatozoon* sp. gamonts were observed in blood smears from the blue tree monitor, with various developmental stages identified in hepatic tissue. Given the phylogenetic divergence from GenBank 18S rDNA sequences, we propose a new species, *Hepatozoon macraei* sp. nov. In the anemic plumed basilisk, multiple *Plasmodium* sp. trophozoites were observed within erythrocytes, and a meront was present in the thrombocyte cytoplasm. After molecular cloning and phylogenetic analysis of cytochrome *b*, *COI*, and 18S rDNA genes, the newly obtained sequences formed a distinct monophyletic clade, which was phylogenetically distant from sequences available in GenBank. This study provides molecular and morphological evidence of hemoprotozoan diversity in reptilian pets in Taiwan, highlighting the need for further research into host-pathogen interactions and their impact on native fauna in the context of the global exotic pet trade.

9: FIBROUS OSTEODYSTROPHY IN A DOMESTIC RABBIT WITH PRIMARY HYPERPARATHYROIDISM

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Background: Fibrous osteodystrophy (FOD) is a metabolic bone disease resulting from primary or secondary hyperparathyroidism in domestic species. In primary hyperparathyroidism, a parathyroid neoplasm secretes parathyroid hormone in an unregulated manner, resulting in hypercalcemia (by bone, kidney, and indirect gut-mediated mechanisms).

Objective: To characterize a case of FOD in a 9-year-old Dutch-belted rabbit with a parathyroid adenoma and metastatic mineralization submitted for necropsy.

Results: Clinically, the rabbit had a 4-week history of hypercalcemia (total calcium 17.8-15.1mmol/l) and a 5-day history of azotemia (creatinine 3.0 mg/dl, BUN 109 mg/dL) with hyperphosphatemia (10.2 mg/dl) prior to euthanasia. During gross examination, the mandible fractured into multiple fragments while manipulating the jaw and complete diaphyseal femoral fractures with hemorrhage were identified. The right extracapsular parathyroid gland was enlarged (6 x 4 x 3 mm); the other parathyroid glands were not grossly identified. Histopathologic examination of the mandible revealed replacement of the bone by fibrous connective tissue and active osteoclast-mediated bony resorption/lysis, consistent with FOD. Histopathology of the right external parathyroid gland was consistent with an adenoma. Histologically, the left extracapsular parathyroid gland measured 1.5 x 3 mm, while the internal parathyroid glands were not identified. Bilaterally, the kidneys had lesions consistent with acute and chronic injury. Metastatic mineralization was present in multiple tissues including the lungs, kidneys, heart, and stomach.

Conclusions: To our knowledge, this is the first report of fibrous osteodystrophy in a rabbit associated with a parathyroid adenoma. Metastatic mineralization from marked hypercalcemia and hyperphosphatemia is attributable to concurrent renal injury.

10: INTESTINAL ADENOCARCINOMA WITH PERITONEAL CARCINOMATOSIS IN A DONKEY (EQUUS ASINUS)

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Intestinal adenocarcinoma is rarely reported in donkeys. A three-year-old, castrated male donkey (*Equus asinus*) was presented to Auburn University Veterinary Teaching Hospital for evaluation of inappetence and lethargy for two weeks. Physical examination revealed reduced gastrointestinal sounds in all quadrants and 6 liters of net enterogastric reflux were retrieved. Abdominal ultrasound revealed a significantly increased amount of heterogeneous free peritoneal fluid and diffuse thickening of the small intestinal walls. Based on these findings, a presumptive diagnosis of peritonitis and pleuritis was made. Due to the poor prognosis, the owners elected euthanasia, and a postmortem examination was performed.

Grossly, there was approximately 2-L of peritoneal effusion. Numerous white to tan, up to 1-cm in diameter, round to irregular plaques were scattered throughout the peritoneal wall, diaphragm, mesentery, greater omentum, serosal surface of the urinary bladder, liver, spleen, small intestine, large colon, and small colon. At the ileocecal junction, the ileal wall was thickened and transmurally effaced by a firm, tan, 13 cm x 12 cm x 5 cm, mass. The ileal mass was histologically confirmed as an adenocarcinoma with lymphovascular invasion. The neoplastic cells had a strong cytoplasmic immunolabeling for cytokeratin and lacked immunolabeling for vimentin. Similar neoplastic cells were widely spread to multiple organs within the peritoneal cavity (carcinomatosis). Although

rarely reported, this case report highlights the importance of including intestinal adenocarcinoma as a differential diagnosis when an intestinal mass is clinically reported in donkeys.

11: IMMUNOCIDIN TREATMENT-ASSOCIATED PYOGRANULOMATOUS DERMATITIS IN A HORSE

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Immunocidin is a well-known immunotherapeutic agent utilizing Mycobacterium Cell Wall Fraction (MCWF) which is commonly implemented as a part of the treatment of equine sarcoids in an attempt to induce immunostimulation and sarcoid regression. Here, we report multifocal pyogranulomatous and lymphocytic dermatitis in multiple sections of tissue submitted for biopsy from cutaneous nodules on a horse being treated with Immunocidin for cutaneous sarcoids of the left midline and left inguinal areas. The animal was treated with locally injected Immunocidin four times over two months with reported subjective decrease in size of the sarcoids. Histologically, sections of skin revealed multifocal dermal and subcutaneous nodular and perivascular granulomatous to pyogranulomatous inflammation occasionally surrounded by variable numbers of fibroblasts and mature fibrocollagenous tissue. Within the center of the inflammatory nodules were variably sized, clear, lipid vacuoles frequently encompassed by neutrophils. Low numbers of extracellular, acid-fast positive bacilli or bacterial fragments were present in regions containing lipid vacuoles. Histopathologic features indicative of equine sarcoid were not observed in the submitted tissue. These findings are consistent with a reaction to MCWF therapy.

12: VIVOLOCK: A NOVEL 3-D SCAFFOLD FOR OPTIMAL FORMALIN PRESERVATION OF GASTROINTESTINAL TISSUE IN A PORCINE MODEL

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Accurate histopathological evaluation relies on optimal tissue preservation, particularly of tissues in the gastrointestinal tract (GIT) prone to rapid autolysis. Conventional formalin fixation methods frequently lead to tissue shrinkage, distortion, and artifact. Here, we introduce VivoLock, a reusable 3-D printed scaffold device specifically designed to enhance formalin fixation by maintaining tissues in anatomically neutral positions, thereby reducing histologic artifacts and shrinkage. This study compares the preservation efficacy of VivoLock across multiple segments of the porcine GIT, including stomach fundus, duodenum, jejunum (proximal and distal), ileum, cecum, spiral colon, descending colon, and rectum. Compared to standard fixation, tissues preserved using VivoLock exhibited enhanced structural integrity, reduced shrinkage, and fewer histologic artifacts. This scaffold-supported fixation method offers a promising advancement in preservation of GIT tissues and may improve histologic detail across diverse diagnostic and comparative pathology applications.

13: A GRANULOMATOUS RIDE: TRULY GENERALIZED SARCOIDOSIS IN A MARE

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Equine sarcoidosis is a rare, systemic granulomatous disease in horses characterized by the formation of non-caseating granulomas in multiple tissues. A 15-year-old Quarter Horse mare with a history of cutaneous sarcoidosis was euthanized after radiographs revealed several lytic lesions of the distal limbs. Initial biopsies ruled out the presence of infectious organisms and supported the diagnosis of sarcoidosis. Postmortem examination revealed multifocal areas of alopecia and thickening over the ventral, thoracic, head, and neck skin; Multifocal tan to beige discoloration of skeletal muscle fibers as well as nodules at the root of the mesentery. Additionally, several limbs had round, gray cortical bone lesions, consistent with those seen in radiographs. Histopathological examination revealed granulomatous inflammation in the skin, lymph nodes, kidneys, liver, skeletal muscle, spleen, mesentery, colon, cerebellum, and bones. Highlighted here are some of the histological findings in a case that presented with cutaneous, visceral, and osseous lesions, for a truly generalized presentation.

The authors declare that portions of the content presented in this abstract are based on findings from a forthcoming article.

14: MYCOPLASMA BOVIS INFECTION IN A HERD OF BISON IN NORTHERN INDIANA

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Background: First isolated in 1961, *Mycoplasma bovis* is a major pathogen of cattle, causing various syndromes including bronchopneumonia, mastitis, keratoconjunctivitis, and arthritis. *Mycoplasma bovis* is an emerging pathogen in herds of bison reported with high morbidity and mortality, with clinical manifestations including pneumonia, polyarthritis, reproductive failure, and necrotizing pharyngitis.

History: Four adult bison were submitted for necropsy at the Indiana Animal Disease Diagnostic Laboratory in November 2024, with reported clinical signs of weakness, difficulty walking, weight loss, and self-isolation from the herd over a duration of two to three weeks. One bison was observed with a deep cough, but no respiratory signs were reported in the other three bison submitted.

Description: The gross necropsy findings among the four animals included fibrinous pleuritis, pulmonary abscesses, caseous bronchopneumonia, and laryngeal ulceration. Histopathologic findings included extensive necrotizing to pyogranulomatous bronchopneumonia, pyogranulomatous lymphadenitis, and ulcerative laryngitis. In each bison, PCR testing identified *Mycoplasma bovis* from samples of lung (Ct values: 18.50, 34.39, 20.71, 18.46). The lesions and ancillary testing in these bison were diagnostic for

respiratory mycoplasmosis. As infections with *M. bovis* are primarily reported from the Western United States, this case is notable as an outbreak in a herd in Indiana.

15: CHRONIC DERMATITIS IN AM AMERICAN FLAMINGO (PHOENICOPTERUS RUBER) WITH INTESTINAL LYMPHOMA

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Background: Lymphoma is occasionally reported as a spontaneous disease in zoo species, including birds of prey and parrots. B-cell lymphoma is most common and typically presents as chronic wasting and inappetence. Paraneoplastic dermatitis is associated with thymic lymphomas and hepatobiliary or pancreatic carcinomas in mammals. While mammal dermatitis can occur secondary to severe, chronic liver inflammation or decreased liver function, this type of dermatitis is not commonly reported in birds despite a high frequency of chronic hepatitis.

Case description: A geriatric, male, American flamingo with a history of chronic alopecia and hyperplastic dermatitis was received for histology after necropsy by zoo veterinarians. Feathered skin including the keel and ventrum were most severely affected. Proximal legs and ventral wings were less severely affected. On histology, the long-standing dermatitis lacked apparent infectious organisms. Previous biopsy has similar findings with few, superficial bacteria (Staphylococcus and Enterobacter sp). The patient was vaccinated for West Nile Virus. On histology, the flamingo had widespread, small cell lymphoma in small intestine sections and mild to moderate, portal hepatitis with sinusoid protein accumulation. Neoplastic lymphocytes were confined to the mucosa and obscured enterocytes with intraepithelial clusters.

Discussion: Dermatitis in birds is typically associated with self-trauma, viral infections not reported in flamingos, or infectious causes ectoparasite, fungal, or bacterial infections. This patient had severe, chronic dermatitis with three primary differentials, severe atopy/immune regulatory dermatitis, hepatopathy dermatitis, or paraneoplastic dermatitis. This presentation will discuss the likelihood of these differentials and encourage discussion of non-infectious dermatitis in zoo species.

16: SKELETAL DYSPLASIA AS A CAUSE OF HYDROPS FETALIS IN AN INFANT AFRICAN GREEN MONKEY (CHLOROCEBUS AETHIOPS)

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A 550-gram full-term male African green monkey (*Chlorocebus aethiops*) was delivered deceased via Cesarean section with anasarca, shortening of the spine and limbs, and scoliosis. The thoracic cavity was small, the heart had eccentric hypertrophy, and the lungs were hypoplastic. On histology, the alveoli were small, separated by prominent mesenchyme, and lined by cuboidal and flattened epithelial cells, consistent with the canalicular stage of development. The bones of the fingers had short growth plates without prehypertrophic chondrocytes in the proliferating zones, and fibrous connective

tissue filled what should have been joint spaces. The metaphyses contained scant normal bone marrow and the diaphyses were mostly composed of solid bone interspersed with small spaces containing loose connective tissue. Nonimmune hydrops fetalis was diagnosed and attributed to skeletal dysplasia. Failure of endochondral ossification and stunting of long bone development contributed to thoracic hypoplasia which in turn arrested pulmonary development and obstructed venous and lymphatic return to the heart leading to widespread edema. Skeletal dysplasia is a heterogeneous group of disorders characterized by abnormal bone and cartilage development. Histologically, this case was most consistent with chondrodysplasia as reported in cattle and experimentally induced mouse models, however to the author's knowledge, cases in nonhuman primates have not been reported.

17: PLEIOMORPHIC FACIAL TUMOR IN A DOMESTIC RAT

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A 2-year-10-month-old male castrated domestic rat (breed unspecified) presented with a two-week history of a rapidly growing subcutaneous mass along the left side of the head (grew to 4-5x original size in that time), and euthanasia was elected. At gross necropsy, the mass was located immediately adjacent to the left submandibular and sublingual glands, 4x3x3cm, soft, and multicavitated, containing a mixture of viscous, clear, straw-colored fluid and hemorrhagic fluid. Histologically, tumor cells were pleiomorphic, exhibiting spindle, stellate, and round cell morphologies arranged in streams and whorls along the periphery vs. sheets at the tumor center, interspersed with occasional tubular structures (r/o remnant ducts). Perineural invasion was evident, along with local invasion into subcutis, masticatory muscle, and salivary gland. Mitotic count was high, with an average of 20 per 2.37mm^2 and frequent bizarre mitoses. Along the tumor margin and scattered throughout the tumor were low numbers of mast cells, lymphocytes, plasma cells, and rare neutrophils. Differential diagnoses included malignant myoepithelioma, mixed malignant tumor of the salivary gland (or less likely exorbital lacrimal gland), or other sarcoma not otherwise specified. Differentiation between malignant myoepitheliomas and mixed malignant tumors is often difficult based on histomorphology alone; immunohistochemistry for CK5/6, SMA, calponin, p63, and IBA-1 were pursued for further characterization. Salivary myoepitheliomas are occasionally reported in mice, and are typically unencapsulated, invasive, often pleiomorphic, and can have central degeneration and necrosis leading to pseudocyst formation. To our knowledge, salivary gland myoepitheliomas have not been previously reported in rats.

18: H5 HIGHLY PATHOGENIC AVIAN INFLUENZA IN 3 CATS IN NEW JERSEY

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Three adult, intact female cats—one Siamese, one domestic shorthair (DSH), and one domestic longhair (DLH)—were submitted to the New Jersey Animal Health Diagnostic Laboratory for postmortem examination. Clinical signs among the animals ranged from

anorexia to varying degrees of upper respiratory disease. The DSH and DLH cats were found deceased, while the Siamese cat was euthanized due to severe respiratory distress. In total, nine cats from the same property died or were euthanized.

Gross examination revealed pulmonary edema in all three cats; otherwise, necropsy findings were unremarkable. Tissues were submitted for histopathologic evaluation, which revealed systemic disease in all cases, with animal variability in lesion distribution and severity. Notable findings included necrotizing hepatitis in the DSH and DLH cats, pulmonary inflammation in the Siamese and DLH cats, myocardial inflammation in the Siamese cat, and meningoencephalitis in the DLH cat. The Siamese cat also exhibited mild bacterial nephritis and pulmonary nematodiasis, which may have contributed to overall morbidity.

Nasal swabs from all three cats tested non-negative for influenza A virus (IAV) and H5 highly pathogenic avian influenza (HPAI) by RT-PCR. Confirmatory testing at the National Veterinary Services Laboratory (NVSL) detected both IAV and H5 HPAI in all three cases.

To our knowledge, this represents the first reported case series of HPAI infection in felines in New Jersey. The affected cats had no known exposure to infected poultry, livestock, raw milk, or raw meat, but were free-roaming and may have encountered infected wild birds or other animals.

19: HISTOLOGIC CHARACTERIZATION OF NEPHROBLASTOMA IN A CAPTIVE COLONY OF NORTHERN TREE SHREWS

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Background: The northern tree shrews, *Tupaia belangeri,* native to Southeast Asia, are of increasing interest in biomedical research as alternative models for human research. Neoplasia occurs in aged, captive tree shrews, with mammary adenocarcinoma, lymphoma, squamous cell carcinoma and pulmonary adenoma/carcinoma most commonly noted. In this study we report on 7 tree shrews (5 Male+ 2 Female) diagnosed with spontaneous, nephroblastoma over a 15-year period.

Objective: Histologic description of nephroblastoma in captive, northern tree shrews (average age at diagnosis - 1.64 years).

Methods: Specimens were fixed (10% NBF), decalcified (Formical) if needed, then processed in paraffin. Tumor type was diagnosed based on gross and H+E microscopic findings by board certified veterinary anatomic pathologists. Special stains (Mason's Trichrome and Von Kossa) were performed as needed to evaluate fibrosis and osseous metaplasia.

Results: Tree shrews reported with gross evidence of an abdominal mass (43%) +/- hemorrhage (29%) or were subclinical with incidental diagnosis (43%). Tumors were unilateral (100%), multi-lobulated, tan, irregular, +/- ossifeus metaplasia (14%) and found compressing remaining renal parenchyma (57%). Histologically, tumors contained

mixtures of stromal (fusiform cells arranged in bundles surrounded by variably dense ECM), epithelial (columnar-cuboidal cells arranged in tubules), and blastemal (basophilic polygonal cells arranged in streams and bundles) cells often compressing or obliterating normal renal parenchyma. Variable amounts of hemorrhage, fibrin thrombi, intersecting bands of collagen and/or irregular osseous matrix was present within and around tumor tissue.

Conclusions: Clinical presentation and tumor morphologies are consistent with first reports of nephroblastoma diagnosed in captive northern tree shrews.

20: GANGLIONEUROMA IN AN EASTERN ROSELLA (PLATYCERCUS EXIMUS): A CASE REPORT

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A 21-year-old Eastern Rosella (Platycercus eximus) presented with a 1.0 cm, soft, tan, subcutaneous mass on the left lateral neck, identified during routine examination and surgically excised one week later. On histologic evaluation, the mass was incompletely excised, densely cellular, multilobular, and partially encapsulated by fibrous connective tissue, composed of mature ganglion cells interspersed within a fibrillar stroma of spindle cells morphologically consistent with Schwann cells. Prominent, densely cellular aggregates of small, round cells surrounded ganglion cell clusters and neuropil-rich regions, initially thought to be neuroblasts.

Immunohistochemistry (IHC) was performed using archived FFPE brain tissue from a conspecific as a control. Neuron-specific enolase (NSE) was strongly positive in ganglion cells, confirming neuronal differentiation. The small round cells were immunoreactive for CD3 and negative for Pax5, indicating T-cell lymphocytes rather than neuroblasts. Synaptophysin did not produce reliable staining in this avian tissue. Based on IHC findings and the patient's advanced age, the mass was diagnosed as a ganglioneuroma.

While likely incidental in this individual, the case underscores the importance of immunohistochemistry in the diagnosis of neuroectodermal tumors in birds, for which the literature remains limited, and highlights both the utility and limitations of IHC in exotic avian species.

21: A CASE STUDY OF SEPTIC ARTHRITIS IN SHOREBIRDS FROM VIRGINIA COAST

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Background: Colonial shorebirds are globally at risk of population decline due to habitat degradation and resource depletion; while they provide ecological services such as nutrient cycling and plant dispersal. In summer 2024, during routine nest monitoring of barges with artificial nesting substrate in Virginia, juvenile shorebirds were observed with swollen, crusty feet and later died or were dispatched.

Objective: To determine the causes and contributors to morbidity and mortality among young shorebirds during a localized outbreak.

Methods: Royal tern (Thalasseus maximus; n=2), black skimmer (Rynchops niger; n=1), and common tern (Sterna hirundo; n=7) carcasses from this outbreak underwent gross and histologic evaluation with ancillary diagnostic testing at the Southeastern Cooperative Wildlife Disease Study.

Results: 50% of affected birds were juveniles; 50% were fledglings (n=5 each). 60% (n=6) were male, 30% (n=3) were female, and sex was unknown for one bird. Most (80%, n=8) had skin wounds and bacterial arthritis, tenosynovitis, and/or pododermatitis. The most affected joints included the tarsometatarsal-phalangeal (30%, n=3), proximal interphalangeal (n=1), metatarsal-phalangeal (n=1), and intertarsal (n=3). Staphylococcus aureus was the predominant bacterial agent cultured from affected joints. Comorbidities and coinfections included aspergillosis (n=3), circovirus infection (n=3), bacterial coelomitis (n=1), and avian pox (n=1).

Conclusions: Morbidity and mortality in wild shorebirds are often anthropogenic in origin or influence. In this case, artificial nest structures were created to encourage and support successful breeding but inadvertently may have adversely impacted health through abrasive surfaces that facilitated bacterial infection of skin and joints and predisposed to secondary infections.

22: CHROMOBACTERIUM VIOLACEUM INFECTION IN A DOG – A COLORFUL CONUNDRUM

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A 2.5-year-old spayed female pit bull was euthanized following a one-week history of worsening ulcerative dermatitis with rapid emergence of multiple draining skin ulcers, fever (105.0°F), and dependent edema. Clinical deterioration was acute and refractory to antimicrobial and anti-inflammatory treatment. Necropsy revealed approximately a dozen cutaneous ulcers distributed over the head and body, ranging from 1.5 x 1.0 x 0.5 cm to 2.5 cm in diameter. Lesions exuded serosanguinous to purulent discharge and were associated with deep subcutaneous tract formation. Edema extended along the subcutis of the ventrum and proximal forelimbs.

Microscopically, epidermal ulceration was contiguous with areas of deep dermal to subcutaneous necrosis containing abundant neutrophils, macrophages, fibrin, and hemorrhage. Dermal and subcuticular blood vessels exhibited thrombosis and fibrinoid

necrosis. Additional lesions included random hepatocellular necrosis with histiocytic and neutrophilic inflammation (microabscesses), perifollicular histiocytic splenitis, and pulmonary edema, suggestive of sepsis.

Gram staining of affected tissues was negative. However, aerobic culture of skin swabs yielded moderate growth of violet-pigmented colonies on MacConkey agar. MALDI-TOF analysis identified the isolate as *Chromobacterium violaceum*, a saprophyte endemic to tropical and subtropical environments. This oxidase positive, Gram-negative rod is an opportunistic pathogen in mammals associated with cutaneous ulceration, visceral abscesses, and septicemia. Transmission likely occurs when cutaneous lesions are exposed to stagnant water or soil, or when the organism is ingested or inhaled. Although rare, infections are rapidly progressive and often fatal. Observation of characteristic violet-pigmented colonies on bacterial culture should prompt consideration of this uncommon but virulent entity.

23: SKELETAL-EXTRASKELETAL ANGIOMATOSIS IN A FEMALE BOXER PUPPY

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A 3-month-old, female, Boxer puppy presented for bilateral inguinal lymph node enlargement, vulvar swelling, and hip pain. Pelvic radiographs revealed moth-eaten to permeative lytic lesions affecting the right hemipelvis and right femoral head and neck. An incomplete pathologic fracture of the cranial proximal right femur, an impending right femoral neck fracture, and a suspected folding fracture of the right ischium were also noted. The dog was euthanized due to a poor prognosis. The fractures were confirmed at necropsy and were surrounded by extensive dark red areas in the skeletal muscle and subcutaneous tissues. Additional gross findings included dark red and enlarged inguinal and iliac lymph nodes, dark red foci in the lungs, and a swollen vulva. Histopathology revealed an infiltrative proliferation of vascular channels lined by bland to mildly hypertrophied CD31-positive and variably PROX1-positive endothelial cells. Affected tissues included the right femur and ischial arch, adjacent gluteus medius muscle, multiple lymph nodes, lungs, and reproductive tract. The findings are consistent with skeletal-extraskeletal angiomatosis, a rare proliferation of vascular structures involving both the bone and soft tissues. In children, this condition is thought to represent a vascular malformation. The few reports of this entity in dogs have all involved the distal phalanx and surrounding soft tissue, with this case being the first report in the femur and visceral organs, to the best of our knowledge.

24: DESIGN AND VALIDATION OF A NOVEL RNASCOPE IN-SITU HYBRIDIZATION PROBE FOR APICOMPLEXAN PROTOZOA IN THE FAMILY SARCOCYSTIDAE (TOXOPLASMA GONDII, NEOSPORA SPP., SARCOCYSTIS SPP.)

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Background: Apicomplexan protozoa in the family *Sarcocystidae* (*Toxoplasma gondii, Neospora* spp., and *Sarcocystis* spp.) cause a wide array of pathologic changes in animals. Consequently, identifying the specific etiology is difficult. These organisms cannot be differentiated on routine histologic examination, and immunohistochemical assays have proven non-specific; thus, RNAscope in-situ hybridization (ISH) was explored to differentiate organisms in formalin-fixed paraffin-embedded (FFPE) tissue.

Objective: Designing and validating RNAscope ISH probes for *Sarcocystidae.*

Material and Methods: A retrospective analysis of protozoal infections diagnosed at the Animal Health and Diagnostic Center (Cornell University) over a 9-year period was completed. Cases with visible protozoal cysts were selected, and FFPE tissue was subjected to nucleic acid isolation, pan-*Sarcocystidae* RT-PCR, sequencing, and analysis. The ARB-Silva database was used as a comprehensive reference collection of rRNA sequences for in-silico design of probes targeting *Toxoplasma gondii*, *Neospora* spp., and *Sarcocystis* spp. RNAscope ISH probes were created based on these sequences and tested on 9 cases.

Results: A total of 855 cases were identified and pan-Sarcocystidae RT-PCRs were completed on 35/855 cases. 17/35 (48.6%) cases were positive for apicomplexan protozoa; 3 distinct ISH probes with 100% in-silico sensitivity and specificity were designed. In-vitro, *Toxoplasma gondii* and *Neospora* spp. probes had strong cross-reactivity; the *Sarcocystis* spp. probe exhibited hybridization signal only in cases positive for *Sarcocystis* spp.

Conclusions: In this pilot study, *Toxoplasma gondii* and *Neospora* spp. ISH probes exhibited significant cross-reactivity, but the *Sarcocystis* spp. probe was specific to *Sarcocystis* spp. hence can improve the current diagnostics of protozoal infections.

25: WIDESPREAD POORLY DIFFERENTIATED SARCOMA IN A WARMBLOOD HORSE: A CASE REPORT

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A 16-year-old intact male Warmblood horse was presented with signs of colic, depression, and reduced appetite. Bloodwork revealed leukocytosis and hypercalcemia of unknown origin. Despite the administration of significant volumes of fluids and antibiotics, the horse’s condition deteriorated, progressing to bilateral hind limb swelling, generalized subcutaneous edema, and gastrointestinal hypomotility. Extensive follow-up examinations ruled out renal and metabolic diseases, raising suspicion for paraneoplastic hypercalcemia of malignancy. However, no overt neoplastic masses were identified ante-mortem. Exploratory abdominal laparotomy revealed large, invasive sublumbar masses, and humane euthanasia was subsequently elected.

Necropsy revealed multiple firm masses along the sublumbar region involving the sublumbar lymph nodes and both adrenal glands, with metastases to the myocardium, intercostal musculature, and rib bones. Cytological evaluation of impression smears showed moderate cellularity with individual spindle to round, pleomorphic neoplastic cells exhibiting moderate to high nuclear-to-cytoplasmic ratios. The neoplastic cells had vacuolated cytoplasm with indistinct cell borders, round to ovoid nuclei with multiple prominent nucleoli, and features consistent with a malignant process, including binucleation, anisocytosis, and anisokaryosis. Histopathology demonstrated a widespread, invasive mesenchymal neoplasm, most consistent with a sarcoma. Immunohistochemistry showed positivity only for vimentin and negativity for CD3, Melan-A, synaptophysin, and chromogranin A, leading to a diagnosis of poorly differentiated sarcoma. Liposarcoma remained a differential diagnosis.

This case highlights the diagnostic challenges of paraneoplastic hypercalcemia in horses and underscores the value of postmortem investigation in identifying occult neoplasia.

26: NECROSUPPURATIVE NEISSERIAL PNEUMONIA IN A DOMESTIC SHORTHAIR CAT

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Background: Neisseria species are small, gram-negative cocci that seldomly cause pneumonia in cats. Pulmonary infection can pose a diagnostic challenge given nodular interstitial pattern in the lungs on thoracic radiographs with more common differentials including fungal or neoplastic disease. The prognosis is typically poor with rapid decline and death which can occur within hours of clinical onset. The pathogenesis is poorly understood; however, hematogenous spread, particularly from the oral cavity, is considered a possibility since it is considered a part of the commensal flora in the region. Here, we describe a case of Neisseria sp. causing pneumonia in a domestic shorthair cat.

Case Description: A 6-year-old, male castrated, domestic shorthair cat was submitted for necropsy at the Louisiana Animal Disease Diagnostic Laboratory after having a fatal seizure at a veterinary clinic. The cat had been lethargic and anorexic with unusual behavior during the previous three days, and acute dyspnea. On gross examination, the lungs were mottled red, dark red, and purple with widespread, multifocal to coalescing, tan to dark red, irregularly contoured, umbilicated nodules within all lobes that bulge on the cut surfaces. Histologically, these nodules corresponded to clusters of degenerate neutrophils and fewer macrophages mixed with abundant necrotic debris, acute hemorrhage, fibrin, and myriads of gram-negative cocci. Aerobic bacterial culture of the lungs has pure and heavy growth for Neisseria sp.

Summary: This is a case of fatal necrosuppurative neisserial pneumonia with pure Neisseria sp. growth that has progressed to sepsis in a domestic shorthair cat.

27: MASS MORTALITY IN JUVENILE FARMED ALLIGATOR SNAPPING TURTLES (MACROCHELYS TEMMINCKII) DUE TO CUTANEOUS FUSARIOSIS (F. SOLANI SPECIES COMPLEX [FSSC]) AND CITROBACTER FREUNDII

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Background:

Fusarium spp. are ubiquitous fungi infecting marine animals with increasing reports in sea turtles and alligators. *F. solani* is considered the main etiological agent in sea turtles and part of a monophyletic species complex (*F. solani* species complex [FSSC]) with some reported isolates being *F. falciforme* and *F. keratoplasticum.*

Citrobacter freundii is a major component of septic cutaneous ulcerative disease (SCUD), a variably fatal disease; other gram-negative bacteria have been implicated. SCUD is due to bacterial colonization and translocation through cutaneous wounds with other factors including poor husbandry/water quality, microbial infections, or decreased immunocompetence.

Case Description: In an alligator snapping turtle hatchery, juveniles hatched in 2023 and 2024 were suddenly lethargic with keratin loss and had escaping behaviors after using environmental water from a nearby bayou. Several hundred died and four cohorts including both euthanized and dead turtles were submitted for necropsy. These had multifocal to coalescing, brown, friable, and easily torn skin on the extremities and head. Histologically, there was erosive to ulcerative dermatitis with numerous fungal hyphae and gram-negative bacilli. Bacterial culture of the skin grew various bacteria including *C. freundii* and other gram-negative bacteria which were also isolated from the liver. Fungal culture of the skin grew heavy *F. solani*, and fungal PCR against internal transcribed spacer region (ITS) revealed DNA from FSSC, identifying most closely as *F. solani* and *F. keratoplasticum*.

Summary: Primary erosive to ulcerative fungal dermatitis from FSSC caused bacterial colonization, translocation, and mass mortality in juvenile farmed alligator snapping turtles.

28: DUODENAL MACROPHAGES AND THEIR ROLE IN EQUINE INFLAMMATORY BOWEL DISEASE (IBD): A PRELIMINARY STUDY

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Background: Intestinal macrophages are key regulators of gastrointestinal homeostasis and inflammation in several species, but their role in equine IBD remains poorly understood.

Objectives: The study aimed to 1) Compare macrophage count between duodenal biopsies and their full thickness counterpart, 2) Quantify duodenal macrophages in horses with IBD.

Methods: Five horses were examined postmortem and 30 horses (with and without IBD) underwent duodenal biopsies at the equine hospital (CHUV). Full-thickness and endoscopic biopsies of the duodenum were obtained postmortem. Duodenal biopsies were performed in 30 horses by gastroduodenoscopy with endoscopic forceps. Tissues were stained immunohistochemically to identify M2 macrophages (CD204) and pan-macrophages (Iba-1). Histomorphometric analysis quantified macrophages within the lamina propria of villi (a), the deep lamina propria (b) and the sub-cryptal area (c).Agreement between biopsies and full-thickness tissues was assessed with intra-class correlation, while T-tests compared macrophage count between IBD and non-IBD horses.

Results: Data revealed that biopsies tend to underestimate CD204 and Iba-1 positive macrophages compared to full-thickness tissues. IBD horses showed significantly higher number of Iba-1 positive macrophages in the lamina propria of villi ($t = 3.44$, $p = 0.003$) and in all three lamina propria layers combined (a+b+c; $t = 2.34$, $p = 0.028$), compared to horses without IBD. Proportion of M1 macrophages (pro-inflammatory) is higher in horses with IBD than those without.

Conclusions: IBD horses have significantly higher numbers of duodenal mucosal macrophages, which highlights their potential importance in the diagnostic process and clinical management.

29: PUTATIVE POST-INTUBATION TRACHEAL PSEUDOMEMBRANE FORMATION IN A BARRED OWL (STRIX VALARIA)

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An adult, intact female barred owl (*Strix varia*) was submitted for postmortem examination after developing fatal dyspnea 25 days post-endotracheal intubation for a surgical procedure following a motor vehicle collision. Grossly, a 1-cm segment of tan opacity and mucus was present in the proximal to mid-trachea. Histologically, the tracheal mucosa was multifocally ulcerated with attenuation, erosion, and squamous metaplasia. Affected areas were lined by mucus, degenerate or hyperplastic epithelial cells, hemorrhage, fibrin, and nuclear debris. Broad-based polypoid projections and thick membranes composed of fibroblasts, collagen, and mixed leukocytes protruded from ulcerated mucosa. Similar inflammation and vasculitis were present in the lamina propria. No organisms were detected on Gram, GMS, or Ziehl-Neelsen stains, and PCR testing was negative for Avian Influenza Virus, Paramyxovirus-1, and West Nile Virus. Findings support fibrinous and fibroblastic tracheal pseudomembrane formation, likely a consequence of prior intubation. Obstructive fibrinous tracheal pseudomembrane (OFTP) is a rare, potentially fatal complication of endotracheal intubation reported in humans and animals, where mucosal trauma incites exudate accumulation and

potential airway obstruction. This case represents an atypical chronic presentation of fibrinous tracheal pseudomembrane formation. While similar (sub)acute airway obstruction lesions have been described, the presence of fibroblasts and collagen in this case indicates a chronic process not commonly reported in the literature. Although endotracheal intubation remains a highly suggestive inciting factor given the lesion distribution and clinical history, definitive attribution remains putative. Nevertheless, this case highlights a rare chronic sequela of post-intubation tracheal injury and expands the known temporal spectrum of this condition.

30: MYCOPLASMA BOVIS LIMB CELLULITIS IN DAIRY CATTLE: CASE INVESTIGATION AND A RETROSPECTIVE STUDY

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Mycoplasma bovis is a well-known bovine pathogen primarily associated with bronchopneumonia and otitis. Its involvement in cellulitis has not been previously described. Our study reports several cases of *M. bovis* limb cellulitis in cattle, based on a case investigation and a ten-year retrospective study. In the case investigation, four dairy cows from a single herd were presented with forelimb swelling and lameness. Postmortem examination revealed chronic necrosuppurative cellulitis and tenosynovitis. *M. bovis* was detected in affected tissues of all 4 animals by culture and immunohistochemistry, while no other bacteria or fungi were isolated. A retrospective study of bovine cases submitted to the California Animal Health and Food Safety Laboratory between 2014 and 2024 identified 12 additional cases of *M. bovis* infection associated with cellulitis, primarily in the limbs and also in the prepuce (n=1) and soft tissues surrounding lymph nodes (n=1). In these 12 cases, *M. bovis* was identified by immunohistochemistry, culture, and/or PCR. Concurrent bronchopneumonia (n=6), otitis media (n=2), and mastitis (n=1) were observed. This study provides evidence of a novel role for *M. bovis* as a cause of limb cellulitis in cattle. Findings support its inclusion as a differential diagnosis in cattle with lameness and subcutaneous inflammation. Further studies are needed to elucidate the pathogenesis, potential routes of infection, and the impact of co-infections.

31: SYSTEMIC FIBRINOGEN A ALPHA-CHAIN AMYLOIDOSIS IN AGED MANCHESTER TERRIERS

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Background: Amyloidosis in dogs comprises well-known entities such as familial systemic AA amyloidosis in Shar-Peis and senile cardiovascular Fibrinogen Aα-chain (FibA) amyloidosis. We identified a novel systemic amyloidosis in Manchester Terriers.

Objective: To elucidate the pathological characteristics of systemic amyloidosis in Manchester Terriers.

Materials and Methods: A retrospective analysis of the University of Missouri's Veterinary Medical Diagnostic Laboratory database from 2010–2019 identified 12 Manchester Terriers diagnosed with amyloidosis, all dogs originated from a single client. FFPE samples were available for five cases diagnosed since 2015. Histological analysis (H&E and Congo red staining) and immunohistochemistry for serum amyloid A (SAA) and fibrinogen Aα-chain (FibA) were performed. LC-MS/MS was conducted on amyloid deposits in the kidney and/or heart in four cases.

Results: All dogs were aged (mean age: 14.58 ± 2.12 years, range: 9–17 years). Amyloid deposits were most prominent within the kidneys (12/12), with additional involvement noted in the small intestinal submucosa (9/12), spleen (6/12), intramyocardial arterial walls (5/8), atrioventricular valves (2/3), and the intrahepatic arterial wall (1/12). In the kidneys, amyloid was deposited exclusively in the glomeruli. LC-MS/MS confirmed FibA in high score in all four analyzed cases. Immunohistochemistry showed positive for FibA and negative for SAA in all amyloid deposits.

Conclusion: We identified a novel, likely familial form of systemic FibA amyloidosis in aged Manchester Terriers. This study expands the spectrum of canine breed-specific amyloidosis and underscores the utility of immunohistochemistry and proteomic analysis for definitive amyloid typing, which is essential for accurate diagnosis and understanding of disease pathogenesis.

32: DIAGNOSTIC INSIGHTS FROM 120 CANINE STIFLE JOINT BIOPSIES OVER 15 YEARS

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Joint biopsies are performed in dogs to investigate intra-articular pathology, yet large-scale studies evaluating their indications, diagnostic yield, and histopathological correlation remain limited. This retrospective study reviewed 120 canine stifle joint biopsies collected over a 15-year period. Of these, 76 (63.3%) were obtained during surgical intervention for orthopedic conditions, most commonly cranial cruciate ligament rupture (CCLR) (n=65). The majority of these revealed hyperplastic, lymphoplasmacytic synovitis. Notable exceptions included synovial myxoma (n=1), synovial lipomatosis (n=1), *Blastomyces dermatitidis* infection (n=1), and suspected immune-mediated polyarthritis (IMPA) (n=1). The second most common indication was investigation of post-CCLR surgery complications (n=17), including lameness, pain, and/or infection, with a mean interval of 4 months postoperatively (range: 10 days to 1 year). Septic arthritis was diagnosed in 13 cases (10.8%) with *Streptococcus pseudintermedius* (n=4)

being the most isolated bacteria. Eleven biopsies were performed for clinically suspected joint neoplasm, yielding diagnoses of intra-articular sarcomas (n=4), histiocytic sarcomas (n=2), synovial myxomas (n=2), chronic synovitis/osteoarthritis (OA) (n=2), and fungal arthritis and osteomyelitis (*Aspergillus* sp., n=1). Additional indications included joint swelling (n=8), lameness (n=7), and chronic pain (n=1). Among these, chronic synovitis/OA (n=12), septic arthritis (n=1), and suspected IMPA (n=1) were diagnosed while two cases showed no significant histologic lesions. Overall, stifle joint biopsies provided clinically valuable diagnostic information and occasionally revealed unexpected findings, including infectious, neoplastic, and immune-mediated conditions. These results highlight the diagnostic utility of biopsy in characterizing the pathologic process occurring within the stifle joint in dogs.

33: CYCLOSPORINE-INDUCED DERMATOSIS IN A DOG

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Cyclosporine is a calcineurin inhibitor commonly used in veterinary medicine for the treatment of canine atopic dermatitis with rarely reported dermatologic side effects. A 1.5-year-old castrated male Great Dane was presented to the Auburn Dermatology Service with a history of non-seasonal pruritus since eight weeks of age. Despite medical management with an allergen-specific immunotherapy, a hypoallergenic diet trial, prednisone, and topical therapies, the patient became increasingly pruritic. Modified cyclosporine (400mg PO q24h) was added, however the dog experienced weight loss and developed progressive pustular lesions on the face, feet, and tail. On physical examination, the patient had multifocal to generalized erythematous and crusted nodules across the body including muzzle, pinnae, limbs, groin, paw pads, tail, and gingiva. Histopathology of haired skin punch biopsies included a multifocally markedly hyperplastic epidermis, predominantly forming broad and stunted papillated projections and occasional plaques, covered with marked compact-to-lamellar ortho- and, occasionally, parakeratotic hyperkeratosis, with subcorneal and intraepidermal pustules. These findings, in conjunction with clinical observations and previous cyclosporine administration, were consistent with cyclosporine-induced dermatosis, or psoriasiform-lichenoid-like dermatosis. Resolution of lesions was achieved by 11 weeks after withdrawal of the cyclosporine. In this case, a lichenoid band of dermal inflammation was not prominent, with the papillated hyperplasia, neutrophilic migration to the epidermis forming pustules, and parakeratotic hyperkeratosis being the most comparable findings to other previously reported cases. Despite the medical term (psoriasiform-lichenoid), the classic pattern may be inconsistent in cyclosporine-associated dermatologic conditions, with this case highlighting the variable clinical presentation associated with cyclosporine use.

34: OBSTRUCTIVE UROLITHIASIS IN THREE GUINEA PIGS (CAVIA PORCELLUS) AND UROLITH ANALYSIS BY INFRARED SPECTROSCOPY

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Urolithiasis is a common condition in guinea pigs (*Cavia porcellus*), frequently involving calcium carbonate uroliths that may form anywhere along the urinary tract. Obstruction can result in varied clinical signs and, in severe cases, sudden death.

This report describes the pathological findings in three guinea pigs from different locations, each presenting with sudden death and submitted to the anatomic pathology unit of the Centro de Diagnóstico Veterinario Bajío. Gross examination revealed bilateral hydronephrosis and hydroureter consistent with urinary tract obstruction.

Microscopically, kidneys displayed marked architectural disruption, with significant loss of corticomedullary differentiation and replacement of parenchyma by clear, fluid-filled spaces, indicating chronic obstructive damage. Uroliths recovered from the urinary system were subjected to infrared spectroscopy, which identified calcium carbonate as the primary mineral component.

The combination of gross, histological, and spectroscopic findings supports a diagnosis of chronic obstructive urolithiasis leading to renal failure and death. This condition underscores the need for preventive measures in guinea pigs, including dietary management and regular monitoring to detect early signs of urinary dysfunction.

Infrared spectroscopy proved to be a reliable diagnostic method for characterizing urolith composition, contributing valuable information for treatment and prevention strategies. These findings highlight the pathological consequences of delayed diagnosis and reinforce the importance of comprehensive clinical evaluation in small mammals.

35: COMPARISON OF HAIR VS. BLOOD AS A GERMLINE REFERENCE TISSUE IN CANINE LYMPHOMA

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Blood is the preferred germline tissue to assess somatic mutations within tumor tissue. However, the presence of circulating neoplastic cells as well as clonal hematopoietic variants could obscure identification of somatic variants in hematopoietic tumors/dogs with lymphoma. Various alternative sources of germline DNA including buccal swabs, saliva, skin biopsies, hair bulbs, urine, nails, CD3+ T cells, and skin swabs have been explored. However, many have limitations in DNA yield or quantity, or leukocyte contamination. Hair bulbs provide a non-invasive source of DNA with less potential for leukocyte contamination. We have recently established a protocol to successfully extract DNA from canine hair bulbs resulting in average fold coverage (30X-39X) sufficient to resolve heterozygosity. In this study, plucked hairs, peripheral blood, and

lymph node aspirate samples were collected from dogs with B-cell lymphoma confirmed via cytology and flow cytometry. Four patients were selected based on their variable proportions of circulating neoplastic cells (0, 332,4292, 47,765 cells/μL) enumerated via flow cytometry using the total white count on the peripheral blood. Whole genome sequencing was performed on hair, peripheral blood and lymph node derived DNA using a standard 350bp DNA library (Illumina). Sequences were aligned to canfam4 and peripheral blood and hair bulb variants compared to that of the lymph node for somatic variant detection using the Integrated Genome Viewer. Our preliminary analysis indicates that somatic variants are present in DNA derived from blood but not hair, suggesting hair may serve as a superior surrogate for germline tissue when circulating neoplastic cells are present.

36: PARAPLEGIA DUE TO SPINAL HISTIOCYTIC SARCOMA IN A PYGMY SLOW LORIS (XANTHONYCTICEBUS PYGMAEUS)

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An 11-year-old captive male pygmy slow loris presented for history of hind limb paraparesis. A CT (computed tomography) scan was performed but was within normal limits. The pygmy slow loris was treated for presumed intervertebral disc disease for six months but clinically progressed to being paraplegic and was euthanized. On histopathology, the spinal cord contained an extramedullary and intradural mass composed of sheets of round cells with moderate to abundant eosinophilic, slightly granular cytoplasm and round to ovoid, open, slightly eccentric nuclei. Anisocytosis and ansiokaryosis were mild with occasional binucleation and there were 0-1 mitotic figures per 2.37mm^2. The differential diagnosis included granular cell meningioma, extramedullary plasmacytoma, and histiocytic sarcoma. Special stains and immunohistochemistry (IHC) were performed. The neoplastic cells were positive for Iba-1 and Vimentin on IHC and negative for S100b, MUM-1, E-cadherin, Claudin-1 and periodic acid-schiff stain. The results support a diagnosis of histiocytic sarcoma. This is the first report of spinal histiocytic sarcoma in a prosimian species.

37: UNIQUE GROSS AND HISTOPATHOLOGIC APPEARANCE OF A FATAL MEDICATION ADMINISTRATION ERROR

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An 8-year-old neutered male Great Pyrenees was presented to MSU-CVM Diagnostic Laboratory for postmortem examination following sudden death during hospitalization for severe melena and anemia. During hospitalization, the dog was diagnosed with a gastric ulcer and received oral barium sulfate every six hours. On postmortem exam, the coronary vessels were diffusely prominent, raised, stark white, and firm. The right auricle was markedly distended and contained pasty, white material that was impacted between the pectinate muscles and adhered to a postmortem clot. All observed postmortem clots throughout the body were coated with similar material. The liver had fine, thread-like, arborizing, white vessels entering and exiting the centrilobular regions. Within the stomach, at the level of the pylorus, there was a 3x2cm chronic ulcer.

Notably, there was no barium within the stomach. The predominant histopathological finding was occlusion of vascular spaces by numerous, rhomboidal, clear, birefringent bright white, <1-20um crystals with rough edges in all major organs. Additionally, there was widespread thromboembolism consistent with disseminated intravascular coagulopathy (DIC). Following gross and microscopic findings, radiographs were taken of the heart, liver, and lungs, which revealed diffuse, radiopaque vascular patterns in each organ. Sections of liver were sent to Michigan State University for GC/MS which was negative for all compounds. A mineral analysis specific for barium was also performed, and barium levels were measured at 230.52 µm/g. The cause of death was attributed to intravascular administration of barium sulfate, which led to vascular occlusion and acute cardiovascular collapse.

****Content based on forthcoming publication****

38: TESTING PARAMETERS FOR HISTOLOGIC DETECTION OF EDEMA-LIKE ARTIFACTS IN LUNG

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Non-cardiogenic, pulmonary edema is a lesion associated with acute lung injury and diffuse alveolar disease. Etiologies can include infections (viral, bacterial, fungal), toxicities (paraquat), and systemic disorders (sepsis) to name a few. Pulmonary edema is microscopically identified by pools of eosinophilic liquid within alveoli and airspaces. The use of various protein products in some histology techniques can produce edema-like changes in lung and we wanted to clarify this potential. In the first experiment, we first evaluated use of gelatin in water baths, as a reported aid to adhere tissue sections onto slides. We tested gelatin concentrations (0.0-1.0% solutions), glass slide types (glass, Plus), positions on slide (top or bottom), and use of multiple washes (2-10x) on normal lung tissues to evaluate for pulmonary edema-like lesions. Edema-like artifacts in alveoli were observed within some airspaces at >0.6%. Yet eosin staining along and exterior to the pleural border were present as low as 0.2%. No significant differences were noted in slide type, tissue position or in multiple washes. To further investigate, we studied lung tissues from mice euthanized as part of other IACUC-approved studies. Lung tissues were insufflated with bovine serum albumin (BSA) to study the effects of protein concentration (0%-18%) in the lung. Higher concentrations of BSA had strong edema-like staining whereas lowest concentrations of BSA had no detectable staining. We propose that pathologists, especially those evaluating lung tissues, be aware of protein sources in histologic techniques and monitor appropriate controls to identify artifacts to prevent false positives of pulmonary edema.

39: ARGINASE-1 IMMUNOHISTOCHEMISTRY LABELS CANINE HEPATOCELLULAR CARCINOMAS

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Arginase-1 (ARG-1) is a widely used immunohistochemical marker for hepatocellular carcinoma in humans, while in veterinary medicine, its use has only been documented in sheep. In this study, 30 canine hepatocellular carcinomas were evaluated for ARG-1 immunohistochemical labeling. Of these, 28 of them showed diffuse, strong, cytoplasmic labeling with frequent, concurrent moderate to strong nuclear staining. The same pattern was seen in the adjacent non-neoplastic liver parenchyma in all cases in which it was available, providing a reliable internal positive control. Both tumors without immunolabeling were highly pleomorphic, however other tumors with strong immunoreactivity showed similar degrees of pleomorphism. In both of these cases, the non-neoplastic parenchyma had appropriate immunolabeling. Macrophages infiltrating areas of collapse and necrosis had consistent, diffuse, moderate cytoplasmic labeling for ARG-1. Increased ARG-1 expression has been described in M2-activated macrophages, consistent with a clean-up reaction. Endothelial cells in veins and arteries had inconsistent, moderate, cytoplasmic immunolabeling. Less than 10% of cholangiocytes had weak cytoplasmic immunolabeling, usually in small clusters or individual cells in 17 out of 30 cases. Nonspecific, weak to moderate labeling of intravascular plasma and mucus was also present in all cases. These results show that ARG-1 is a sensitive marker for canine hepatocellular carcinomas. Testing ARG-1 immunohistochemical staining on non-hepatocellular neoplasms is forthcoming to further validate the diagnostic value of this marker.

40: CORNEAL PLAQUES WITH FEATURES SUGGESTIVE OF CANINE PAPILLOMAVIRUS INFECTION IN THREE DOGS

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Canine papillomaviruses are double-stranded DNA viruses reported to cause a variety of clinical syndromes in dogs, including canine oral papillomatosis, cutaneous exophytic papillomas, inverted papillomas, and pigmented plaques and papules. Presently, there are at least 24 reported canine papillomaviruses. Little is reported regarding ocular lesions associated with canine papillomavirus infection. Reported lesions include corneal viral papillomas and conjunctival epithelial hyperplasia. Herein, we describe three cases of corneal plaque lesions that exhibit histologic changes classically associated with canine papillomavirus infection. Clinically, the patients presented with well demarcated, paraxial, grey, minimally raised corneal lesions. Two patients presented with an associated area of corneal ulceration. Histologic lesions were well demarcated with areas of epithelial dysplasia, prominent keratohyalin granules, koilocytes, parakeratotic hyperkeratosis, and rare eosinophilic intranuclear inclusions. In addition to these described changes, one patient sample also had a squamous cell carcinoma in situ adjacent to the area of aforementioned epithelial dysplasia. All corneal lesions were negative for papillomavirus via immunohistochemistry. Corneal lesions exhibited increased frequency of immunolabeling for Ki67 and p53. Two cases were negative for papillomavirus genes E1 and L1 via PCR, while one case was positive. These histologic lesions, in addition to the IHC and PCR results, support canine papillomavirus as one potential cause of corneal plaques in dogs.

41: STASIS DERMATITIS IN A CYNOMOLGUS MACAQUE

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Background: Stasis dermatitis is a cutaneous manifestation of venous hypertension from chronic impaired venous return. Increased hydrostatic pressure and venous stasis promotes local accumulation of edema fluid and inflammatory cells. This stimulates remodeling of the extracellular matrix and results in the proliferation of fibrovascular tissue.

Objective: To report a case of stasis dermatitis in a cynomolgus macaque.

Methods: An 8-year-old, 6kg, female, cynomolgus macaque (*Macaca fascicularis*-Chinese) with a history of a right hindlimb edema, alopecia, prominent varicose veins, dermatitis and a palpable arteriovenous fistula (AVF) of the right femoral triangle underwent necropsy.

Results: The right hindlimb was swollen and had thickened, dry, flaky skin over the dorsolateral surface of the ankle. Histologically, the epidermal surface was irregular, thickened, hyperkeratotic with nuclear retention, and covered in fibrin, neutrophils, necrotic cellular debris, and mats of bacteria. Edema fluid with vesicles containing neutrophils, lymphocytes, and homogeneous eosinophilic material expanded keratinocytes. Many narrow rete pegs penetrated the underlying superficial dermis, which was replaced by dense, fibrovascular connective tissue that extended to the deep dermis, around adnexal structures, medium to large caliber arteries, and prominent veins. The skeletal muscle had randomly distributed, coalescing areas of mature fibrous connective tissue forming whorls, intermixed with many small capillaries, hemorrhages, dilated lymphatic vessels and hemosiderin-laden macrophages. Adjacent myocytes were small, with fragmented cytoplasm, a loss of cross striations, indistinct nuclei, and pale amphophilic, vacuolated sarcoplasm.

Conclusions: Statis dermatitis is a reported sequela of arteriovenous fistula attributed to repeated blood draws from the area.

42: PERFORMANCE OF VETSCAN IMAGYST® AI MASSES ALGORITHM FOR CANINE AND FELINE LYMPH NODES

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Background:

Cytology is a rapid, minimally invasive, and cost-effective diagnostic commonly used in veterinary practice to evaluate skin/subcutaneous masses. Cytologic slides are generally sent to reference laboratories, delaying results and subsequent treatment. Additional barriers in private practice include cost, limited staff training, and restricted access to clinical pathologists.

Objective:

Evaluate the performance of Vetscan Imagyst AI Masses (VS-I-Masses), an artificial intelligence (AI) algorithm designed to screen lymphadenopathy cytology samples and classify them into one of five possible clinical profiles. AI clinical profile classifications were compared to consensus clinical profile classification of board-certified clinical pathologists (CPs).

Method:

300 retrospectively collected digital slides from Zoetis Imagyst Digital Cytology service were pre-screened for five clinical profiles: Reactive Lymphoid Hyperplasia (RLH), Lymph Node with Inflammation, Large Cell Lymphoma (LCL), Non-Lymphoid Tissue, and Indeterminate. Sixty slides per clinical profile (canine, 50%; feline, 50%) were enrolled. Ten CPs were divided into two groups assuring parity of experience. Each group reviewed half of the total slides (n = 150) and all CPs within a group read the same slides. Agreement between CPs and between consensus CP results and VS-I-Masses was analyzed.

Results:

Agreement between pathologists: Fleiss' Kappa: 0.784 and 0.705 (substantial agreement). Agreement between consensus pathologist and VS-I-Masses: Cohen's Kappa: 0.7834 (substantial agreement). VS-I-Masses accuracy: LCL: 97%, RLH: 87%, Lymph node with inflammation: 90%, Non-lymphoid Tissue: 95%, Indeterminate: 91%.

Conclusion:

VS-I-Masses demonstrated substantial agreement with expert pathologists and high accuracy across profiles, supporting its value in enabling rapid, in-house cytologic screening to improve diagnostic access in practice.

43: NASOLABIAL ADENOCARCINOMA FACILITATING LOCALIZED AND PULMONARY DISSEMINATION OF SCHIZANGIELLA SERPENTIS IN A CAPTIVE COACHWHIP (MASTICOPHIS FLAGELLUM)

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Introduction: A euthanized, captive 12-year-old male coachwhip (Masticophis flagellum) was prosected and submitted in 10% neutral-buffered formalin. One month prior, the snake had left eye exophthalmos, right nare swelling with epistaxis, and dermal/subcutaneous nodules. CT revealed right attenuating, soft tissue nodule causing bony lysis of the right nasal and maxillary bones and turbinates. Differentials included abscess, granuloma or neoplasia. CT favored the dorsal body subcutaneous nodule as an abscess or granuloma.

Methods: Postmortem examination, histopathology, histochemical staining, panfungal PCR on paraffin-embedded head tissue.

Results: The snake had two infections and two different neoplasms: a right-sided nasolabial mucus gland adenocarcinoma causing clinical epistaxis and swelling of the right nare/eye; a dermal/subcutaneous soft tissue sarcoma of dorsal body wall; hepatic granulomas with intralesional acid fast bacilli; multifocal granulomatous fungal pneumonia and marked granulomatous fungal stomatitis, myositis, and osteomyelitis of the right caudodorsal oral cavity. Fungi were PAS positive and morphology was consistent with Schizangiella serpentis. Formalin-fixed paraffin-embedded head tissue was negative on three attempts of panfungal PCR performed by the University of Florida's Molecular Fungal ID Lab due to prolonged formalin fixation that interfered with PCR amplification.

Conclusions: S. serpentis is an uncommon cause of granulomatous mycosis in captive and free-ranging colubrids/viperids. Route of infection and risk factors are unknown though likely represent opportunistic infection acquired from the environment. The coachwhip's chronic primary nasolabial tumor probably facilitated localized fungal infection with aerogenous spread to the lungs. This describes the fourth case in a snake, and potentially the first report in a coachwhip.

44: UNDIFFERENTIATED RHABDOMYOSARCOMA WITH INFILTRATION INTO THE CERVICOTHORACIC VERTEBRAL COLUMN AND COMPRESSION OF THE SPINAL CORD IN A YOUNG GOAT

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Introduction: A 6-month-old female Nigerian dwarf goat was submitted for necropsy after acute onset of progressive lameness and completely non-weight bearing in the front limbs caused by a large infiltrative mass over the right shoulder.

Methods: Postmortem examination, histopathology, immunohistochemistry, and transmission electron microscopy (TEM) were pursued to characterize the neoplasm.

Results: Gross examination revealed a neoplasm extending from the musculature overlying the right dorsolateral neck into the vertebral canal at the cervicothoracic junction, compressing the spinal cord at C6-T1. An onslaught of immunohistochemical stains and TEM were pursued to help confirm the diagnosis of rhabdomyosarcoma, undifferentiated rhabdomyoblastic variant.

Conclusions: Rhabdomyosarcomas are rare neoplasms of humans and domestic animals, with a wide range of gross morphologies with histologic and cellular phenotypic variations. In goats, this neoplasm is exceptionally rare. None of the previous case reports in goats describe the degree of invasiveness of the neoplasm, nor infiltration causing neurologic deficits and compromise, as described herein. In this case, the neoplastic population was also diffusely positive for chromogranin A and punctate positive for cytokeratin AE1/AE3. To our knowledge, there are no published reports of rhabdomyosarcomas in domestic animals which describe immunoreactivity to

chromogranin A, nor cytokeratin AE1/AE3, though both have been described to be aberrantly expressed in human alveolar rhabdomyosarcomas. This present case highlights TEM as an exceptional method to diagnose rhabdomyosarcoma, the importance of considering rhabdomyosarcoma as a differential diagnosis for neck masses in young goats, and emphasizes their immunohistochemical complexity due to considerable variation in immunoreactivity.

45: SYSTEMIC XANTHOGRANULOMATOSIS IN EUROPEAN GLASS LIZARD (PSEUDOPUS APODUS) WITH DETECTION OF MYCOBACTERIUM AVIUM

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Background: Xanthogranulomatosis is a chronic inflammatory condition characterized by nodular aggregates of lipid-laden macrophages, extracellular lipid, and granulomatous inflammation. Mycobacterium infection has been reported in reptiles and attributed to several species such as *M. avium, M. chelonae, M. fortuitum, M. intracellulare, M. marinum, M. phlei, M. smegmatis and M. ulcerans*. However, xanthogranulomatosis accompanied by Mycobacterial infection has not been recorded yet. Here, we describe a case of systemic xanthogranulomatosis in a European Glass Lizard (*Pseudopus apodus*) and a possible association with a concurrent Mycobacterial infection.

Objective: To describe the histopathologic findings of systemic xanthogranulomatosis in a European glass lizard and evaluate a potential association with *Mycobacterium avium* infection.

Methods: A European glass lizard was submitted to the Connecticut Veterinary Medical Diagnostic Laboratory (CVMDL) for postmortem examination. Tissue sections from various organs were stained with Hematoxylin and Eosin (H&E), Ziehl Neelsen stain, and Fites stain. Polymerase Chain Reaction and amplicon sequencing of the lung was performed at CVMDL.

Results: Histopathologic examination revealed multisystemic xanthogranuloma formations within the brain, lung, and liver, whereas granulomatous inflammation was observed within the ovary. Polymerase Chain Reaction and amplicon sequencing of the lung detected *Mycobacterium avium*. Ziehl-Neelsen stain revealed acid-fast bacteria within the ovary.

Conclusions: This case represents the first reported instance of systemic xanthogranulomatosis in a European Glass Lizard associated with a confirmed *Mycobacterium avium* infection. These findings suggest a potential link between chronic mycobacterial infection and xanthogranulomatous inflammation in reptiles, highlighting the importance of including mycobacterial testing in similar cases.

46: DISSEMINATED NEISSERIA ANIMALORIS INFECTION IN AN AMERICAN BADGER (TAXIDEA TAXUS) FROM KANSAS, USA

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Background: The American badger is a widespread, keystone species throughout the USA and extending into Mexico and Canada, where they are ecosystem engineers and contribute to rodent and snake population control and soil turnover. Species morbidity and mortality data are limited.

Objective: To describe gross, histopathologic, and microbiological findings associated with a systemic *Neisseria animaloris* infection in an American badger.

Methods: A free-ranging, adult, female badger wandered aimlessly with limited awareness of its surroundings near a power plant. It was dispatched, and the carcass was submitted to the Southeastern Cooperative Wildlife Disease Study for postmortem examination.

Results: Nutritional condition was poor. Grossly, both kidneys adhered to the dorsal body wall via numerous, pus-filled abscesses. The right kidney was markedly enlarged, had a severely dilated pelvis filled with abundant, clear, tan fluid with tan granules, and a focal abscess. Histologically, remnant right renal tissue exhibited severe lymphoplasmacytic and necrotizing tubulointerstitial nephritis with small colonies of gram-negative cocci within Splendore-Hoeppli material. Similar bacteria were within the perirenal abscesses, lung, and stomach. Aerobic bacterial culture of kidney yielded heavy growth and isolation of *N. animaloris*.

Conclusion: *Neisseria* spp. infections are rarely reported in free-ranging wildlife. *N. animaloris* is considered a commensal of the oral cavity of domestic dogs and cats. In humans, it is sporadically implicated in infections of dog and cat bite wounds. The initial infection nidus in this badger remains unclear; however, we hypothesize that a previous bite from a domesticated or wild felid or canid played a role.

47: RENAL NEUROENDOCRINE CARCINOMA WITH PULMONARY METASTASIS IN RICHARDSON'S GROUND SQUIRREL (UROCITELLUS RICHARDSONII)

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Background and Objective: Primary neuroendocrine carcinoma (NEC) of the kidney is extremely rare in domestic animals. This study describes the histopathological and immunohistochemical characteristics of renal NEC with pulmonary metastasis in a Richardson's ground squirrel (*Urocitellus richardsonii*).

Signalment: A 2-year-old female Richardson's ground squirrel weighing 455 g presented with anorexia. Abdominal ultrasound examination revealed a vascular-invasive mass. The animal died two days later, and a necropsy was performed by a clinician. Representative organs were submitted for histopathological examination.

Results: Grossly, an abdominal mass measuring approximately 7 cm in diameter was observed, replacing most of the right kidney parenchyma, and a cyst was observed in the renal cortex. Histologically, the mass was poorly demarcated, infiltrative, and composed of polygonal cells arranged in sheets, nests and occasional pseudo-glandular structures within eosinophilic fibrous stroma. Approximately 70% of neoplastic cells were positive for synaptophysin, focally positive for cytokeratin AE1/AE3 and vimentin, and negative for PAX8, Iba-1, and neurofilament. Grimelius stain showed partial positivity, indicating the presence of intracytoplasmic argyrophilic granules. These findings supported the diagnosis of neuroendocrine carcinoma. In addition, metastatic lesions were observed in the lungs.

Conclusion: This is the first case of primary renal NEC with pulmonary metastasis in Richardson's ground squirrel. Renal NEC is composed of various tissue forms, requiring comprehensive evaluation using histology and immunohistochemistry for diagnosis. The origin and distribution of neuroendocrine cells in the kidney are unclear, therefore, further accumulation of cases across animal species and comparative studies is needed to clarify the pathogenesis of renal NEC.

48: THE PRACTICE OF PATHOLOGY INCREASES SELF-PERCEIVED COMPETENCE AND CONFIDENCE IN FINAL YEAR VETERINARY STUDENTS

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Background: Performing a postmortem examination involves the use of surgical instruments, anatomical dissection, tissue and organ handling, good observation and interpretation skills and the use of previously acquired knowledge on disease pathogenesis. This project aimed to investigate whether the pathology rotation, part of the curriculum in many institutions, may contribute to increased student self-perceived confidence in their knowledge and clinical skills and self-perceived competency.

Methods: Students undergoing their final year pathology rotation (n=46) at the Nottingham Vet School, UK, were invited to voluntarily participate in the study. Students were invited to complete one survey at the start (S1), and a second survey (S2) and a short interview at the end of the rotation.

Results: 17 students completed S1, 16 students completed S2. All 46 completed the interview. Students self-perceived competence and confidence in surgically accessing the abdominal cavity of companion animals ($p=0.04$), in identifying and examining major abdominal organs ($p=0.007$ and $p=0.003$, respectively), in their dissection skills ($p=0.001$) and anatomy knowledge ($p=0.019$) was significantly increased at the end of

the rotation. Some students (25%) also felt that it improved how confident they will feel when approaching medical cases and about having to perform surgery.

Conclusion: This study highlights the importance of veterinary pathology teaching in increasing students' self-confidence and self-perceived competency in knowledge, clinical abilities and in promoting a can-do attitude towards clinical procedures. Student imprinting in veterinary pathology is crucial; high-quality, engaging, and contextual pathology teaching can lay a foundation for excellence in both pathology and clinical practice.

49: INTERACTIVE XERTE-BASED VETERINARY PATHOLOGY QUIZ USING PHOTOGRAMMETRY FOR GEN Z VETERINARY STUDENTS ('ZTUDENTS')

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Background: Photogrammetry-based animations represent a transformative tool in veterinary pathology and in the biomedical sciences more broadly. By converting real-world specimens into accurate, interactive 3D models, they enhance teaching by allowing students to explore complex pathological lesions and anatomical details from multiple perspectives. Many Z-generation students (Ztudnets) use smartphones as an everyday tool, integrating them seamlessly into both their personal and academic lives.

Methods: Photogrammetry based animations developed with Polycam™ using smartphones or tablets were embedded into the open source UoN developed software Xerte (https://www.nottingham.ac.uk/xerte/). A short self-paced learning package was created with slides featuring embedded interactive 3D models which students can explore in real time before working through a short multiple-choice quiz to assess learning. The resources are optimised for smartphones and mobile devices.

Results: The media-rich quiz was easy to develop and characterised by immersivity, interactivity and adapt to students with diverse learning approaches and styles.

Conclusion: A smartphone-based interactive quiz in veterinary pathology can be highly effective for teaching. The versatility of Xerte allows to integrate photogrammetry quizzes into live lectures, workshops, self-directed learning and practical teaching sessions as well as for asynchronous self-direct learning experiences. It offers an immersive, hands-on experience that aligns with the Ztudnets' learning approaches, promoting engagement, self-directed exploration, and deeper retention of complex pathological concepts. The authors plan to enhance the quiz through the use of augmented reality integration and pilot with students.

50: MATRIX METALLOPROTEINASE 14 (MMP-14) EXPRESSION IN CANINE CUTANEOUS AND SUBCUTANEOUS SOFT TISSUE TUMORS

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Background: Matrix metalloproteinases are proteolytic enzymes that are involved in extracellular matrix remodelling, degradation and mediate several changes in the tumour microenvironment. MMP-14 (MT1-MMP) is a membrane-type metalloproteinase that is up-regulated in different types of human cancer and promotes neoplastic cell invasion and metastasis. It is highly expressed in different types of human sarcomas, with high expression being associated with poor prognosis in osteosarcomas, but there is a lack of knowledge regarding its expression in canine soft tissue tumours (STTs).

Objective: This study analysed the immunohistochemical expression of MMP-14 in 38 canine cutaneous and subcutaneous appendicular STTs with a minimum of 40 days follow up post surgical excision.

Methods: A semiquantitative analysis evaluated the intensity of immunostaining and percentage of MMP-14 positive cells. Using an immunoreactive score (IRS), an overall score of intensity of staining was also calculated.

Results: MMP-14 was expressed in all cases, with a median percentage of positive cells of 90%. Intensity of immunolabeling, percentage of positive cells and IRS did not correlate with histological grade or survival time. In all cases in which recurrence occurred (n=9; 26.68%), a significantly higher percentage of MMP-14 positive cells in tumours with shorter time to recurrence was observed (p=0.032), however statistical significance was not observed when considering all tumours.

Conclusion: To our knowledge this is the first study reporting MMP-14 expression in canine STTs. Further larger studies are necessary to investigate its high expression as a potential negative prognostic factor when predicting the chances of local recurrence.

51: HISTOPATHOLOGIC AND IMMUNOHISTOCHEMICAL FEATURES OF CANINE OLFACTORY GANGLIONEUROBLASTOMA: A CASE REPORT OF A RARE ENTITY

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A 9-year-old, male, neutered, Siberian Husky was presented for panting and decreased airflow through both nasal passages. According to the owner, the dog had left-sided nasal swelling with occasional epistaxis for the past month. Radiographic imaging revealed an invasive sinonasal mass extending into the frontal sinus and nasopharynx with destruction of the nasal turbinates. The rhinoscopic examination demonstrated a nearly obstructive, invasive, firm, white mass filling both nasal cavities, from which a

biopsy was obtained. Histologically, the expansile nasal mass was composed of variably cellular, biphasic neoplastic population within a fibrovascular stroma featuring scattered glandular-like structures. The neoplastic cells included neuroblastic cells arranged in anastomosing cords, trabeculae and lobules with occasional rosette formation, separated by abundant neurofibrillary stroma. Intermixed with these neuroblastic cells were polyhedral ganglion cells containing peripheral Nissl substance. Immunohistochemical staining was performed to further characterize the neoplastic population. The neuroblastic cells were immunolabeled with pancytokeratin (AE1/AE3), whereas the ganglion cells and stroma showed strong immunoreactivity to synaptophysin. Additionally, TuJ-1 and MAP-2, markers with higher specificity for canine olfactory neuroblastomas, demonstrated positive staining: TuJ-1 labeled neuroblasts, ganglia, neuronal processes, and stroma, while MAP-2 staining was positive in ganglion cells and neuronal processes. Based on the presence of both immature neuroblasts and well-differentiated ganglion cells within a neurofibrillary stroma, along with supportive immunohistochemical findings (TuJ-1, MAP-2, and synaptophysin immunopositivity), a diagnosis of olfactory ganglioneuroblastoma was established in this case.

52: VISCERAL AND PERITONEAL CYSTICERCOSIS IN A GOAT

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A 1-year-old Alpine goat was submitted to the Tulare branch of the California Animal Health and Food Safety Laboratory for necropsy. The goat was from a dairy farm with approximately 500 goats of various ages grazing on alfalfa pastures shared with five mixed-breed guardian dogs. Seven animals showed reduced milk production, decreased food and water intake, and fever (40-40.5°C). The main gross findings included about 2 L of clear yellow fluid, mixed with numerous free-floating 5.0-10.0 mm semi-translucent white cylindrical parasitic cysts in the abdominal cavity, along with extensive necrohemorrhagic linear parasitic track migrations on the liver and lungs. Histopathology revealed extensive concentric areas of necrosis, with hemorrhage, fibrin deposition, and variable infiltrates of eosinophils, neutrophils, lymphocytes, plasma cells, and macrophages. Multiple longitudinal and cross sections of encysted larval cestodes (cysticerci) measuring 2.4 mm to 2.6 mm in diameter were displacing the parenchyma. Diagnosis of acute cysticercosis often relies on gross findings and the presenceof parasites during necropsy. A common differential diagnosis is hydatid disease caused by Echinococcus granulosus and Echinococcus multilocularis, although the morphology of Echinococcus cysts differs from C. tenuicollis. Additional diagnostic methods include histopathology, ultrasonography, and molecular assays. Controlling cysticercosis in sheep and goats involves managing infection in the definitive host and preventing the host from ingesting carcasses of infected intermediate hosts.

53: TWO UNREPORTED GONADAL NEOPLASMS IN NEOTROPICAL PRIMATES

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Genital neoplasia in neotropical non-human primates is uncommon. Tumors of the female reproductive tract comprise up to 15% of all neoplasms in neotropical primates, with uterine leiomyomas most commonly reported. Tumors of the male reproductive tract are rare, and largely consist of seminomas and interstitial cell tumors. Here, we describe two previously unreported gonadal tumors in neotropical non-human primates. An 18-year-old, female squirrel monkey (*Saimiri boliviensis*) was found dead in her enclosure. At necropsy, an incidental, 1.5x1.3x1.1 cm, white, firm, irregular mass was found in the left ovary. Histologically, the mass was well demarcated, unencapsulated, and composed of streams and bundles of mesenchymal cells with moderate eosinophilic cytoplasm and elongate, blunt ended nuclei. Mitotic figures were rarely seen. Neoplastic cells stained red with trichrome, and 100% of neoplastic cells showed strong, cytoplasmic immunolabelling for smooth muscle actin (SMA), consistent with an ovarian leiomyoma. A 15-year-old, male owl monkey (*Aotus nancymaae*) developed a 1.8x1.4x1.4 cm, tan, soft, multinodular mass in the right testicle. Histologically, the mass was poorly demarcated, partially encapsulated, and composed of polygonal cells arranged in cords, tubules, and rare rosettes, supported by abundant fibrous stroma. Neoplastic cells often palisaded along the stroma. Neoplastic cells showed 100% weak cytoplasmic immunolabelling for alpha inhibin, and 30% of neoplastic cells showed weak to moderate cytoplasmic immunolabelling for anti-Mullerian hormone (AMH), consistent with a Sertoli cell tumor. While ovarian leiomyomas are rarely described in human and veterinary literature, our findings represent the first report of either neoplasm in their respective species.

54: NASAL CARCINOMA WITH PERIORBITAL AND CRIBRIFORM PLATE INVASION, VON WILLEBRAND DISEASE, AND PRESUMPTIVE FIBROCARTILAGINOUS EMBOLISM IN A 7-YEAR-OLD ROTTWEILER

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Background: Nasal tumors in dogs, such as carcinoma, frequently cause epistaxis and can be locally invasive. Von Willebrand Disease (vWD) can exacerbate bleeding tendencies. Fibrocartilaginous Embolism (FCE) is an uncommon cause of acute myelopathy.

Objective: To describe the clinical findings, diagnostics, management, and outcome of a Rottweiler with extensive nasal carcinoma, Von Willebrand factor (vWF) deficiency, and a presumptive FCE.

Methods: A 7-year-old male Rottweiler was evaluated for epistaxis. The initial diagnostic protocol included blood analysis (with vWF measurement), rhinoscopy with biopsy sampling, for histopathology and immunohistochemistry analyses (Ki67 antigen,

monoclonal mouse, anti-human, clone MIB-1, Agilent Technologies Singapore, 1:100). Subsequently, a head computed tomography (CT) scan was performed to assess tumor extent and prognosis. Acute neurological deficits post-CT were investigated.

Results: Blood tests showed vWF deficiency (40.5%). Rhinoscopy revealed a left nasal mass; histopathology confirmed nasal carcinoma (adenomatous/solid pattern). Head CT imaging suggested a malignant infiltrative process (differentials: Chondrosarcoma, Carcinoma, Sarcoma), with extensive bone lysis invading the periorbital region, cribriform plate (meningeal/olfactory bulb contact), nasopharyngeal meatus, and left choanae. The left mandibular lymph node was reactive (metastasis vs. inflammation). Post-CT, acute posterior paresis (clinical FCE) occurred; physiotherapy was initiated. Analyzed samples showed marked immunopositivity for Ki67 in the nuclei of neoplastic epithelial cells.

Conclusion: This case illustrates a locally highly invasive nasal carcinoma with a guarded prognosis confirmed by CT, complicated by vWD. The marked Ki67 positivity suggests a high replicative capacity. The presumptive FCE further worsened the condition. Intractable epistaxis, aggravated by vWD and advanced malignancy, led to euthanasia.

55: POLYNEURITIS EQUI-LIKE SYNDROME IN AN ADULT ALPACA

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Background: Polyneuritis Equi (PNE) is an infrequent progressive neurological disease characterized by nonsuppurative neuritis of the sacrocaudal and/or cranial nerve roots in equids and other animals, likely of immune-mediated origin. PNE has not been previously reported in alpacas. This report describes a PNE-like syndrome in this species.

Objective: To describe the clinical and pathological findings of a progressive hindlimb paralysis resembling PNE in an adult alpaca previously challenged to produce SARS-CoV-2 antibodies as part of a research study.

Methods: A 10-year-old female alpaca with progressive non-ambulatory paraplegia, urinary/fecal incontinence, and perineal hypoesthesia underwent clinical, neurological, radiographic, and laboratory examinations. Following euthanasia, a complete autopsy and histopathology were performed.

Results: Clinically**,** neurological deficits were localized to a diffuse lesion of the lumbosacrocaudal (L6-Cd5) spinal cord and/or associated spinal nerves roots and spinal nerves. Radiographic examination of this segment was unremarkable. A mild elevation in CK was noted. At necropsy, there was a moderate and asymmetric swelling of many nerve roots at the lumbosacral region. Microscopically, lumbosacral nerve roots were heavily infiltrated with large numbers of lymphocytes and plasma cells, and moderate numbers of histiocytes, with myelin dilation, swollen axons (spheroids) and

multifocal areas of hemorrhage. The inflammatory process extended to the meninges of the spinal cord, perivascular spaces and into the associated skeletal muscle.

Conclusions: Progressive polyradiculoneuritis and meningomyelitis are findings consistent with PNE in horses. To the best of our knowledge, this is the first description of a PNE-like case in an alpaca, presumably associated with an immune-mediated component.

56: CANINE DIROFILARIOSIS IN BOGOTA D.C., COLOMBIA

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A case of heartworm disease is reported in an 8-year-old French Poodle: The dog spent 5 years in Coveñas (2 m above sea level, (a. s. l.), low-altitude tropic), Sucre. The pet was then moved to Bogota D.C. (2,625 m a. s. l., high-altitude tropic) in December 2024. In January 2025, signs of right heart failure and severe respiratory distress were observed. Clinical examination revealed lethargy, pale and dry mucous membranes, tachypnea, hypothermia, and hypotension; clinical pathology revealed thrombocytopenia (60 $x10^9$/L), anemia (RBCs 4.2 $x10^{12}$/µL), hematocrit: 30.6%, hemoglobin: 12.4 g/dL), and leukocytosis (30.3 $x10^9$/L). Echocardiography revealed values consistent with pulmonary hypertension, various parasitic structures in the right atrium, chronic mitral and tricuspid valve disease. Due to poor prognosis, euthanasia was elected. At necropsy, ascites, dilation and severe congestion of the vena cavae were observed; in the right atrium, severe dilation caused by 35 adult nematodes in the lumen, severe edema, and atrial endocardial thrombi measuring 10 mm of diameter; the liver was enlarged with an accentuated lobular pattern. Histopathology revealed pulmonary proliferative endarteritis, adventitial fibrosis, perivascular lymphohistiocytic and eosinophilic pneumonia, vasculitis, fibrinoid necrosis, and severe hemosiderosis. In the liver, there was moderate centrilobular necrosis and atrophy, intense congestion, severe lymphoplasmacytic phlebitis, and extramedullary hematopoiesis. Parasitology classified the adult specimens as *Dirofilaria* spp., and qPCR detected *Dirofilaria immitis* (2400 copies/µL). This case highlights the epidemiological risk of introducing heartworm into non-endemic highland tropical areas when preventive measures are not taken. This is the first integrative report of canine dirofilariosis in Colombia.

57: PRIMARY HISTIOCYTIC SARCOMA IN TWO DOGS

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Two cases of primary splenic and pulmonary histiocytic sarcoma in two canines are described. Diagnosis was made by exploratory laparotomy, computed tomography (CT), cytology, and histology, and confirmed by immunohistochemistry (IHC). 1) An 8.5-year-old male Bernese Mountain dog showed progressive weight loss, listlessness, and an abdominal mass on palpation. Laparotomy revealed a 6-cm diameter, yellowish-white, solid, and friable mass in the spleen. 2) A 10-year-old female Schnauzer showed progressive respiratory distress. Clinical examination revealed an abnormal growth in the thoracic cavity. CT showed a 6-cm diameter solid mass located in the middle and caudal lobes of the right lung, with invasion of the tracheobronchial lymph nodes. At 30-day follow-up, multiple peribronchial and subcutaneous tissue masses were observed in the left chest. Cytology described a population of round cells, some multinucleated, with severe pleomorphism and anisocytosis, severe anaplasia, and karyomegaly. Histologically, populations of non-encapsulated neoplastic cells were observed in the spleen and the lung. The cells were round with widely vacuolated cytoplasm and multiple projections, phagocytosed material, severe anisocytosis and cellular pleomorphism; binucleated and multinucleated cells, severe anaplasia. The nuclei were round or cleft, central to paracentral, with severe nuclear anisocytosis and pleomorphism. The mitotic count was 31 (case 1) and 8 (case 2) mitotic figures in 2.37 mm^2; some were aberrant, and intravascular neoplastic cells were observed. IHC for CD1, CD68, and CD163 were positive for all antibodies; immunostaining was intracytoplasmic and moderately granular. Clinical, morphological, and IHC findings confirmed histiocytic sarcoma in both dogs.

58: MYCOTIC BALANITIS AND DERMATITIS OF SCROTUM IN A JUVENILE MALE RHESUS MACAQUE

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Male genital yeast infection is very rare in rhesus macaques. We report a rare case of balanitis and dermatitis of the scrotum caused by a dimorphic fungal organism in a juvenile male rhesus macaque (RM). The 0.58-year-old RM presented to the clinic at the Tulane National Primate Research Center for severe diarrhea/dehydration. The animal was treated with probiotics, and parenteral palliative fluid therapy, and a rectal swab was collected. Two days post the initial presentation, the animal developed severe scrotal and preputial edema. Bacterial culture from the rectal swab recovered *Yersinia enterocolitica,* among other pathogenic bacteria. Consequently, the animal was given antibiotics. The animal was euthanized a few days later due to lack of improvement and poor prognosis. On necropsy, the animal had dehydration and diarrhea. The scrotum and prepuce were severely edematous. Histologically, the dermis of the scrotum and prepuce was markedly expanded by edema and fibrin and infiltrated by moderate numbers of neutrophils and eosinophils, and lesser numbers of mononuclear inflammatory cells. The epidermis was ulcerated and covered by a mat of a dimorphic fungal organism. The yeast form was oval with 3-4.6 um diameter thin-walled spores, frequently forming short chains (pseudohyphae). The hyphal form was scarce and better demonstrated by fungal special stains. The hyphae were thin, 1-2 um in diameter with parallel walls, non-branching, and septate. Panfungal PCR test on a FFPE sample

identified the fungus as *Trichosporon sp*. This is a rare and unique case of “genital mycosis” occurring in a juvenile male RM.

59: AGGRESSIVE CANINE B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA WITH BCL-6 EXPRESSION

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Background: B-cell chronic lymphocytic leukemia (BCLL) is generally a slowly progressive disease in aged dogs. In humans, a high Ki67 index, unmutated IgV_H gene, and expression of bcl-6 has been associated with shorter treatment-free intervals.

Objective: Characterize the clinical, hematologic, cytologic, histologic, and immunophenotypic features of aggressive BCLL without Richter’s transformation in a dog.

Results: The dog was presented with a history of anorexia, lethargy, anemia, and severe CD21-positive small-cell lymphocytosis, consistent with BCLL, 6-weeks prior at the referring veterinarian. Two-weeks before presentation to the University of Wisconsin Veterinary Care (UWVC) oncology service, the dog was treated with cyclophosphamide, followed by chlorambucil for maintenance. At presentation, the important CBC findings were severe anemia (Hct 16% (RI:39 – 57%)), thrombocytopenia (47 $\times 10^6$/µL; (RI: 175 – 500 $\times 10^6$/µL)), and lymphocytosis (60.4 $\times 10^3$/µL (RI: 0.7 – 4.3 $\times 10^3$/µL)). Blood smear and lymph node cytology showed a predominant population of small lymphocytes with round to cleaved nuclei, coarse chromatin, and small amounts of pale blue cytoplasm. Histologically, lymph nodes, bone marrow, and spleen were infiltrated by neoplastic lymphocytes arranged in sheets or large to confluent proliferation centers. The neoplastic cells labeled with CD20, bcl-2, and bcl-6. The Ki67 index was 26%. The dog was euthanized 47 days after the initial presentation.

Conclusions: Bcl-6 expression could serve as a cost-effective and efficient clinical diagnostic marker, as well as a potential negative prognostic indicator, in canine BCLL. In addition, this finding has therapeutic implications, particularly in light of recent developments in bcl-6 inhibitors for bcl-6-mutated lymphomas.

60: DERMAL VASCULITIS IN FELID HERPESVIRUS-1 DERMATITIS IN CATS

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Background: Felid herpesvirus-1 (FeHV-1) is a common pathogen of cats that is associated mainly with ulcerative dermatitis, stomatitis, conjunctivitis, and rhinitis.

Objectives: To report dermal vasculitis in FeHV-1 dermatitis in cats.

Methods: Cases of feline herpesviral dermatitis were retrospectively searched from the Athens Veterinary Diagnostic Laboratory biopsy archives between 2010 and 2025.

Results: We retrieved 8 biopsy submissions that were confirmed by FeHV-1 immunohistochemistry. Affected cats were 5 castrated males and 3 spayed females. Domestic Short Hair cats were affected more often (7 cases) with one Domestic Medium-Hair cat. The age of patients ranged from 6 months to 10 years (median age = 8.5 years). Affected sites included the nose (5 cases), perinasal area (one case), right flank (one case), and right elbow, right shoulder, and tarsal area (one case). Histologic changes were typical of FeHV-1 infection in all cases. In addition, dermal vasculitis, characterized by fibrinoid necrosis, infiltration of inflammatory cells into the vessel wall, and thrombosis, affected small to medium-sized vessels in the superficial, mid, and deep dermis were observed in all cases. In 2 cases, dermal vasculitis was associated with dermal necrosis.

Conclusions: Although not previously reported, vasculitis may occur in cases of FeHV-1 dermatitis.

61: BILATERAL LENS ANOMALY IN A FREE-RANGING AMERICAN BLACK BEAR (URSUS AMERICANUS) CUB FROM NORTH CAROLINA, USA

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Background: American black bears are a species of great ecological, recreational, and cultural importance across North America. Reports of ocular disease in free-ranging wildlife, including black bears, are limited.

Objective: To describe the histopathologic and immunohistochemical findings associated with a bilateral lens anomaly in a free-ranging black bear cub.

Methods: An approximately 6-month-old, male black bear cub was observed stumbling and acting disoriented. It was dispatched, and the carcass was submitted to the Southeastern Cooperative Wildlife Disease Study for postmortem evaluation.

Results: Bilaterally, the lens capsule and epithelium (anteriorly) were disrupted by multiple 75-150 µm diameter, round, patchily eosinophilic to amphophilic, globular but solid concretions. These concretions appeared to be continuous with the lens capsule on a periodic acid-Schiff hematoxylin reaction. Alpha A crystallin immunohistochemistry was performed. Immunolabeling was inconsistent with some areas within a concretion demonstrating weakly positive immunolabeling consistent with lens fibers, while other areas were immuno-negative consistent with lens capsule.

Conclusion: Given the clinical observations, we suspect this cub's vision was impaired by these lens concretions. The impetus for their development is unclear; however, a congenital defect is suspected due to this animal's young age. Concurrently, this cub

was emaciated and had ursicoptic mange, indicating it had been doing poorly for some time.

62: PATHOLOGIC CHARACTERIZATION AND CLINICAL SIGNIFICANCE OF LYMPHOVASCULAR INVASION IN CANINE THYROID CARCINOMAS

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Background/Objective. Microscopic lymphovascular invasion (LVI) is recognized in canine thyroid carcinoma (cTC). Associations between LVI and clinical outcomes have been poorly characterized, and a standard definition of LVI is lacking. This study morphologically characterizes LVI and evaluates the relationship between LVI and outcomes.

Methods. Clinical and histopathologic data were collected from 30 thyroidectomized dogs with 35 cTC from the University of Pennsylvania (2011-2020). Submission forms and follow-up questionnaires supplied clinical data. Histologic parameters included cTC subtype, nuclear pleomorphism, mitotic count, necrosis, mineralization, capsular invasion, and margins. Extracapsular or capsular LVI was categorized as: nonendothelialized LVI with adherent fibrin, three morphologic variations of endothelialized LVI (bulging, adhered, floating), pseudoinvasion, or absent. CD31 and PROX-1 immunohistochemistry determined blood or lymphatic vascular origin, respectively. Descriptive statistics included means with standard deviations or medians with ranges, and categorical variables were summarized using frequencies and percentages. Survival analysis was conducted using univariate and multivariate Cox proportional hazards models.

Results. Endothelialized LVI were identified in 26 samples; ten were within extracapsular vessels. On multivariate analysis, endothelialized/extracapsular LVI (ET/EC) was associated with a 3-fold increase in the likelihood of death (HR 3.15, 95% CI 1.27-7.83, P=0.013). Endothelialized LVI were most common in blood vessels (CD31+; n=17), and one case of PROX-1+ lymphatic vessels. No histopathologic or LVI parameters were associated with tumor metastasis or recurrence.

Conclusion. ET/EC is associated with worse outcomes in cTC, whereas other morphologic variations of LVI are not. Further study is warranted on a larger population of dogs with standardized treatment and follow-up.

63: FIRST REPORT OF PHASIANUS CHAPHAMAPARVOVIRUS-1 ASSOCIATED WITH HEPATIC NECROSIS IN PHEASANTS (PHASIANUS COLCHICUS) IN THE UNITED STATES

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Parvoviruses are small, icosahedral, non-enveloped, single-stranded DNA viruses. Phasianus chaphamaparvovirus-1 (PhChPV-1) has been associated with hepatic necrosis, enteritis, and nephritis in young pheasants in countries other than the US. We describe a mortality event associated with PhChPV-1 in pheasants in Northern California. A total of 118 out of 1,500 young pheasants died following a history of anorexia on a rearing farm (8% mortality). Eight carcasses (two males and six females) were submitted for necropsy. Gross findings included hepatic pallor and random, red, pinpoint foci throughout the liver in all birds. Similar foci were observed in the heart in 2 of 8 cases. Histologically, there was multifocal and often pan-lobular hepatocellular degeneration and necrosis, moderate ductular reaction, and mild histiocytic and lymphoplasmacytic portal infiltrates. Occasional hepatocellular karyomegaly with intranuclear inclusion bodies was observed. Additional findings included lymphoplasmacytic nephritis and myocarditis with hemorrhage and rare necrosis. Ultrastructural examination revealed Parvoviridae viral particles in the liver of all birds. Whole-genome sequencing of liver samples identified a chaphamaparvovirus with 94.4% nucleotide identity to PhChPV-1. To our knowledge, this is the first report of chaphamaparvovirus-like causing hepatic necrosis in pheasants in the United States. Given that these pheasants were reared for hunting, the potential spread of PhChPV-1 into wild populations warrants consideration.

64: DRACUNCULUS OESOPHAGEUS-ASSOCIATED CHRONIC MYOSITIS IN A RED-TAILED BOA

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Background: *Dracunculus* spp. are filarial nematodes with zoonotic potential, capable of infecting a wide range of mammals, amphibians, fish, and reptiles. While *Dracunculus medinensis* and *insignis* are well-known in humans and mammals, clinical disease associated with *Dracunculus* infection in reptiles is rarely reported.

Objective: To describe a case of chronic myositis associated with *Dracunculus oesophageus* infection in a red-tailed boa (*Boa constrictor constrictor*).

Methods: A captive-born juvenile female red-tailed boa was presented to the Louisiana State University Teaching Hospital for anorexia and regurgitation. On clinical

examination, she had reduced muscle tone and an absent righting reflex in the caudal two-thirds of the body. The right coelomic musculature near the cardiac region was soft on palpation. Radiographs revealed no spinal abnormalities. Due to poor prognosis, the animal was euthanized.

Results: Postmortem examination revealed poor body condition and discoloration of the right coelomic musculature. Histopathology revealed multiple cross-sections of degenerated nematodes with large uteri containing larvae, embedded in granulomatous inflammation, fibrosis, and atrophic myocytes. Inflammation extended into adjacent nerve bundles. PCR amplification and sequencing of the 18S rRNA gene from paraffin-embedded tissue identified the nematodes as *Dracunculus oesophageus* (97.29% match with the NCBI GenBank database accession AY852269.1). No significant spinal cord lesions were noted.

Conclusions: This case documents *D. oesophageus* as a cause of chronic myositis with nerve involvement in a red-tailed boa. The neuromuscular lesions correlated with clinical signs. To the authors' knowledge, this is the first reported case of clinical disease caused by *D. oesophageus* in this species.

65: EXTRAINTESTINAL PATHOGENIC ESCHERICHIA COLI (EXPEC)-ASSOCIATED HEMORRHAGIC PNEUMONIA IN TWO DOGS FROM THE SAME SHELTER: INVESTIGATING STRAIN RELATEDNESS AND PREDISPOSING FACTORS

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Background:
Fatal hemorrhagic pneumonia in dogs associated with hemolytic extraintestinal pathogenic *Escherichia coli* (ExPEC) has been previously documented. However, its epidemiology in shelter settings remains unclear. Strain transmission and the role of extra-respiratory coinfections or other comorbidities as predisposing factors warrant further investigation.

Objective:
To describe two cases of fatal hemorrhagic pneumonia associated with toxigenic *E. coli* from a shelter in Louisiana, focusing on strain relatedness and possible predisposing factors.

Methods:
Two dogs from the same shelter died within 24 hours after developing severe respiratory signs. Both presented initially with vomiting and signs of upper respiratory infection; one had a recent history of lower urinary tract infection. Postmortem examinations, histopathology, aerobic bacterial culture, PCR for virulence factors, and antimicrobial susceptibility testing were performed. Whole genome sequencing (WGS) is pending to determine clonal similarity.

Results:
Both dogs had severe acute hemorrhagic pneumonia with multifocal colonies of short rods. The second dog also had cystitis with hematuria. Aerobic cultures from lung tissue (both dogs) and urine (second dog) yielded hemolytic *E. coli*, and all isolates were positive for the cytotoxic necrotizing factor 1 (CNF1) gene. However, antimicrobial susceptibility profiles differed substantially among the three isolates, raising questions about their genetic relatedness.

Conclusion:
These cases highlight the need to better understand ExPEC-related pneumonia in shelters, including transmission risk and potential predisposing factors. Divergent antimicrobial susceptibility profiles suggest that infections may not have arisen from a single strain, though genomic analysis is ongoing.

66: STREPTOCOCCAL PNEUMONIA IN THREE RHESUS MACAQUES (MACACA MULATTA)

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History: Three Indian-origin, male rhesus macaques ranging in age from 4-6 years old presented for necropsy. Clinically, two of the three animals had respiratory signs including dyspnea and were experimentally naïve. One animal was part of an SIV study and reached the study endpoint with no clinical respiratory abnormalities.

Description: Grossly, all three animals had firm regions of pulmonary lobar consolidation and two had pleural adhesions. The brain was grossly unremarkable in all three cases. Microscopically, lung lesions consisted of fibrinosuppurative bronchopneumonia with variable hemorrhage, edema, and type II pneumocyte hyperplasia. Two cases had fibrinosuppurative pleuritis with areas of pleural fibrosis. Culture of the lung yielded *Streptococcus pneumoniae* in all three cases. *S. pneumoniae* is a common causative agent of bronchopneumonia in rhesus macaques, as well as meningitis and brain abscesses.

67: ANOMALOUS RIGHT AND LEFT CORONARY ARTERIES FROM THE PULMONARY ARTERY

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A 60.0kg (134lb), 3-week-old, Black Angus heifer calf was found dead. Gross findings include thoracic and abdominal serosanguineous effusion; mild bilateral bronchopneumonia and pulmonary edema; globose heart with markedly ectatic left and right ventricles and atria (interpreted as eccentric hypertrophy), patent foramen ovale, absence of the origin of the right and left coronary arteries at the aortic root[BP1] sinuses, and presence of a 1.5-2cm diameter vascular lumen within the pulmonary artery root progressing and extending along the left and right coronary grooves of the heart; and an enlarged liver with enhanced lobular pattern. Heart and liver histologic findings include chronic centrilobular hepatocellular degeneration and necrosis with early centrilobular fibrosis, and midzonal to periportal hepatocellular vacuolar (lipid-type)

degeneration; chronic subendocardial myocardial necrosis and degeneration with replacement fibroplasia and mineralization. These changes are interpreted as anomalous origin of both right and left coronary arteries from the pulmonary artery (ARLCAPA) with ensuing congestive heart failure leading to pulmonary edema, bi-cavitary effusion, and centrilobular hepatocellular necrosis. Although anomalous origin from the left coronary artery from the pulmonary artery (ALCAPA) in calves has been described, there are no published cases of ARLCAPA.

68: ANAPLASTIC ASTROCYTOMA IN THE FRONTOTEMPORAL REGION OF THE BRAIN, GRADE III

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This case describes the cytohistological diagnostic approach to a cystic lesion in the brain identified in a 5-year-old cat, whose neurological examination revealed ataxia with increased intracranial pressure due to the presence of a cystic lesion in the frontotemporal region of the brain, identified by contrast-enhanced MRI. Cytological evaluation with simultaneous incisional biopsy sampling and subsequent immunohistochemistry led to its characterization as a Grade III astrocytoma (WHO, 2016).

Astrocytomas are glial tumors classified as either circumscribed or diffuse, with several histological variants. Their grading system (WHO, 2016) consists of four levels, based on criteria such as cellularity, necrosis, mitosis, histological pattern, and neovascularization.
They commonly appear in the frontotemporal regions, show intra-axial-supratentorial growth without ventricular involvement, and are more frequently diagnosed in geriatric patients.

Anaplastic astrocytomas fall under the diffuse subtype and are classified as Grade III tumors. Cytologically, they exhibit mitotic activity and atypia, frequent bare nuclei, and sometimes a fibrillar background.[3] In our case, cytology revealed a high cellular population with fusiform/polygonal appearance, marked anisocytosis, anisokaryosis, nuclear atypia, aberrant mitotic figures, and bare nuclei. Histological examination showed Rosenthal fibers and a fibrillar background. Immunohistochemistry confirmed the diagnosis with positive GFAP & OLIG-2 staining.

In cats, meningioma is the most common primary brain tumor; astrocytic tumors are rarely diagnosed, occurring in approximately 3.5 out of every 100,000 cases. Although advances have been made in clinical treatment, their low incidence still limits the ability to accurately correlate findings with human grading systems for a more precise prognosis.

69: FIBRINOUS PERICARDITIS AND MYOCARDITIS CAUSED BY LISTERIA MONOCYTOGENES IN A BEARDED DRAGON (POGONA VITTICEPS)

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Background: Bacterial myocarditis is rarely reported in reptiles. This case documents severe fibrinous pericarditis and myocarditis due to *Listeria monocytogenes* in a bearded dragon, expanding the frequency and differentials for pericardial and myocardial disease in exotic companion animals.

Case Presentation: A 1-year-old male bearded dragon presented with progressive lethargy and inappetence. Imaging identified a cranial coelomic soft tissue opacity and moderate pericardial effusion. Pericardiocentesis yielded yellow, viscous fluid. The patient was treated supportively but declined and died three days later.

Gross Findings: Necropsy revealed severe fibrinous pericarditis and epicarditis with minimal residual pericardial fluid. The liver was diffusely yellow and friable. The animal was emaciated and dehydrated, with mild coelomic effusion.

Histopathology: There was extensive fibrinous, heterophilic and histiocytic pericarditis and myocarditis with myofiber degeneration, necrosis and loss; edema, vascular thrombosis, and fibrosis. Gram stain revealed intralesional and intrahistiocytic gram-positive coccobacilli. Hepatic lipidosis and mild portal fibrosis were also present. Other tissues were unremarkable.

Microbiology: Aerobic culture of a pericardial swab yielded a small number of *Listeria monocytogenes* colonies, confirming the etiologic agent.

Conclusion: This case demonstrates *Listeria monocytogenes* as a cause of severe, fatal cardiac disease in a bearded dragon. Diagnostic integration of imaging, fluid analysis, histopathology, and bacterial culture were essential. While rare, *Listeria monocytogenes* should be considered in cases of reptilian pericarditis and myocarditis. This case builds on limited published reports and provides valuable insight into the diagnostic challenges of infectious cardiac disease in reptiles. Further investigation into transmission routes and susceptibility in captive reptiles is warranted.

70: EASTERN EQUINE ENCEPHALITIS VIRUS IN A FOAL WITH LYMPHOHISTIOCYTIC CENTRAL NERVOUS SYSTEM PERIVASCULAR INFILTRATES

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A 5-month-old Quarter Horse filly was presented to the Mississippi State University College of Veterinary Medicine Equine Emergency Service for head pressing, ataxia, and circling. CSF collected from the atlanto-occipital joint reavled a mildly increased protein (135.0 mg/dl) and moderate neutrophilic pleocytosis (232 cells/ul). Postmortem examination revealed diffuse cerebral and cerebellar vascular congestion, multifocal brainstem petechial hemorrhage, and multifocal hemorrhage and malacia of the cervical and thoracic spinal cord. Histologically, the brain and spinal cord contained multifocal regions of lymphohistiocytic and neutrophilic perivascular cuffing with concurrent gliosis, neuronal necrosis, intravascular fibrin thrombi, and hemorrhage. Given these histologic

lesions, prioritized differential etiologic agents included rabies, neurotrophic herpes, and eastern equine encephalitis (EEE). Direct fluorescent antibody testing of brain tissue was negative for rabies. PCR of whole blood and brain tissue was negative for equine herpesvirus 1. PCR of cerebrospinal fluid was negative for equine herpesvirus 1 and 4. PCR of brain tissue for EEE was positive (Ct: 23.0). EEE is a mosquito-borne, neuroinvasive arbovirus primarily found in North America and the Caribbean. Susceptible hosts such as equids, livestock, and humans are usually dead-end hosts. The histologic lesions appreciated in this case diverged somewhat from those typically associated with EEE. Unlike most EEE cases that primarily display neutrophilic inflammation, the predominance of mononuclear cell infiltrates coupled with vascular thrombosis in this foal heightened the concern for neurotropic herpesvirus. While lymphohistiocytic inflammation is not considered a classic lesion for this disease, this may be indicative of a more chronic disease manifestation.

71: UNRAVELING THE MYSTERY: A CASE OF HPAI H5N1 INFECTION IN A DOMESTIC CAT POSSIBLY LINKED TO CAT-TO-CAT TRANSMISSION

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Background: Highly pathogenic avian influenza (HPAI) H5N1 transmission between species has been reported. To our knowledge, there have been no other cases of cat-to-cat transmission of H5N1.

Objective: To characterize necrotizing bronchointerstitial pneumonia in association with H5N1 in a 13-month-old castrated male cat submitted for necropsy.

Results: Grossly, lung lobes were multifocally red with pale pink regions on the pleural surface. Histopathology revealed necrotizing bronchointerstitial pneumonia and fibrinosuppurative interstitial pneumonia consistent with a viral infection and acute respiratory distress syndrome, respectively. Necrotizing hepatitis, splenitis, and lymphadenitis were also observed. Testing was negative for tularemia (PCR), feline infectious peritonitis (IHC), and SARS-CoV-2 (PCR) and positive for avian influenza virus (PCR). The National Veterinary Services Laboratories confirmed H5N1 positivity (PCR) on lung, spleen, brain, and urine. Immunohistochemistry for influenza A virus and RNAscope™ in situ hybridization using probe for H5N1 had positive immunolabeling and signal amplification, respectively, in necrotic areas of the lungs, liver, and spleen. Whole genome sequencing and analysis of the lung and brain classified the H5N1 strain as genotype B3.13 within the 2.3.4.4b clade. This was a clinic cat with no exposure to production animals, high-risk foods/treats, or the outdoors. The cat had contact with a staff member who previously handled a febrile cat on a raw food diet recalled for H5N1 contamination, the same cat's pet carrier, and the IVC placement treatment area of this cat prior to decontamination.

Conclusion: This may be the first known case of a naturally infected HPAI H5N1 domestic cat via cat-to-cat transmission.

72: FELINE PULMONARY CARCINOMA WITH SYSTEMIC METASTASES AND AORTIC THROMBOSIS PRESENTING AS PARAPLEGIA

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A 12-year-old, female spayed, American Shorthair cat exhibited acute onset paraplegia, tachypnea, and a history of anorexia and constipation. Neurological examination revealed absent hindlimb pulses and pain sensation. Supportive care with anticoagulants resulted in transient pulse return. Abdominal and cardiac ultrasound excluded cardiac disease but identified bilateral renal masses and an abdominal mass. Thoracic radiographs showed alveolar and interstitial patterns. Despite therapy, impairment of function of the hindlimbs was noted and euthanasia was performed due to poor prognosis.

Grossly, multiple firm white to beige masses were noted in lungs, kidneys, pancreas, and mediastinal lymph nodes. The lumen of abdominal aorta was occluded by a firm, red thrombus. Histopathology revealed carcinoma characterized by neoplastic epithelial cells forming tubular, papillary, and sheet-like structures with necrosis and suppurative inflammation in lung, kidneys, pancreas, mediastinal lymph nodes, and choroid of the right eye. Immunohistochemistry the neoplastic cells were positive for cytokeratin, and TTF-1, suggestive of a pulmonary origin of the neoplasm.

This case highlights a feline pulmonary carcinoma with aggressive metastases to multiple organs and aortic thrombosis leading to ischemic hindlimb paralysis. The findings are consistent with the reported “Feline MODAL syndrome” (muscle/ocular/digit/aorta/lung) pattern of metastatic spread. The aortic thrombosis is likely contributed to clinical signs in hindlimb and might be caused by paraneoplastic hypercoagulability.