Diagnostic Pathology Focused Scientific Session I

Sunday, 1:30 pm-5:00 pm
Pedro Pinczowski, DACVP (AP); Neha Mishra, MS MVSc PhD DACVP (AP)

NEURODEGENERATIVE DISEASE IN A TRANSGENIC MOUSE MODEL LACKING MICROGLIA

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Background:

This case involves two experimentally naïve 15-month-old female transgenic mice with selective deletion of the *fms*-intronic regulatory element (FIRE) in the colony-stimulating factor 1 (*Csf1r*) locus, resulting in depletion of tissue-specific macrophages. *Csf1r*$^{\Delta FIRE/\Delta FIRE}$ mice presented with a history of distended abdomens, wet fur along the ventrum, and periodic dragging of the hind limb (n=1/2).

Results:

In both mice, post-mortem examination revealed marked distention of the urinary bladder. Urethras were patent and a urine culture was negative for bacterial growth (n=1/2). The bladders were microscopically unremarkable. Histologic evaluation of the central nervous system (CNS) identified severe intramyelinic edema throughout the brain and spinal cord with spheroid formation and digestion chambers. Acute neuronal necrosis was also evident bilaterally. The cause of decline was attributed to demyelination, axonal degeneration, and hippocampal neuronal necrosis with subsequent neurogenic atony of the urinary bladder and monoparesis (n=1/2).

Conclusion:

The current case is a unique report of neurodegenerative disease affecting the brain and spinal cord of two 15-month-old transgenic *Csf1r*$^{\Delta FIRE/\Delta FIRE}$ mice who conventionally lack microglia. Leukoencephalopathy with spheroid formation has been described in human patients with a mutation in CSF1R. Various mouse models exist to mirror this condition. While global knockout of CSF1R often results in early lethality, heterozygosity leads to a similar age-related demyelinating disease. However, *Csf1r* +/- heterozygous mice reportedly have normal to *increased* densities of brain microglia. Therefore, the present diagnostic case supports a growing body of literature highlighting the complex role of colony stimulating factor and microglia in myelin formation and neurogenesis.

Discipline: Anatomic pathology, **Topic:** Comparative pathology, **Species:** Rodents, **Systems:** Nervous

CUTANEOUS LAGENIDIUM DECIDUUM IN THE TAIL OF A KITTEN

Christina Middendorf[1], Aline Rodrigues Hoffmann[2], Maiara Goncalves Ramos[2], Johnny Kaldis[3], Mayane Faccin[1]
[1]Texas A&M University, College of Veterinary Medicine & Biomedical Sciences, College Station, TX, USA, [2]University of Florida College of Veterinary Medicine, Gainesville, FL, USA, [3]Animal Medical Center of the Village, Houston, TX, USA

A seven-month-old male castrated domestic shorthair kitten presented for biopsy of a 4x2 cm alopecic and pruritic cutaneous mass on the proximal dorsal tail. Treatment with antibiotics and steroids showed minimal improvement over three months. Histology of the mass showed multifocal to coalescing nodular inflammatory infiltrates composed of epithelioid macrophages, multinucleated giant cells, variably degranulated eosinophils, fewer neutrophils, lymphocytes, and plasma cells. Within the center of the nodules were numerous 7-12 um in diameter negatively to pale basophilic staining, poorly septate, argyrophilic (GMS stain) hyphae with non-parallel walls and irregular branching. The hyphae also extended to involve hair follicles. Panfungal PCR was performed on formalin-fixed, paraffin embedded tissue. The internal transcribed spacer (ITS) was targeted, amplified and sequenced with Nanopore. The resulting 1011 bp contig sequence was analyzed with the NCBI BLAST database, and it matched *Lagenidium deciduum* with 98.32% identity. *Lagenidium* is a genus composed of numerous oomycete (water mold) species, including pathogenic mammalian species such as *L. giganteum* f. *caninum* and *L. deciduum* that can cause progressive cutaneous disease, which is most commonly diagnosed in temperate and tropical climates such as the Gulf Coast, southeastern, midwestern, and western regions of the United States. *L. giganteum* f. *caninum* is most often diagnosed in dogs and rarely in cats. Cutaneous infection by *L. deciduum* has been reported in an immunosuppressed person and has been seen in two cats from Louisiana (data not published). Clinical descriptions of this specific species are lacking in veterinary medicine.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Feline, **Systems:** Integument

CONCURRENT ACUTE LEUKEMIA AND DISSEMINATED LISTERIOSIS IN AN ADULT DOG

Danielle Ahmed, Kari Frankhouse, W. Shane Sills, Megan Schreeg, Laurie Millward, Maxey Wellman, Hamideh Esmaeilzadeh
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A 6-year-old male neutered mixed-breed dog presented with clinical signs of fever, lethargy, vomiting, weight loss, and hyporexia. Complete blood count revealed mild lymphocytosis (5.64 x 10^9/L, RI: 0.7-3.20) composed of large, atypical cells, and moderate thrombocytopenia (44 x 10^9/L, RI: 145 – 463). Bone marrow aspiration revealed a monomorphic blast population comprising 70% of all nucleated cells, marked toxic changes, and occasional intracellular, rod-shaped bacteria. The bone marrow aspirate was submitted to Colorado State University for flow cytometry analysis and polymerase chain reaction for antigen receptor rearrangement (PARR). PARR analysis detected a clonal rearrangement of both the T-cell receptor (TCR) gene and B-cell immunoglobulin gene (Ig), while flow cytometric analysis revealed an expansion of CD34+ cells consistent with acute leukemia (AL). Sequencing of the 16S rRNA amplicon isolated from bone marrow cytology slides resulted in forward and reverse sequences identical to *Listeria monocytogenes.* After one month of antimicrobial therapy there was marked clinical improvemnt; however, the circulating neoplastic population progressed, peaking at 29.71 x 10^9/L. Bone marrow evaluation performed 2 months after diagnosis revealed near effacement, with the neoplastic population comprising 90% of all nucleated cells. Analyses of blood via PARR and flow cytometry revealed the same clonal rearrangements and CD34+ populations as

in the bone marrow. In the case report herein, we hypothesize that the disseminated infection led to early detection of the AL, prolonging patient survival. This case highlights the importance of cytology as an important, in-hospital screening tool in guiding patient diagnosis and clinical management.

Discipline: Clinical pathology, **Topic:** Infectious disease, **Species:** Canine, **Systems:** Hematopoietic

USING GENE EXPRESSION PROFILING TO DIAGNOSE CANINE CUTANEOUS ROUND CELL TUMORS WITH NANOSTRING TECHNOLOGY

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Background: Canine cutaneous round cell tumors (CCRCTs) are neoplasms with frequently overlapping histologic features, making differentiation challenging. CCRCTs include epitheliotropic T-cell lymphoma (ETCL), plasma cell tumor (PCT), mast cell tumor (MCT), histiocytoma (HA), histiocytic sarcoma (HS), and melanoma, each with different prognoses. Differentiation necessitates immunohistochemistry (IHC) delaying diagnosis and adding cost.

Objective: Use gene expression patterns of CCRCTs to classify tumor type with a single comprehensive assay.

Methods: Fourteen tumors (training set) of each CCRCT type (84 total) were evaluated using a custom NanoString gene panel based on their predicted expression. ANOVA identified 70 significantly differentially expressed genes. The top 30 differentially expressed genes were selected as the feature set for classification. Eight classification algorithms were evaluated using 5-fold cross-validation to estimate internal model performance and guide model selection. The best-performing model was applied to an independent validation cohort of an additional 10 tumors of each CCRCT type (60 total), profiled with another NanoString panel comprising the same 30 genes.

Results: Across eight classifiers, training 5-fold cross-validation accuracy ranged from 64.3% to 75.1%. Logistic Regression achieved the highest accuracy (72.7% ± 5.7%). Per-class accuracy for Logistic Regression was: HA 100%, PCT 90%, ETCL 70%, HSA 70%, MCT 70%, and Melanoma 60%.

Conclusions: Early data shows promising accuracy of the gene panel and algorithm for classifying CCRCTs. This panel could aid in diagnosis of difficult to differentiate CCRCTs based on histopathology and may be an alternative to extensive IHC profiling or a tool to use in conjunction with more targeted IHC.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Canine, **Systems:** Other (Cutaneous round cell neoplasia)

DISTINCT STREPTOCOCCUS EQUI SSP. ZOOEPIDEMICUS SEQUENCE TYPES ASSOCIATED WITH CONCURRENT DISEASE IN DOGS AND CATS DURING A SHELTER OUTBREAK IN INDIANA

Chelsea C. Pulter[1], Nelly Elshafie[1], Rachel C. Soltys[1], David C. King[2], Maria Paz Buman Ruiz Diaz[1], Alec Daniels[1], Mario Sola[1], Nobuko Wakamatsu Utsuki[1]
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In 2025, an outbreak of *Streptococcus equi* ssp. *zooepidemicus* (*S. zooepidemicus*) affected 10 dogs and 7 cats in an overcrowded Indiana shelter. Clinical signs included hemorrhagic nasal discharge, hemoptysis, and frequently sudden death. Disease in cats began one month after onset in dogs. We hypothesized that affected dogs and cats were infected with the same *S. zooepidemicus* strain because they originated from the same shelter environment. This study compared the pathology and bacterial genomics of isolates from affected animals.

One dog and two cats were submitted for necropsy. The dog had necrotizing hemorrhagic pneumonia, whereas cats had myocarditis (2/2), suppurative meningitis and rhinitis (1/2), and suppurative bronchopneumonia (1/2). One cat also had concurrent feline infectious peritonitis. Aerobic culture yielded pure *S. zooepidemicus* from the dog's lung and one cat's brain, and mixed growth of *S. zooepidemicus* and *Pasteurella multocida* from the lung of the second cat. All cases were PCR-negative for other respiratory pathogens.

Whole genome sequencing unexpectedly identified the canine isolate as sequence type (ST) 507 and both feline isolates as ST78. Three additional feline shelter isolates and one local feline control isolate were also ST78, indicating regional subclinical carriage. Contrary to our hypothesis, canine and feline isolates belonged to distinct sequence types, suggesting separate introductions or concurrent circulation of multiple lineages. These findings demonstrate that genetically distinct *S. zooepidemicus* strains can cause severe disease during the same shelter outbreak and suggest that host and environmental stressors may play a greater role in disease emergence than sequence type alone.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Feline, **Systems:** Respiratory

PRIMARY CEREBELLAR GRANULAR CELL DEGENERATION IN A MINATURE DACHSHUND PUPPY WITH NOVEL GENETIC VARIANT

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An 11-month-old male neutered miniature Dachshund presented with progressive cerebellar dysfunction and was euthanized due to poor prognosis. Gross examination revealed the cerebellum was decreased in sized and had increased space between the cerebellar folia on cut section. Histologically, cerebellar folia were markedly thinned and hypocellular with severe selective loss of the cerebellar granular cell layer with relative preservation of Purkinje cells. These findings are consistent with primary cerebellar granular cell degeneration, a rare form of cerebellar cortical abiotrophy that has not been previously described in miniature Dachshunds. The pathogenesis of this condition is unknown, but a hereditary origin is suspected as a separate puppy from a previous litter produced by the same dam and sire presented with similar clinical signs and cerebellar atrophy identified on magnetic resonance imaging. Antemortem EDTA blood from this puppy and frozen samples of liver, kidney, and brain from the necropsied puppy were sent to PennGenn Laboratories. Both dogs were found to carry a rare variant in the CASK gene, an x-linked recessive gene that encodes calcium/calmodulin-dependent serine protein kinase, a protein vital for neuronal development and function. In humans, CASK gene mutations are associated with neurodevelopmental disorders including microcephaly with pontine and cerebellar hypoplasia and x-lined intellectual disability with or without nystagmus. These findings indicate that the CASK gene variant may be associated with primary cerebellar granular cell degeneration in miniature Dachshunds. Primary cerebellar granular cell degeneration should be considered as a differential diagnosis in puppies with progressive clinical signs of cerebellar dysfunction.

Discipline: Anatomic pathology, **Topic:** Degenerative, **Species:** Canine, **Systems:** Nervous

RETROSPECTIVE STUDY OF SPONTANEOUS NEOPLASMS IN A CLOSED RESEARCH COLONY OF NORTHERN GREATER GALAGOS (OTOLEMUR GARNETTII)

Catherine O. Black, Katherine N. Gibson-Corley, Michael M. McKinney, Nicholas M. Tataryn, Katherine A. Shuster, Tzushan S. Yang
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The Northern greater galago (*Otolemur garnettii*), a small prosimian primate, has served as a valuable mammalian model to study evolutionary brain development and sensory systems. As documentation on background lesions in galagos is limited, this survey consists of a retrospective review of spontaneous neoplasms from a closed colony between 2015 and 2026. Of the 21 animals submitted for necropsy, twelve animals (ten females, two males), from ages 4 to 16 years old, were diagnosed with benign and/or malignant neoplasms. Four animals were diagnosed with mammary tumors, which included phyllodes-like tumor (2), cystadenoma (1), and mixed mammary tumor (1). All mammary tumors occurred in females and were either histologically benign or showed no evidence of metastasis. Four animals had focal or metastatic round cell tumors. In one animal, the mass was localized to the left ear pinna, an unusual primary site, with strong CD3 immunoreactivity and no evidence of metastasis or extension into the middle or inner ear. The remaining three round cell tumors, one of which was metastatic, remained unclassified despite extensive attempts at characterization through immunohistochemistry. Additional neoplastic or tumor-like lesions included ovarian mixed germ cell-sex cord-stromal tumor (1),

clitoral sebaceous adenoma (1), dermal fibroma (1) and lipoma (2). These tumor types, sites of identification, and ages of occurrence help to highlight areas for future clinical surveillance in aging galago colonies and to provide more comprehensive information on natural disease not previously reported in Northern greater galagos.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** NHP, **Systems:** Systemic

TOXIC EPIDERMAL NECROLYSIS IN AN ENGLISH SPRINGER SPANIEL DOG: HISTOPATHOLOGIC AND IMMUNOHISTOCHEMICAL CHARACTERISTICS OF LETHAL IMMUNE-MEDIATED CYTOTOXIC DERMATITIS

Rahul Kumar[1], Mary Ann Lee[1], Sonika Patial[2], Yogesh Saini[1], Ina Herrmann[1], Keith Linder[3], Brigid Troan[4]
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Background: Toxic epidermal necrolysis (TEN) is a rare, typically drug-induced, immune-mediated cytotoxic dermatitis with an acute onset of erythema, erosions, ulcers, and epidermal detachment. The underlying mechanism remains poorly defined in dogs.

Objective: To provide detailed pathological features of TEN in a 4-year-old, female spayed English Springer Spaniel dog presented with a history of acute epidermal detachment on the ventrum, paw pads, mucosa, and mucocutaneous junctions following phenobarbital administration for idiopathic epilepsy.

Methods: Histopathology, immunohistochemistry, and an algorithm for assessment of drug causality in epidermal necrolysis (ALDEN) scoring were performed.

Results: Grossly, there were multifocal erosions to ulcerations in the nasal planum, esophagus, mucocutaneous junctions, oral mucosa, ventral abdomen, tongue, pinnae, and paw pads involving approximately 35% of the body surface area. Phenobarbital was associated with a ‘very probable’ ALDEN score of 6. Histologically, transepidermal keratinocyte apoptosis with mild lymphocytic satellitosis, progressing to laminar and full-thickness epidermal necrosis was present in all skin sections. Similar lesions were present in the esophageal mucosa and submucosal glands. Terminal deoxynucleotidyl-transferase–mediated deoxyuridine triphosphate nick-end labeling (TUNEL) and cleaved caspase 3 (CC-3) staining were immunoreactive in epidermal keratinocytes and infundibular regions of hair follicles, as well as the mucosal epithelial and submucosal mucus cells of the esophagus. CD-3+ lymphocytes were found infiltrating the regions of CC-3-positive cells in the skin and esophagus.

Conclusions: These findings supported the diagnosis of TEN and the role of apoptosis in disease development. Future work will explore the spatial relation between apoptosis and immune response using multiplex immunofluorescence.

Discipline: Anatomic pathology, **Topic:** General pathology, **Species:** Canine, **Systems:** Integument

PANCREATITIS, POLYARTHRITIS, AND PANNICULITIS SYNDROME IN A CANINE WITH PANCREATIC ADENOCARCINOMA

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An approximately 11-year-old, spayed female, German Shepherd mix was presented for postmortem examination following an extensive workup after a ten-month history of shifting hindlimb lameness and swollen joints. Previously, three biopsy submissions were obtained, all of which revealed necrotic adipose tissue without evidence of infection or neoplasia. Immune-mediated steatitis was therefore presumptively diagnosed, and immunosuppressive therapy was initiated. However, the lameness progressed to an inability to walk, prompting referral to the Mississippi State University Animal Health Center for further evaluation. During the intake examination, the animal was febrile (104.9°F/40.5°C), and all the joints along the distal extremities were erythematous, swollen, and painful. During hospitalization, purulent draining tracts developed over multiple affected joints. Humane euthanasia was elected because of severe pain and poor quality of life. Postmortem examination revealed fibrinosuppurative polyarthritis, multifocal necrotizing panniculitis of the neck and right lateral flank, and a large, infiltrative, cystic pancreatic mass. On histopathology, the pancreatic mass represented an adenocarcinoma with associated chronic, necrotizing pancreatitis. Aerobic and anaerobic cultures of multiple joints, urine, and blood yielded no bacterial growth, supporting a diagnosis of paraneoplastic pancreatitis, polyarthritis, and panniculitis (PPP) syndrome. PPP syndrome is infrequently documented in the veterinary literature, and diagnosis depends on a combination of clinical signs, histopathological results, and evidence of a sterile process confirmed by multiple cultures.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Canine, **Systems:** Endocrine

CYTOLOGIC AND DIAGNOSTIC IMAGING FINDINGS OF PRESUMED SPONTANEOUS REGRESSION OF MULTIFOCAL METASTATIC OSTEOSARCOMA IN A GERMAN SHEPHERD DOG

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Texas A&M University, College of Veterinary Medicine & Biomedical Sciences, College Station, TX, USA

A 6-year-old, female spayed, German Shepherd dog presented for a monthly recheck for palliative care of metastatic osteosarcoma. Osteosarcoma of the right distal tibia was previously diagnosed via histopathology following amputation one year ago. After completion of carboplatin chemotherapy, the patient developed presumed metastatic pulmonary lesions. All oncologic treatments were discontinued upon progression of pulmonary lesions.

At this appointment, a large, subcutaneous mass at the right lateral cervical area was noted. Cytologic evaluation of this mass was most consistent with metastatic osteosarcoma, displaying a dense population of highly atypical neoplastic mesenchymal cells which exhibited consistent cytoplasmic labelling for alkaline phosphatase. The pulmonary lesions were mildly progressive.

One month later, the subcutaneous cervical mass was significantly smaller. Cytologic evaluation showed a similar population of malignant neoplastic mesenchymal cells, with an additional, overt increase of small and small to intermediate lymphocytes. Thoracic radiographs revealed significantly smaller pulmonary lesions. Six months have passed since first noticing the cervical mass, which is now completely regressed, and all pulmonary lesions continued to decrease in size despite no treatment administered. Further follow-up is pending.

Spontaneous regression of primary and metastatic bone neoplasms is an extremely rare occurrence that has been described in humans and dogs. The increased lymphocytes in this case prompts discussion of immune-mediated resolution of cancerous lesions. Tumor-associated lymphocytes have been discussed in numerous human cancers, and their increase may be associated with more favorable prognoses. Their role in metastatic osteosarcoma remains unclear, yet possibly pivotal, in this case of an unusual disease outcome.

Discipline: Clinical pathology, **Topic:** Oncology, **Species:** Canine, **Systems:** Other (Immunologic, integument and respiratory)

SPINDLE CELL SQUAMOUS CELL CARCINOMA OF THE MANDIBLE IN A HORSE

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Squamous cell carcinoma (SCC) is a malignant epithelial neoplasm and among the most common neoplasms in horses, including the most common oral malignancy. Ultraviolet light exposure, depigmented skin, and sparse hair coat are risk factors associated with SCC development and contribute to the predilection of development on the head, eyes, and genitals. Spindle cell squamous cell carcinoma (scSCC) is a rare histologic variant characterized by elongated spindle-shaped epithelial cells and has been infrequently reported in cats, dogs, and horses. Here we describe a case of scSCC in a 19-year-old American Quarter Horse gelding with two-month history of progressive mandibular swelling, suspected to be secondary to a tooth root pathology, unresponsive to appropriate dental care. Incisional biopsies of mandibular soft tissue and bone revealed spindle cells arranged in bundles and streams infiltrating adjacent tissue, accompanied by severe suppurative osteomyelitis, generating top differentials of fibrosarcoma or scSCC. The gelding was euthanized and submitted for necropsy where a 15 x 14 x 10 cm firm left mandibular mass with cavitated pockets of purulent material was identified. Histologically, the mass consisted of polygonal to fusiform neoplastic cells arranged in interlacing bundles and streams with moderate pleomorphism and multinucleation. Replicate sections immunostained with pancytokeratin revealed strong cytoplasmic reactivity in 60% of the neoplastic cell population leading to a diagnosis of scSCC. This case highlights a rare histologic variant of equine SCC and emphasizes the value of cytokeratin immunohistochemistry in differentiating scSCC from fibrosarcoma and other mesenchymal spindle cell neoplasms of the oral cavity and mandible.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Equine, **Systems:** Bone and joints

ACQUIRED FANCONI'S SYNDROME SECONDARY TO COPPER-ASSOCIATED HEPATOPATHY IN A DOG

Kari Frankhouse, Anna Foley, Megan Schreeg, John Rowe, Maxey Wellman
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A 4-year-old spayed female mixed-breed dog was evaluated for a three-week history of progressive hyporexia and vomiting. Clinicopathologic abnormalities included mild azotemia, hyperchloremic metabolic acidosis characterized by decreased bicarbonate concentration and a normal anion gap, mild hypophosphatemia, mild hypokalemia, increased alanine aminotransferase and aspartate aminotransferase, and normoglycemia. Urinalysis demonstrated inadequate urinary concentration with a specific gravity of 1.015, marked glucosuria, mild proteinuria, and ketonuria. The combination of normoglycemic glucosuria, electrolyte derangements, and hyperchloremic metabolic acidosis was consistent with generalized proximal renal tubular dysfunction. Abdominal ultrasonography revealed no clinically significant abnormalities; however, the increase in liver enzymes warranted additional ancillary diagnostics. Fine-needle aspiration of the liver yielded hepatocytes with mild vacuolar change consistent with lipid accumulation and coarse pale green to turquoise cytoplasmic granules, suggestive of copper. These findings prompted laparoscopic liver biopsy. Histopathologic examination identified chronic hepatitis with severe hepatocellular copper accumulation, and quantitative hepatic mineral analysis confirmed marked hepatic copper accumulation (5,995 µg/g dry weight; reference interval, 137–400 µg/g), with mild concurrent increases in hepatic iron and zinc concentrations. The clinicopathologic abnormalities, histopathologic findings, and quantitative hepatic copper analysis supported a diagnosis of acquired Fanconi syndrome secondary to copper-associated hepatopathy. Although acquired Fanconi syndrome is most commonly associated with nephrotoxic agents or ingestion of commercial jerky treats, copper-associated hepatopathy is a rarely recognized cause of generalized proximal tubular dysfunction and should be considered in dogs with persistent normoglycemic glucosuria and concurrent evidence of hepatocellular injury in order to initiate appropriate therapy.

Discipline: Clinical pathology, **Topic:** Metabolic disease, **Species:** Canine, **Systems:** Urinary

RENAL DYSPLASIA WITH CONCURRENT NUTRITIONAL SECONDARY HYPERPARATHYROIDISM IN A PEMBROKE WELSH CORGI PUPPY

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Background: Renal dysplasia is an uncommon developmental cause of renal failure in dogs. Concurrent nutritional secondary hyperparathyroidism (NSHP) may complicate interpretation of mineral abnormalities.

Objective: To describe the clinicopathologic and histopathologic findings of renal dysplasia with concurrent NSHP in a puppy.

Methods: A 3-month-old intact male Pembroke Welsh Corgi presented to the Purdue University Veterinary Hospital with lethargy, abdominal distension, facial edema, and increased respiratory effort. Complete blood count, serum biochemistry, urinalysis, endocrine testing, necropsy, and histopathology were performed.

Results: Clinicopathologic abnormalities included mild nonregenerative anemia (hematocrit, 30%; RI, 37–55%), severe azotemia (BUN, 182 mg/dL; RI, 7–32 mg/dL; creatinine, 2.1 mg/dL; RI, 0.5–1.5 mg/dL), hyperphosphatemia (24.3 mg/dL; RI, 2.2–7.9 mg/dL), hypocalcemia (5.9 mg/dL; RI, 9.7–12.3 mg/dL), hypercholesterolemia (500 mg/dL; RI, 125–301 mg/dL), isosthenuria (USG, 1.011), and 3+ proteinuria. Marked hypocalcemia prompted endocrine testing, which revealed increased PTH (31.9 pmol/L; RI, 0.5–5.8 pmol/L), decreased ionized calcium (0.65 mmol/L; RI, 1.25–1.45 mmol/L), and decreased 25-hydroxyvitamin D (26 nmol/L; RI, 109–423 nmol/L), consistent with NSHP secondary to an unbalanced raw diet. Necropsy revealed bilaterally small kidneys. Histologically, kidneys contained fetal glomeruli, tubular degeneration and ectasia, interstitial fibrosis, glomerulosclerosis, and multifocal mineralization, consistent with renal dysplasia. Fibrous osteodystrophy and metastatic mineralization were also present.

Conclusions: Renal dysplasia was the primary cause of renal failure, while concurrent NSHP exacerbated mineral abnormalities. This case highlights the importance of integrating clinicopathologic, endocrine, histopathologic, dietary, and clinical history findings to recognize concurrent disease processes.

Discipline: Both, **Topic:** Metabolic disease, **Species:** Canine, **Systems:** Urinary

PAPILLOMAVIRUS-INDUCED CERVICAL SQUAMOUS CELL CARCINOMA WITH LUNG METASTASIS IN A RHESUS MACAQUE (MACACA MULLATA)

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Papillomaviruses (PV) are ubiquitous epitheliotropic double-stranded DNA viruses with oncogenic potential. High-risk mucosal human papillomaviruses (HPVs), such as HPV16 and 18, cause malignant orogenital neoplasms in humans and are responsible for more than 70% of cervical cancers in women. Of the animal species affected by PV-induced neoplasia, mucosal neoplasms similar to those in humans are only reported regularly in other primates. In this study, the necropsy of a 27-year-old female rhesus macaque with a history of urinary retention revealed a homogeneously off-white neoplasm expanding the cervix and obstructing urinary outflow. Similar masses were attached to the serosal surface of the neck of the urinary bladder and occurred multifocally throughout the lungs. Histopathology was consistent with squamous cell carcinoma, with neoplastic cells within the lumina of cervical and pulmonary vessels indicating vascular invasion and metastasis. Immunohistochemistry with P16 and P53 supported likely PV-infection. *Macaca fascicularis* papillomavirus-5 was then isolated from the primary and metastatic tumors, which suggested that PV integration had contributed to carcinogenesis and metastasis. The findings in this case are comparable to high-risk HPV infection in women, wherein the lung is the most common site of metastasis from cervical carcinoma, and to the authors' knowledge, represent the first confirmed report of

pulmonary metastasis from PV-induced cervical carcinoma in a non-human primate (NHP). These findings suggest that the oncogenic mechanism of PVs across primates is conserved and thus highlights the significance of NHPs as comparative models for viral oncogenesis.

The content of this presentation is based on a forthcoming publication.

Discipline: Anatomic pathology, **Topic:** Comparative pathology, **Species:** NHP, **Systems:** Reproductive

POORLY DIFFERENTAITED SINONASAL CARCINOMA WITH CRIBRIFORM PLATE AND INTRACRANIAL INVASION IN A BOBCAT (LYNX RUFUS)

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Kansas Veterinary Diagnostic Laboratory, College of Veterinary Medicine / Diagnostic Medicine / Pathobiology, Manhattan, KS, USA

Primary nasal neoplasms are rarely reported in nondomestic felids. We describe a poorly differentiated sinonasal carcinoma with localized invasive growth involving the cribriform plate and rostral frontal cortices in a bobcat (*Lynx rufus*) presenting with acute neurologic dysfunction.

A 15-year-old captive bobcat was euthanized following onset of acute progressive neurologic signs with an inability to eat or drink. Rabies virus was not detected in cross sections of cerebellum, brainstem and cerebrum by fluorescent antibody testing. A 3–4 cm firm white expansile poorly demarcated mass effaced the caudal nasal turbinates, extended through and partially replaced the cribriform plate, and infiltrated into the rostral frontal cerebral cortices. Mild chronic bilateral otitis media was also observed.

The neoplasm was composed of polygonal epithelial cells arranged in solid sheets and nests, with multifocal tubule and acinar differentiation supported by fine fibrovascular stroma. Neoplastic cells exhibited moderate anisocytosis and anisokaryosis, and a high mitotic count of 29/2.37 mm². Neoplastic cells infiltrated through the cribriform plate and into the subjacent olfactory bulbs, and frontal cerebral cortical neuroparenchyma; this was accompanied by hemorrhage, necrosis, gliosis, neocapillarization, and mild perivascular lymphocytic inflammation.

Neoplastic cells displayed diffuse moderate to strong cytoplasmic immunoreactivity for vimentin and patchy weak to moderate immunoreactivity for pancytokeratin, but no immunoreactivity for chromogranin A, synaptophysin, or neuron-specific enolase. These data support a diagnosis of poorly differentiated sinonasal carcinoma.

To our knowledge, this is the first reported case of poorly differentiated sinonasal carcinoma with cerebral invasion in a nondomestic large captive felid.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Wildlife/Zoo, **Systems:** Respiratory

EPIZOOTIC HEMORRHAGIC DISEASE VIRUS IN CATTLE IN OREGON: ABORTIONS AND FETAL CENTRAL NERVOUS SYSTEM LESIONS

Aarathi Sureshkumar[1], Kristine Carter[2], Vaughn Taylor-Lehman[1], Christiane Löhr[1], Rachael Gruenwald[1]
[1]Gary R. Carlson, MD, College of Veterinary Medicine, Oregon State University, Corvallis, OR, USA, [2]Oregon Department of Agriculture – Animal Health Program, Salem, OR, USA

Introduction: Epizootic hemorrhagic disease virus (EHDV) is a Culicoides-borne orbivirus that causes severe hemorrhagic disease in wild ruminants. In contrast, cattle mostly develop subclinical disease. However, recent outbreaks suggest increased EHDV virulence, including reproductive loss. This case series characterizes manifestations of transplacental EHDV infection in Oregon cattle during 2026.

Methods: Between February and March 2026, seven calves (1 necropsy; 6 fixed and fresh tissues) were submitted to the Oregon Veterinary Diagnostic Laboratory from herds experiencing abortions, stillborn calves, and live-born calves with neurologic abnormalities. Diagnostics included gross necropsy (when applicable), histopathology, bacterial cultures, and molecular testing. EHDV and Bluetongue virus multiplex PCR was performed on all submissions.

Results: Four calves had central nervous system (CNS) lesions, including hydrocephalus and/or hydranencephaly (n=3), cerebellar hypoplasia (n=1), and anencephaly (n=1). Additional lesions included placentitis, myocardial necrosis, meningitis, pulmonary edema, enteritis, and hepatic necrosis. EHDV was detected in all submissions; bluetongue virus, bovine viral diarrhea virus, and bovine herpesvirus type 1 were not detected. EHDV was identified in the blood of 15 of 16 affected dams, including serotype 2 in six dams; serotypes 1 and 6 were not detected.

Conclusion: This case series expands the recognized spectrum of EHDV-associated disease in cattle by characterizing congenital CNS lesions and reproductive loss associated with transplacental infection during a regional outbreak in Oregon. It highlights EHDV as an emerging infectious abortifacient in cattle and supports consideration of its inclusion in routine abortion diagnostic workups as Culicoides distribution and EHDV epidemiology evolve, potentially influenced by climate change.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Bovine, **Systems:** Nervous

METASTATIC PRIMITIVE NEUROECTODERMAL TUMOR IN A BUDGERIGAR (MELOPSITTACUS UNDULATUS)

Madison Callicott, Sarah Linn-Peirano
University of Tennessee College of Veterinary Medicine, Knoxville, TN, USA

An adult male intact Budgerigar (*Melopsittacus undulatus*) was presented to the University of Tennessee Avian and Exotics Department for labored breathing with audible clicks. The bird died while being anesthetized for euthanasia. Filling the right nasal cavity, compressing the nasal septum, and displacing the overlying nasal turbinates was a 7x5x5mm, distinct, firm, lobular, mottled pale tan to brown mass.

There was a 6x5x5mm distinct, firm, round, homogenous pale tan mass in the right liver lobe, and similar masses in the left liver lobe (1mm in diameter) and right kidney (2mm in diameter). Histologically, the masses were composed of polygonal to spindle cells arranged in packets, cords, pseudorosettes, and rare rosettes in a fine fibrovascular stroma, consistent with a primitive neuroectodermal tumor. There was also infiltration of neoplastic cells into the tongue, and intravascular neoplastic cells in the lung. Given the size and location within the nasal cavity, the presumed primary site and origin is olfactory neuroepithelium. Olfactory neuroblastoma, also called esthesioneuroblastoma, is rarely reported in domestic animals and has not been reported as a metastatic neoplasm in birds.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Avian, **Systems:** Nervous

DUCHENNE MUSCULAR DYSTROPHY IN A MALTESE DOG

Chi-Hsuan Sung[1], Yu-Ya Liang[2], Kazuyuki Uchida[3], Raquel Rech[1]
[1]Texas A&M University, College Station, TX, USA, [2]Utah Valley University, Orem, UT, USA, [3]The University of Tokyo, Tokyo, Japan

Duchenne muscular dystrophy (DMD) is an X-linked myopathy caused by loss-of-function mutations in the *DMD* gene, leading to absence of dystrophin and progressive degeneration of skeletal and cardiac muscle. The *DMD* gene spans approximately 2.3 Mb across 79 exons. In human DMD, deletions and frameshift variants predominate. In dogs, DMD has been sporadically reported in more than 10 mostly medium-to-large breeds, due to various types of mutation, including point insertion or deletion or large deletions. We describe DMD in a 5-year-old male Maltese dog with lethargy, slow ambulation, and lateral recumbency. Serum biochemistry showed hyperphosphatemia (13 mg/dL), hypocalcemia (6.5 mg/dL), elevated ALT (590 U/L), and markedly increased creatinine above assay limits despite dilution. Histologically, appendicular skeletal muscles, diaphragm, tongue, esophagus, perilaryngeal muscles, and panniculus carnosus muscles exhibited marked, polyphasic myofiber degeneration and necrosis with regeneration and fibrosis. Cardiomyocytes were also affected. Kidneys exhibited myoglobin-induced acute tubular injury. Dystrophin immunohistochemistry with two different antibodies for the rod domain demonstrated complete absence of the normal sarcolemmal staining pattern in affected muscles. Slow- and fast-myosin immunolabeling showed partial preservation, making neurogenic atrophy unlikely. Histologic and immunohistochemical findings support a diagnosis of DMD. Whole-genome sequencing identified a 2-bp insertion in exon 79, the terminal coding exon of DMD, and focal read-depth reductions across multiple exons suggestive of sub-resolution structural variation. Because terminal-exon frameshifts typically escape nonsense-mediated mRNA decay, the absence of dystrophin likely indicates post-translational degradation of the altered protein, highlighting a previously undocumented non-canonical mechanism in canine DMD.

Discipline: Anatomic pathology, **Topic:** Genetic/hereditary disorders, **Species:** Canine, **Systems:** Muscle

Veterinary Student Platform Presentations Session

Sunday, 12:15 pm-1:15 pm

Santhipriyadarsini Sridharan, BVSc MVSs PhD MVeTSc DACVP (AP) DABT, Stacey Piotrowski, DACVP (AP)

HISTOPATHOLOGIC DESCRIPTION OF AVIAN PHILOPHTHALMIASIS: LESIONS AND IMPLICATIONS

Kristina Camarena[1], Vaughn Taylor-Lehman[1], Hakon Jones[2], Michael Yabsley[2], Elizabeth A. Ihms[3]

[1]Gary R. Carlson, MD, College of Veterinary Medicine, Oregon State University, Corvallis, OR, USA, [2]Southeastern Cooperative Wildlife Disease Study, UGA College of Veterinary Medicine, [3]TRAP Labs, LLC, Corvallis, OR, USA

Philophthalmus gralli is a zoonotic ocular trematode that can be found in waterfowl worldwide. Philophthalmiasis has been described in only a few species of birds and a fully comprehensive histopathologic description of its lesions has not been performed to date. A full description of these lesions would help further elucidate the pathogenesis and life cycle of *P. gralli*. American coots from the Southeastern Cooperative Wildlife Disease Study in Georgia were found to be infected with *P. gralli.* Eleven samples were submitted to TRAP labs for histopathology and photomicrographs of characteristic lesions were obtained. Six out of the 11 coots were infected with the trematode, with the most common site of infection in the conjunctiva. Pathological lesions ranged from mild epithelial hyperplasia to lymphocytic and heterophilic conjunctivitis with hyperplasia and severe edema. Interestingly, flukes were also found in the infraorbital sinus of some birds, which is not a commonly reported site of infection. Molecular genomics were used to confirm that these flukes were also *P. gralli.* The flukes found in the infraorbital sinus all appeared to be large and gravid, which could indicate that this is the primary site of colonization and maturation of the infective metacercariae stage. As *P. gralli* is a zoonotic trematode that is increasing its range worldwide, understanding its life cycle and the pathologic lesions it causes is essential in order to avoid exposure and diagnose potential infections in humans and other avian species, as infection has been documented in chickens.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Wildlife/Zoo, **Systems:** Eye/ear

ARTIFICAL INTELLIGENCE-BASED WHOLE SLIDE IMAGE ANALYSIS FOR CLASSIFICATION OF LYMPH NODE METASTASIS IN CANINES WITH MAST CELL TUMORS

Lydia Zermuehlen, Lilian Oliveira, Samantha Schlemmer

University of Georgia College of Veterinary Medicine, Athens, GA, USA

Introduction: The Histologic Node (HN) classification system has facilitated standardized assessment of lymph node metastasis in canines with mast cell tumors (MCT) and is generally associated with prognosis and clinical outcome. However, histologic classification remains subject to interobserver variability. Whole-slide imaging (WSI) and artificial intelligence (AI)-based approaches offer the potential to improve reproducibility through comprehensive, quantitative evaluation of histologic specimens. This study evaluated the feasibility of a WSI AI-based classifier for HN classification.

Methods: Fifty extirpated lymph nodes with pathologist-assigned HN class were assessed. WSIs from 5, 40, and 5 cases were used for annotation, training, and validation, respectively, using DenseNet-V2, Nuclear Segmentation, and Object Phenotyper models in Halo AI (v4.2, Indica Labs). A five-step pipeline was developed: (1) tissue vs. background (broad classifier); (2) node vs. other tissue (node classifier); (3) nuclear segmentation for cell identification; (4) mast cell vs. non-mast cell classification; and (5) a Python-based algorithm applying established HN criteria to assign a HN class.
Results: Preliminary data shows the broad classifier achieved precision of 0.990, recall of 0.993, and F1 score of 0.992; the node classifier achieved a precision of 0.924, recall of 0.980, and F1 score of 0.950, both exceeding the 0.70 F1 threshold.
Conclusions: These preliminary results support the feasibility of an AI-assisted WSI pipeline for HN classification in canine MCT, with sub-classifiers reliably distinguishing tissue and nodal boundaries. Remaining steps, mast cell quantification, and comparison to pathologist-assigned HN classifications, are underway. This approach may ultimately reduce observer variability and improve classification reproducibility.

Discipline: Anatomic pathology, **Topic:** Methods/technology, **Species:** Canine, **Systems:** Hematopoietic

ASSESSMENT OF FEMORAL HEAD TISSUE CHANGES IN RESPONSE TO DRILLING IN A PIGLET MODEL OF LEGG-CALVÉ-PERTHES DISEASE
Viktorie Menna, Ashton Amann, Ferenc Toth, Casey Johnson, Alexandra Armstrong
University of Minnesota College of Veterinary Medicine, Saint Paul, MN, USA

Background: Legg-Calvé-Perthes Disease (LCPD) causes ischemic damage to the femoral head in children and toy-breed dogs, leading to necrosis of the bone, marrow, and growth cartilage. Surgical drilling of the ischemic femoral head is one treatment option to initiate bone healing and new bone formation. The exact mechanism of this healing response is not well characterized. We hypothesized that the presence of fibrovascular tissue and osteoid (immature unmineralized bone) within the epiphysis of the femoral head will be increased near the drill tract vs. far from the drill tract and as compared to the contralateral control femoral head.

Methods: Samples were collected from drilled piglet femoral heads and contralateral controls at 1-3 weeks post-drilling (3-5 weeks post-ischemia). Samples were fixed in 10% NBF, decalcified in 10% EDTA, and sections were stained with H&E and RGB-Trichrome. A tissue classifier was trained to categorize and quantify the area of mineralized bone, unmineralized bone (osteoid), cartilage, marrow, and fibrovascular tissue within the femoral head from scanned slides using HALO.

Results: A repeated-measures ANOVA and/or Friedman test (based on normality of data for each tissue type) showed significant differences in the percentage area of mineralized bone, cartilage, marrow, and fibrosis across groups.

Conclusion: Compared to control, drilling of the ischemic femoral head did cause an increase in fibrovascular tissue near the drilling tract but did not alter the amount of unmineralized (new) bone in the near or far samples. Marrow and mineralized bone decreased in ischemic femoral heads and cartilage area increased.

Discipline: Anatomic pathology, **Topic:** Comparative pathology, **Species:** Porcine, **Systems:** Bone and joints

DOMESTIC CATS HAVE VARIABLE SEROPOSITIVITY FOR SARS-COV-2 OMICRON B.1.1.529 AND WUHAN-HU-1 VARIANTS SEVERAL YEARS AFTER VIRUS EMERGENCE (2024, 2026)

Taylor Crisologo[1], Mary Nehring[1], Treana Mayer[1], Sachithra Gunasekara[2], Craig Miller[2], Jennifer Rudd[2], Angela Bosco-Lauth[1], Tyler Sherman[3], Ian Zander[3], Christie Mayo[3], Sue VandeWoude[1]
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Background: SARS-CoV-2 has evolved into several variant lineages since emerging in December 2019, and sub-variants of Omicron still circulate today. Domestic cats are susceptible to multiple variants following exposure to infected people or other cats. While the risk of cat-to-human transmission is low, cross-species spillover results in higher mutation rates and potential variant emergence.
Objective: Estimate variant-specific seroprevalence of SARS-CoV-2 in domestic cats using novel Omicron seroassays.
Methods: We assessed seroprevalence in owned domestic cats using sera samples submitted to the Colorado State University Veterinary Diagnostic Laboratory in 2024 and 2026 (n=350). We developed enzyme-linked immunosorbent assays to detect feline antibodies against spike (S1+S2) and receptor binding domain (RBD) antigens for Omicron B.1.1.529 (Omicron) and Wuhan-Hu-1 (Wuhan) antigens.
Results: We found 4.6% (16/350) SARS-CoV-2 seroprevalence for Omicron and Wuhan variants combined. Several cats were positive for the Wuhan variant only (n=8/16), and the majority of cats positive for Omicron were also positive for Wuhan (n=7/8). One individual was positive for the Omicron variant only.
Conclusion: Our results indicate Wuhan-based assays have enhanced sensitivity compared to Omicron. This may imply Wuhan antigens are more immunodominant, point to Omicron's immune evasion properties, or suggest cats infected earlier in the pandemic may have generated persistent antibodies. Most feline seroprevalence studies during the peak of human infections found less than 5% feline seroprevalence. Our study using contemporary samples found seroprevalence levels similar to those at the height of the pandemic, highlighting the importance of including domestic cats in future One Health disease monitoring.

Discipline: Clinical pathology, **Topic:** Immunology, **Species:** Feline, **Systems:** Systemic

A RETROSPECTIVE ANALYSIS OF REPRODUCTIVE TRACT LESIONS AND RISK FACTORS IN ZOO-MANAGED FEMALE URSIDS

Grace Sherlitz[1], Anneke Moresco[1,2], Chelsey Yob[1], Jamie Douglas[1], Dalen Agnew[1,2]
[1]Michigan State University, East Lansing, MI, USA, [2]Reproductive Health Surveillance Program, East Lansing, MI, USA

Background: In many species, reproductive tract lesions have been associated with age, parity, and progestin contraceptives. Few studies have characterized

reproductive lesions in zoo-managed bears or identified demographic and management factors that increase the likelihood of lesion development.
Objective: To describe the prevalence of lesions found in the female ursid reproductive tract and identify associated risk factors.
Methods: Tissue samples from 51 bears across 6 species were utilized from the Reproductive Health Surveillance Program (RHSP) archive. Five µm histologic sections of the ovaries and uterus were routinely prepared and examined, with ovarian activity and lesion presence recorded. Observations were assessed for associations with potential risk factors via logistic regressions and Fisher's exact tests.
Results: Increased age was significantly associated with both uterine (p=0.0007) and ovarian (p=0.0006) lesions, while longer parturition gap was associated with ovarian lesions only (p=0.0232). Hyalinization of uterine blood vessels was associated with having at least one litter (p=0.0050). Progestin use was associated with clinically significant endometrial hyperplasia (p=0.0254). Unlike other species, all spectacled bears (*Tremarctos ornatus*) examined were found to have endometrial hyperplasia (9/9). Being not of the *Ursus* genus (3/6 species) was associated with adenomyosis (p=0.0156).
Conclusion: Caution should be exercised when using progestin contraceptives in ursids and alternative contraceptive methods should be considered. Controlled studies isolating the effects of risk factors and further investigating species differences would be important next steps. Through better understanding of reproductive disease in ursids, facilities housing bears can improve their care and breeding management while minimizing reproductive consequences.

Discipline: Anatomic pathology, **Topic:** General pathology, **Species:** Wildlife/Zoo, **Systems:** Reproductive

UROPERITONEUM SECONDARY TO PUBIC EXOSTOSIS IN A HORSE

Emma Adams, Victoria Shuster, Lina Crespo Bilhalva, Isidora Navarro Münchmeyer, Fabio Brum Rosa, Joyce Knoll, Amanda Prisk, Francisco Conrado
Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA, USA

A 10-year-old Warmblood gelding presented for persistent and progressive acute abdominal discomfort and a 2-day history of stranguria. Abdominal ultrasound revealed increased free peritoneal fluid. Abdominocentesis yielded fluid with a low TNCC (340/µL) and total solids (<2 g/dL). However, the fluid creatinine (12.8 mg/dL) was more than double the serum creatinine (5.8 mg/dL) and contained calcium carbonate crystals, consistent with uroperitoneum. Initial cystoscopy identified a full-thickness tear on the caudoventral surface of the urinary bladder. Perineal urethrotomy was performed for further evaluation and attempted intervention, and repeat cystoscopy revealed a bony projection extending through the bladder defect. The patient's discomfort progressed further and, with the bony projection raising concern for potential pelvic trauma, humane euthanasia was pursued. On necropsy, approximately 7 L of pale-yellow fluid, consistent with urine, was present in the abdominal cavity, alongside a full-thickness tear of the urinary bladder. Directly underneath the defect, a 3 cm long x 0.8 cm wide irregular, pointed osseous outgrowth projected dorsally from the pubic symphysis. Histologically, this

outgrowth consisted of mature lamellar bone covered by periosteum, consistent with an exostosis. A second, smaller osseous outgrowth not associated with bladder injury was discovered and diagnosed as an osteochondroma due to the presence of cartilage. The final anatomic diagnosis was an acute urinary bladder perforation caused by the exostosis, with an incidental osteochondroma. We report the first known case of an exostosis rupturing the urinary bladder of an equine, a rare event previously documented only once with an osteochondroma.

Discipline: Both, **Topic:** General pathology, **Species:** Equine, **Systems:** Urinary

THE COLLABORATIVE CROSS MOUSE MODEL REVEALS GENETIC BASIS FOR SUSCEPTIBILITY AND RESISTANCE TO PRIMARY AMEBIC MENINGOENCEPHALITIS

Kayla Floyd, Joseph Dainis, Dennis Kyle
College of Veterinary Medicine, UGA, Athens, GA, USA

Background: *Naegleria fowleri (Nf)* is the causative agent of Primary Amebic Meningoencephalitis (PAM), a disease with a >97% mortality rate in infected individuals, with an average of five cases per year in the United States. Previous work in the lab showed that genetically diverse mice from the Collaborative Cross mouse model had differing survival outcomes post-*Nf* challenge as assessed by both survival time and detected amoebic burdens.
Objectives: To explore gross pathology and immune response variations between genetically distinct mice challenged with lowly and highly virulent *Nf* amoebae.
Methods: Seven mouse lines representing a gradient of previously phenotyped PAM survival outcomes were challenged with either V067 (5,000) or Villa Jose (500) parasites. Mice were euthanized according to approved AUP parameters based on symptomology (greater than 20% weight loss or neurological symptoms). Whole brains were formalin-fixed, paraffin-embedded, and H&E stained for pathological analysis via QuPath to compare their immune responses.
Results: Refractory mice (CC032, CC058) showed survival patterns suggesting improved outcomes in comparison to more susceptible mice (CC039, CC012, AJ). This is especially true for CC058, which showed improved survival outcomes with both lowly (V067) and highly (Villa Jose) virulent *Nf* isolates. Histopathology showed notable differences in neutrophil responses between refractory, susceptible, and control C57/Bl6J mice.
Conclusion: Genetically diverse mice had survival outcomes comparable to previous studies. Differences in neutrophil response may play a notable role in survival outcomes. Future studies will further characterize how these responses correlate with improved or reduced survival outcomes.

Discipline: Clinical pathology, **Topic:** Infectious disease, **Species:** Rodents, **Systems:** Nervous

FLAVIVIRAL OCULAR PATHOLOGY IN EXPERIMENTALLY INFECTED PASSERINE BIRDS

Jasmine Mackley, Angela Bosco-Lauth, Jennifer Buczek
Colorado State University College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO, USA

West Nile Virus (WNV), St. Louis encephalitis virus (SLEV), and Usutu Virus (USUV) are mosquito-borne flaviviruses that can cause neurologic disease and mortality in birds. Ocular lesions for naturally acquired WNV and USUV have previously been described in gallinaceous birds and raptors; however, there is a paucity of literature that describes flavivirus-associated ocular lesions in passerines. In two studies investigating infection kinetics of WNV, SLEV, and USUV in experimentally infected passerine birds, ocular pathology was evaluated in the birds that succumbed to either WNV or USUV. Four American robins (*Turdus migratorius*) were infected with USUV, and 16 house sparrows (*Passer domesticus*) were infected with WNV and/or SLEV in singular or heterologous infections. Observed pathology in both American robins and sparrows included mild to moderate lymphocytic pectenitis (2/4 robins, 50%; 8/16 sparrows, 50%), lymphocytic choroiditis (2/4 robins, 50%; 1/16 sparrows, 6.25%), and mild vacuolization of the optic nerve (1/4 robins, 25%; 1/16 sparrows, 6.25%). These findings generally mirror those seen in prior studies assessing ocular lesions among naturally infected birds, suggesting that experimental infection recapitulates natural pathogenesis; however, choroiditis was less commonly observed compared to the pre-existing literature. Additionally, moderate ulcerative or non-ulcerative lymphocytic conjunctivitis was found in 10% of birds but is likely unrelated to primary viral infection; rather, it was deemed likely secondary to chronic *Mycoplasma* spp. infection or an environmental irritant. Future studies could leverage different timepoints of infection to assess severity and lesional distribution over time, and immunohistochemical staining can be integrated to illustrate virus localization.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Avian, **Systems:** Eye/ear

A FORENSIC INVESTIGATION OF THE PANCREAS: HOW RELIABLE ARE ZYMOGEN GRANULES IN DECOMPOSED VERSUS STARVATION CASES.

Christian York, Ching Yun Hsu, Araceli Ordunez, Nanny Wenzlow
Texas Tech University, School of Veterinary Medicine

Introduction: In veterinary forensic pathology, starvation cases are overrepresented with 60% of causes of reported cruelty committed against dogs. Starvation is an extreme nutritional state resulting from long term insufficient intake of proteo-caloric food. Some cases will consist of bodies in variable stages of decomposition, and the pathognomonic histological findings of zymogen granule depletion in starvation cases can be compromised.

Objectives: The hypothesis of this study is that zymogen granules will become indistinct in canine pancreatic tissue with increasing postmortem intervals (PMI) ranging from 0 to 120 hours. The specific aims are to (1) identify the PMI at which zymogen granules become indistinct on H&E staining; and (2) identify potential special stains capable of identifying granules at later time points postmortem.

Methods: Pancreas from ten known-fed dogs, euthanized for reasons unrelated to this project, were collected postmortem at T0h, T4h, T8h, T24h, T48h, T72h, T96h and T120h preserved at room temperature. Routine FFPE 4um slides were stained with H&E, Masson Trichrome, Toluidine Blue and Periodic Acid-Schiff. Zymogen granule detectability and autolysis were assessed using a semi-quantitative scoring

system.

Results: Autolysis of pancreas was moderate at 24-48h and marked by 72h postmortem. Zymogen granules visibility decreased markedly by 48h postmortem and became indistinct by 72h on H&E, as well as all special stains.

Conclusion: Zymogen granules were indistinct in tissues decomposed for more than 72h. No special stain outperformed H&E. In decomposed tissues, the diagnosis of starvation cannot be made solely based on pancreas histopathology.

Discipline: Anatomic pathology, **Topic:** Forensic, **Species:** Canine, **Systems:** Endocrine

TUMORS IN PARADISE: A RETROSPECTIVE STUDY OF NEOPLASIA IN CARIBBEAN AFRICAN GREEN MONKEYS

Jaypal Bedi, Thaiza Savaris, Diana Bochynska, Matthew Valentine, Amy Beierschmitt, Pompei Bolfa, Candita Chapman, David Hilchie
Ross University School of Veterinary Medicine, Basseterre, Saint Kitts and Nevis

A retrospective histopathologic study was conducted to characterize spontaneous lesions in African green monkeys (*Chlorocebus sabaeus*) from two colonies in St. Kitts and Nevis between 2010 and 2026. Nineteen lesions were identified in 18 animals, with one animal diagnosed with two distinct lesions. Age information was available for 18 animals, with affected animals ranging from 9 to 33 years (mean, 19.4 years). Lesions occurred predominantly in aged animals (>15 years; 13/18, 72.2%), followed by young adults (4–8 years; 3/18, 16.7%) and adults (8–15 years; 2/18, 11.1%); no juveniles (0–4 years) were affected. Twelve affected animals (66.7%) were female and six (33.3%) were male. Colony history included 11 colony-born animals (57.9%), 7 wild-caught animals (36.8%), and 1 animal of unknown origin (5.3%).

Seventeen lesions were neoplasms, comprising 8 benign (47.1%), including papilloma, leiomyoma, pheochromocytoma, and ameloblastoma, and 9 malignant (52.9%), including squamous cell carcinoma, mammary gland carcinoma, cutaneous melanoma, histiocytic sarcoma, malignant pheochromocytoma, and microcystic meningioma; 2 lesions (10.5%) were diagnosed as calcinosis circumscripta. The integumentary system was the most frequently affected organ system (7/19, 36.8%), followed by the reproductive system (3/19, 15.8%), alimentary system (3/19, 15.8%), adrenal gland (2/19, 10.5%), respiratory system (2/19, 10.5%), musculoskeletal system (1/19, 5.3%), and central nervous system (1/19, 5.3%). These findings establish baseline data on the spectrum and distribution of spontaneous neoplastic and tumor-like lesions in captive African green monkeys and contribute to the comparative pathology literature for this species.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Non-rodent lab animals, **Systems:** Systemic

Clinical Pathology Focused Scientific Session I

Monday, 1:30 pm-5:00 pm
Lisa Schlein, DACVP (CP); Robert, DACVP (CP)

MALDI-TOF MS TO DETECT MELANOMA CIRCULATING TUMOR CELLS IN DOGS: A PROOF-OF-CONCEPT ANALYSIS

Fwu Shing Teng[1], Priscila Serpa[2], Samya Bowen[3], Alexandra Fox[4], Bettina Heid[4]
[1]School of Veterinary Medicine and Animal Science, São Paulo State University, Botucatu, Brazil, [2]Cornell University College of Veterinary Medicine, Ithaca, NY, USA, [3]Hampton University, Hampton, VA, USA, [4]Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, USA

Canine oral melanoma (COM) is the most prevalent oral neoplasm in dogs, characterized by high metastatic potential. Blood-based liquid biopsy offers a novel approach to detect systemic dissemination via circulating tumor cells (CTCs). While Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) Mass Spectrometry (MS) is well-established for differentiating microbiological samples, its use for other sample types remains underexplored in oncology practice. This pilot study aims to demonstrate the capability of MALDI-TOF MS to distinguish the mass spectra of canine white blood cells (WBC) from COM cell lines. Blood samples were obtained from three healthy dogs, and WBC were isolated. A biological triplicate of a COM cell line (TLM-1) was used to define the melanoma spectrum. Ten technical replicates per sample were analyzed to obtain an average spectrum for each sample. Exploratory analysis demonstrated a clear difference between the groups. Principal component analysis revealed high variance in PC1 (92.8%), and hierarchical clustering showed similar robust separation. In total, 192 peaks were detected. Statistical analysis using the *limma* method identified 137 peaks with differential intensities (log2 fold-change> 2, FDR < 0.01), of which 100 showed higher intensity in melanoma cells. The significant differences in mass spectra between COM cells and WBC provide a strong proof of concept for the use of MALDI-TOF MS in liquid biopsies. To advance this diagnostic tool, future studies will focus on establishing the assay's limit of detection using serially diluted, CTC-spiked blood samples and on developing machine learning algorithms to classify CTCs presence from proteomic fingerprint spectra.

Discipline: Clinical pathology, **Topic:** Oncology, **Species:** Canine, **Systems:** Gastrointestinal

MAST CELL TUMOR OF THE TONGUE IN A HORSE WITH LYMPHOVASCULAR INVASION

Sofía Dazarola, Krysten Schmidt, Caroline Gillespie-Harmon, Mario Sola, Camila Benaduce Emanuelli Mello
Purdue University College of Veterinary Medicine, West Lafayette, IN, USA

Background: Mast cell tumors (MCTs) are uncommon in horses and most frequently reported in the skin. Occurrences within the oral cavity, particularly those involving the tongue, are exceedingly rare and have been sparsely documented.

Case description: A 13-year-old Warmblood mare was presented to Purdue University Veterinary Hospital for evaluation of ulcerative lesions affecting the tongue and gingiva. Physical examination identified a 2.5 cm ulcer on the left tongue surrounded by fibrous, whitish tissue. Cytological imprints were highly cellular and composed predominantly of well-differentiated mast cells, with a moderate eosinophilic infiltrate, consistent with mast cell tumor (MCT). Histologically, one fragment from the ulcer was composed of sheets of neoplastic round cells with

distinct borders, a moderate amount of amphophilic cytoplasm, round to reniform nuclei, stippled chromatin, and one faint nucleolus. Moderate anisocytosis and anisokaryosis, and 5 mitotic figures in ten 400x HPF (2.37 mm^2) were observed. Rows of similar neoplastic round cells within the muscular connective tissue and lymphovascular invasion were also identified in the remaining fragments. Neoplastic cells stained positively with Toluidine blue and Giemsa, confirming MCT. Previously reported equine lingual MCTs have demonstrated benign biological behavior. In the present case, however, lymphovascular invasion suggested malignant potential. Interesting, although complete surgical excision was not achieved, aggressive debridement of the ulcerated lesion resulted in healing, with no gross evidence of recurrence 12 months after diagnosis. The relationship between the gingival lesions and the lingual MCT could not be determined.

Discipline: Clinical pathology, **Topic:** Oncology, **Species:** Equine, **Systems:** Gastrointestinal

PROGNOSTICATION OF CANINE CUTANEOUS MAST CELL TUMORS VIA FLOW CYTOMETRIC QUANTIFICATION OF KI-67

BinXi Wu[1], Deborah Molehin[1], Sylvia Ferguson[1], Benjamin Hulsey[1], Paula Bartolome[1], Jared Jaffey[1], Amir Kol[2], Fulvio Riondato[3]
[1]Midwestern University, Glendale, AZ, USA, [2]UC Davis School of Veterinary Medicine, Davis, CA, USA, [3]University of Turin, Turin, Italy

Background

Canine cutaneous mast cell tumor (MCT) is a common neoplastic disease that has a wide range of biologic behavior. Prediction of its behavior is mainly based on histopathologic grading, which is not commonly done prior to surgical treatment. Therefore, histopathologic grading has limited utility in guiding the surgical management of this disease. Flow cytometry, on the other hand, is a well-established method that can utilize pre-operative fine needle aspirate (FNA) specimen, which may help guide surgical treatment. The specific aim of this study is to determine if flow cytometric quantification of mast cells Ki-67 expression (Ki-67%) in FNA samples of canine cutaneous MCT correlates with the cytologic and histologic grade.

Methods

Flow cytometric analysis was performed on cytologically confirmed MCT samples. Ki-67% was determined on viable, CD117+, CD11b weak cells, and compared to the patient's Camus cytologic grade and 2-tiered (Kiupel) histologic grade.

Results

11 cases met the enrollment criteria. Two cases were excluded because either the cell recovery was low or the neoplastic cells did not express CD117. The Ki-67% of the high-grade cutaneous MCT (n=4) are 9.92%, 19.3%, 21.3%, and 27.5% with a mean of 19.51% [CI 4.95-34.07] and the Ki-67% of the low-grade cutaneous MCT (n=5) are 3.82%, 9.71%, 8.96%, 11.4%, and 15.9% with a mean of 9.95% [CI 1.24-18.68].

Conclusion

Flow cytometric assessment of canine cutaneous MCT Ki-67% is a promising modality to predict the tumor grade. However, more cases are still needed and enrollment is ongoing.

Discipline: Clinical pathology, **Topic:** Oncology, **Species:** Canine, **Systems:** Integument

CIRCULATING TUMOR DNA CAPTURES A BROADER MUTATIONAL SPECTRUM AND CORE ONCOGENIC SIGNALING IN CANINE B-CELL LYMPHOMA COMPARED TO SINGLE-SITE TUMOR BIOPSIES

Priscila Serpa[1], Javad Omidi[1], Sai Navya Vadlamudi[2], Kevin Lahmers[2], Shawna Klahn[3]
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This study presents a genome-wide characterization of matched plasma-derived cell-free DNA (cfDNA) and tumor DNA from five dogs with B-cell lymphoma using whole-genome sequencing. The analysis revealed substantial inter-sample heterogeneity in mutational burden and compartment-specific alterations. Of all mutated genes, 174 were shared between compartments (103 annotated, the remainder LOC loci), alongside 446 plasma-restricted and 2,267 tumor-restricted genes. Key recurrently mutated genes identified across both compartments included MUC6, NIP7, and EPPK1, which were not previously characterized in canine lymphoma, as well as LINS1, AHNAK, RAI1, and immunoglobulin-related loci (LOC100683065, LOC106559118). Additionally, known canonical lymphoma drivers, including MYC, POT1, and TRAF3, were detected in selected higher-burden cases. Variant-level concordance between plasma and tumor remained limited (Jaccard similarity 0.37–7.78%), with plasma samples frequently exhibiting higher mutation burdens, capturing alterations not detected in matched tumor samples. Genome-wide profiling identified 712 copy-number events (426 gains, 286 losses), with amplifications predominantly in cfDNA and deletions enriched in tumor tissue, including highly recurrent events on chromosomes 8, 13, and 17. Despite limited sequence-level overlap, both compartments converged on shared functional pathways, including PI3K-Akt signaling, Pathways in cancer, JAK-STAT signaling, Signal Transduction, Signaling by Receptor Tyrosine Kinases, and Rho GTPase signaling. These findings demonstrate that cfDNA provides a complementary, biologically meaningful representation of the tumor genomic landscape, capturing recurrent gene alterations and functional programs while reflecting broader, partially distinct tumor heterogeneity than single-site tissue sampling. Together, these results position liquid biopsy as a complement to (not a replacement for) tissue profiling in canine lymphoma.

Discipline: Clinical pathology, **Topic:** Oncology, **Species:** Canine, **Systems:** Hematopoietic

SPATIAL TRANSCRIPTOMIC ANALYSIS OF LOW-GRADE INTESTINAL T-CELL LYMPHOMA AND LYMPHOPLASMACYTIC INFLAMMATORY BOWEL DISEASE IN CATS

Laura Victoria Quishpe Contreras, Priscila Serpa
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Background: Low-Grade Intestinal T-Cell Lymphoma (LGITL) and Inflammatory Bowel Disease (IBD) are relevant entities in feline medicine with overlapping clinical, imaging, cytological, and even histopathological features. Unraveling molecular differences at the transcriptional level can bring relevant information to better understand both entities' physiopathology and identify candidate genes that allow for more accurate differentiation.

Objectives: To identify transcriptional differences in formalin-fixed paraffin-embedded (FFPE) samples of cats with LGITL or IBD using Spatial transcriptomics.

Methods: Jejunum and ileum FFPE samples from cats with histological diagnoses of LGITL and IBD were retrospectively evaluated; samples with PARR results in concordance with the histological diagnosis were subsequently selected. Three ileum samples of cats without intestinal diseases were collected as controls. Spatial transcriptomics was performed using the platform GeoMx (Bruker) and a commercial canine panel (Canine Cancer Atlas). Gene expression of CD3-positive and CD20-positive cells was assessed and compared between disease groups, and between tissue areas: superficial mucosa (SM), deep mucosa/submucosa (DM), and Peyer patches (PP) on ileum.

Results: Twelve samples (6 LGITL, 6 IBD) were analyzed. A total of 26 genes were differently expressed between T-cells of LGITL and IBD groups. Genes included PIK3CD, CLDN7, and BCL2. Variable gene expression between tissue areas was present, examples including APOA1 and ID1 on IBD (SM vs DM, CD3-cells) or increased TXNIP and CFD on LGITL (DM vs PP, CD20-cells).

Conclusion: Spatial transcriptomics can be applied to cat samples and allowed to identify differently expressed genes in LGITL and IBD, paving the way to identify potential biomarkers.

Discipline: Clinical pathology, **Topic:** Oncology, **Species:** Feline, **Systems:** Gastrointestinal

PROOF-OF-CONCEPT STUDY OF MICRORNA EXPRESSION IN INTESTINAL SAMPLES FROM CATS WITH LOW-GRADE INTESTINAL LYMPHOMA AND LYMPHOPLASMACYTIC INFLAMMATORY BOWEL DISEASE

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Background: Low-grade intestinal T-cell lymphoma (LGITL) and lymphoplasmacytic inflammatory bowel disease (IBD) present a significant challenge in veterinary clinical practice, as both share overlapping findings in clinical presentation, routine laboratory and imaging exams. Differences in microRNA (miRNA) expression may help identify minimally invasive biomarkers to distinguish IBD from LGITL.

Objectives: To identify differentially expressed miRNAs on formalin-fixed paraffin-embedded (FFPE) samples of cats with LGITL, IBD, and without intestinal disease.

Methods: Jejunum and ileum FFPE samples from cats with histological diagnosis of LGITL (n = 6) and IBD (n = 6), and PARR results in concordance with the histological diagnosis were chosen. Three ileum samples from cats without intestinal diseases were prospectively collected as controls. RNA was sequenced and, given the absence of a cat miRNA database, annotations were performed using canine (cfa), human (hsa), and murine (mmu) miRNA databases. MiRNAs were ranked by integrating the magnitude of difference between groups, p-value, overall expression level, and detection across samples.

Results: Of the 1,928 miRNAs detected, 477 were consistently detected in at least three samples. When comparing IBD with LGITL, highly expressed miRNA in IBD included NovelmiRNA-1671 and cfa-miR-361, the latter being downregulated in lymphoma in other species. Other miRNAs, such as cfa-miR-150, cfa-miR-363, and NovelmiRNA-1287, showed higher expression in LGITL. Interestingly, cfa-miR-150 has been found downregulated in aggressive B-and T-cell lymphoma, but studies on indolent lymphomas are lacking.

Conclusion: Candidate miRNAs differentially expressed between IBD and LGITL were identified and will be validated for further investigation as potential diagnostic biomarkers.

Discipline: Clinical pathology, **Topic:** Oncology, **Species:** Feline, **Systems:** Gastrointestinal

ACCURACY OF FINE NEEDLE ASPIRATION CYTOLOGY FOR DETECTION OF METASTATIC DISEASE IN 189 CERVICAL LYMPH NODES FROM DOGS WITH ORAL TUMORS

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Background: Evaluation of cervical lymph nodes for metastatic disease is a crucial component of staging dogs with oral tumors. Fine needle aspiration cytology (FNAC) is often utilized as it can potentially negate the need for nodal dissection for histopathologic staging. However, the true predictive value of cytology for staging oral neoplasia in dogs has not been well characterized.

Objective: Determine the accuracy of FNAC for detecting metastatic disease and other pathologic features in cervical lymph nodes in dogs with oral tumors.

Methods: This prospective study included 189 lymph nodes samples from 36 dogs with primary neoplasms of the oral cavity. Fine needle aspiration was performed *ex vivo* immediately following lymph node dissection; lymph nodes were also evaluated histologically. Sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) were calculated for a cytologic diagnosis of metastasis and for other relevant cytologic features of lymph nodes, using histopathology as gold standard.

Results: Corresponding cytology and histopathology results were available for 189 lymph nodes. Sensitivity, specificity, NPV, and PPV for a cytologic diagnosis of metastatic disease were 32%, 100%, 92%, and 100%, respectively. Cytology had good accuracy for detecting hemosiderin and necrosis, but there were high proportions of false positives for other nodal pathologic features including lymphoid reactivity and inflammation.

Conclusions: FNAC of cervical lymph nodes had very poor sensitivity but good specificity for detecting metastatic disease in dogs with primary oral tumors. Results suggest that histopathologic evaluation is necessary for accurate determination of metastatic status in clinically negative nodes.

Discipline: Clinical pathology, **Topic:** Oncology, **Species:** Canine, **Systems:** Systemic

RETROSPECTIVE ASSESSMENT OF LYMPHOCYTE SUBTYPE POPULATIONS IN FELINE LYMPHOCYTE-RICH EFFUSIONS AND CHALLENGES ASSOCIATED WITH EFFUSION IMMUNOCYTOCHEMISTRY

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Background:

Management of feline lymphocyte-rich effusions (LREs), including chylous effusions, can entail repeated centesis, which may negatively impact the patient's nutritional and immune status. Human literature reports a predominance of T-cells in chylous effusions, with selective loss of CD4+ lymphocytes in chyle compared to peripheral blood. A single analysis of feline chylous effusion was T-cell predominant and in proportion to peripheral blood.

Objective:

To retrospectively characterize lymphocyte subsets in feline LREs using immunocytochemistry.

Methods:

Four antibodies were validated for use in previously stained and coverslipped cytology samples. CD3, CD4, CD8, and CD20 antibodies were utilized to determine the proportions of T cells, T subtypes (T helper cells and cytotoxic T cells) and B cells, respectively, in archived cytospin preparations of feline LREs prepared with a tissue transfer technique.

Results:

40 samples were stained, however, 13 were eliminated due to problems with tissue transfer. There was no CD3 immunoreactivity in 44% of the remaining 27 samples, and convincing immunoreactivity for CD4 and CD8 was only observed in 7.4% of samples. The percentage of CD20+ lymphocytes ranged from 0-95%. B lymphocytes were the predominant cell type in 85% of samples.

Conclusions:

The inherent characteristics of biological fluids, along with preanalytical factors that could not be controlled for and analytical limitations, likely contributed to the erroneous results. This emphasizes the importance of interpreting immunocytochemistry with knowledge of context and understanding of specimen characteristics. Retrospective immunocytochemistry of feline LREs is not recommended.

Discipline: Clinical pathology, **Topic:** General pathology, **Species:** Feline, **Systems:** Systemic

Diagnostic Pathology Focused Scientific Session II

Monday, 8:00 am-12:00 pm
Pedro Pinczowski, DACVP (AP); Neha Mishra, MS MVSc PhD DACVP (AP)

EXTRACRANIAL ORBITAL MENINGIOMA WITH OSTEOCARTILAGINOUS METAPLASIA

Juan Pablo Velasco Montes de Oca[1], Miguel Ortega Guerra[1], Jose Manuel Guzman Gudiño[2]
[1]Diagnóstico Veterinario Del Valle, CDMX, Mexico, [2]CVE 24hrs, CDMX, Mexico

A 6-year-old mixed-breed dog presented for ophthalmologic evaluation. Findings included corneal opacity, corneal neovascularization, vision loss, increased intraocular pressure, and retinal detachment. Ultrasonography identified a periorbital proliferative mass, and exenteration was subsequently performed for biopsy.

Gross examination revealed a poorly defined, homogeneous retrobulbar mass with invasion of the optic nerve. Microscopic examination demonstrated an infiltrative, unencapsulated, densely cellular neoplasm composed of whorls and short streams of polygonal to spindle-shaped cells with moderate amounts of cytoplasm and oval nuclei containing bar-shaped chromatin and occasional pseudoinclusions. Anisocytosis and anisokaryosis were marked. Five mitotic figures were counted in ten high-power fields (40×; adjusted to 2.37 mm²). Islands of fibrocartilaginous matrix were observed and highlighted by Masson's trichrome stain. More than 90% of neoplastic cells exhibited positive immunoreactivity for vimentin, whereas osteonectin, CKAE1/AE3, Melan-A, S100, and EMA were negative.

In dogs, meningiomas are reported most frequently in Golden Retrievers, German Shepherds, Terriers, Collies, and Boxers, without an apparent sex predisposition. Orbital meningiomas characteristically exhibit a conical growth pattern arising from the optic nerve and/or intraorbital meningothelial cells, extending into the orbit and/or through the orbital foramen. Similar to their intracranial counterparts, they may exhibit diverse histologic patterns and, rarely, areas of myxomatous or osteocartilaginous metaplasia. Immunohistochemically, they are typically positive for vimentin and variably positive for cytokeratins, epithelial membrane antigen (EMA), S100 protein, and neuron-specific enolase (NSE).

In the present case, no metastases were detected during postoperative follow-up.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Canine, **Systems:** Eye/ear

KNUFIA EPIDERMIDIS–ASSOCIATED ULCERATIVE DERMATITIS IN A VEILED CHAMELEON (CHAMAELEO CALYPTRATUS)

Ladislav Novotny[1], Barbora Kopecka[2], Jan Misik[1], Steven Tillis[3], Martin Kveton[4], Robert Ossiboff[3]
[1]St. George's University, True Blue, Grenada, [2]Veterinary University Brno, Brno, Czech Republic, [3]University of Florida College of Veterinary Medicine, Gainesville, FL, USA, [4]Charles University, Prague, Czech Republic

Background: Melanized fungi are increasingly recognized as important pathogens of reptiles; however, *Knufia epidermidis* has not previously been reported as a cause of disease in reptiles.

Objective: To describe the clinicopathologic features, histopathology, and molecular diagnosis of *K. epidermidis* infection in a veiled chameleon.

Methods: A 2.5-year-old male veiled chameleon developed progressive multifocal ulcerative dermatitis refractory to topical and systemic treatment. Skin biopsies and postmortem tissues were examined histologically. Pan-fungal PCR targeting the ITS2 region followed by Oxford Nanopore sequencing was performed on ethanol-fixed skin.

Results: Histopathology revealed severe necrotizing and ulcerative dermatitis containing abundant pigmented septate fungal hyphae and sporangium-like structures, consistent with phaeohyphomycosis. Molecular analysis identified *Knufia epidermidis* with 98.3–100% sequence identity to reference isolates. Despite systemic voriconazole, pradofloxacin, and topical antiseptic therapy, the chameleon deteriorated and was euthanized. Necropsy demonstrated bacterial necrotizing myocarditis and hepatitis consistent with septicemia, but no evidence of fungal dissemination, indicating death was most likely due to secondary bacterial sepsis following disruption of the cutaneous barrier.

Conclusions: This represents the first confirmed case of *Knufia epidermidis* infection in a reptile and the first report of phaeohyphomycosis caused by this fungus in a chameleon. The case expands the spectrum of fungal pathogens causing reptile dermatomycosis, emphasizes the importance of molecular diagnostics for accurate fungal identification, and highlights secondary bacterial septicemia as a potentially fatal complication of severe cutaneous mycoses.

Declaration: The content of this abstract is based on a forthcoming manuscript that is currently in preparation for publication.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Wildlife/Zoo, **Systems:** Integument

PREVALENCE OF LISTERIOSIS, PESTE DES PETITS RUMINANTS, AND FOOT-AND-MOUTH DISEASE IN GOATS AND SHEEP OF NOWSHERA AND SWABI DISTRICTS, PAKISTAN

Luqman Alam

The Agriculture university of Peshawar, Nowshera, Pakistan

Background:

Small-ruminant production plays a vital role in the livestock sector of Pakistan. Infectious diseases, including listeriosis, Peste des Petits Ruminants (PPR), and Foot-and-Mouth Disease (FMD), continue to adversely affect animal health and productivity.

Objective:

To determine the prevalence of listeriosis, PPR, and FMD in goats and sheep from Nowshera and Swabi districts, Pakistan.

Methods: A cross-sectional study was conducted from November 2025 to February 2026. The study population consisted of 2,650 goats and 1,540 sheep. A total of 1,400 samples were collected from clinically suspected animals. Clinical examinations and laboratory investigations were performed at the Civil Veterinary Hospital Laboratory. Data were analyzed to determine disease prevalence and distribution.

Results:

Of the 1,400 samples examined, 421 (30.1%) were positive for PPR, 319 (22.8%) for FMD, and 240 (17.1%) for listeriosis. PPR was the most prevalent disease, followed by FMD and listeriosis. Affected animals exhibited characteristic clinical signs, including respiratory and gastrointestinal involvement in PPR, vesicular lesions in FMD, and neurological manifestations in listeriosis. The findings demonstrate a substantial burden of infectious diseases among small ruminants in the study region.

Conclusions:

Listeriosis, PPR, and FMD remain significant health challenges in goats and sheep of Nowshera and Swabi districts. Strengthened disease surveillance, improved vaccination programs, and enhanced biosecurity practices are recommended to reduce disease burden and improve livestock productivity

Discipline: Both, **Topic:** Infectious disease, **Species:** Bovine, **Systems:** Respiratory

NECROTIZING BACTERIAL PNEUMONIA IN A SALMON JUNGLE BOA ASSOCIATED WITH A MIXED INFECTION OF STENOTROPHOMONAS MALTOPHILIA AND BOWDENIELLA/ACTINOMYCES NASICOLA

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Bacterial pneumonia is not uncommon in snakes and is usually attributed to Gram-negative organisms, such as *Pseudomonas* and *Aeromonas*. We describe a Salmon jungle boa with severe caseating pneumonia that was associated with a mixed bacterial population including a Gram-positive filamentous, nonacid-fast organism consistent with an actinomycete. Culture of lung tissue yielded heavy growth of a Gram-negative rod identified as *Stenotrophomonas maltophilia (*formerly *Pseudomonas maltophilia)* by 16S gene sequencing. In addition, a more fastidious Gram-positive branching bacillus was also isolated and identified by sequencing as *Bowdeniella/Actinomyces nasicola*. *S. maltophilia* is part of the normal oral microbiota in snakes but is recognized as an opportunistic pathogen associated with a variety of infections in snakes although not well documented. To our knowledge, this is the first report of *B. nasicola* associated pneumonia in snakes. This boa was also PCR positive for reptarenavirus and viral associated immunosuppression may have been a contributory factor in the development of this mixed bacterial pneumonia. Recently, oral, cloacal and hemipenal actinomycosis due to an unclassified *Actinomyces* has been reported in ball pythons associated with granulomatous lesions. While the lesion in this boa differs in morphology, these reports raise the question whether actinomycetes may represent emerging pathogens in snakes. Actinomycosis is typically a polymicrobial infection, and the presence of heavy growth of *S. maltophilia* may have obscured detection of the more fastidious actinomyces on initial culture. This underscores the importance of correlating pathologic findings with microbiologic techniques to facilitate recovery and identification of fastidious organisms such as actinomycetes.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Wildlife/Zoo, **Systems:** Respiratory

CUTANEOUS PHAEOHYPHOMYCOSIS IN HORSES

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Background:

Phaeohyphomycosis is an infection caused by opportunistic melanin-pigmented fungi. Morphologically, they consist of variably branching septate hyphae, often in a toruloid arrangement, or thick-walled sclerotic bodies.

Objective:

Although cutaneous phaeohyphomycosis has been reported in horses, this characterizes the lesions and the fungal species involved in equine cutaneous phaeohyphomycosis in a larger series of cases.

Methods:

The databases of three institutions were searched for cases of equine cutaneous phaeohyphomycosis. H&E and GMS stains were examined in each case. DNA was extracted from paraffin-embedded tissues and the fungal ITS and LSU conserved regions were amplified using PCR. The sequenced products were compared to the NCBI BLAST database.

Results:
Thirty-four cases were selected. Of 26 cases that had an anatomic location, nodules involved the face and neck (12/26), limbs (6/26), abdomen (4/26) thorax (3/26), and scrotum (1/26). Lesions were dermal nodules composed of pyogranulomatous (19/34 cases) and lymphocytic inflammation (15/34). Infrequently, the fungi formed aggregates consistent with eumycotic mycetoma (5/24). Speciation was achieved in 20 cases. *Curvularia* spp. was most common (13/20), including 3 cases of *Curvularia spicifera*, a case of *Curvularia hawaiiensis*, 5 cases in which the species was not determined, and 4 cases that matched to both *Curvularia* and *Bipolaris*. *Cladosporium* sp. was detected in 4 cases; and *Nigrograna mackionii*, *Alternaria* sp., and *Epicoccum* sp. were detected in individual cases. The fungi were indistinguishable based on histomorphology.

Conclusions:
Speciation through molecular analysis is needed for accurate identification of the fungi involved in equine cutaneous phaeohyphomycosis.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Equine, **Systems:** Integument

EFFECTS OF PLASTIC INGESTION IN PENGUINS STRANDED ALONG THE CHILEAN COAST

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Background: Between 2024 and 2026, 18 necropsies were performed on Humboldt (*Spheniscus humboldti*) and Magellanic (*Spheniscus magellanicus*) penguins at LiCiVet, Universidad del Alba, Chile, to investigate the presence of plastic debris and its association with mortality.

Methods: Nine Humboldt and nine Magellanic penguins that stranded alive or dead along the Chilean coast were examined. Live-stranded individuals were initially admitted to the Humboldt Conservation Center and, following death and authorization by SERNAPESCA, underwent necropsy. Plastic debris was identified, classified, measured, and evaluated for its potential contribution to death. Gastrointestinal areas with adhered plastic were sampled for histopathological examination.

Results: Plastic debris was detected in four penguins, including three Magellanic and one Humboldt penguin. The largest fragment measured 32.97 × 4.73 × 0.1 cm. In one Magellanic penguin, plastic intertwined with feathers and fishing line completely obstructed the cloaca, causing edema and mucosal thickening. Another case represented an incidental finding without associated lesions. In the Humboldt penguin, a rigid plastic fragment adhered to the gastrointestinal mucosa, resulting in ulceration and mucosal thickening. Histologically, lesions were characterized by a foreign-body reaction with lymphoplasmacytic inflammation, macrophages,

multinucleated giant cells, and fibroplasia, consistent with chronic inflammation and tissue repair.

Conclusions: Plastic debris can occur in the digestive tract of Humboldt and Magellanic penguins and may cause obstruction or mucosal injury associated with chronic inflammatory responses. The true frequency of plastic ingestion is likely underestimated due to regurgitation or fecal elimination. Further studies on microplastics and standardized criteria for assessing their pathological significance are needed.

Discipline: Anatomic pathology, **Topic:** Comparative pathology, **Species:** Wildlife/Zoo, **Systems:** Gastrointestinal

ENHANCING ONE HEALTH SURVEILLANCE: HARMONIZING MILITARY WORKING DOG DIAGNOSTIC TERMINOLOGY WITH NONCLINICAL AND HUMAN CLINICAL CODING SYSTEMS

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Background/**Objective:** Veterinary anatomic pathology morphologic diagnoses are not inherently interoperable with human clinical coding systems, limiting the ability to integrate animal health data into broader comparative or translational research frameworks. As military working dogs (MWDs) share environments and potential exposures with service members, they can serve as valuable sentinels for health risks. Aligning their health data with human systems is a critical step toward a "One Health" approach to surveillance for both populations. We evaluated the feasibility of mapping routine diagnostic findings from MWDs to human medical terminologies (SNOMED-CT, NCIt, and ICD-10) and INHAND.

Methods: Each MWD diagnosis was reviewed for the presence of an equivalent or hierarchical parent concept within SNOMED-CT, NCIt, ICD-10, and INHAND.

Results: We assessed 2,922 MWD pathology records, including >23,000 post-mortem and >5,900 biopsy diagnoses. Over 90% of veterinary pathology diagnoses had a directly applicable or hierarchically appropriate SNOMED-CT term, demonstrating substantial cross species terminological compatibility. Lower agreement was observed with other systems. We identified challenges in areas where veterinary specific entities lacked human analogs, where lesion granularity differed between systems, or where anatomic or etiologic descriptors diverged.

Conclusion: This work provides a practical framework for veterinary diagnosticians seeking to align pathology data with human clinical coding systems. Improved terminological harmonization will enhance the utility of veterinary datasets—particularly those from high value populations such as MWDs—for comparative pathology, translational research, and integrated health surveillance. This enhanced data integration can serve as an early warning for environmental health threats to military service members.

Discipline: Anatomic pathology, **Topic:** General pathology, **Species:** Canine, **Systems:** Systemic

DENTAL PATHOLOGY ASSOCIATED WITH ELEVATED GROUNDWATER FLUORIDE CONCENTRATIONS IN SEEDSTOCK ABERDEEN ANGUS BULLS IN ARGENTINA

Santiago Diab[1], Germán Cantón[2], Ignacio Álvarez[3], Federico Giannitti[4], Jorge García[5]
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Background: Dental fluorosis results from chronic ingestion of excessive fluoride during tooth development, leading to enamel hypomineralization and premature dental wear. We describe an outbreak of dental fluorosis in seedstock Angus bulls associated with elevated fluoride concentrations in drinking water.

Case description: Thirty-five Angus bulls purchased from a seedstock operation in central Argentina, at 2–2.5 years of age, were found one year later to have severe premature dental wear, variably associated with misshapen and/or malaligned incisors, tan to brown mottling, and tooth cracking. Because permanent teeth develop between 6 and 36 months of age, fluorosis was suspected based on the characteristic dental lesions. No skeletal abnormalities were reported, and the bulls were otherwise apparently healthy.

Results: In a water chemistry analysis, fluoride concentrations in the three wells used during tooth development were 2.7, 3.9, and 8.0 ppm; the latter was used for 8 months during this susceptible period. Recommended drinking-water fluoride concentrations for growing cattle are 2.5–4 ppm, although some reports suggest chronic exposure to concentrations exceeding 2 ppm may be toxic.

Conclusions: These findings support a diagnosis of dental fluorosis based on the epidemiology tooth pathology and elevated concentrations of fluoride in groundwater in this cohort of seedstock bulls. This investigation highlights the importance of thorough dental examination during prepurchase and prebreeding soundness evaluations, including fluorosis in the differential diagnosis of premature dental wear in young cattle, and routine water-quality testing in regions with elevated groundwater fluoride to prevent substantial economic losses.

Discipline: Anatomic pathology, **Topic:** Toxicology, **Species:** Bovine, **Systems:** Other (Oral or tooth pathology)

PHALARIS ANGUSTA INTOXICATION IN RUMINANTS FROM CENTRAL ARGENTINA: A RETROSPECTIVE CASE SERIES

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Background: *Phalaris angusta* (narrow canarygrass) is an annual grass native to South America that causes neurological disease in herbivores due to the production of tryptamine alkaloids. Subacute to chronic neurological signs can develop weeks or months after cattle are removed from the toxic pasture.

Objective: To describe 17 fatal outbreaks of natural *P. angusta* intoxication in cattle and sheep in central Argentina.

Methods: Epidemiological, clinical, and pathological information from 17 cases of *P. angusta*- poisoning diagnosed in 2005-2024 in Buenos Aires and La Pampa provinces were retrospectively analyzed.

Results: Cases occurred in cattle (n=15) and sheep (n=2), mainly during spring (n=11), followed by winter (n=4), fall (n=1), and summer (n=1). Mean morbidity (16 outbreaks), and mortality and lethality (11 outbreaks) risks were 10.2% (range 1.1-96.6%), 4.0% (range 0.7-47.1%), and 47.3% (range 13.5-100%), respectively. Affected animals grazed native grasslands with abundant *P. angusta*. Clinical disease was characterized by subacute and/or chronic neurologic signs. Gross lesions included greenish-gray pigmentation of the brainstem (n=9) and/or kidneys (n=5) and traumatic hematomas in the chest and submandibular area secondary to falls caused by severe ataxia (n=5). Histologically, granular golden-brown pigment accumulated within neurons of the brainstem and renal tubular epithelial cells.

Conclusions: Epidemiological, clinical, and pathological findings were consistent with *P. angusta* poisoning. This is a sporadic neurotoxic disease in grazing ruminants of central Argentina has a clear winter-spring seasonal pattern and can have devastating effects on affected herds. Currently, there is no way to predict when pastures containing *P. angusta* will become toxic.

Discipline: Anatomic pathology, **Topic:** Toxicology, **Species:** Bovine, **Systems:** Nervous

A QUANTITATIVE ANALYSIS OF EFFICIENCY AND ACCURACY GAINS IN AI-ASSISTED VS. MANUAL CANINE MAST CELL TUMOR GRADING

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Background:
Mast Cell Tumours (MCTs) are among the most common malignant cutaneous neoplasms in dogs and exhibit a wide spectrum of clinical behaviour. Accurate tumour grading is essential for prognosis and treatment planning. In the context of increasing diagnostic workloads in veterinary pathology, manual grading can be time-intensive and may be subject to inter observer variability. This study introduces a two-stage artificial intelligence (AI) framework to assist pathologists, aiming to

improve diagnostic efficiency, reduce turnaround time (TAT), and standardize tumour grading.

Materials and **Methods:**
A vision transformer-based deep learning system was developed using multi-source data from five independent cohorts, comprising 700 whole-slide images (WSIs) for MCT vs. non-MCT classification and 252 WSIs for grading. To assess operational utility, a concordance study was performed involving three veterinary pathologists. Cases were independently graded in two phases: manual and AI-assisted grading, enabling intra-observer comparison of diagnostic accuracy, and turnaround time.

Results:
The concordance study demonstrated that integration of the AI framework improved agreement with the consensus reference standard to 97.6% (Cohen's κ = 0.95), compared with 88.9% (Cohen's κ = 0.78) using manual grading alone. The greatest benefit was observed in complex and borderline cases, where the system reduced TAT and supported more consistent decision-making by streamlining hotspot selection and reducing subjective uncertainty.

Conclusion:
Integration of this AI framework into veterinary pathology workflows enhances tumour grading efficiency and consistency, while reducing TAT. The system provides targeted decision support during challenging cases and demonstrates potential to improve diagnostic throughput in high-volume laboratory settings.

Discipline: Clinical pathology, **Topic:** Oncology, **Species:** Canine, **Systems:** Integument

RECTAL MUCOSAL MACROPHAGE DIFFERENTIATION AND DISTRIBUTION IN EQUINE INFLAMMATORY BOWEL DISEASE (IBD)

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Background – Despite its significant clinical and economic impact, the immunopathology of IBD remains poorly understood, with macrophages potentially driving disease through pro- (M1) and anti-inflammatory (M2) differentiation.

Objective – To characterize rectal macrophage populations in horses with IBD by comparing density and distribution of pan-macrophages (Iba-1$^+$) and M2 macrophages (CD204$^+$) with those of horses without IBD.

Methods – Twenty-nine horses with chronic colic (19 with IBD and 10 without IBD), admitted to the Equine Veterinary Teaching Hospital between January 2022 and April 2024, underwent rectal biopsies. Endoscopy-guided rectal biopsies were collected under sedation, fixed in 10% formalin, paraffin-embedded, and sectioned for Hematoxylin–Eosin–Phloxine–Saffron (HEPS) staining. Histopathology was performed blinded by board-certified anatomic pathologists. Macrophages positive on Iba-1 and CD204 immunohistochemistry were quantified histomorphometrically in the apical-basal-subcryptal lamina propria, and in the epithelium. Intra-observer

reproducibility was assessed using an intraclass correlation coefficient, and macrophage densities were compared between groups using Mann–Whitney U tests.

Results – Horses with IBD had a significantly higher median density of Iba-1$^+$ macrophages in the basal area of lamina propria compared with horses without IBD (respectively 37 and 20 pan-macrophages/10,000 µm²; p = 0.02). Although not significant, IBD horses showed increased numbers of Iba-1 positive macrophages in the apical and sub-cryptal areas.

Conclusions – Horses with IBD have increased rectal mucosal macrophage densities with variable distribution within the lamina propria, suggesting a role in disease pathogenesis and the potential to improve the accuracy of histological diagnosis.

Discipline: Anatomic pathology, **Topic:** Immunology, **Species:** Equine, **Systems:** Gastrointestinal

A RETROSPECTIVE STUDY OF TONSILLAR LESIONS IN 128 DOGS (2010-2024)

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Tonsillar biopsies are a relatively uncommon type of sample submission in our surgical biopsy service. Our objective of this study was to characterize the frequency and types of tonsillar lesions in dogs. A retrospective search was performed at the Athens Veterinary Diagnostic Laboratory (AVDL) biopsy archives for tonsillar lesions in dogs between 2010 and 2024. A total of 128 biopsies were retrieved. Diagnoses included non-neoplastic lesions (77 cases or 60% of all biopsies), which consisted of inflammatory lesions (8 cases or 10.4% of non-neoplastic lesions), tumor-like proliferative lesions (67 cases or 87% of non-neoplastic lesions), and anatomic anomalies (2 cases or 2.6% of non-neoplastic lesions). The most common neoplasms were epithelial neoplasms (37 cases or 63.8% of neoplastic lesions), with a high prevalence of squamous cell carcinoma (31 cases or 83.7% of epithelial neoplasms). The second most common neoplasms were round cell neoplasms (11 cases or 19% of neoplastic lesions) with a high prevalence of lymphoma (9 cases or 81.8% of round cell neoplasms). The average age of affected dogs was 9.3 years with a slightly higher percentage (58%) of males. Mixed breed dogs, Cocker Spaniels, and Terriers were the most reported breeds. Our study highlights the high frequency of neoplasms, particularly squamous cell carcinomas, in tonsillar biopsies of dogs submitted to our surgical biopsy service.

Discipline: Anatomic pathology, **Topic:** General pathology, **Species:** Canine, **Systems:** Other (Immune system)

CHARACTERIZATION OF SYNOVIAL AMYLOIDOSIS IN A KOMODO DRAGON (VARANUS KOMODOENSIS) USING ULTRASTRUCTURAL, BIOPHYSICAL, AND IMMUNOCHEMICAL TECHNIQUES

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Amyloidosis has rarely been confirmed in reptiles. A 7-year-old Komodo dragon was euthanized due to progressive hindlimb weakness. Autopsy identified a lumbosacral fracture with spinal cord compression and severe degenerative joint disease. Histologically, synovial villi were expanded by abundant eosinophilic amorphous material suggestive of amyloid; however, the deposits lacked Congo red (CR) positivity and birefringence under polarized light. Glutaraldehyde-fixed synovium was examined by transmission electron microscopy (TEM), revealing extracellular aggregates of randomly arranged, nonbranching fibrils measuring 8-10 nm in diameter and lacking periodic banding, consistent with amyloid. Frozen synovium was homogenized using lysis buffer, mortar and pestle, and sonication. Total protein concentration was 8.3 mg/mL by the Lowry method. Proteins were separated by16% SDS-PAGE and transferred to nitrocellulose membranes for Western blotting (WB). CR staining of the synovial protein extract demonstrated a characteristic spectral shift detected by visible spectrometry and polarized light microscopy. Thioflavin T binding showed a 37-fold increase in fluorescence compared with baseline. Similar CR spectral shifts and Thioflavin T fluorescence increases were observed with serum amyloid A1 fibrils used as a positive control. TEM and WB confirmed fibrillar structures in both the synovial protein extract and the positive control. WB identified serum amyloid A1, serum amyloid P component, and apolipoprotein A1 within the deposits. This case demonstrates synovial amyloidosis in a Komodo dragon and highlights the value of complementary ultrastructural, biophysical, and immunochemical techniques when routine CR histochemistry is inconclusive. The severe degenerative joint disease may have provided a pro-inflammatory microenvironment, favoring localized amyloid deposition.

Discipline: Anatomic pathology, **Topic:** General pathology, **Species:** Wildlife/Zoo, **Systems:** Bone and joints

THE EFFECT OF DECALCIFICATION METHODS ON RNASCOPE IN SITU HYBRIDIZATION SIGNAL AMPLIFICATION IN TURTLE SHELLS

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In situ hybridization (ISH) techniques are commonly utilized to study disease pathogenesis; however, application can be complicated by processing required for certain tissue types. One such tissue type is turtle shell, which is composed of bone that requires decalcification and scutes comprised of thick keratin. We compared three decalcification methods (10% ethylenediaminetetraacetic acid (EDTA), 15% formic acid, and Morse solution) and assessed their effects on decalcification time, histomorphology, 18S rRNA preservation, and RNAscope ISH signal amplification of 18S rRNA and GAPDH genes in turtle shells (n=10). Decalcification time (days) means were longer in EDTA ($p<0.001$) than formic acid and Morse solution. EDTA sections had better overall histomorphology mean scores ($p<0.001$) when compared to formic acid and Morse solution. RNA ISH signal for 18S rRNA was higher in

samples decalcified with EDTA than the other solutions ($p<0.0003$), and while signal for GAPDH was lower overall than that of 18S rRNA, it was also significantly higher in shells decalcified with EDTA compared to formic acid ($p=9.66x10^{-05}$) and Morse solution ($p=3.72x10^{-05}$). Similarly, amplifiable 18S rRNA Cq values were significantly lower in formalin-fixed paraffin-embedded samples decalcified with EDTA than formic acid ($p=4.6x10^{-04}$) or Morse solutions ($p=1.2x10^{-03}$). Our results demonstrate that EDTA shell decalcification offers robust nucleotide preservation and histomorphology suitable for future *in situ* gene expression studies, but prolonged decalcification time may preclude use in routine diagnostics.

Discipline: Anatomic pathology, **Topic:** Methods/technology, **Species:** Wildlife/Zoo, **Systems:** Bone and joints

BOVINE ABORTION & NEONATAL LOSS ATTRIBUTED TO HIGHLY PATHOGENIC AVIAN INFLUENZA (HPAI) H5N1 CLADE 2.3.4.4B

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The highly pathogenic avian influenza (HPAI) H5N1 virus clade 2.3.4.4b, which has been circulating in thousands of wild and domestic birds since January 2022, first spilled over into dairy cattle in the United States in March 2024. Anecdotally there were reports of HPAI associated dairy cattle abortions in mid-to-late gestation that were thought to be secondary to fever and the effects of an exaggerated cytokine response. Here we describe HPAI H5N1 virus associated abortion outbreaks in five separate US dairies and subsequent neonatal losses at calf ranches. Thirty-two fetuses were submitted from all lactation stages, and gestation ranges were from 3 months to term. The fetuses grossly demonstrated marked bicavitary effusion, marked myocardial streaking, pulmonary interlobular edema, and a cobblestone liver. Histopathology revealed varying degrees of random lymphocytic myocarditis, myocardial necrosis, and mineralization, lymphocytic interstitial pneumonia, necrotizing bronchiolitis, and in some cases lymphocytic encephalitis. Lung swabs were positive via reverse transcriptase (RT-qPCR) tests targeting the matrix, H7 and H5 genes and a cleavage site within the H5 gene specific for the Eurasian lineage goose/Guangdong H5 clade 2.3.4.4b of avian influenza virus. Twelve 1–5-day old neonates or tissues from neonates were also submitted and had similar pathology to the fetuses. Influenza A immunohistochemistry revealed immunoreaction within many of the lesions. This is the first report of HPAI H5N1 viral induced mammalian fetal and neonatal lesions in the literature.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Bovine, **Systems:** Reproductive

ABERRANT MIGRATION OF ANGIOSTRONGYLUS CANTONENSIS IN THE CENTRAL NERVOUS SYSTEM OF A CAPYBARA

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In June of 2025, a 14-month-old, female, capybara (Hydrochoerus hydrochaeris) in human care at a zoological institution was examined due to acute onset of paraparesis and tachypnea. After diagnostic exams and showing initial improvement with empirical treatment, the capybara rapidly declined and euthanasia was elected, one week after first presentation. Examination of the brain and spinal cord revealed severe, multifocal, eosinophilic meningoencaphalomyelitis with multiple cross sections of metastrongyle larval parasites within the spinal cord. Based on histologic morphology, PCR and subsequent DNA sequencing of fresh tissues, these parasites were identified as Angiostrongylus cantonensis. The parasite was also identified in the lungs, and eosinophilic inflammation was noted in multiple major organs. The normal parasitic life cycle of A. cantonensis involves maturation in the lungs of rats; however, larval migration in the central nervous system has been reported in multiple aberrant host species, primarily primates. Previous reports of angiostrongyliasis from this same zoological institution include several species of non-human primates, a capybara, a Patagonian mara, and a Coscoroba swan, which were primarily diagnosed via PCR. This is the first known report in a capybara with histologic demonstration of the organism. This case represents an excellent example of A. cantonensis aberrant migration in an unusual species and adds to the growing knowledge of susceptible hosts.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Wildlife/Zoo, **Systems:** Nervous

DISCOVERY OF A PUTATIVE NOVEL BASAL ENTAMOEBA SPECIES IN A CAPTIVE BEARDED DRAGON

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A 4-year-old intact male bearded dragon (*Pogona vitticeps*) was presented for evaluation of chronic, recurrent diarrhea that had been present intermittently since adoption at 5 months of age. Previous fecal testing identified coccidiosis and *Salmonella* spp., both successfully treated with ponazuril, fenbendazole, and ceftazidime, with subsequent negative follow-up testing. A cloacal swab was also positive for Agamid adenovirus 1, confirmed by sequencing. Intermittent lymphocytosis was documented on multiple CBCs, including the most recent evaluation (14.85 x10³/µL; RI: 0.9–8.4 x10³/µL). During the current presentation, coelomic ultrasonography revealed diffuse small-intestinal wall thickening consistent with enteritis and scant anechoic peritoneal effusion that could not be sampled due to its low volume. Zinc sulfate fecal flotation identified a few oxyurid eggs, small flagellated protozoa, and occasional 16–20 µm spherical organisms with multiple uniform nuclei, morphologically consistent with *Entamoeba* spp. cysts. Fecal PCR targeting *Entamoeba* spp. was positive, and Sanger sequencing generated a 123-bp sequence representing a putative novel *Entamoeba* species, likely basal to currently characterized members of the genus based on preliminary phylogenetic analysis.

Following treatment with metronidazole, repeat fecal examination was negative for *Entamoeba*, although intermittent diarrhea persisted. While *E. invadens* is the principal pathogenic species reported in reptiles, the clinical significance of this novel *Entamoeba* remains uncertain, particularly given the concurrent adenovirus infection. Nevertheless, this case expands the known diversity of reptilian *Entamoeba* and highlights the utility of molecular diagnostics in identifying previously unrecognized protozoan species. Further investigation is warranted to determine the epidemiology and pathogenic potential of this organism.

Discipline: Clinical pathology, **Topic:** Infectious disease, **Species:** Wildlife/Zoo, **Systems:** Gastrointestinal

A RARE PRESENTATION OF A LUNG LOBE TORSION ASSOCIATED WITH CHYLOUS PLEURAL EFFUSION IN A CAT

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An 8-year-old neutered male domestic long-haired cat presented to the Kansas State University (KSU) Veterinary Health Center (VHC) for increased respiratory effort and pleural effusion. Four months prior, the patient had been diagnosed with bronchopneumonia and treated with antibiotics, but clinical signs persisted. Three weeks after initial presentation, an echocardiogram revealed normal cardiac structure and function, although TFAST identified mild bilateral pleural effusion and collapse of the right cranial lung lobe. Despite medical management, coughing and pleural effusion persisted. At KSU, thoracocentesis was performed, and the collected fluid was grossly red, cloudy, triglyceride-rich, and composed predominantly of small lymphocytes, consistent with chylous effusion. Thoracic CT was performed, revealing right cranial lung lobe torsion with necrosis and enlargement of cranial mediastinal lymphatic vessels. The patient underwent subtotal pericardiectomy, right cranial lung lobectomy, and pleural port placement. Histopathologic examination of the excised lung lobe revealed acute-to-subacute severe lobar pulmonary hemorrhage, edema, and alveolar septal necrosis, supporting the diagnosis of lung lobe torsion. Mild-to-moderate chronic eosinophilic and lymphoplasmacytic peribronchial/peribronchiolar and perivascular interstitial inflammation was also present, which could correlate with chronic feline asthma, potentially predisposing to lung lobe torsion. After surgery, pleural fluid accumulation decreased substantially over time, and no respiratory signs were noted at 1 week post-surgery. This report describes a rare presentation of lung lobe torsion associated with chylous pleural effusion in a cat. Whether the effusion preceded the torsion or developed secondary to it remained unclear.

Discipline: Both, **Topic:** Other (Uncommon clinical condition), **Species:** Feline, **Systems:** Respiratory

Industrial and Toxicologic Pathology Focused Scientific Session

Monday, 1:30 pm-5:00 pm
Predrag Novakovic, DABT DVM MSc PhD DACVP (AP)

HYDROXYSTEROID 17-BETA DEHYDROGENASE 7 IS REQUIRED FOR PROPER NEURAL DEVELOPMENT

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Background: Cholesterol biosynthesis disorders are a heterogenous suite of genetic diseases with potential life-threatening consequences for early development and a range of craniofacial and CNS clinical features. Cholesterol supplementation has limited effect on clinical progression; cholesterol precursor buildup secondary to cholesterol biosynthesis disruption may contribute to disease. *Hydroxysteroid 17-Beta Dehydrogenase 7* (*Hsd17b7*) encodes for a cholesterol biosynthesis enzyme. **Objectives:** Investigate consequences of *Hsd17b7* deletion on the CNS. **Methods:** Cre-Lox system is leveraged to delete *Hsd17b7* in *Wnt1* and *Emx1* cell lineages. Long-read DNA whole-genome sequencing confirmed integration of LoxP sites around *Hsd17b7* Exon 4. *Wnt1cre* embryonic timepoint (E)18.5 embryos were collected for skeletal preparation, imaged, and measured. Double-fluorescent cre reporter mT/mG was crossed into the *Wnt1* model and embryos were collected, imaged, and cleared for whole-mount CC3 IHC. Brains from postnatal timepoints (P) and embryos from *Emx1cre* and *Wnt1cre* mice were collected for histological examination. Antibodies against TUJ1 were applied to E14.5 *Emx1cre* cerebrums, TBR2 to E12.5 and E14.5 cerebrums, and CC3, PHH3, and PAX6 to E10.5, E12.5, and E14.5 cerebrums. **Conclusions:** *Wnt1cre/wt;Hsd17b7flox/wt* mice reveal cerebellar dysgenesis linked to changes in the midbrain-hindbrain boundary, hydrocephaly linked to malformation of the subcomissural organ, and craniofacial anomalies. *Emx1cre/wt;Hsd17b7flox/wt* mice have a hypoplastic forebrain. Discussion: Loss of *Hsd17b7* leads to early apoptosis of the *Wnt1* and *Emx1* cell lineage, compromising development of the cerebellum, craniofacial primordia, and cerebrum. Buildup cholesterol precursors are hypothesized to cause early loss of pertinent developmental cell lineages. We show *Hsd17b7* is required for proper CNS development.

Discipline: Anatomic pathology, **Topic:** Genetic/hereditary disorders, **Species:** Rodents, **Systems:** Nervous

ACUTE COBALT TOXICOSIS IN CUTTHROAT TROUT (ONCHORHYNCHUS CLARKII): LATERAL LINE INJURY, BRANCHITIS, AND MORTALITY

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Cobalt is rarely monitored in Colorado but is found downstream of multiple legacy mines. This element is toxic to neuromasts of the fish lateral line, a critical sensory organ that enables fish to navigate their environment. To investigate the effect of acute aqueous exposure to cobalt, Colorado River Cutthroat Trout (CRC, average standard length 34.2 +/- 1.5 mm; average weight 0.472 +/- 0.055 g) were exposed to nominal concentrations of cobalt (0, 15.3, 30.4, 60.5, 120.1, 238.6, 477.2, 947.8,

1882.7, 3739.6, and 7428 µg/L) for 96 hours. The lateral line was evaluated with 4-Di-2-ASP vital dye, and fluorescence microscopy images were assessed with image analysis software to discern normalized neuromast number, average staining intensity, and percent neuromast area by region. 100% mortality occurred above 1882.7 µg/L within 48 hrs, and the lateral line of these fish were not assessed. Histopathological examination revealed that CRC exposed to ≥ 1882.7 µg/L had moderate proliferative branchitis with occasional individual necrotic cells. Mean neuromast number, intensity, and area were significantly decreased in CRC exposed to 947.8 and 477.2 µg/L cobalt versus control. Concentrations of aqueous cobalt below reported LC50 values cause lateral line damage in CRC. This sublethal pathology has potential to manifest into species lost by negatively influencing swimming energetics, foraging ability, and predator avoidance.

Discipline: Anatomic pathology, **Topic:** Toxicology, **Species:** Aquatic, **Systems:** Other (lateral line, gills (respiratory))

PATHOLOGY OF EXPERIMENTAL ORGANOMETALLIC LUBRICANT ADDITIVE TOXICITY IN SPRAGUE DAWLEY RATS

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Organometallic additives are chemical compounds that contain at least one direct bond between a metal atom and a carbon atom. They constitute a family of oil additives that function to enhance engine performance, prevent oxidation, and reduce engine wear by forming a protective layer on metal surfaces. A 90-day dietary study in Sprague Dawley rats was conducted to determine the potential toxicity of an organometallic test substance. Male and female rats were fed a basal diet or the test substance (TS) diet at concentrations of 0, 1400, 3000, or 9000 ppm for 90 days, followed by a 28-day recovery period. The study was performed in accordance with 40 CFR Part 792, Good Laboratory Practice Standards. There were no test substance effects on survival. Test substance microscopic findings at the terminal euthanasia were noted in the pancreas, kidney, and prostate at ≥ 1400 ppm. Pancreatic findings consisted of acinar apoptosis. The spectrum of renal findings was consistent with alpha 2u-globulin nephropathy. Prostatic findings consisted of mixed cell inflammation, which correlated macroscopically with focal discoloration. Test substance-related organ weight changes consisted of higher mean liver and thyroid/parathyroid gland weights with no microscopic correlates. Findings were considered nonadverse due to low severity grade and absence of correlating clinical pathology values. At recovery, there was persistence of microscopic changes in the prostate gland and kidney, but findings in the pancreas, liver, and thyroid/parathyroid gland had resolved.

Discipline: Anatomic pathology, **Topic:** Toxicology, **Species:** Rodents, **Systems:** Systemic

COMPARISON OF WHOLE-LUNG AND LOBE-SPECIFIC ACUTE LUNG INJURY SCORING FOR PREDICTING RESPIRATORY FUNCTION IN AN LPS-INDUCED RAT MODEL

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Acute lung injury (ALI) scoring systems are widely used to quantify pulmonary injury in experimental models; however, no standardized approach exists regarding whether lungs should be evaluated as a single unit or by individual lung lobes. This study compared whole-lung and lobe-specific ALI scoring approaches and evaluated their association with respiratory function in a rat lipopolysaccharide (LPS)-induced ALI model.

Male rats received intratracheal vehicle or LPS. Twenty-four hours after dosing, lungs were evaluated histologically using a standardized ALI scoring system based on neutrophilic infiltration, hyaline membranes, proteinaceous alveolar debris, and alveolar septal thickening. Each animal received a whole-lung score and individual scores for the left, right cranial, right middle, right caudal, and accessory lobes. Mean lobe scores were compared with whole-lung scores. Respiratory function was assessed by whole-body plethysmography. Associations between histopathologic scores and respiratory parameters were analyzed using Spearman correlation.

Both scoring approaches demonstrated dose-dependent increases in ALI severity. Mean lobe scores showed stronger correlations with respiratory rate ($r = 0.317$, $P = 0.131$) and tidal volume ($r = –0.323$, $P = 0.124$) than whole-lung scores (respiratory rate: $r = 0.206$, $P = 0.335$; tidal volume: $r = –0.250$, $P = 0.239$). Correlations with minute volume were weak for both mean lobe ($r = 0.138$, $P = 0.520$) and whole-lung ($r = 0.013$, $P = 0.951$) scores.

These findings demonstrate lobe-specific ALI scoring more effectively captures the heterogeneous distribution of pulmonary lesions, providing better concordance with respiratory function than conventional whole-lung assessment, supporting its application in nonclinical toxicology studies.

Discipline: Anatomic pathology, **Topic:** Toxicology, **Species:** Rodents, **Systems:** Respiratory

FIBROTIC LUNG REMODELING ALTERS TUMOR TARGET EXPRESSION AND SHAPES SAFETY RISK IN LUNG-DIRECTED CANCER THERAPIES

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Target-directed lung cancer therapies—including antibody–drug conjugates, radioligand therapies, immune cell engagers, and adoptive cell therapies—are increasingly used in patients with pre-existing chronic lung disease. However, safety assessments are typically based on target expression in normal lung, potentially overlooking unanticipated disease-associated changes that may increase on-target/off-tumor (OTOT) toxicity risk.

Chronic fibrosing lung conditions, including idiopathic pulmonary fibrosis (IPF), radiation-induced pulmonary fibrosis (RIPF), and the fibrotic phase of acute respiratory distress syndrome (ARDS), arise from distinct etiologies but converge on shared pathological features, including aberrant epithelial repair, fibroblast activation, and fibrotic architectural remodeling. Whether these conditions induce common or context-specific alterations in tumor target expression remains unclear.

To address this, we selected mesothelin (MSLN) and folate receptor alpha (FOLR1) as potential lung cancer targets and evaluated their immunohistochemical expression in lung biopsies from patients with RIPF (n=10), IPF (n=9), and fibrotic ARDS (n=9). Across all conditions, fibrotic remodeling was associated with increased FOLR1 expression and de novo or ectopic MSLN expression within the epithelial compartment, independent of the initiating insult.

These expression changes localized predominantly to regions of aberrant epithelial remodeling, particularly areas exhibiting bronchiolization and honeycomb architecture. Bronchiolization represents a maladaptive regenerative process characterized by persistence of transitional epithelial states without terminal differentiation.

Collectively, these findings indicate that altered target expression is a shared feature of fibrotic lung remodeling rather than injury-specific biology. This has important safety implications, as such remodeling may create previously unrecognized OTOT risk hotspots, potentially amplifying toxicity in patients with underlying chronic lung disease.

Discipline: Anatomic pathology, **Topic:** Toxicology, **Species:** Humans, **Systems:** Respiratory

CROSS-MODEL AGILITY FOR DEEP LEARNING DETECTION OF HEPATOCELLULAR NECROSIS ACROSS VIRAL HEMORRHAGIC FEVERS

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Introduction: Viral hemorrhagic fevers caused by Ebola virus (EBOV) and Lassa virus (LASV) share pathological features, including multifocal liver necrosis with minimal local inflammatory responses. We investigated whether a U-Net model trained exclusively on EBOV-associated necrosis could detect LASV-associated necrosis, testing model generalizability across distinct viral diseases. Materials and **Methods:** A U-Net model trained to recognize necrosis exclusively on archived histopathology slides from livers of lethally EBOV-exposed non-human primates (NHPs) was used to segment hepatocellular necrosis on slides (n=12) from LASV-exposed animals. U-Net-generated predictions were compared to pathologist-generated ground truth masks; segmentation performance was evaluated for accuracy, sensitivity (Sn), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV). **Results:** Model performance was precise and reliable in distinguishing LASV-associated necrosis, with 98% accuracy, 97% Sp, 90% PPV, and 96% NPV. Slightly lower Sn (65%) for LASV-associated necrosis was comparable to model Sn for EBOV-associated necrosis. **Conclusion:** Although

trained only on livers from an NHP model of lethal EBOV infection, a U-Net model retained accuracy in segmentation of hepatocellular necrosis caused by a different virus. This study demonstrates the potential for deep learning models to identify pathologic features across viral infection models and explore common threads of pathogenesis. Funding: This research was supported by the Intramural Research Program of the National Institutes of Health (NIH) and with federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. 75N91019D00024.

Discipline: Anatomic pathology, **Topic:** Comparative pathology, **Species:** NHP, **Systems:** Hepatobiliary

CATHETER OVER NEEDLE (OTN) TAIL VEIN INFUSIONS ARE WELL-TOLERATED AND SUCESSFUL NOVAL METHOD IN C57BL/6 MICE UP TO 13 WEEKS

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Longer-term intravenous (IV) infusion studies are in demand for preclinical safety assessment. Traditional surgically altered animals (through-the-needle [TTN] tail vein catheters or jugular vein catheters) are often riddled with procedural-related patency complications and morbidity/mortality. Since C57BL/6 mice are the preferred model for infusion studies, we implemented our refined IV infusion method in this strain using a 26-gauge ½ inch over-the-needle (OTN) pediatric catheter. Our objectives were to determine the maximum feasible duration (as defined by patency) of OTN tail vein infusion dosing in C57BL/6 mice when administered once weekly for the first 4 weeks, then once monthly or twice monthly, for up to 13 weeks with a saline dose volume infusion rate of 50 mL/kg/hour for 30 minutes. Our method resulted in 100% tail vein patency and minimal mortality in all catheterized mice at the end of the study. Furthermore, there was low incidence of expected procedure-related vascular changes at the infusion site that included vascular mixed cell inflammation, hemorrhage, intimal proliferation, thrombi, and/or fibroplasia/fibrosis, of which severity did not exceed mild. Overall, tail vein infusions are well-tolerated and successful in C57BL/6 mice at multiple administration frequencies by the developed OTN method for up to 13 weeks.

Discipline: Anatomic pathology, **Topic:** Methods/technology, **Species:** Rodents, **Systems:** Cardiovascular

Natural Disease Focused Scientific Session II

Monday, 3:30 pm-5:00 pm
Mason Jager, DVM PhD DACVP (AP)

HAND IN PAW: A COMPARATIVE APPROACH TO DECIPHERING THE IMMUNOPATHOGENESIS OF CANINE MENINGOENCEPHALITIDES OF UNKNOWN ORIGIN

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Canine meningoencephalitides of unknown origin (MUO) comprise three neuroinflammatory diseases: granulomatous meningoencephalitis (GME), necrotizing meningoencephalitis (NME), and necrotizing leukoencephalitis (NLE). GME is presumed to be immune-mediated and recapitulates pathogenetic features of multiple sclerosis (MS), suggesting that canine patients may benefit from advances in MS therapeutics. Notably, the roles of Th17 cells and the glymphatic system/perivascular (PV) space, both implicated in MS pathogenesis, remain largely unexplored in dogs. We thus investigated 100 MUO cases (66/100 GME, 22/100 NME, 12/100 NLE) by combining immunohistochemistry (IHC), fluorescent *in situ* hybridization (FISH), and spatial lipidomics by Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI-MSI) with a focus on Th17 cells and PV spaces.

Histopathological features were compared to the immune landscape within and between MUO subtypes. Whole-slide IHC targeted T-cells (CD3), B-cells (CD20, PAX5), plasma cells (MUM1), macrophages (CD204, IBA1, MAC387), astrocytes (GFAP), endothelium (von Willebrand Factor), and myelin (MOG), with quantification in QuPath. Multiplex FISH assessed Th17 cells (*CD3, CD4, CD8, IL17RC, IL17A, RORC*). Spatial lipidomics analysis combined annotated digitized H&E slides (QuPath) with metabolite heatmaps (SCiLS™ Lab).

Histologically, GME and NLE had overlapping inflammatory patterns, and all conditions were T-cell-rich (p-adj > 0.99). GME and NLE had B- and plasma-cell-rich PV cuffs and meningeal infiltrates. GME was enriched in MAC387+ macrophages and had intra-parenchymal, PV, and meningeal Th17 cell infiltrates. All conditions exhibited altered lipidomic profiles.

We conclude that Th17 cells and altered lipid metabolism are important features of GME. Our findings strengthen the value of a translational research approach for canine patients.

Discipline: Anatomic pathology, **Topic:** Immunology, **Species:** Canine, **Systems:** Nervous

IN SITU SLAMF1 SPLICE VARIANT CORRELATES WITH ESCHERICHIA COLI-ASSOCIATED GRANULOMATOUS COLITIS OF BOXERS AND FRENCH BULLDOGS

Donghyun K. Lee[1], Gerald E. Duhamel[1], Shawna R. Cook[2], Jacquelyn M. Evans[2], Jessica J. Hayward[1], Alexandria J. Shumway[1,3], Praveen Sethupathy[1], Belgin Dogan[1], Kenneth W. Simpson[1]
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The histological diagnosis of *Escherichia coli*-associated granulomatous colitis of Boxers and French bulldogs (EGCBF) is based on mucosal infiltration by macrophages that accumulate periodic acid–Schiff (PAS)-positive material along with the presence of invasive *Escherichia coli* (Ec). EGCBF is thought to be a consequence of a genetic defect that impairs macrophage uptake and phagocytosis

of Ec. Previous studies in murine models have directly linked the transmembrane receptors signaling lymphocytic activation molecule family 1 (SLAMF1/CD150) and family 2 (SLAMF2/CD48), to selective sensing, uptake, and intracellular killing of Ec. In the current study, we assessed SLAMF1 wild-type (WT) and mutated splice variant (Mut) expression in colonic biopsies from BF diagnosed with EGCBF (n = 5) or lymphocytic-plasmacytic colitis (LPC; n = 6) using mRNA-specific probes and BASEscope® *in situ* hybridization (ISH). All EGCBF dogs (5/5; 100%) hybridized with the Mut probe in areas where PAS-positive macrophages and Ec were present, indicating the presence of a risk allele. In conclusion, the SLAMF1 mutated splice variant co-localized with PAS-positive macrophages and intracellular Ec in EGCBF but not in BF with LPC.

Discipline: Anatomic pathology, **Topic:** Genetic/hereditary disorders, **Species:** Canine, **Systems:** Gastrointestinal

PREVALENCE, TISSUE DISTRIBUTION AND DISEASE ASSOCIATION OF FELINE BOCAPARVOVIRUS SPECIES IN A POPULATION OF DOMESTIC CATS

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Bocaparvovirus is a genus of parvovirus within the Parvovirinae subfamily. Well described bocaparvoviruses like canine minute virus (CPV-1), bovine parvovirus 1, porcine bocavirus and human bocavirus 1 are associated with enteric and respiratory illnesses that are more likely to cause severe disease in neonatal individuals. Bocaparvovirus infection is common in many species and can have lifetime, subclinical persistence.

There are three identified feline bocaviruses: FBD1, FBD2, and POR1. Their prevalence and the potential that these viruses can cause disease individually or as co-infections is unknown. The objective of this study is to identify the prevalence of bocaparvovirus species in cats submitted to UC Davis for necropsy, demonstrate the tissue distribution of the viral antigens in a subset of cases, and uncover potential disease associations.

Post-mortem sampling of forty-eight cats, ranging in age from 1 week to 18 years old, was conducted during a seven-month collection period. Twenty-nine of these cats were either singly or dually infected with FBD2 or POR1 species of feline bocaparvovirus as detected by PCR on splenic tissue. Species specific infection sequelae of feline bocaparvoviruses were explored by a combination of PCR, sequencing, electron microscopy, and in situ hybridization. In three dual-infected kittens systemic distribution of virus was associated with necrosis, inflammation, and intranuclear inclusions in pancreatic exocrine cells and hepatocytes.

Bocaparvovirus infection, including dual infections with FBD2 and POR1 bocaparvovirus species, were common in the examined population. Occasional cases of multi organ necrosis and inflammation were associated with bocaparvovirus, particularly in kittens.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Feline, **Systems:** Systemic

HISTOLOGICAL FEATURES OF DOMESTIC CAT HEPATITIS B VIRUS (DCHBV)-POSITIVE HEPATOCELLULAR CARCINOMA

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Background: Hepatitis B virus causes more than 50% of all hepatocellular carcinomas (HCC) in humans. Evidence supports a causal role for domestic cat hepatitis B virus (DCHBV) in approximately 25% of feline HCC. No studies have compared the morphology of DCHBV-positive and DCHBV-negative HCCs.

Objective: To describe and compare histological features of DCHBV-positive (cases) versus DCHBV-negative (controls) feline HCCs.

Methods: A scoring system was developed according to the published Veterinary Cancer Guidelines and Protocols. Parameters evaluated included mitotic and multinucleation count (cells/2.37mm^2), histological pattern, nuclear and cytoplasmic morphology, and extracellular matrix. Histopathological review of archived formalin-fixed paraffin-embedded HCCs was performed by a board-certified pathologist blinded to DCHBV status.

Results: Eighty-three HCCs were evaluated, including 18 (21.7%) cases and 65 (78.3%) controls. Trabecular was the most common pattern observed in both DCHBV-positive (10/18, 55.5%) and DCHBV-negative (38/65, 58.5%) HCCs. Distribution of the patterns did not differ significantly between cases and controls (Fisher's exact p=0.3662). None of mitotic and multinucleation counts (Wilcoxon p=0.2531 and p=0.9880 respectively) nor nuclear and cytoplasmic morphology (Wilcoxon p=0.2495 and p=0.1208 respectively) were significantly different between cases and control. For extracellular matrix, cases were classified as fibrovascular [15/18 (83.3%) in the case-group and 54/65 (83%) in the controls group] or fibrous [3/18 (16.7%) cases; 11/65 (16.9%) controls]. Again, no difference was observed in type of matrix between groups (Fisher's exact p=1.00).

Conclusion: DCHBV-positive and DCHBV-negative HCCs showed similar histological features. This may indicate that histopathology alone is insufficient for distinguishing DCHBV positive and negative tumors.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Feline, **Systems:** Hepatobiliary

COMPARATIVE HISTOLOGIC SURVEY AND TRANSCRIPTOMIC INVESTIGATION INTO CANINE PROSTATE CARCINOMA

Nathan Hoggard[1], Said Elshafae[2], Darshan Chandrashekar[3], Nigel Daniels[4], Jonathan Young[1], Chris Premanandan[5], John Echols[3], Blake Hildreth[3], Michael Haffner[6,7], Thomas Rosol[1,8]
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Background: Dogs share features in prostate gland anatomy, physiology, and pathology with men. However, human and canine prostate carcinoma (PC) have histologic and molecular differences. Particularly, the histogenesis of canine PC (cPC) is unclear. **Objective:** This study investigated the origin of cPC using histopathology and transcriptomics with comparison to men. **Methods:** Prostate glands retrospectively and prospectively collected from 445 dogs (approximately 95% autopsy samples) were surveyed for early carcinomas and preneoplastic lesions, particularly high-grade prostatic intraepithelial neoplasia (HGPIN) due to its role in the pathogenesis of PC in men. Lineage gene signatures defining prostate luminal epithelium and urothelium were identified for inter- and intraspecies RNA-sequencing comparisons, including between cPC and canine urinary bladder urothelial carcinoma (UC). **Results:** Postmortem prostate lesion frequencies were similar to previously reported canine studies. Intraductal/intra-acinar growth (31/35; 88.6%) was common in representative samples of cPC. Prostate epithelial changes consistent with HGPIN in men were not observed. Proliferative lesions and early carcinomas were rare (7/445; 1.6%). Patterns in prostate and urothelium marker gene expression signatures differed between human and canine PC. Compared to non-neoplastic prostate gland, cPC had significantly decreased prostate-specific and increased urothelium gene signatures. **Conclusions:** The results suggest many cases diagnosed as cPC are UC or have urothelial differentiation and thus differ from PC in men, with important implications for canine tumor classification and translational studies.

Discipline: Anatomic pathology, **Topic:** Comparative pathology, **Species:** Canine, **Systems:** Reproductive

TOLL-LIKE RECEPTOR 4 EXPRESSION IN CANINE OSTEOSARCOMA: A PILOT STUDY

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Background

Osteosarcoma (OSA) is the most common primary bone tumor in dogs and is associated with a poor prognosis despite current treatment strategies. Interestingly, dogs that develop post-operative bacterial infections survive longer than dogs that do not, implicating immune reprogramming in improved survival. Toll-like receptors

(TLRs) are innate immune receptors that exhibit antitumor activity in several cancers. Expression of TLRs in canine OSA is largely unexplored.

Objective

Determine whether spontaneously arising canine primary OSA tumors express TLR4.

Methods

Tissue microarrays were constructed from 17 archived formalin-fixed, paraffin-embedded canine patient primary appendicular OSAs and normal canine lymph nodes (control). RNAscope^{TM} was performed to evaluate mRNA expression of canine TLR4, peptidylprolyl isomerase B (PPIB; positive control), and DapB (negative control). Immunohistochemistry for CD18, Iba-1, and CD3 was performed to identify immune cell infiltrates. The hybridization signal and the degree of immune cell infiltration were evaluated by microscopy.

Results

The positive control, PPIB, was detected in neoplastic cells from 13/17 tumors. Within this cohort, neoplastic cell-rich regions displayed moderate to strong TLR4 expression in 5/13 tumors. In 6/13 tumors, neoplastic cell-rich areas had inconsistent weak TLR4 expression. Neoplastic cell-rich regions from 2/13 tumors did not display TLR4 expression.

Conclusion

Canine OSA appendicular tumors display heterogeneous TLR4 mRNA expression, suggesting potential use as a prognostic biomarker or immunotherapeutic target. Future studies are warranted to determine TLR4 expression and prognostic significance across distinct OSA subtypes and to expand these findings to evaluate expression of other TLRs.

Discipline: Clinical pathology, **Topic:** Immunology, **Species:** Canine, **Systems:** Bone and joints

RETROSPECTIVE REVIEW OF SYNGNATHID BROOD POUCH CHANGES

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The family Syngnathidae comprises seahorses, pipefish and seadragons; species in this family display a unique evolutionary adaptation in which males maintain postzygotic embryo care within specialized brood pouches. Compilation of syngnathid necropsy submissions between 2008-2025 to North Carolina State University were assessed for brood pouch changes. Included in the study were sixty-two male syngnathids (*Hippocampus erectus, Hippocampus abdominalis, Syngnathus fuscus, Syngnathus scovelli, Syngnathus floridae,* and unknown seahorse and pipefish species) with histologically examined brood pouches. Approximately 40.3% of brood pouches examined were considered normal. Brood pouch changes often display overlapping features, grouping into multiple categories. The following microscopic changes were observed within the brood pouches:

hyperplasia (33.9%; 21/62), infectious disease (27.4%; 17/62), edema (16.1%; 10/62), neoplasia (3.2%; 2/62), embryos (3.2%; 2/62), emphysema (3.2%; 2/62) and inflammation of unknown etiology (1.6%; 1/62). Hyperplastic changes can be broken down into cystic hyperplasia and melanophore hyperplasia. Cystic hyperplasia was a common finding in adult male syngnathids in varying degrees of severity, and the cause and significance are currently unknown. Infectious cases included acid-fast bacteria (i.e. *Mycobacterium spp*.), non-acid fast bacteria, ciliated protozoa, trematodes, and fungi. Neoplasia included a round cell tumor and a teratoma. Emphysema supported suspicion for gas bubble disease in affected individuals. In 30.6% (19/62) of cases, the brood pouch lesions were considered related to the primary cause of patient decline and/or death.

Discipline: Anatomic pathology, **Topic:** General pathology, **Species:** Aquatic, **Systems:** Reproductive

DESCRIPTION OF 341 TERATOMAS IN MAMMALS, BIRDS AND REPTILES

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Teratomas are neoplasms arising from at least two germinal cell layers, including ectoderm, mesoderm, and/or endoderm. These neoplasms are reported in many animal species, with dogs and horses disproportionately represented. A multi-institutional retrospective and comparative study was performed to better characterize the epidemiologic, clinical, gross, and microscopic features and pathologic significance of teratomas in animals. A total of 341 teratoma cases from over 20 species was evaluated. Teratomas were composed of tissues derived from

three germinal cell layers in 34% (116/341) of cases and two germinal cell layers in 63% (215/341) of cases, with ectoderm and mesoderm as the most common combination. Commonly reported tissues include ectodermal adnexa, skin, and neural tissue; mesodermal cartilage, adipose tissue, fibrous connective tissue, and bone; and endodermal respiratory epithelium. The most common intratumoral lesions were cysts, inflammation, necrosis, and hemorrhage. Malignant teratomas represented 6.5% (22/341) of cases, with metastasis in 46% (10/22). Teratomas were more commonly reported in dogs (n=213), horses (n=33), chickens (n=27), and cats (n=22). In dogs, 99.1% (211/213) teratomas were reported in females, with 43% (91/211) being incidental. Clinical signs were reported in 20% (43/211) of females, and uterine lesions were described in 23% (48/211). In horses, teratomas were most common in Quarter Horse cryptorchid males (42%; 14/33). In chickens, 26% (7/27) of teratomas were malignant. Teratomas were occasionally diagnosed in non-human primates, small mammals, and reptiles. Teratomas were most commonly identified in dogs, horses, chickens, and cats, with the majority composed of tissues derived from two germinal cell layers.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Equine, **Systems:** Reproductive

SLC40A1-ASSOCIATED (TYPE 4B) HEMOCHROMATOSIS IN RHESUS MACAQUES: DISCOVERY OF A LARGE-ANIMAL MODEL AND CLINICOPATHOLOGIC CHARACTERIZATION

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Background: Pathogenic variants in SLC40A1, encoding the iron exporter ferroportin (FPN1), cause autosomal-dominant iron overload with two principal phenotypes: (i) classical ferroportin disease (loss-of-function; reticuloendothelial iron retention) and (ii) SLC40A1-related hemochromatosis (Type 4B; gain-of-function; hepcidin resistance) with hepatocellular iron loading.

Objective: To characterize a naturally occurring SLC40A1 Type 4B hemochromatosis phenotype in rhesus macaques (*Macaca mulatta*) and establish its translational relevance.

Methods: We retrospectively analyzed an extended pedigree, integrating clinical records, necropsy and histopathology (H&E, Perls' Prussian blue), iron scoring, hepatic elemental iron quantification, and WGS interrogation (mGAP) focusing on iron-homeostasis genes.

Results: We identified a missense SLC40A1 variant equivalent at the protein level to human p.Gly204Ser (G204S),a residue within TM6 previously associated with hepcidin resistance and parenchymal iron loading, which segregated with disease; no pathogenic variants were detected in HFE, HJV, HAMP, or TFR2. Among 85 evaluable animals (51 carriers; 34 wild-type), carriers had markedly higher Total Iron Scores (TIS) (mean 19.47/60) than wild-type (2.70/60). Hepatocellular iron was zonally accentuated (Rappaport zones 1–2) and iron was also evident in Kupffer cells; portal iron occurred in a subset with higher TIS. "Siderotic nodules" and

iron-free foci of hepatocytes appeared in higher-burden animals. Hepatic elemental iron was substantially elevated in carriers (mean 13,668 µg/g dry weight; n=16) versus wild-type (mean 669 µg/g; n=5).

Conclusions: This rhesus macaque pedigree represents the first large-animal model of SLC40A1 Type 4B hemochromatosis, reproducing hepatocellular iron loading as in humans and providing a powerful platform to study genotype–phenotype relationships, ferroportin biology, and interventions.

Discipline: Anatomic pathology, **Topic:** Genetic/hereditary disorders, **Species:** NHP, **Systems:** Hepatobiliary

PATHOLOGICAL SURVEY OF SLOTHS: (2001-2026)

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Sloths belong to the order Pilosa, including three-toed sloths (genus *Bradypus*) and two-toed sloths (genus *Choloepus).* A limited number of single-case reports or small

case series have described diseases in sloths. To address the limited information on disease prevalence in these species, a multi-institutional study was performed to provide a detailed characterization of sloths' diseases. A total of 227 pathology submissions from sloths were retrieved from 16 diagnostic laboratories in Costa Rica, the USA, and Brazil; 27.3% (62/227) from *Bradypus* spp. and 72.7% (167/227) from *Choloepus* spp. 57.3% (118/206) of the submitted animals were female, and 42.7% (88/206) were male. The age ranged from neonates to 35 years of age, and 62.5% (142/227) were older than 1-year-old. Respiratory (32.1%; 73/227), gastrointestinal (29.0%; 66/227), urinary (16.3%; 37/227), and cutaneous (11.4%; 26/227) diseases were most commonly reported. The most common respiratory lesions were pneumonia (54.8%, 40/73), caused by Canine distemper virus (6.8%, 5/73) or mixed bacterial infection (aspiration; 17.8%, 13/73). Bacterial enterocolitis (9.0%, 6/66) and alimentary mycotic infections (25.7%, 17/66) predominated in gastrointestinal tract diseases. Chronic kidney disease with soft tissue mineralization was frequently reported. Dermatitis was caused by bacteria, acari (*Sarcoptes* sp.), or fungi (*Malassezia* sp.). Septicemia-related lesions were documented in 6.6% (15/227) of the submissions. Neoplasms in sloths were reported in only 5.3% (12/227) of cases, with most being incidental benign neoplasms and three cases of urothelial carcinoma. Our findings provide insights into disease frequency in sloths to improve the husbandry and conservation of captive and free-ranging individuals.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Wildlife/Zoo, **Systems:** Respiratory

HISTOLOGIC CHARACTERIZATION OF GILL LESIONS ASSOCIATED WITH PARVICAPSULA MINIBICORNIS IN SOCKEYE SALMON (ONCORHYNCHUS NERKA) FROM THE LAKE WASHINGTON WATERSHED (WASHINGTON, USA)

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Sockeye Salmon in the Lake Washington watershed are a hatchery-supplemented population suffering from increased en route and prespawn mortality. To reduce mortality, hatchery practices capture fish entering freshwater and transport them to the hatchery, bypassing their natural migration. *Parvicapsula minibicornis,* a myxozoan parasite that typically affects the kidneys of salmonids, occurs at high prevalence in adult Lake Washington Sockeye Salmon during spawning migration in freshwater. In the kidney, high parasitic loads can lead to tubular degeneration and glomerulopathies. Fish health monitoring of this population in collaboration with the Washington Department of Fish and Wildlife detected this parasite associated with branchitis in 2023; however, the histologic features remain undocumented. We examined and described the histologic distribution and severity of disease associated with *P. minibicornis* in gills and kidneys of fish collected in 2025. Naturally migrating fish collected from the Cedar River (n=64) were compared to low/unexposed transported population (n=30). H&E kidney and gill samples were blindly evaluated and scored for the presence of *P. minibicornis* within associated lesions. In naturally migrating fish, the most common lesions in the gills associated with P. minibcornis trophozoites included lamellar fusion (n=52, 81%), neutrophilic

filamentous branchitis (n=51, 78%), neutrophilic lamellar branchitis (n=36, 56%), and hemorrhage (n=40, 63%). The parasite was not detected in the kidney or gill of transported fish, although multifocal, neutrophilic branchitis was noted in 9 fish (30%). In summary, detection of *P. minibicornis* should be considered significant due to the association with a variety of gill lesions.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Aquatic, **Systems:** Respiratory

PARASITE DISTRIBUTION AND LESION SEVERITY IN CAPTIVE CHELONIANS WITH TESTUDINE INTRANUCLEAR COCCIDIOSIS (TINC)

Keegan Lim, Brianna Ferrer, Ana Herrera, Steven Tillis, Justin Stilwell, Robert Ossiboff
Department of Comparative, Diagnostic and Population Medicine, University of Florida College of Veterinary Medicine, Gainesville, FL, USA

Testudine intranuclear coccidiosis (TINC), caused by an Eimeria-like parasite, is an important cause of morbidity and mortality in captive tortoises and turtles. Despite its growing clinical significance and often fatal disease progression, the spectrum of microscopic lesions in affected chelonians and the tissue distribution of the causative agent are poorly characterized. To address these knowledge gaps, we evaluated >300 tissue samples from 30 tissue types collected during necropsy of chelonians (n=23), including both Old and New World tortoises and Asian box turtles. All tissues were scored for inflammation using a semiquantitative microscopic evaluation system. To assess parasite distribution and abundance, RNAscope in situ hybridization (ISH) using a ZZ-probe complementary to a portion of the parasite 18S rRNA gene was performed. ISH slides were analyzed digitally with an open-source digital pathology software (QuPath) to quantify the number of ISH-positive parasites per unit area. Pathogen-associated lesions were present in nearly all tissue types examined, with pronounced epitheliotropism in all cases. The gastrointestinal, respiratory, and urinary tracts were most consistently affected, with prominent involvement of the oral and nasal cavities. There was a clear disparity in organism detection between ISH and routine light microscopy, with ISH detecting up to 10-fold more organisms. This study provides a systematic analysis of complete tissue sets from tortoises and turtles with TINC, highlights the significance of this systemic disease in captive chelonians, and emphasizes the poor sensitivity of organism detection by light microscopy alone. Ancillary diagnostics may be necessary for definitive diagnosis of this debilitating disease.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Wildlife/Zoo, **Systems:** Systemic

Clinical Pathology Focused Scientific Session II

Tuesday, 8:00 am-12:00 pm
Lisa Schlein, DACVP (CP); Robert, DACVP (CP)

APPLICABILITY OF EXPLAINABLE AI IN CYTOPATHOLOGICAL EVALUATION OF CANINE LYMPHOMA

Matthew Vardas[1], Chanel Shum[1], Donghee Lee[2], Karthik Karnam[2], Samantha Masessa[1], Hyunji Jo[2], Keerthana Chinthala[2], Kevin Hall[1], Shir Gilor[1], Jong Kim[2], Cleverson Souza[1]

[1]Department of Comparative, Diagnostic and Population Medicine, University of Florida College of Veterinary Medicine, Gainesville, FL, USA, [2]Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA

Background: Explainable artificial intelligence (AI) can improve transparency in image-based diagnostic models, but its clinical utility remains underexplored in veterinary oncology and cytology. This study aimed to develop and evaluate explainable image-based classification models using cytological images from canine lymphoma and reactive lymphoid hyperplasia (RLH).

Methods: A total of 5,378 cytological images were obtained from 182 dogs, including 2,284 lymphoma images from 105 dogs and 3,094 RLH images from 77 dogs. Images were stratified by patient and divided into training, validation, and test subsets. Model robustness was assessed using 10-fold cross-validation. Binary classification models were developed using the ResNet-50 architecture. Clinical interpretability was evaluated using Gradient-weighted Class Activation Mapping (Grad-CAM).

Results: The model achieved strong image-level performance, with test accuracy, weighted precision, weighted recall, and weighted F1-score each reaching 0.945. The area under the receiver operating characteristic curve was 0.979, and the area under the precision-recall curve was 0.981. At the patient level, 18 of 20 dogs with lymphoma were correctly classified, and all 16 dogs with RLH were correctly classified, corresponding to a sensitivity of 0.900, specificity of 1.000, positive predictive value of 1.000, and negative predictive value of 0.889. Grad-CAM highlighted regions relevant to lymphoid cell morphology, providing visual explanations for model predictions.

Conclusions: These findings support the potential use of explainable AI for cytological classification of canine lymphoma and RLH in diagnostic decision support. Grad-CAM improved interpretability by identifying diagnostically relevant image regions, suggesting that explainable AI may provide both classification support and pathologically meaningful visual feedback.

Discipline: Clinical pathology, **Topic:** Methods/technology, **Species:** Canine, **Systems:** Hematopoietic

DEVELOPMENT OF A MODEL OF INTERVAL LIKELIHOOD RATIOS: A PARALLEL PATH TO PATHOLOGIST PROBABILITY-MODIFYING TERMS

Helen Michael, Kimberly Yore, Heidi Peta, Jesse Faller, Corie Drake
IDEXX Laboratories, Westbrook, ME, USA

Background: Cytology reports often contain probability-modifying terms, and a need for more clarity in describing subjective confidence has been established. Newly available automated artificial intelligence-based cytology applications have similar challenges communicating the confidence of results within the constraints of cytology as a diagnostic tool.

Objective: Define confidence of IDEXX inVue Dx™ Cellular Analyzer automated mast cell tumor (MCT) results at different certainty thresholds.

Methods: The analyzer automatically produces discrete result categories reflecting increasing certainty of MCT. Automated inVue Dx results range from no mention of mast cells, “low numbers of mast cells,” “MCT suspected,” to “MCT detected.” A comprehensive reference truth was developed for 656 cutaneous/subcutaneous masses using all available pathology data. The interval-specific likelihood ration (iLR) represents the scalar change in odds of disease following a diagnostic result. Therefore, an iLR < 1 indicates a decrease in odds of MCT while an iLR > 1 indicates an increase in odds of MCT (e.g., iLR=10 would indicate a 10-fold increase in odds of MCT post-test).

Results: Different analyzer confidence categories showed strong discriminatory performance. “MCT detected” had a very high likelihood ratio (iLR 189.2, 90% CI: 84.6–660.7), followed by “MCT suspected” (iLR 73.5, 90% CI: 32.3–394.3). “Low numbers of mast cells” indicated limited likelihood of MCT (iLR 3.7, 90% CI: 0.9–13.2), while “not discussed” had a decreased likelihood of MCT (iLR 0.2, 90% CI: 0.1–0.3).

Conclusion: Using iLRs to describe performance of automated cytology results with varying levels of confidence enables first-of-its-kind, standardized clinical pathology reporting of objective confidence.

Discipline: Clinical pathology, **Topic:** Methods/technology, **Species:** Canine, **Systems:** Integument

MALASSEZIA-ASSOCIATED ANAL SACCULITIS WITH ANAL SAC RUPTURE IN A DOG

Lina Crespo Bilhalva, Victoria Shuster, Janey Oline, Emily Brinker, Francisco Conrado
Tufts University, North Grafton, MA, USA

A 13-year-old spayed female Rhodesian Ridgeback was presented for evaluation of an acute episode of hematochezia and vomiting. Physical examination was unremarkable except for obesity (BCS 8/9) and a firm 3.5 cm left anal sac region mass. Fine-needle aspiration yielded a highly cellular sample with moderate blood contamination, keratin, cholesterol crystals, and nuclear streaming. Nucleated cells consisted predominantly of degenerate neutrophils, with fewer macrophages, mixed lymphocytes, occasional reactive fibroblasts, and rare mast cells. Numerous intra- and extracellular, 1.5 x 5.0 µm, ellipsoidal, bilobed yeasts consistent with Malassezia spp. were present. Two days later, the dog was re-presented with weakness, lethargy, and decreased appetite. Abdominal ultrasonography, complete blood count, and serum biochemistry revealed no significant changes. Supportive outpatient management was elected; however, the patient deteriorated further and was euthanized the following day because of progressive hind limb weakness and declining clinical status. At necropsy, the left anal sac was filled with black, pasty, malodorous material and was surrounded by fibrosis. Histologically, yeasts were present in the anal sac corneal layer. The connective tissues surrounding the anal sac were effaced by pyogranulomatous inflammation with similar yeasts and free keratin, consistent with anal sac rupture. Fungal culture of the anal sac wall yielded Malassezia pachydermatis, identified by MALDI-TOF. This case demonstrates yeast-associated anal sacculitis with anal sac rupture, supported by unusually high numbers of Malassezia yeasts within the anal sac lumen and within the surrounding

pyogranulomatous inflammation, highlighting Malassezia as a clinically relevant opportunistic pathogen.

Discipline: Both, **Topic:** Infectious disease, **Species:** Canine, **Systems:** Integument

PHAEOHYPHOMYCOSIS CAUSED BY EXOPHIALA BERGERI INFECTION IN A CAT

Jelena Palić[1], Marrit Prins[1], Els Acke[1], Ursula Mayer[1], Maria Elena Turba[2]
[1]IDEXX Laboratories, Kornwestheim, Germany, [2]Genefast, Forlì, Italy

Background: A 10-year-old spayed female domestic shorthair cat from the Netherlands presented for diagnostic workup of a lesion on the bridge of the nose suspected to be self-induced or caused by a scratch. The cat was in good general condition, with access to outdoors and no travel history. A large nodular lesion was found, spanning from the planum nasale to the point between the eyes, 2 cm in diameter and 2 cm in height. **Objective:** Describe cytologic features of the *Exophiala bergeri* infection in the cat.
Methods: Fine-needle aspirates from the nodule were collected for evaluation.

Results: Macrophages and multinucleated giant cells contained round to oval yeast-like cells measuring 2-4 µm in diameter, with thin clear wall and small basophilic nucleus. Yeast-like cells were exhibiting budding. Rare larger structures, measuring approximately 7-8 µm in length were seen. A series of PCR assays were performed after DNA extraction from cytologic preparations. The resulting sequence was compared with publicly available nucleotide databases, which revealed alignment consistent with fungal organism *Exophiala bergeri*. *Exophiala* species initially grow on culture as brown-black, yeast-like colonies, and are therefore often referred to as “black yeasts”. As the fungal cultures mature, septate brown-black hyphae are formed.
Conclusion: Pheohyphomycosis should be included in the differential list even in cases in which most of the organisms detected in macrophages are small budding yeast-like forms resembling *Histoplasma spp*., and without obvious dark pigment present in the cell wall. Molecular identification with DNA extraction and PCR is recommended for accurate species identification.

Discipline: Clinical pathology, **Topic:** Infectious disease, **Species:** Feline, **Systems:** Integument

DISSEMINATED MYCOBACTERIUM AVIUM INFECTION IN A DOG WITH PROGRESSIVE GASTROINTESTINAL DISEASE

Danielle Ahmed, Aaron Dunkerson-Kurhumov, Christopher Premanandan, Laurie Millward
College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA

A 6-year-old spayed female Siberian Husky presented with a one-month history of progressive gastrointestinal disease characterized by lethargy, fever, anorexia, weight loss, abdominal pain, and diarrhea that began after killing a skunk. The patient had a history of killing small mammals, including rabbits and opossum. Initial treatment with broad-spectrum antimicrobials resulted in improvement, but the patient’s symptoms returned and the onset of hematochezia prompted referral to The

Ohio State University internal medicine service. A complete blood count identified a moderate, non-regenerative, normocytic, normochromic anemia (HCT 26%) and leukocytosis (21,000/µL) characterized by mature neutrophilia and mild lymphopenia. Abdominal ultrasound revealed an enlarged spleen with a honeycomb pattern and mildly heterogeneous liver. Fine needle aspirates of spleen and liver were submitted for cytologic evaluation, which revealed marked macrophagic inflammation and numerous, negatively staining, intracellular rods consistent with mycobacteria. Bacterial culture subsequently confirmed Mycobacterium avium. Despite aggressive antimicrobial therapy, the patient was euthanized due to disease severity. Post-mortem evaluation demonstrated multifocal, severe, granulomatous hepatitis, splenitis, nephritis, and lymphadenitis, with perivascular granulomatous infiltrates in the lung. Acid-fast rod-shaped bacteria were identified.

Mycobacterium avium complex (MAC) is a group of intracellular bacteria with a lipid coat that provides the characteristic negative staining on cytology. While the disease is usually self-limiting in adult dogs, it is difficult to treat when disseminated because of its inherent resistance to beta-lactam antibiotics. This case demonstrates the utility of cytologic evaluation in guiding diagnosis and clinical management.

Discipline: Clinical pathology, **Topic:** Infectious disease, **Species:** Canine, **Systems:** Gastrointestinal

THEILERIA ORIENTALIS IN AN ADULT BEEF COW: AN EMERGING TICK-BORNE DISEASE

Kari Frankhouse, Andrew Niehaus, Matthew Caldwell, Risa Pesapane, Laurie Millward, Hamideh Esmaeilzadeh, Ally Bonney, Maxey Wellman
The Ohio State University, Columbus, OH, USA

An 8-year-old Aberdeen Angus cow from southern Ohio presented for stumbling and weight loss after calving 4 days prior to presentation. Several other cows on the same farm had recently aborted and several of those cows died. Physical examination revealed tachycardia, tachypnea, and pale, icteric mucous membranes. A CBC showed marked, regenerative anemia. A blood smear review revealed numerous small, irregularly shaped intraerythrocytic structures consistent with *Theileria orientalis*, which was confirmed by PCR. *Theileria orientalis* is a protozoal parasite that causes hemolytic anemia, pyrexia, icterus, malaise, ill thrift, sporadic abortions, and death in cattle of all ages. The parasite is transmitted by the Asian longhorned tick, *Haemaphysalis longicornis*. Infections can be economically devastating due to decreased milk production, reproductive losses, morbidity, and mortality. There currently is no effective treatment. The farmer also noted that many deer had been dying on multiple farms in the area, prompting screening for additional infectious diseases. The cow was positive for Enzootic Hemorrhagic Disease based on PCR. Enzootic Hemorrhagic Disease is an orbivirus transmitted by biting midges, particularly *Culicoides sonorensis*, which were prevalent due to recent drought conditions. Enzootic Hemorrhagic Disease causes severe, often fatal disease in white-tailed deer, but typically causes subclinical infection in cattle. However, co-infection with *Theileria sp*. has the potential to cause serious clinical disease in cattle, especially as tick populations continue to spread across the United States. This case highlights the importance of monitoring infectious diseases that may have an increasing economic impact on the cattle industry.

Discipline: Clinical pathology, **Topic:** Infectious disease, **Species:** Bovine, **Systems:** Hematopoietic

ELECTROPHORETIC URINE PROTEIN BANDING PATTERNS IN HEALTHY CATS AND CATS WITH KIDNEY DISEASE

Kate Sarkan[1], Catherine Yeoman[2], Katelyn Brusach[3], Sarah Shropshire[4], Rachel Cianciolo[5], Jessica Quimby[1], Jessica Hokamp[2]
[1]College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA, [2]Texas A&M University, College of Veterinary Medicine & Biomedical Sciences, College Station, TX, USA, [3]Mayo Clinic, Department of Quantitative Health Sciences, Rochester, MN, USA, [4]Colorado State University College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO, USA, [5]Niche Diagnostics, LLC, Columbus, OH, USA

Background: Determining the cause of proteinuric renal disease in cats is a diagnostic challenge and often requires renal biopsy for definitive diagnosis. The use of urine electrophoretic banding patterns to determine origin of renal proteinuria is underexplored in cats.

Objective: Determine the urine protein banding patterns of clinically healthy cats and compare to cats with histopathology-confirmed kidney disease.

Methods: Biobanked urine supernatant was analyzed from 20 clinically healthy cats and 45 cats with histopathology-confirmed kidney disease: 13 stable chronic kidney disease (CKD) with tubulointerstitial (TI) damage; 20 immune complex-mediated glomerulonephritis (ICGN); 12 non-ICGN glomerular disease (amyloidosis, podocytopathy, focal segmental glomerulosclerosis). Urine proteins were separated with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Low (LMW, <36.5 kDa), intermediate (IMW, 36.5-66.3 kDa), high (HMW, 66.3-200 kDa), and very high (VHMW, >200 kDa) molecular weight bands were enumerated.

Results: Healthy cat urine contained protein bands at molecular weights consistent with albumin, cauxin, and occasionally uromodulin. The number of LMW and IMW bands were significantly greater in ICGN and non-ICGN glomerular disease than healthy cats and in ICGN cats compared to TI CKD cats. HMW and VHMW bands were significantly increased in ICGN and non-ICGN glomerular cats compared to healthy and TI CKD cats. ICGN cats had the highest median number of VHMW bands, and >2 VHMW bands were 100% sensitive and 78% specific for ICGN.

Conclusions: Urine SDS-PAGE differentiates cats with glomerular disease from healthy cats and TI CKD cats and has modest to high sensitivity and specificity for identifying ICGN cats.

Discipline: Anatomic pathology, **Topic:** General pathology, **Species:** Feline, **Systems:** Urinary

EVALUATION OF DIRECT ANTIGLOBULIN TESTING FOLLOWING ALLOGENEIC BLOOD TRANSFUSION IN DOGS

Bridgette Murphy[1], Dana LeVine[1], Maria Naskou[1], Maria Matilde Merodio[2], Clémence Visseaux[3], Marie-Claude Blais[3], Austin Viall[4], Erin McQuinn[2]
[1]Auburn University, Auburn, AL, USA, [2]Iowa State University, Ames, IA, USA, [3]Université de Montréal, Saint-Hyacinthe, QC, Canada, [4]UC Davis School of Veterinary Medicine, Davis, CA, USA

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 the 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 of conversion from DAT negative to DAT positive, in transfusion naïve dogs following allogeneic blood transfusion. To characterize the time interval between blood transfusion and DAT conversion. To inform clinicians regarding the reliability of the DAT for diagnosis of IMHA in previously transfused dogs.

Methods: Transfusion naïve dogs requiring blood transfusion have a pre-transfusion DAT and major crossmatch performed using polyspecific canine anti-globulin/anti-complement gel column technology (Alvedia). Patients with a positive pre-transfusion DAT, history of steroid administration, blood product transfusion, and suspected autoimmune disease are excluded. Samples are collected 24 hourly for DAT testing, depending on patient availability.

Preliminary results: In 49 patients, one positive DAT has been detected (T24), which was followed by further negative results (T36, T48). Post-transfusion DATs range from T24 to T1080 (45 days).

Conclusions: Preliminary results suggest allogenic blood transfusion does not cause sustained DAT seroconversion, and positive results should not be dismissed as the result of allogeneic blood transfusion in suspected IMHA patients.

Discipline: Clinical pathology, **Topic:** Immunology, **Species:** Canine, **Systems:** Hematopoietic

EVALUATION OF LARGE LANGUAGE MODELS FOR SUPPORTING CLINICAL PATHOLOGY DATA INTERPRETATION IN NON-CLINICAL SAFETY ASSESSMENT

Valerie Wong[1], Corinne Jones[2], David Ledieu[2], Megumi Onishi-Seebacher[2], Dominique Brees[2]
[1]Novartis, Cambridge, MA, USA, [2]Novartis, Basel, Switzerland

Background: There is limited published information on the utility of artificial intelligence (AI) for supporting clinical pathology data interpretation in non-clinical safety assessment.

Objective: To evaluate the performance of three large language models (LLMs; GPT-5.4, Claude Opus 4.6, and Gemini 2.5 Pro) and their collective insights ("Colin") for determining test article-related effects (TAREs) in clinical pathology datasets.

Methods: Hematology, coagulation, and clinical chemistry data from twenty-two rat studies were annotated with dose, sex, and timepoint and presented to the LLMs in an analysis-ready format. The prompt comprised four publications on toxicologic clinical pathology (TCP) and instructions for the LLMs and Colin. The instructions mainly called for the AI entities to determine TAREs and non-TAREs, while stating the confidence level and rationale. The AI outputs were compared to the human interpretation using pairwise agreement statistics (Fisher's exact test). Performance metrics, such as negative predictive values and accuracy, were computed.

Results: All four AI entities generated outputs relevant to TCP. Agreements with the human interpretation were high (GPT: 94.1%; Claude: 94.1%; Gemini: 92.9%; Colin: 95.0%). The median number of test article-related effects per study for each sex was 4.0, and the negative predictive values (NPVs) were high (GPT: 98.8%; Claude: 98.7%; Gemini: 98.5%; Colin: 98.4%).

Conclusion: When prompted with the appropriate context and clearly annotated data, all AI entities performed similarly to human clinical pathologists in determining TAREs. The high NPVs of the AI outputs demonstrated an opportunity for efficiency gains via AI screening of negative results prior to human reviews.

Discipline: Clinical pathology, **Topic:** Other (Artificial intelligence), **Species:** Rodents, **Systems:** Systemic

Experimental Disease Focused Scientific Session

Tuesday, 1:30 pm-5:00 pm
Jessica Fortin, DACVP (AP)

EFFECTS OF CHRONIC LOW-DOSE RADIATION ON LIVER FUNCTION: A STUDY TO SUPPORT MILITARY READINESS IN AIR AND SPACE TRAVEL

Elise LaDouceur[1], Lisa Crevoiserat[1], Sarah Hay[2], Brendon Bagley[2], Maura Rabbette[2], Liem Phan[2], Robert Rigor[2]
[1]Joint Pathology Center, Silver Spring, MD, USA, [2]60th MDG, Clinical Investigation Facility, Travis AFB, CA, USA

Introduction: This study investigates if chronic, low-dose ionizing radiation, experienced by military personnel in environments like high-altitude and high-latitude air travel, Navy nuclear submarines, battlefield exposures to radiation, and space travel, causes liver damage.

Methods: 12 male, 6-month-old, Yucatan minipigs were utilized to model chronic solar radiation exposure. Six treatment animals were subjected to an 8-week fractionated x-ray series, delivering a frontal-directed, whole-body total dose of 1 Gy (n=2), 2 Gy (n=2), or 4 Gy (n=2), followed by a 4-week recovery. Two negative controls (no radiation, identical time course) and four positive controls (unfractionated 2 Gy dose followed by 4-week [n=2] or 12-week recovery [n=2]) were included. Weekly blood samples were collected prior to radiation for alanine transaminase (ALT), aspartate amino-transferase (AST), and alkaline phosphatase (ALP) analysis. At week 12, liver histopathology was assessed.

Results: All positive controls animals (n=4) had moderate zonal (portal) hepatocellular atrophy, and lack of PAS-positive cytoplasmic granules, which were present in all treatment groups and negative control animals. Treatment groups with chronic 2 Gy and 4 Gy had the highest mean elevations of ALT (57.15 U/L and 61.5 U/L), though marginally above the control mean (48.65 U/L), and only the 4 Gy group was above reference intervals (31-58 U/L).

Conclusions: These findings suggest that cumulative doses up to 4 Gy may induce subtle physiological shifts, but do not cause appreciable hepatocyte damage across the doses and time course of this study, providing a baseline for assessing long-term operational readiness in chronic low-dose radiation exposure environments.

Discipline: Anatomic pathology, **Topic:** Comparative pathology, **Species:** Porcine, **Systems:** Hepatobiliary

INTEGRATED IMAGING-PATHOLOGY FRAMEWORKS FOR TISSUE LOCALIZATION, VALIDATION, AND BIOLOGIC INTERPRETATION IN EXPERIMENTAL DISEASE MODELS

Caralyn Labriola[1,2], Margaret Terry[2], Rachele Bochart[1], Aaron Barber-Axthelm[1], Kimberly Armantrout[1], Ava Dickinson[1], Gavin Zilverberg[1], Rachel Dannay[1], Louis Picker[1,2], Michael Axthelm[1,2]
[1]Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, OR, USA, [2]Oregon Health and Science University, Beaverton, OR, USA

Imaging-pathology correlates offer significant opportunities to improve localization and interpretation of biologically relevant tissues and lesions in experimental pathology. Imaging and pathology provide complementary data, with imaging offering spatial and temporal context, and pathology affording definitive tissue characterization. The objective was to develop imaging-guided pathology frameworks integrating multiple imaging modalities with targeted tissue collection to enhance localization, annotation, validation, and biologic interpretation across experimental disease models. Two representative imaging-guided pathology applications are presented. First, in vivo and post-mortem near-infrared (NIR) imaging was used to map lymphatic vessel (LV) drainage, assess LV meristic and morphologic characteristics, and identify LV-rich regions of interest (ROIs) for targeted sample collections, with fluorescent signal confirmation in tissue blocks and histologic sections, across peripheral, mesenteric, and cerebrospinal fluid-associated lymphatic structures. Second, serial thoracic CT was spatially correlated with gross and histopathology to localize pulmonary tuberculosis lesions at necropsy, validate subtle radiographic abnormalities, and enable retrospective temporal evaluation of granuloma development. Across multiple models, imaging-guided tissue localization

improved identification and biologic profiling of regions challenging to evaluate via imaging or pathology alone. Post-mortem NIR imaging detected LV-rich ROIs corresponding to those observed in vivo and enabled precise harvest of lymphatic structures for downstream analyses. CT-guided lesion localization enabled confirmation of small lesions at necropsy while, reciprocally, gross and histopathology validated radiographic findings, establishing lesion-specific imaging-pathology correlations for longitudinal assessment of disease progression. Integrated imaging-pathology frameworks improve data collection, interpretation, and validation and have broad applications in experimental disease research with potential adaptability to oncology and diagnostic pathology.

Discipline: Anatomic pathology, **Topic:** Methods/technology, **Species:** NHP, **Systems:** Systemic

INTRANEURONAL MUTANT SERPINA1 EXPRESSION IN ALPHA-1-ANTITRYPSIN DEFICIENCY MOUSE MODELS

Amy Flis, Kelly Keefe, Tatyana Taksir, Hong Ma, Colleen Maloney, John Bladon, June Baik, Christophe Lechauve, Caroline Morel, Ingeborg Langohr, Christian Mueller, Can Kayatekin, Dinesh Bangari
Sanofi, Cambridge, MA, USA

Background: Alpha-1-antitrypsin (A1AT) deficiency is a genetic disorder in which mutant A1AT accumulates in hepatocytes, leading to liver damage from gain-of-toxic-function, and is not released into circulation, leading to pulmonary disease from loss-of-function. The most common cause of severe disease is the PiZ mutation of A1AT-encoding gene *SERPINA1*. Transgenic PiZ mouse models recapitulate hepatic disease, but transgene-associated pathology in other tissues is not as well-characterized.

Objective and **Methods:** To identify mutant A1AT accumulation and associated pathology, we used H&E, PAS-diastase, and IHC and/or ISH for human *SERPINA1* (*hSERPINA1*) to evaluate a multicopy PiZ mouse model (Jax #035411) and two single-copy PiZ mouse models with *mSerpina1* knockout (*hSERPINA1* full-length model PiZ-101 and optimized model PiZ-124). To evaluate neuromuscular function, we performed wire hang and grip strength tests on multicopy-PiZ mice.

Results: Within neurons of dorsal root and trigeminal ganglia of multicopy-PiZ mice, we observed PAS-positive, diastase-resistant eosinophilic cytoplasmic globules, which were hSERPINA1-positive by IHC. Cytoplasmic *hSERPINA1* ISH signal was present in globule-laden neurons, as well as spinal cord neurons. While neither single-copy PiZ model had neuronal globules, PiZ-101 mice had cytoplasmic *hSERPINA1* ISH signal in neurons in dorsal root ganglia, trigeminal ganglia, and spinal cord ventral horns. By wire hang, multicopy-PiZ mice had faster latency to fall compared to C57BL/6J matched mice.

Conclusion: We identified neuronal expression of mutant *hSERPINA1* in two PiZ mouse models, along with its functional effect in the multicopy-PiZ model. Further work will evaluate neuronal *SERPINA1*'s relevance for A1AT deficiency patients through human samples and human-based models.

Discipline: Anatomic pathology, **Topic:** Comparative pathology, **Species:** Rodents, **Systems:** Nervous

HISTOPATHOLOGIC EVALUATION OF EXPERIMENTAL OLIGOFRUCTOSE-INDUCED EQUINE LAMINITIS FOLLOWING CLOPIDOGREL ADMINISTRATION

Krysten Schmidt[1,2], Timothy Lescun[2], Sandra Taylor[2], Abigail Cox[1,2]
[1]Purdue University, West Lafayette, IN, USA, [2]Purdue University College of Veterinary Medicine, West Lafayette, IN, USA

Background: Laminitis is a common, debilitating disease of horses, donkeys, and ponies. The oligofructose-induced laminitis model reliably produces acute developmental and early active-phase laminitis characterized by epidermal lamellar detachment, basement membrane disruption, dermal inflammation, and vascular microthrombus formation. Because platelet aggregation contributes to microvascular dysfunction, clopidogrel has been investigated as a potential therapeutic due to its inhibitory effects on platelet activation and thrombus formation.

Objective: To characterize the histopathologic lesions and compare morphometric and semiquantitative histologic outcomes in horses with experimental oligofructose-induced laminitis treated with clopidogrel or placebo.

Methods: Eleven horses were enrolled at the time of this abstract submission in a randomized, blinded study evaluating clopidogrel as a potential treatment for oligofructose-induced laminitis. Morphometric outcomes included secondary epidermal lamellar (SEL) length, width, and angle. Semiquantitative histologic grading assessed basement membrane integrity, dermal inflammation, vascular and microthrombotic lesions, and lamellar detachment.

Results: Histopathologic lesions consistent with acute oligofructose-induced laminitis were observed in both groups and included epidermal lamellar detachment, basement membrane disruption, dermal inflammation, vascular lesions, and microthrombi. No differences were identified between the clopidogrel- and placebo-treated horses for composite scores of basement membrane integrity, dermal inflammation, vascular and microthrombotic lesions, and lamellar detachment (Mann-Whitney $U = 21.5$, $p = 0.26$, rank-biserial $r = -0.43$).

Conclusion: Clopidogrel treatment was not associated with detectable differences in the histopathologic or morphometric outcomes evaluated. However, additional investigations incorporating clinical, physiologic, and molecular outcomes are needed to more fully assess its potential effects in laminitis.

Discipline: Anatomic pathology, **Topic:** General pathology, **Species:** Equine, **Systems:** Integument

COMPARATIVE PATHOLOGY AND GENETIC FEATURES OF JAPANESE HIGH PATHOGENICITY AVIAN INFLUENZA VIRUS ISOLATES DURING THE 2025–2026 SEASON

Hayate Nishiura, Takao Iketani, Hiroshi Iseki, Yoshihiro Takadate, Saki Sakuma, Junki Mine, Ryota Tsunekuni, Yuko Uchida, Miki H Maeda, Kazuhiro Kojima, Tomoyuki Shibahara, Kohtaro Miyazawa
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Background and Objective
Clade 2.3.4.4b high pathogenicity avian influenza viruses (HPAIVs) have caused outbreaks in Japan every year since 2020. In Japan, HPAIVs are further classified by the hemagglutinin gene into subgroups such as G2c (Asian group) and G2d (group distributed in North America, the Bering Sea, the Russian Far East, and Japan). We genetically analyzed G2c and G2d HPAIVs and pathologically evaluated G2c isolates from Japan during the 2025–2026 season.
Methods
Whole-genome sequences of the isolates were analyzed phylogenetically. Isolates representing newly identified genotypes were evaluated by experimental infection in chickens (n = 5/group) inoculated intranasally with 10^3–10^6 50% Egg infectious dose (EID_{50}) of virus, and carcasses were examined histopathologically.
Results
Three new G2c genotypes (G2c-19, G2c-20, and G2c-21), one G2d genotype (G2d-0), and one unassigned European genotype were identified. Experimental infections involved only G2c genotypes. 50% chicken lethal dose values for G2c-19, G2c-20, and G2c-21 were 4.50, 4.17, and 4.50 $\log_{10}$ EID_{50}, respectively, with mean death times of 2.0, 3.1, and 3.5 days. Histopathologically, multifocal necrosis was observed in multiple systemic organs in all groups. Severe interstitial pneumonia was observed in G2c-19-infected chickens, but not in 2/5 G2c-20-infected chickens or any G2c-21-infected chickens; in these seven birds, viral antigen was not detected in systemic endothelial cells. Some chickens without pneumonia had severe histiocytic myocarditis.
Conclusion
Genetic diversity of HPAIVs was confirmed in Japan during the 2025–2026 season. Although virulence in chickens was comparable among genotypes, pathological findings indicated genotype-dependent differences in pathogenesis.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Avian, **Systems:** Systemic

VARIATION IN SUB-STRAIN PATHOGENESIS AND CONSPECIFIC TRANSMISSION OF HIGHLY PATHOGENIC AVIAN INFLUENZA (H5N1, CLADE 2.3.4.4B) IN DOMESTIC CATS

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Background: Transmission of H5N1 highly pathogenic avian influenza (HPAIV) remains poorly understood in domestic cats. The primary assumption is that cats acquire HPAIV by consuming contaminated food. However, transmission may occur between cats through social behavior. Mutations in viral genes associated with mammalian adaptation have been identified in isolates of H5N1 derived from B3.13 and D1 genotypes, while these mutations are absent in B3.1.

Objective: Our goal is to assess genotype differences in pathogenesis and transmission between mammalian and poultry adaptive H5N1.

Methods: Three groups of 5 cats were intranasally and orally infected with genotypes B3.1, B3.13, and D1.1. After 2 days, contact animals were introduced and allowed to free roam with infected animals. Viral shedding was monitored 3 times per week through the study. At end of study, or following euthanasia due to disease

progression, animals were necropsied and tissues collected for virus isolation, PCR and sequencing, histopathology, and immunohistochemistry.

Results: We identified variable pathogenesis in cats infected with each genotype. Cats inoculated with B3.13 developed severe clinical disease necessitating euthanasia, while those infected with B3.1 developed mild clinical disease and recovered. Cats in the D1.1 group demonstrated mild to moderate clinical disease. Within the D1.1 group, one contact cat contracted H5N1 and shed of infective virus, confirming conspecific transmission of D1.1 in domestic cats.

Conclusion: The D1.1 genotype of H5N1 allows for conspecific transmission between cats with less severe disease than seen in genotype B3.13, suggesting viral adaptation to domestic cats.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Feline, **Systems:** Systemic

EQUINE HERPESVIRUS 1 IMMEDIATE-EARLY PROMOTER-ASSOCIATED LONG NON-CODING RNA IS COEXPRESSED WITH ITS IE GENE, IN VIVO

Paul Valcorza[1], Fuka Takahashi[1], Yosuke Sato[2], Keisuke Aoshima[1], Takashi Kimura[1]
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Background. Although herpesviruses encode numerous non-coding RNAs (ncRNAs), few have been identified in equine herpesvirus 1 (EHV-1) infections. Recently, our group demonstrated that a 998-nucleotide ncRNA antisense to the EHV-1 immediate-early (IE) gene promoter, named *IE pancRNA*, is expressed in a rat neuronal cell line, and functions as transactivator of the IE gene.

Objective. This study further demonstrates the *in vivo* expression of IE pancRNA, providing novel insights into its potential role during natural EHV-1 infection.

Methods. Rat neuronal cell line (RN33b-A68B2M) and BALB/c mice were infected with EHV-1 Ab4 strain *in vitro* and *in vivo*, respectively. Archived paraffin blocks from PCR-confirmed equine abortion cases were also examined.

Results. Histological analysis of tissues from EHV-1-infected mice and naturally-aborted equine fetuses revealed distinct intranuclear inclusion bodies and lymphocytic infiltration. RT-qPCR detected both IE pancRNA and IE mRNA in the lungs, brains, and trigeminal nerves of infected mice. RNA fluorescence *in situ* hybridization (RNA-FISH) demonstrated co-expression of both transcripts in EHV-1-infected RN33B-A68B2M cells, infected mice, and aborted equine fetuses. Notably, characteristic spherical structures formed by IE pancRNA were consistently observed within the nuclei in both *in vitro* and *in vivo* settings. Furthermore, combined RNA-FISH and immunofluorescence showed no distinct colocalization between IE pancRNA and chromatin modification domains.

Conclusions. These findings demonstrate that IE pancRNA and IE mRNA are co-expressed both *in vitro* and *in vivo* during active phase of EHV-1 infection. This

supports the hypothesis that IE pancRNA may contribute to the promotion or maintenance of IE gene expression during productive infection.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Equine, **Systems:** Systemic

THE POTENTIAL FOR JAMACIAN FRUIT BATS TO TRANSMIT MONKEYPOX VIRUS

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Monkeypox virus (MPXV) is a zoonotic pathogen that has caused multiple recent outbreaks of human Mpox in non-endemic regions of the world. It is important to identify potential animal reservoirs of MPXV to better understand and prevent its spread. To assess susceptibility of Jamaican fruit bats (*Artibeus jamaicensis*) to MPXV, viral replication was first evaluated in vitro. MPXV clade IIa replicated to similar titers in Jamaican fruit bat fibroblasts and susceptible monkey epithelial cell lines. Next, four Jamaican fruit bats were inoculated intranasally with 10^6 foci-forming units of MPXV clade IIa. As controls, two bats were mock-inoculated with saline. The bats were weighed daily and monitored for clinical signs of disease. Nasal and rectal swabs were collected daily, blood was collected weekly, and a necropsy was performed after humane euthanasia on Day 28 post-inoculation. The swabs were analyzed using qPCR with three primer-probe sets targeting MPXV DNA. No weight loss, lesions, or visible disease signs occurred; however, qPCR testing detected viral DNA in nasal swabs from two of four MPXV-infected bats and in rectal swabs from all four MPXV-inoculated bats up to 8 days post-inoculation. These results suggest that, despite the absence of clinical disease, MPXV-infected bats may contribute to viral transmission via shedding in oronasal secretions and feces.

Discipline: Clinical pathology, **Topic:** Infectious disease, **Species:** Wildlife/Zoo, **Systems:** Systemic

Natural Disease Focused Scientific Session I

Tuesday, 1:30 pm-5:00 pm
Mason Jager, DVM PhD DACVP (AP)

PREDATORS UNDER PRESSURE: PATHOBIOLOGY OF HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS VERSUS WEST NILE VIRUS IN NORTH AMERICAN RAPTORS

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Background: Unique biology among raptors predisposes them to health risks, including infectious diseases. West Nile virus (WNV) was introduced to North America in 1999, while clade 2.3.4.4b highly pathogenic avian influenza viruses (HPAIV) were introduced in 2021. Avian mortality events and efficient intracontinental spread followed both introductions. Alongside public and veterinary health risks, potential impacts of these viruses on wild birds are concerning.

Objective: We compared pathogenesis and lesion patterns for these viruses in North American raptor species.

Methods: We reviewed raptor diagnostic cases at the Southeastern Cooperative Wildlife Disease Study since each respective virus introduction.

Results: Both viruses caused fatal systemic and neurologic disease of varied, and sometimes overlapping, lesion patterns. West Nile disease was diagnosed in 44 raptors (11 species), most commonly red-tailed hawks (14) and bald eagles (11). HPAI was diagnosed in 324 raptors (9 species), most often in black vultures (188) and bald eagles (83). Both viruses caused myocardial necrosis and/or lymphoplasmacytic myocarditis, which were more consistent and severe with WNV; and neuronal necrosis and/or lymphoplasmacytic meningoencephalitis, which were more consistent and severe with HPAIV. Multiorgan necrosis was widespread and common with HPAIV, while necrosis and inflammation were less common outside of brain and heart for WNV. Coinfections occasionally were diagnosed in raptors infected with each virus.

Conclusion: Despite differing pathogenesis between WNV and HPAIV, lesion patterns overlap, while species-specific disease manifestation varies. Both viruses are now established in North America, underscoring the need for continued monitoring as host–virus interactions evolve and potential conservation impacts emerge.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Wildlife/Zoo, **Systems:** Systemic

OCULAR MANIFESTATION OF SARCOCYSTOSIS IN A COLONY OF WHITE-FRONTED BEE-EATERS

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Clinically significant *Sarcocystis* infection in birds is often due to a pulmonary or neurologic form of the disease. This study describes the pathologic features of an unusual manifestation of avian sarcocystosis primarily affecting the eye. Over a period of 8 months (October 2025–May 2026) at a single zoological institution, 11 cases of sarcocystosis occurred in a colony of 34 white-fronted bee-eaters (*Merops bullockoides*) resulting in death or euthanasia (32.4% mortality rate). Of these mortalities, 7 young adults (0.6–1.8 years of age, mean 1.0 year) had ocular sarcocystosis identified as the most significant disease leading to death (n = 2) or euthanasia (n = 5). Acute pulmonary sarcocystosis was the cause of death in 3 birds. Neurologic sarcocystosis led to euthanasia in 1 case. In the cases of ocular sarcocystosis, clinical signs frequently included lethargy, weight loss, and vision deficits. Gross examination of fixed globes in 3 cases revealed an opaque vitreous, geographic chorioretinal scarring, and occasionally a thickened pecten. Histologically, 5 of 6 cases with both globes available for examination had bilateral changes consisting primarily of a proteinaceous exudate in the vitreous, choroiditis, pectenitis, retinitis, and retinal degeneration, atrophy, necrosis, and metaplasia. Protozoal schizonts and merozoites were found in the retina of 4 of 7 cases. *Sarcocystis* PCR on Davidson's- or formalin-fixed paraffin-embedded eye scrolls and subsequent sequencing in 3 of 4 cases detected *S. falcatula*. Ocular sarcocystosis

should be considered a differential diagnosis in this species with ocular abnormalities.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Wildlife/Zoo, **Systems:** Eye/ear

CHARACTERIZATION OF SIN NOMBRE HANTAVIRUS TISSUE TROPISM IN NATURALLY INFECTED DEER MICE USING RT-QPCR AND RNASCOPE IN SITU HYBRIDIZATION

Rebecca Radisic, Alexandra Stone, Ernesto Rojas-Sanchez, Reena Grewal, Erin Howell, Tracy Drazenovich, Alexandre Tremeau-Bravard, Jalika Joyner, Olivia Cords, Allison Huske, Ken Jackson, Patricia Pesavento, Brian Bird
University of California, Davis, Davis, CA, USA

Background: The North American deer mouse (*Peromyscus maniculatus*) is the primary reservoir of Sin Nombre virus (SNV, *Orthohantavirus sinnombreense*), the etiologic agent of hantavirus cardiopulmonary syndrome in humans. The role of reproductive tissues in SNV maintenance and transmission in rodents remains unclear.

Objective: Characterize SNV tissue distribution in naturally infected deer mice, with emphasis on reproductive tissues.

Methods: Free-ranging *P. maniculatus* (n=169) were collected from an SNV-endemic site. Oral and urogenital swabs, testes or ovary, kidney, and pooled spleen-liver-lung were evaluated by SNV RT-qPCR. A subset with SNV-positive reproductive tissues underwent histopathology, RNAscope in situ hybridization (ISH, targeting positive sense SNV RNA), and SNV RNA strand specific RT-qPCR.

Results: Overall, 48.5% (n=82/169) of individuals were RT-qPCR positive in at least one specimen. Reproductive tissues were RT-qPCR positive in 32% (54/169) of mice. SNV strand-specific testing revealed higher genomic (negative sense) RNA loads compared to anti-genomic (positive sense) in reproductive tissues versus kidney and pooled spleen-liver-lung. ISH was performed on eight rodents (four RT-qPCR positive and four negative). SNV RNA signal was not detected in negative animals. Among positive animals, signal localized within lung, spleen, and kidney, with no signal detected in reproductive tissues.

Conclusions: SNV RNA was detected in multiple tissues by RT-qPCR and ISH. Absence of positive-sense ISH signal in reproductive tissues despite RT-qPCR positivity, combined with lower levels of positive-sense RNA by strand-specific PCR, suggests reproductive tissues differ from other organs in SNV replication and tissue localization. Analyses of epididymis, seminal vesicles, uterus, and vagina are in progress.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Wildlife/Zoo, **Systems:** Systemic

THE PATHOLOGY, HOST RANGE, AND GEOGRAPHIC DISTRIBUTION OF EMERGING ZOONOTIC THELAZIA CALLIPAEDA IN PENNSYLVANIA, USA

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Thelaziosis is a zoonotic orbital disease caused by a spirurid nematode of the genus *Thelazia.* Their definitive hosts include people and a variety of domestic and wild animals. Originating in Asia with subsequent spread to Europe, *Thelazia callipaeda* has been implicated in most cases of thelaziosis. In 2020 the first autochthonous case in the United States of America (USA) was reported in a domestic dog from New York; since then, the incidence of thelaziosis has expanded. The parasite's novel range and naive hosts raise concerns about the potential for clinical disease and spillover events. Our objectives were to investigate the host range of *T. callipaeda*, characterize associated histologic lesions in the definitive hosts, and provide insights into the pathogen's epidemiology and ecology in Pennsylvania, USA. During the 2025/2026 hunting season in Pennsylvania, USA, wildlife were opportunistically examined at check stations for evidence of infection with *Thelazia* spp. Infected hosts included 118 black bears, 13 coyotes, two fishers, and one red fox. Cases were predominantly located in northeastern Pennsylvania with occasional cases as far as the southwestern part of the state. The eyes from six of these animals were fixed in 10% NBF and routinely processed with H&E. Histologically, lesions consisted of mild to moderate regional to multifocal chronic ulcerative conjunctivitis and blepharitis with occasional hemorrhage. Our findings reflect the establishment and increased prevalence of *T. callipaeda* in non-endemic regions, further highlighting the necessity for continued research into the clinical significance and extent of disease in humans, domestic animals, and wildlife.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Wildlife/Zoo, **Systems:** Eye/ear

Posters: Diagnostic Pathology

1: A RARE VARIANT OF MULTICENTERIC LYMPHOMA WITH MOTT CELL DIFFRENTIATION IN A 6-YEAR-OLD POTBELLIED PIG (SUS SCROFA DOMESTICUS)

Sahar Abdelrahman[1], Zi-An Yu[1], Matt Miesner[2], Matthew Aviles[2], Nicolette Cassel[2], Nathan Jackson[2], Clay Hallman[2], Lorelei Bojorquez[2], Ji-Hang Yin[1]
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Lymphoma with Mott cell differentiation (MCL) is an uncommon variant of lymphoma with limited reports in veterinary medicine. A 6-year-old castrated male Potbellied pig (*Sus scrofa domesticus*) was presented to the Veterinary Health Center at Kansas State University with a 3-week history of suspected respiratory disease. On presentation, the patient was in respiratory distress. Thoracic and abdominal ultrasonography revealed effusion in both cavities and multiple mesenteric nodules.

Radiographs showed loss of serosal detail and splenomegaly. Due to the poor prognosis, humane euthanasia was elected.

Gross examination revealed generalized lymphadenomegaly and multifocal pale tan to white, firm, variably sized nodules scattered throughout the lungs, liver, kidneys, spleen, intestines, and urinary bladder. Impression smears of a mesenteric lymph node revealed a heterogeneous lymphoid population composed of approximately 40-50% variably sized lymphoid cells and 50-60% mature plasma cells with abundant Mott cell differentiation.

Histopathologic evaluation revealed an unencapsulated, infiltrative, densely cellular neoplasm that extensively effaced multiple organs. The neoplasm contained two distinct cellular populations: (1) pleomorphic small to large-sized lymphocytes (2) well-differentiated Mott cells containing abundant Russell bodies. Immunohistochemistry revealed diffuse, strong membranous CD20 immunoreactivity in approximately 90% of neoplastic lymphocytes and Mott cells, whereas approximately 30% of both populations exhibited immunoreactivity for CD79a. Neither cell population demonstrated nuclear immunoreactivity for MUM1.

A diagnosis of multicentric MCL was made given the distribution, and histologic and immunohistochemical features. To the authors' knowledge, this is the first report of MCL in a potbellied pig, and this variant appears to exhibit aggressive biological behavior.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Porcine, **Systems:** Hematopoietic

2: DIGITAL KI-67 QUANTIFICATION IN CANINE ORAL AND CUTANEOUS MELANOCYTIC TUMORS: COMPARISON WITH MANUAL ASSESSMENT AND PROGNOSTIC PERFORMANCE

Andrea von Däniken[1], Sohvi Blatter[1], Leonore Aeschlimann[1], Maria-Ines Crescio[2], Camille Hanot[3], Chiara Brachelente[4], Ilaria Porcellato[4], Sven Rottenberg[1], Sara Soto[1], Simone De Brot[1]
[1]Institute of Animal Pathology Bern, Bern, Switzerland, [2]National Reference Center of Veterinary and comparative Oncology (CEROVEC), Genoa, Italy, [3]Médi-Vet SA, Lausanne, Switzerland, [4]Department of Veterinary Medicine, Perugia, Perugia, Switzerland

Background: The proliferation marker Ki-67 is a well-established prognostic marker in canine oral and cutaneous melanocytic tumors. However, established protocols of manual Ki-67 quantification are labor-intensive and subject to interobserver variability. Digital Ki-67 quantification analyses may be more efficient and standardized. They also enable whole-slide assessment.

Objective: To assess the agreement of manual and digital Ki-67 quantification methods and evaluate their prognostic performance in canine cutaneous and oral melanocytic tumors.

Methods: Ki-67 immunohistochemistry was quantified in 71 cutaneous and 74 oral canine melanocytic tumors. Manual Ki-67 quantification was compared with three semi-automated digital workflows: (1) automated hotspot detection followed by Ki-67

quantification, (2) manual hotspot selection followed by Ki-67 quantification, and (3) Ki-67 quantification across the entire tumor section. Prognostic performance was assessed using clinical follow-up data.

Results: The hybrid method demonstrated the highest agreement with manual Ki-67 quantification, exceeding interobserver agreement in cutaneous tumors and matching or exceeding it in oral tumors. The workflow using automated hotspot detection showed lower agreement, likely due to inflammatory infiltrates affecting hotspot selection. Whole-slide analysis showed good agreement with manual assessment, while inflammatory and necrotic areas had no significant impact on its performance.

Conclusions:

Digital Ki-67 quantification combining manual hotspot selection with automated counting closely reproduces established manual assessment. Whole-slide analysis minimizes the impact of biasing factors such as inflammation and provides a promising basis for the future development of fully automated Ki-67 quantification in canine melanocytic tumors.

Discipline: Anatomic pathology, **Topic:** Methods/technology, **Species:** Canine, **Systems:** Integument

3: SENSITIVITY AND SPECIFICITY OF SPECIAL AT-RICH SEQUENCE-BINDING PROTEIN 2 (SATB2) IMMUNOHISTOCHEMISTRY IN THE DIAGNOSIS OF CANINE AND FELINE OSTEOSARCOMAS

Katherine Bederka, Rebecca Smedley
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Histopathologic diagnosis of osteosarcomas (OSA) in domestic animals is complicated by neoplastic osteoblast pleomorphism and variable matrix production. Immunohistochemistry (IHC) for special AT-rich sequence-binding protein 2 (SATB2) was examined previously in numerous types of canine neoplasms. This study aimed to further validate SATB2 as a diagnostic antibody for canine OSAs, and to determine its efficacy in feline OSAs. All IHC labeling for SATB2 was performed at Michigan State University Veterinary Diagnostic Laboratory utilizing a rabbit monoclonal SATB2 antibody (BioSB) performed on Autostainer Link 48 (DAKO). IHC evaluation was performed on nineteen OSAs (subtypes including multilobular tumor of bone, giant cell, telangiectic, chondroblastic, fibroblastic, and extraskeletal), four canine chondrosarcomas, one canine well-differentiated maxillary-mandibular fibrosarcoma, two histiocytic sarcomas, six hemangiosarcomas, one salivary carcinoma, nine unspecified soft tissue sarcomas, and six melanomas. Additionally, nine feline OSAs and ten feline soft tissue sarcomas were evaluated. Specimens were assigned a score between 0-4 based on percentage of cells with strong nuclear immunolabeling; specimens with a score ≥2 were considered positive for SATB2. SATB2 demonstrated 94% and 77% sensitivity in detecting canine (18/19) and feline OSAs (7/9) respectively and had a specificity of 93% in the canine tumor set and 90% in the feline tumor set. Cautious interpretation is warranted in cases of chondrosarcoma, melanomas with osteocartilaginous differentiation, and feline soft tissue sarcomas. A panel of antibodies to rule out differentials is always recommended.

Discipline: Anatomic pathology, **Topic:** Methods/technology, **Species:** Other (Canine and feline), **Systems:** Bone and joints

4: OUTBREAK OF FATAL TOXOPLASMOSIS IN CAPTIVE NONHUMAN PRIMATES: A CASE SERIES

Steven Bronson, Barbara Alexiou, Felix Valles Feliciano, Franco Matias Ferreyra, Brett Fisher, Shannon Gonsoulin, Luan Henker, Rakshith Kumar, Brittany Rasche, Stephanie Cruz Rosado, Yianelly Rodriguez Ruiz, Kristen Hill Thimmesch, Ji-Hang Yin
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Toxoplasma gondii is an apicomplexan protozoan parasite that can cause acute, often fatal disease in nonhuman primates, with multiple outbreaks of toxoplasmosis reported in captive colonies. This case series describes the gross and histologic lesions associated with an outbreak of fatal toxoplasmosis in a zoological collection submitted to the Kansas Veterinary Diagnostic Laboratory between 2025 and 2026. Thirteen nonhuman primates, including five squirrel monkeys (*Saimiri sciureus*), four ring-tailed lemurs (*Lemur catta*), three red ruffed lemurs (*Varecia rubra*), and one golden-headed lion tamarin (*Leontopithecus rosalia*), acutely with non-specific clinical signs including anorexia, lethargy, and not wanting to move . Grossly, all animals (13/13) had diffusely poorly collapsed, lungs with numerous, pinpoint to 4-mm pale tan to white foci. Similar foci were present in the liver (4/13). Splenomegaly (8/13) and mesenteric lymphadenomegaly (4/13) were common findings. Histologically, affected animals exhibited disseminated necrotizing to lymphohistiocytic inflammation involving multiple organs. Numerous intralesional protozoal tissue cysts measuring approximately 20 × 15 µm, with thin eosinophilic cyst walls surrounding numerous 1–2 µm elongate bradyzoites, were identified within affected tissues. Eleven of thirteen cases were confirmed as toxoplasmosis by PCR, while the remaining two were considered suspected cases in which PCR for *T. gondii* was not performed. *Toxoplasma gondii* remains a significant concern in zoological collection due to its wide host range and zoonotic potential. Further investigation into the immunologic status of affected nonhuman primates and the potential role of concurrent infections in other species within the collection is currently ongoing.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** NHP, **Systems:** Systemic

5: ENTEROPATHOGENIC E. COLI SEPTICEMIA LEADING TO TUBULOINTERSTITIAL NEPHRITIS IN THREE COLONY CHINCHILLAS

Maria Paz Buman Ruiz Diaz, Abigail L. English, Krysten Schmidt, Amanda K. Darbyshire, Megan M. Ellis, Rachel C. Soltys, Meaghan M. Broman, Viju V. Pillai, Nobuko Wakamatsu
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Enteropathogenic *Escherichia coli* is not a well-described disease of chinchillas. As this species gains popularity in laboratory animal research, a better understanding of naturally occurring diseases in chinchillas will be necessary. In this case series, we describe an unusual presentation of colibacillosis with ensuing septicemia and subsequent tubulointerstitial nephritis in three chinchillas following brief periods of lethargy and anorexia. All animals were housed within the same colony and perished

within three weeks of each other. Histologically in all 3 chinchillas, intestinal villi were segmentally carpeted by gram-negative, short rod bacteria with multifocal apical enterocyte necrosis and suppurative enteritis. The renal tubules were multifocally moderately to severely filled with neutrophils as was the renal interstitium. In all cases, intestinal cultures yielded few to very many *E. coli* colonies, and samples from two cases were also positive for *eae* toxin by PCR typing. Culture of kidney tissue from one case also yielded *eae*-positive *E. coli*. This case series provides a better understanding of this enteric disease and the renal sequelae that can occur due to enteropathogenic *E. coli* infection and raises awareness of this pathogen as a cause of future outbreaks in this species.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Rodents, **Systems:** Gastrointestinal

6: AMELOBLASTIC CARCINOMA IN A QUARTER HORSE: A CASE REPORT
Emely Castelo, Elis Fisk
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Ameloblastic carcinomas are rare neoplasms in horses with few reports in literature. We describe a case in a 30-year-old Quarter Horse gelding that presented with progressive hyporexia and a right mandibular mass first noted a year prior to presentation. Due to the grave prognosis, humane euthanasia was elected.

Postmortem examination revealed a 21.5 x 15.0 x 9.5 cm poorly circumscribed neoplasm arising from the right mandible with extensive invasion into and destruction of the mandibular body. Molar 411 was absent and molar 410 was loosely rooted within the mass. When incised, the neoplasm was composed of tan to red soft tissue, hard bone spicules, and pockets of hemorrhage, thick brown fluid, and friable debris. Histological examination revealed obliteration of the mandibular bone by an epithelial neoplasm composed of densely packed islands and ribbons within a dense, multifocally hyalinized collagenous stroma. Neoplastic cells exhibited variably prominent odontogenic features including peripheral palisading, antibasilar nuclei, basilar cytoplasmic clearings, and partial dual cytokeratin-vimentin immunoreactivity. There was no gross or histologic evidence of metastasis.

The gross and histologic features in combination with the partial dual cytokeratin-vimentin immunoreactivity are most consistent with an ameloblastic carcinoma. Equine ameloblastic carcinomas are aggressive, locally invasive, and tend to respond poorly to radiation and chemotherapy.

This case highlights the importance of including ameloblastic carcinoma as a differential diagnosis for rapidly growing and locally invasive masses arising from the mandible in horses.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Equine, **Systems:** Other (Odontogenic)

7: A RETROSPECTIVE STUDY OF MORTALITY IN CAPTIVE REINDEER (RANGIFER TARANDUS) AT THE INDIANA ANIMAL DISEASE DIAGNOSTIC LABORATORY: 2013–2026
Seunghee Cho, Meaghan Broman

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In Indiana, reindeer (Rangifer tarandus) are maintained primarily for agritourism, agricultural education, and seasonal holiday exhibitions. A retrospective review of 28 reindeer necropsy cases submitted to the Indiana Animal Disease Diagnostic Laboratory between 2013 and 2026 was conducted to characterize diseases affecting captive reindeer. Gross, histopathologic, microbiologic, and molecular diagnostic findings were reviewed. Cases ranged from aborted fetuses to 16-year-old animals. The most common diagnoses overall were sepsis or suspected sepsis (9/28, 32%) and enterocolitis (6/28, 21%). Two aborted fetuses had no significant diagnostic lesions. Most neonatal calves (0–2 weeks; 5/6) were diagnosed with or suspected of sepsis, including three cases of omphalophlebitis (navel ill); Escherichia coli was the most frequently isolated bacterium (2/5). Enterocolitis occurred predominantly in juveniles younger than 1 year (4/6), followed by suspected sepsis (1/6) and meningoencephalitis (1/6). Ancillary toxin PCR among the enterocolitis cases identified enteropathogenic E. coli (3/4), enterotoxigenic E. coli (2/4), and Clostridium perfringens type A (1/4). Adult reindeer (>1 year) exhibited a broader disease spectrum, including antler abscesses (2/28), meningoencephalitis, tooth abscess, ruminal acidosis, nephritis, babesiosis, epizootic hemorrhagic disease, and gastrolith impaction (1/28 each). Trueperella pyogenes was the most frequently isolated bacterium in adults (3/14), recovered from both antler abscesses and one case of sepsis. These findings demonstrate that sepsis and enterocolitis predominate in juvenile captive reindeer, whereas adults exhibit a broader range of infectious and management-associated diseases, providing baseline diagnostic information to support diagnosis, herd health management, and preventive care.

Discipline: Anatomic pathology, **Topic:** General pathology, **Species:** Other (Cervid), **Systems:** Systemic

8: AN EPIZOOTIC OF BORDETELLOSIS IN FREE-RANGING COMMON RINGTAIL POSSUMS (PSEUDOCHEIRUS PEREGRINUS) IN TASMANIA, AUSTRALIA

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Over the late autumn to mid-winter period in 2024, multiple free-ranging adult Common ringtail possums (Pseudocheirus peregrinus) were found deceased in a semi-rural location in Tasmania, Australia. On macroscopic and histologic post-mortem examination, all possums were in good body condition and had severe, multifocal to coalescing, necrosuppurative bronchopneumonia, tracheitis and bilateral sinusitis with clusters of short bacterial rods. Bordetella bronchiseptica was consistently isolated and identified by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) in samples from the lung, trachea, and nasal turbinates from these possums. Bordetella bronchiseptica has been identified to be a commensal bacterium of the upper respiratory tract and oropharynx in various domestic and wildlife species, with an affinity for ciliated respiratory epithelium. Pre-disposing factors for opportunistic clinical infection of the respiratory tract by this

bacterium include stress, poor husbandry conditions and co-infection with other agents, with young and immunocompromised individuals being more susceptible. To the best of our knowledge, this is the first report of clinical Bordetellosis in Common ringtail possums.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Wildlife/Zoo, **Systems:** Respiratory

9: PITUITARY CARCINOMA RESULTING IN HYPERADRENOCORTICISM FOLLOWED BY ADRENAL CORTICAL ATROPHY IN A DOG

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Pituitary carcinomas are rare neoplasms in dogs. Most of these neoplasms are endocrinologically inactive. The following report describes a six-year-old female spayed Boston terrier with a functional pituitary carcinoma. The presenting complaints included polyuria and polydipsia and inappropriate urination. A minimum database detected isosthenuria, hyperalbuminemia, increased liver enzyme activity with an ALT of 141 U/L and an ALP of 754 U/L. ACTH stimulation was consistent with hyperadrenocorticism. No improvement with trilostane therapy was noted, and the dog developed lethargy and inappetence. Serum biochemistry was repeated and determined persistently increased liver enzyme activity with an ALT of 646 U/L and an ALP of 913 U/L. Lethargy worsened and hind limb ataxia was noted, which progressed to circling when walking. The dog was euthanized and submitted for necropsy at the Utah Veterinary Diagnostic Laboratory. Gross findings included a pituitary mass, and bilateral adrenal cortical atrophy. Histopathologic findings determined that the pituitary neoplasm was a carcinoma, characterized by invasion of the adjacent neuropil, frequent mitotic figures and cellular atypia. Immunohistochemistry of the neoplasm is pending. The clinical progression of this case suggests pituitary carcinoma that was initially functional, resulting in hyperadrenocorticism; however, the adrenal cortical atrophy at the time of death is compatible with adrenal insufficiency. It was initially assumed that an adverse reaction to trilostane had occurred, but further discussion indicated that the treatment was sporadic due to the lack of improvement. The conclusion was that the neoplasm either effaced the pituitary gland or lost its endocrine function over time.

Discipline: Both, **Topic:** Oncology, **Species:** Canine, **Systems:** Endocrine

10: MAMMARY CARCINOMA WITH LYMPH NODE INVASION IN A 26-YEAR-OLD THOROUGHBRED MARE

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Here, we report mammary adenocarcinoma with lymph node invasion in a 26-year-old Thoroughbred multiparous mare which was euthanized and submitted for autopsy at the University of Kentucky Veterinary Diagnostic Laboratory. The left mammary gland was first recognized as being firm and enlarged, with amber, turbid secretions in October of 2025. Mammary secretions and a fine needle aspirate sample submitted for cytology revealed extensive necrosis and many clusters of

pleomorphic epithelial cells admixed with mixed suppurative, histiocytic, and lymphocytic inflammation. Grossly, the left mammary gland is diffusely enlarged (15x13x6.5cm) and expanded by multiple firm tan variably discrete nodules (approximately 0.5x0.5x0.5-2.0x2.0x2.0cm) on cut section. A small amount (approximately 5mL) of brown translucent fluid is exuded from tissue upon sectioning. Histologically, this corresponds with a multinodular poorly demarcated neoplasm composed of epithelial cells forming tubules and solid areas on a scirrhous stroma (mammary adenocarcinoma). Adjacent lymph nodes are variably effaced by large numbers of similar neoplastic cells.

Mammary carcinoma is an uncommon neoplasm in horses, with relatively few reported cases.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Equine, **Systems:** Mammary

11: CHOLELITHIASIS AND HEPATIC ENCEPHALOPATHY IN A 10-YEAR-OLD THOROUGHBRED MARE

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A 10-year-old Thoroughbred mare presented for autopsy. Reported clinical history included treatment for dehydration over a period of two weeks and elevated liver enzymes. The mare was found down in a creek prior to death. The owner was concerned for a chronic disease process. At the time of autopsy, the liver is markedly enlarged with rounded margins and is diffusely firm and tan to yellow with an enhanced reticular pattern. Intrahepatic bile ducts are multifocally distended by numerous variably sized (0.5x0.5x0.5-5x4.5x3cm) yellow to green, variably firm and formed or soft to pasty material (choleliths). Histologically, the enhanced reticular pattern corresponds with severe periportal to bridging fibrosis, ductular hyperplasia, and mixed neutrophilic and lymphoplasmacytic inflammation. Marked cholestasis is observed. Moderate numbers of Alzheimer type II astrocytes are scattered throughout the cerebral gray matter.

Aerobic cultures of the liver and lung yielded numerous E. coli. This, along with histologically evident hepatitis and increased numbers of circulating leukocytes in vessels throughout the body, raises concern for septicemia.

Cholelithiasis is a rare condition in horses which can lead to obstructive cholestasis, widespread hepatic fibrosis, and occasionally septicemia due to ductal compromise.

Discipline: Anatomic pathology, **Topic:** General pathology, **Species:** Equine, **Systems:** Hepatobiliary

12: ATYPICAL RETINAL SPINDLE CELL NEOPLASM ARISING FROM THE OPTIC DISC IN A CAT

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Intraocular neuronal tumors are uncommon in cats and include a broad range of differential diagnoses, such as glial neoplasms, melanocytic tumors, and peripheral

nerve sheath tumors. Immunohistochemistry is often required to establish the cell of origin. This report describes an unusual intraocular spindle cell tumor arising at the optic nerve head and causing retinal detachment and secondary glaucoma.

A 4-year-old domestic shorthair cat presented with retinal detachment and secondary glaucoma of the left eye. Ocular ultrasonography identified a mass at the optic disc, and enucleation was performed. Routine histopathology and immunohistochemistry were conducted using antibodies against GFAP, S100, PNL2, smooth muscle actin (SMA), and factor VIII.

Histologically, a well-demarcated, non-encapsulated spindle cell proliferation arose from the optic nerve head and protruded into the vitreous. Neoplastic cells were arranged in irregular fascicles within a prominent vascular stroma and exhibited mild-to-moderate anisocytosis and anisokaryosis with no identifiable mitotic figures. Diffuse retinal detachment, extensive retinal atrophy, scleral thinning, and dilation of the iridocorneal drainage angle were present, consistent with chronic secondary glaucoma. Neoplastic cells exhibited strong diffuse S100 immunoreactivity and were negative for GFAP, PNL2, and SMA. Factor VIII highlighted the abundant vascular network. These findings excluded the initially suspected glial neoplasm and favored a neoplasm of Schwannian origin, with a peripheral nerve sheath tumor considered the most likely diagnosis.

This case expands the spectrum of feline intraocular spindle cell tumors and highlights the critical role of immunohistochemistry in distinguishing peripheral nerve sheath tumors from glial neoplasms arising at the optic nerve head.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Feline, **Systems:** Eye/ear

13: DISCOVERY OF A PUTATIVE NOVEL BASAL ENTAMOEBA SPECIES IN A CAPTIVE BEARDED DRAGON

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A 4-year-old intact male bearded dragon (*Pogona vitticeps*) was presented for evaluation of chronic, recurrent diarrhea that had been present intermittently since adoption at 5 months of age. Previous fecal testing identified coccidiosis and *Salmonella* spp., both successfully treated with ponazuril, fenbendazole, and ceftazidime, with subsequent negative follow-up testing. A cloacal swab was also positive for Agamid adenovirus 1, confirmed by sequencing. Intermittent lymphocytosis was documented on multiple CBCs, including the most recent evaluation (14.85 x10³/µL; RI: 0.9–8.4 x10³/µL). During the current presentation, coelomic ultrasonography revealed diffuse small-intestinal wall thickening consistent with enteritis and scant anechoic peritoneal effusion that could not be sampled due to its low volume. Zinc sulfate fecal flotation identified a few oxyurid eggs, small flagellated protozoa, and occasional 16–20 µm spherical organisms with multiple uniform nuclei, morphologically consistent with *Entamoeba* spp. cysts. Fecal PCR targeting *Entamoeba* spp. was positive, and Sanger sequencing generated a 123-bp

sequence representing a putative novel *Entamoeba* species, likely basal to currently characterized members of the genus based on preliminary phylogenetic analysis. Following treatment with metronidazole, repeat fecal examination was negative for *Entamoeba*, although intermittent diarrhea persisted. While *E. invadens* is the principal pathogenic species reported in reptiles, the clinical significance of this novel *Entamoeba* remains uncertain, particularly given the concurrent adenovirus infection. Nevertheless, this case expands the known diversity of reptilian *Entamoeba* and highlights the utility of molecular diagnostics in identifying previously unrecognized protozoan species. Further investigation is warranted to determine the epidemiology and pathogenic potential of this organism.

Discipline: Clinical pathology, **Topic:** Infectious disease, **Species:** Wildlife/Zoo, **Systems:** Gastrointestinal

14: SYSTEMIC DISSEMINATION OF MYCOBACTERIUM INTRACELLULARE IN A CAT DIAGNOSED BY HISTOPATHOLOGY AND NEXT-GENERATION SEQUENCING

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A reportedly indoor-only, 2-year-old neutered male domestic medium-haired cat initially presented to its primary veterinarian with fever, anorexia and an abdominal mass identified on abdominal palpation. An exploratory laparotomy revealed numerous splenic abscesses. Empirical doxycycline therapy was initiated; however, the animal deteriorated and died during treatment.

Gross examination revealed marked splenomegaly with multifocal to coalescing disseminated splenic nodules, generalized lymphadenomegaly, and hepatomegaly. Histologically, multifocal granulomatous inflammation was present in the liver, lungs, spleen, several lymph nodes, and kidneys. The splenic parenchyma was extensively effaced by nodular granulomatous inflammation with numerous epithelioid macrophages and multinucleated giant cells. Similarly, lymph nodes were markedly enlarged and effaced by macrophages, which were frequently associated with necrotizing lymphadenitis. Special stains performed on splenic tissue demonstrated numerous intralesional bacilli that were variably Gram-positive, strongly acid-fast, and periodic acid-Schiff (PAS) positive within macrophages and multinucleated giant cells.

To further characterize the infectious agent, next-generation sequencing (NGS) was performed on formalin-fixed paraffin-embedded (FFPE) splenic tissue. Sequencing identified Mycobacterium spp. as the predominant bacterial taxon (118,232 reads), with Mycobacterium intracellulare representing the most abundant species-level assignment. These findings supported a diagnosis of disseminated non-tuberculous mycobacterial (NTM) infection, most consistent with a member of the Mycobacterium avium complex (MAC), specifically M. intracellulare. This case highlights the importance of considering disseminated NTM infection as a differential diagnosis in cats with granulomatous disease and demonstrates the utility of combining histopathology, special stains, and NGS for definitive pathogen identification.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Feline, **Systems:** Systemic

15: BILATERAL ACCESSORY PULMONARY LOBES WITH TORSION IN AN ADULT ALPACA

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An 11-year-old female alpaca with a history of sternal recumbency for 3 days before death was submitted for necropsy. Grossly, a 4 x 5 x 2 cm ovoid mass resembling lung was connected by a 5 cm long and 0.8 cm in diameter twisted stalk to the tip of the right middle lung lobe. A similar 8 x 5 x 3 cm ovoid mass resembling lung was connected by a 2 cm long and 0.8 cm in diameter stalk to the cranial latero-ventral side of the left caudal lung lobe. Hydrothorax, pulmonary atelectasis, cranioventral bronchopneumonia, and fibrinous pleuritis were also present and due to systemic toxoplasmosis. Histologically, the pulmonary masses and their stalks consisted of acutely infarcted lung tissue. These masses were diagnosed as supernumerary or accessory pulmonary lobes with torsion. Accessory pulmonary lobes are extrapulmonary masses of lung tissue with a bronchial connection to the lung, trachea, or bronchi, and have been described in both humans and domestic animals. In this case, however, the attachment to the lung consisted of a pedicle of pulmonary tissue without a bronchus. Hydrothorax and fibrinous pleuritis likely predisposed to torsion and infarction.

Discipline: Anatomic pathology, **Topic:** Other (Congenital anomaly), **Species:** Camelids, **Systems:** Respiratory

16: PRIMARY VISCERAL WELL-DIFFERENTIATED CYSTIC LYMPHANGIOSARCOMA OF THE LIVER AND SPLEEN IN A DOG

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This study presents the clinical, macroscopic, histological and immunohistochemical findings of a primary visceral well-differentiated cystic lymphangiosarcoma in a dog's liver and spleen. A nine-year-old spayed female Vizsla dog was presented with abdominal distension. Ultrasound revealed abdominal effusion, hepatomegaly, multiple hepatic cystic lesions, and a heterogeneous splenic mass. Serology and PCR for Echinococcus spp. were negative. Euthanasia was performed four months after presentation due to progressive clinical deterioration and lack of response to treatment. Necropsy revealed disseminated cystic lesions throughout all liver lobes, which had increased in size since the ultrasonography. Histologically, multiple cysts were lined by well-differentiated endothelial cells with mild atypia. Multifocal, dissecting, infiltrative growth was observed within the hepatic parenchyma. The splenic mass had doubled in size and was histologically characterized by irregular cavernous spaces, lined by flattened and plumped endothelial cells. Occasional incomplete dissected trabeculae and a focal kaposiform arrangement with increased cellularity and moderate cellular pleomorphism were present. Mitotic count was low. Immunohistochemistry revealed moderate to intense immunoreactivity for LYVE-1 and CD31 within the neoplastic endothelial cells confirming their lymphatic

differentiation. No cutaneous neoplastic lesions or lymphoedema were identified. Malignancy was supported by the dissecting infiltrative growth in the liver, areas of increased cellularity and pleomorphism in the spleen, and the aggressive clinical course. To the authors' knowledge this is the first report of a primary lymphangiosarcoma involving the liver and spleen in a dog. This case highlights the diagnostic challenges posed by these neoplasms and the importance of thorough sampling to improve diagnostic accuracy.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Canine, **Systems:** Other (Lymphatic system)

17: CEREBRAL AND NASAL B-CELL LYMPHOMA IN AN AMERICAN BLACK BEAR (URSUS AMERICANUS)

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An adult male American black bear (*Ursus americanus*) was submitted to the New Jersey Animal Health Diagnostic Laboratory for postmortem examination following a 4-day history of persistent circling behavior observed near a private residence. A New Jersey Fish and Wildlife technician documented the neurologic abnormalities; the bear appeared to be in good body condition and exhibited no evidence of lameness. As the animal subsequently became recumbent and was unable to rise, it was chemically immobilized with ketamine and xylazine and humanely euthanized. A brain sample was submitted for rabies testing, and results were negative.

Gross examination revealed a large, tan to brown, firm, necrotic, cavitated mass within the mid-dorsal cerebrum that infiltrated the underlying neuroparenchyma. A similar infiltrative mass was present in the caudodorsal nasal cavity and extended through the basisphenoid bone at the skull base. An additional yellow-tan, smooth, soft to firm mass was identified in the left nasal cavity. Additional findings included an embedded arrow associated with chronic inflammation in the right humerus and a markedly enlarged cervical lymph node containing multiple cavitations filled with tan purulent material. Histopathologic evaluation demonstrated that the cerebral and nasal masses shared similar neoplastic features. Immunohistochemistry performed on representative cerebral and nasal tumors through the Michigan State University Veterinary Diagnostic Laboratory revealed strong CD20 immunoreactivity and negative labeling for CD3 and pancytokeratin, supporting a diagnosis of B-cell lymphoma.

To the authors' knowledge, this represents the first reported case of B-cell lymphoma in an American black bear.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Wildlife/Zoo, **Systems:** Systemic

18: PULMONARY SACCHAROMYCES CEREVISIAE INFECTION IN A BALD EAGLE

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Candidiasis is an opportunistic mycosis of birds, most often involving the upper alimentary tract, and is less often reported in free-ranging raptors. An adult male Bald Eagle (*Haliaeetus leucocephalus*), estimated to be >4.5 years old based on all-white head feathers and rectrices, was submitted for postmortem examination. The carcass was in good body condition. Grossly, yellow, rough exudate covered the trachea, bronchi, and thickened left posterior thoracic air sac. The left caudal lung lobe was diffusely grey-green.

Histopathology revealed multifocal to coalescing accumulations within tracheobronchial lumina, atria, and air capillaries of abundant eosinophilic to amphophilic proteinaceous exudate containing innumerable 8–10 µm round to oval fungal organisms with central to eccentric basophilic puncta. Rare organisms formed short pseudohyphae, and occasional broad- to narrow-based budding was present. The exudate was infiltrated by macrophages, lymphocytes, and heterophils admixed with intra- and extracellular bacterial colonies. Inflammatory cells expanded the pulmonary interstitium and interatrial septa. Multifocal erosions and ulcerations of the tracheobronchial mucosa were covered by similar inflammation extending into the adjacent submucosa. Scattered fibrin thrombi were present within air capillaries and small vessels. Similar lesions were present in the trachea and thoracic air sacs. Gomori methenamine silver and periodic acid-Schiff stains highlighted abundant fungal yeasts, most consistent with *Candida* spp. Aerobic bacterial culture yielded mixed bacteria. Avian influenza virus testing was negative, and liver lead concentrations were within reference intervals. This case documents severe mixed *Candida* and bacterial infection of the lungs, trachea, and thoracic air sacs in a Bald Eagle.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Avian, **Systems:** Respiratory

19: ROSENTHAL FIBERS IN CANINE GIANT AXONAL NEUROPATHY: A DIAGNOSTIC MIMIC OF ALEXANDER DISEASE

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Rosenthal fibers are a rare neuropathologic finding that are classically associated with Alexander disease. We describe two unrelated young Golden Retrievers (1.5-year-old castrated male and 2-year-old spayed female) that presented to the neurology service at the University of Pennsylvania, School of Veterinary Medicine, Ryan Hospital with progressive neurological dysfunction characterized by pelvic limb paresis and ataxia, reduced to absent spinal reflexes, and megaesophagus. Necropsy findings were limited to esophageal dilation and pulmonary consolidation.

Histologically, both cases exhibited bilaterally symmetric axonal degeneration affecting the gray and white matter of the caudal midbrain, brainstem, and spinal cord. Lesions consisted of numerous large spheroids and deposition of abundant,

brightly eosinophilic fibrillar to ovoid, perivascular and periventricular aggregates of Rosenthal fibers. Based on their presence, Alexander disease was diagnosed.

In humans and dogs, Alexander disease is associated with dominant variants in *GFAP*; here commercial genetic testing and whole genome sequencing failed to identify plausible *GFAP* candidates. Filtering assuming a recessive inheritance mechanism revealed a rare homozygous polymorphism in *GAN* in both cases, absent in the Dog10k database barring one heterozygous dog. *GAN* encodes gigaxonin, a protein involved in neurofilament turnover. *GAN* variants cause Giant Axonal Neuropathy, a rare, recessive, progressive neurodegenerative disorder reported in humans and a single line of German Shepherds.

These findings expand the reported neuropathology of canine Giant Axonal Neuropathy and demonstrate the utility of expanded molecular work up of rare diseases. Consequently, Rosenthal fibers alone should not be considered sufficient for a diagnosis of Alexander disease without supporting molecular evidence.

Discipline: Anatomic pathology, **Topic:** Genetic/hereditary disorders, **Species:** Canine, **Systems:** Nervous

20: BLUNT FORCE TRAUMA WITH POSTMORTEM SCAVENGING IN TWO EQUINE SKULLS

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Background: Forensic taphonomy studies postmortem processes that occur between death and body recovery. Distinguishing between taphonomic change and antemortem trauma is crucial in animal cruelty cases to identify foul play.

Case History: The skeletonized heads of two horses, allegedly euthanized with a hammer, were submitted for forensic examination to determine the cause and manner of death.

Case Findings: One skull had a large dorsal defect involving the caudal aspects of bilateral frontal and parietal bones, with multiple irregular fractures extending rostrally. The rostral nasal bones and right zygomatic arch were absent with ragged margins, indicating postmortem scavenging. An “L”-shaped, depressed defect with multiple associated fractures was present in the right frontal bone. The second skull had a large, oval dorsal defect with ragged edges on the left parietal bone and a semicircular fracture communicating with the main defect. An inverted “T”-shaped fracture line on the right parietal bone and an oblique fracture caudal to the right coronal suture were present. The shortened left mandibular coronoid process with ragged edges suggests scavenging.

Discussion: The pattern of large focal defects with associated radiating fractures in both horses’ skulls is consistent with the use of a hammer or similar blunt object. Multiple fracture lines suggest multiple impact points, likely from multiple blows to the

head. The preexisting antemortem lesions provided enhanced access for scavengers, further distorting and enlarging the injuries.

Conclusions: The cause of death in both horses is blunt force trauma, and the manner of death is classified as non-accidental killing.

Discipline: Anatomic pathology, **Topic:** Forensic, **Species:** Equine, **Systems:** Bone and joints

21: AGE-RELATED TRIPLE AMYLOIDOSIS IN A DOG: FIBRINOGEN ALPHA-CHAIN, APOLIPOPROTEIN A-I, AND EFEMP1 AMYLOIDOSIS

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Background

Amyloidosis in animals is classified into 21 types, and two or more types may sometimes coexist within the same individual. Since amyloid deposition patterns differ depending on the precursor protein, understanding the histopathological features of each disease is important for accurate diagnosis. In this report, we describe a case of amyloidosis in an aged dog involving three distinct types of amyloid.

Materials and Methods

A 14-year-old Shetland Sheepdog was examined histopathologically after sudden death. Mass spectrometry-based proteomic analysis and immunohistochemistry were performed to identify the amyloid precursor proteins.

Results

In addition to systemic amyloid deposition, necrotizing suppurative hepatitis and adrenalitis with intralesional Gram-positive bacilli were observed. Death was attributed to be septic shock associated with bacterial infection. Histopathological analysis, mass spectrometry and immunohistochemistry revealed that fibrinogen Aα chain-derived amyloid was deposited in the vascular walls of the liver, glomeruli of the kidneys, mitral valve of the heart, coronary artery walls, and cardiac interstitium; apolipoprotein A-I-derived amyloid was deposited in the pulmonary vascular walls and alveolar septa; and EGF-containing fibrin-like matrix protein 1 (EFEMP1)-derived amyloid was deposited in the submucosa and vascular walls of the gastrointestinal tract. All three of these amyloidoses have been documented as age-related pathology in animals, suggesting that aging may also be involved in the development of triple amyloidosis in this case.

Conclusion

This case demonstrates the diversity and pathological complexity of amyloidosis in aged dogs, and highlights the diagnostic value of tissue-specific amyloid typing.

Discipline: Anatomic pathology, **Topic:** Degenerative, **Species:** Canine, **Systems:** Systemic

22: ACUTE MYELOID LEUKEMIA WITH EOSINOPHILIC DIFFERENTIATION IN A KUNEKUNE PIG (SUS SCROFA DOMESTICUS)

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A 7-year-old Kunekune pig presented with a 12-day history of progressive paraparetic ataxia that failed to respond to aggressive medical therapy and culminated in recumbency. Humane euthanasia was elected, and the animal was submitted for a full postmortem examination. On postmortem examination, the liver and spleen were enlarged, firm, and contained numerous well-demarcated white nodules ranging from 1 to 20 mm in diameter that were disseminated throughout the parenchyma. Multifocally, the thoracic and lumbar vertebral canals contained green, mucoid material and were accompanied by moderate bridging spondylosis of the thoracic spine. Histologically, the vertebral bone marrow, pachymeninges, liver, and spleen were infiltrated by large, densely packed sheets of immature neoplastic cells with finely stippled eosinophilic cytoplasm, a round nucleus containing coarsely stippled to finely clumped chromatin and one to two eosinophilic nucleoli. Approximately 5% of the cells contained large secondary eosinophilic cytoplasmic granules and retained immature nuclear features. This morphology, combined with histochemical stains and immunohistochemistry, supported a diagnosis of acute myeloid leukemia (AML) with eosinophilic differentiation. The extensive meningeal infiltration and associated vertebral canal masses likely accounted for the acute neurologic deficits through spinal cord compression. This condition is rarely reported in pigs; to the authors' knowledge, this is the first reported case of AML in a Kunekune pig, expanding the spectrum of species affected by this uncommon neoplasm and highlighting its potential to cause severe neurologic disease through extensive meningeal involvement.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Porcine, **Systems:** Hematopoietic

23: OSTEOPETROSIS IN A JUVENILE DOMESTIC CAT

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A 5-month-old domestic shorthair cat was presented to a veterinary clinic with a history of recurrent upper respiratory signs, lameness, and polydipsia/polyuria. Physical examination revealed an abnormal skull shape, short limbs, absence of most teeth, and fracture of the right ulna. Due to lack of improvement and poor prognosis, euthanasia was elected. On gross examination, cone-shaped bone extending from the epiphysis to the metaphysis was observed in the humerus. Microscopically, the cone-shaped bone corresponded to trabeculae of primary spongiosa. Given the age of the cat and the absence of concomitant diseases, inherited osteopetrosis was suspected. This case was unique in its presentation with polyuria/polydipsia. It is suspected, due to lack of lesions in other tissues, that the thickening of the bones of the skull included thickening and stenosis of the sella

turcica, which may have applied pressure to the pituitary and induced neurogenic diabetes insipidus.

Discipline: Anatomic pathology, **Topic:** Genetic/hereditary disorders, **Species:** Feline, **Systems:** Bone and joints

24: IDENTIFICATION OF NASAL BPIFB4 AMYLOID DEPOSITION IN DOGS AND CATS

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Background: Localized amyloidosis in nasal tissues, including those associated with nasal neoplasms, remains poorly understood in both humans and animals.

Objective: To elucidate the pathological characteristics of localized nasal amyloid deposition in dogs and cats.

Methods: Thirty-five formalin-fixed paraffin-embedded nasal biopsy samples containing eosinophilic amorphous material based on H&E staining were collected. In all cases, amyloid deposition was confirmed histologically by positive Congo-red staining. Amyloid characterization was performed using transmission electron microscopy (TEM) in 3 cases, liquid chromatography-tandem mass spectrometry (LC-MS/MS) in 8 cases, and immunohistochemistry using anti-BPI fold-containing family B member 4 (BPIFB4) antibody in all cases.

Results: Histologic diagnoses included nasal adenocarcinoma (3 dogs, 30 cats), nasal gland hyperplasia (1 dog), and inflammation (1 cat). Amyloid deposits were identified in the interstitium in 12 cases, within corpora amylacea-like (CA) structures in glandular lumina in 8 cases, and in both sites in 15 cases. TEM identified non-branching fibrils approximately 10 nm in diameter in 1 canine interstitial and 2 feline CA amyloid deposits. LC-MS/MS of all tested samples including 3 Interstitial deposits (2 dogs and 1 cat) and 5 CA amyloid deposits (5 cats), consistently identified peptides derived from BPI fold-containing family B member 4 (BPIFB4). All amyloid deposits demonstrated BPIFB4 immunoreactivity, most notably with an N-terminal antibody.

Conclusion: Our findings demonstrate that BPIFB4 is a novel amyloidogenic protein associated with nasal amyloid deposition in dogs and cats. This discovery expands the spectrum of known amyloidogenic proteins and suggests that additional amyloids remain to be identified in animals.

Discipline: Anatomic pathology, **Topic:** General pathology, **Species:** Other (Canine and Feline), **Systems:** Respiratory

25: BABESIA CONRADAE INFECTION IN KANSAS COYOTE-HUNTING DOGS

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Babesia conradae is an emerging hemoparasite reported in coyote-hunting Greyhounds, but clinicopathologic and pathologic findings remain limited. Here, we describe clinical, molecular, and pathologic findings in three coyote-hunting dogs evaluated for *B. conradae* infection. A 5-year-old male Greyhound-mix dog from a kennel with multiple clinically affected coyote-hunting dogs was evaluated for lethargy, fever, anemia, thrombocytopenia, and bilirubinuria. Blood smear examination of this dog showed anemia, thrombocytopenia with platelet clumping, low numbers of spherocytes, a left shift, and small intraerythrocytic piroplasm-like organisms. Whole-blood PCR confirmed *B. conradae* infection. Two additional male Greyhound or Greyhound-mix hunting dogs from the same geographic region were submitted for necropsy after acute or progressive illness. In one necropsy case, the main histopathologic lesions were marked membranoproliferative glomerulopathy with tubular proteinosis, lymphoplasmacytic interstitial nephritis, pulmonary and vascular mineralization with edema, multifocal myocardial necrosis and mineralization, splenic hemosiderosis, and extramedullary hematopoiesis. The second necropsy case had severe chronic lymphoplasmacytic pyelonephritis with tubular necrosis, multiorgan mineralization, and splenic hemosiderosis. Several necropsy findings, particularly renal disease and systemic lesions, overlapped with reported complications of *B. conradae* infection. To further evaluate, FFPE tissues from both necropsy cases were submitted for Babesia PCR (results were pending). These cases highlight *B. conradae* as an important differential diagnosis in coyote-hunting dogs with anemia, thrombocytopenia, renal disease, or systemic illness as main disease manifestations. Additional testing of affected and exposed coyote-hunting dogs may help to identify and corroborate the distribution of lesions and systems affected, and common associated pathologic findings of this emerging parasite.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Canine, **Systems:** Systemic

26: A RARE CASE OF MULTISYSTEMIC EXTRAMEDULLARY PLASMACYTOMA WITH CENTRAL NERVOUS SYSTEM INVOLVEMENT IN A DOMESTIC SHORTHAIR CAT

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Background: Extramedullary plasmacytomas are uncommon neoplasms of plasma cell origin that arise outside the bone marrow, most frequently involving cutaneous sites in domestic animals. In cats, these tumors are rare and are generally considered biologically indolent with limited reports of systemic dissemination. To our knowledge, central nervous system (CNS) involvement has not been previously reported.

Objective: This report describes a rare case of multisystemic extramedullary plasmacytoma in a domestic shorthair cat with central nervous system involvement,

highlighting its unusual dissemination pattern and emphasizing the importance of considering plasma cell neoplasia in the differential diagnosis of feline CNS diseases.

Results: A 4-year-old spayed female domestic shorthair cat developed progressive ambulatory dysfunction approximately one month after ovariohysterectomy. Grossly, both kidneys were irregular with innumerable, coalescing, light tan, firm nodules measuring 5–25 mm in diameter that bulged from the cortical surfaces and were partially adhered to the renal capsules. Histologically, sheets of plasmacytoid round cells infiltrated multiple organs, including the kidneys, liver, encephalic leptomeninges, and cervical-thoracic spinal cord. Bone marrow was not affected. Immunohistochemically, the plasmacytoid cells exhibited diffuse nuclear immunoreactivity for MUM1, whereas CD79 staining was limited to faint, nonspecific nuclear labeling, and the cells were negative for PAX5. These findings supported a diagnosis of multisystemic extramedullary plasmacytoma.

Conclusions: To our knowledge, this represents a rare case of feline extramedullary plasmacytoma with meningeal and myelonic infiltration and highlights the potential for clinically significant central nervous system involvement despite the absence of gross lesions.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Feline, **Systems:** Systemic

27: INTRA- AND INTER-RATER REPRODUCIBILITY OF THREE HISTOPATHOLOGIC SCORING SCHEMES IN SALMONELLA AND DSS-INDUCED MOUSE COLITIS MODELS

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Background

Mouse colitis models are widely used to study enteric disease. Histopathologic scoring systems vary across studies, limiting reproducibility and cross-study comparisons.

Objective

To analyze whether different published mouse colitis scoring schemes yield comparable trends across raters and within raters over time.

Methods

Two sets of H&E-stained colon slides from experimentally-induced colitis in mice (n=36 Salmonella spp.-induced; n= 38 DSS-induced) were studied. In a preliminary analysis, slides (n=9 each model) were digitally scanned, de-identified, and randomized. An ACVP board-certified pathologist and a graduate student scored each slide using three published schemes differing on how the tissues were assessed: 1) based on the worst-affected area (“spot”), 2) based on affected length of tissue (“length”), and 3) combining intensity and length affected (“combined”). For

intra-rater alignment, raters repeated the “spot” and “combined” scoring. Intra-rater reproducibility and inter-rater alignment were assessed via Pearson correlations.

Preliminary results

All methods showed strong correlation (r=0.82-0.97). Both “combined” and “spot” methods showed strong intra- and inter-rater agreement (r=0.84-0.98). The “length” scheme showed lower inter-rater correlation (Salmonella r=0.79; DSS r=0.88). Overall, “combined” method best balanced the detection of subtle differences between samples with favorable intra-rater reproducibility and inter-rater alignment. “Spot” scoring showed high reproducibility, however, lacked granularity. “Length” scheme was more workflow-intensive but provided higher granularity in terms of length of tissue affected.

Conclusion

The three scoring methods showed strong correlation across two experimentally-induced colitis models, with intra-rater reproducibility remaining high in non-expert raters. Future work incorporating more slides, methods, and clinical data could further validate these findings.

Discipline: Anatomic pathology, **Topic:** Comparative pathology, **Species:** Rodents, **Systems:** Gastrointestinal

28: LISTERIA MONOCYTOGENES SEPSIS IN A NEONATAL FOAL

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A 4-day-old Peruvian Paso colt was presented to the Mississippi State University Animal Health Center for recumbency and anorexia. Examination by the referring veterinarian revealed an IgG concentration of less than 400 mg/dL. On presentation, the foal was comatose with nystagmus and hypoglycemia, consistent with septic shock. Initial treatment with Karo syrup applied to the gums and a shock dose of dextrose-supplemented lactated Ringer solution (LRS) resulted in mild, transient improvement. Additional treatment included total parenteral nutrition, intravenous LRS, and plasma. The foal did not improve by the following day, and euthanasia was elected. At necropsy, all examined joints contained increased volumes of synovial fluid with large fibrin strands. Multifocal, slightly raised, white-to-tan, 2-mm-diameter nodules were present on the renal capsular surfaces and throughout the cortices of both kidneys. Histopathologic examination revealed multifocal necrosuppurative nephritis characterized by renal nodules composed of neutrophils and necrotic debris that effaced and displaced glomeruli and tubules. Similar inflammatory infiltrates were present in multiple tissues. Culture of blood and kidney samples yielded *Listeria monocytogenes*. Gross, histopathologic, and clinical findings were consistent with neonatal sepsis, as confirmed by blood and kidney cultures. Listeriosis affects ruminants more commonly, particularly those exposed to contaminated feed or improperly stored silage. Despite its rarity, listeriosis has been reported in foals, often associated with failure of passive transfer or immunosuppression. Moreover, *L. monocytogenes* has been recovered from a small proportion of septic foals. Therefore, listeriosis should be considered in the differential diagnosis of neonatal and juvenile horses with systemic disease.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Equine, **Systems:** Systemic

29: A TALE OF TWO WOODCHUCKS: SPONTANEOUS COLONIC LEIOMYOSARCOMA CAUSING COLOCOLIC INTUSSUSCEPTION

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Background:
Leiomyosarcoma is an uncommon malignant neoplasm of smooth muscle origin that has rarely been reported in woodchucks (*Marmota monax*), with only a single published case arising in the oral cavity. Colonic leiomyosarcoma has not previously been described in this species.

Objective:
To describe the clinicopathologic and immunohistochemical features of two spontaneous cases of colonic leiomyosarcoma in a laboratory woodchuck breeding colony.

Methods:
Two aged woodchucks (6-year-old female and 6-year-old male) from a laboratory breeding colony were euthanized following development of lethargy, abdominal distension, and palpable abdominal masses. Clinical pathology, complete necropsies, histopathology, and smooth muscle actin (SMA) immunohistochemistry were performed.

Results:
Colocolic intussusception with transmural necrosis, colonic perforation, and suppurative peritonitis was present in both woodchucks. Histologically, infiltrative spindle cell neoplasms arose within the muscularis propria and muscularis mucosae at the site of intussusception. The neoplasms formed interlacing bundles of pleomorphic spindle cells and demonstrated strong cytoplasmic immunoreactivity for SMA, supporting a diagnosis of leiomyosarcoma. One case exhibited extensive vascular invasion with metastases to the liver, lung, heart, and mesentery, whereas the other remained confined to the colon. Marked neutropenia and hyperphosphatemia were identified in each animal, consistent with severe intestinal injury and septic peritonitis. The neoplasms likely disrupted intestinal motility, resulting in intussusception, bowel perforation, septic peritonitis, and ultimately euthanasia.

Conclusion:

These represent the first reported cases of colonic leiomyosarcoma in woodchucks and expand the spectrum of spontaneous neoplasia recognized in this species. Colonic leiomyosarcoma should be considered a differential diagnosis for colocolic intussusception in aged woodchucks.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Rodents, **Systems:** Gastrointestinal

30: COMBINED HEPATOCELLULAR CARCINOMA AND CHOLANGIOCARCINOMA IN A SUGAR GLIDER (PETAURUS BREVICEPS)

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An 11-year-old neutered male sugar glider (*Petaurus breviceps*) was presented for postmortem examination after an acute onset of hindlimb weakness and anorexia, followed by death. Grossly, the liver exhibited many well-demarcated, firm, raised, tan-yellow, 1-15 mm in diameter, multinodular masses. The lungs had similar nodules. Microscopically, the liver was expanded and effaced by an unencapsulated, poorly demarcated, densely cellular, multinodular neoplasm composed of polygonal cells arranged in thick trabeculae supported by the preexisting fibrovascular stroma. Neoplastic cells had abundant, variably vacuolated, eosinophilic cytoplasm and round nuclei with finely stippled chromatin and occasionally prominent nucleoli. Anisocytosis and anisokaryosis were marked, with occasional multinucleated cells. Mitoses were 24 per 2.37 mm^2. These cells displayed faint cytoplasmic HepPar1 immunoreactivity. An adjacent nodule was expanded and effaced by an unencapsulated, poorly demarcated, moderately cellular, infiltrative neoplasm composed of epithelioid cells arranged in tubules separated by abundant desmoplastic stroma. Neoplastic cells were cuboidal with a moderate amount of eosinophilic cytoplasm and a round nucleus with finely stippled chromatin and small nucleoli. Anisocytosis and anisokaryosis were marked. Mitoses were 6 per 2.37 mm^2. These cells displayed strong cytoplasmic cytokeratin 19 immunoreactivity. In some areas, the distinction between these two neoplastic populations was less evident. Postmortem and histopathologic findings support the diagnosis of a combined hepatocellular carcinoma and cholangiocarcinoma. Hepatocellular carcinoma and cholangiocarcinoma have infrequently been documented in sugar gliders, generally as separate entities. Consequently, the simultaneous occurrence of hepatocellular carcinoma and cholangiocarcinoma in this case is atypical and contributes to the scarce literature on hepatic neoplasia.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Wildlife/Zoo, **Systems:** Hepatobiliary

31: NON-EOSINOPHILIC CUTANEOUS LESIONS ASSOCIATED WITH FELINE HERPESVIRUS-1 IN 6 CATS

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Background: Feline herpesvirus-1 (FHV-1), a common upper respiratory pathogen in domestic cats, infrequently manifests as ulcerative dermatitis. Classically, skin lesions occur on the face and are histologically characterized by necrotizing and eosinophilic dermatitis with or without epithelial intranuclear inclusions.

Objective: This retrospective study aims to describe non-eosinophilic dermatologic lesions associated with FHV-1 in cats.

Methods & **Results:** Six cats were diagnosed with non-eosinophilic dermatitis attributed to FHV-1 by postmortem examination or surgical biopsy at the University of California-Davis Weill School of Veterinary Medicine from 2000 to 2026. Lesion locations were the nasal planum (4/6), muzzle (4/6), periocular region (1/6), lips

(1/6), chin (1/6) and face (not otherwise specified; 1/6). When clinical or gross descriptions were available (4/6), the affected skin was eroded to ulcerated with thick crusts. Histologically, there was necrotizing dermatitis and folliculitis characterized by ulceration, furunculosis, and a largely neutrophilic infiltrate along with variable numbers of lymphocytes and histiocytes. Rare to occasional intranuclear inclusions were present (3/6), equivocal (2/6), or not appreciated (1/6) and were most frequently identified within follicular followed by sebaceous, apocrine, and epidermal epithelium. In cases for which immunohistochemistry for FHV-1 was performed (5/6), epithelia adjacent to and within lesions were immunoreactive.

Conclusion: The cutaneous lesions documented in these cats suggest that dermatitis associated with FHV-1 can be necrotizing with a predominantly neutrophilic to lymphohistiocytic infiltrate rather than eosinophilic. Feline herpesvirus-1 should be considered in cases of facial necroulcerative dermatitis and folliculitis even if eosinophils and intranuclear inclusions are absent.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Feline, **Systems:** Integument

32: SCHISTOSOMIASIS IN A DOMESTIC SHORTHAIR CAT

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Schistosomiasis is well documented in wildlife species and dogs but is rarely reported in domestic cats. In addition, cases of schistosomiasis are uncommon in Kansas. A 4-year-old male neutered strictly outdoor domestic shorthair cat residing in Kansas, with no known travel history, was found dead and submitted to the Kansas State Veterinary Diagnostic Laboratory for postmortem examination. At necropsy, the liver was diffusely mottled dark brown to dark red with a markedly accentuated parenchymal reticular pattern. Histologically, lesions predominantly affected the liver and small intestine and composed of multifocal to coalescing eosinophilic to granulomatous infiltrates centering on vasculatures or extending randomly within the parenchyma. Associated granulomas contained moderate numbers of thin-walled, clear to golden-brown trematode eggs and eggshell fragments measuring up to 60 µm in diameter with some eggs containing miracidia. Occasional trematode eggs were identified within hepatic central veins. Mild portal and centrilobular fibrosis and biliary hyperplasia were in the affected regions. In the small intestine, trematode eggs frequently transmurally affected. Trematode eggs were also identified on fecal examination. Given the affected organs, histologic findings with prominent vascular involvement, and fecal examination results, a diagnosis of schistosomiasis was made, with *Heterobilharzia americana* suspected as the likely etiologic agent. Further molecular characterization is currently in progress to identify the *Schistosoma* species. This case report highlights the importance of including schistosomiasis in the differential diagnosis in cases of hepatic and intestinal granulomas in cats.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Feline, **Systems:** Hepatobiliary

33: PHEOCHROMOCYTOMA IN A PARAKEET

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Microscopic examination of a 5-year-old male Parakeet (Melopsittacus undulatus) identified an adrenal pheochromocytoma characterized histologically by an unencapsulated, infiltrative proliferation of medullary-type neuroendocrine cells arranged in nests and packets supported by a fine fibrovascular stroma. In avian species, where cortical (interrenal) and medullary (chromaffin) cells are intermingled rather than anatomically segregated into cortex and medulla, differentiation of adrenal neoplasms can be challenging. Immunohistochemistry is used to differentiate cortical tumors from medullary ones. This mass demonstrated variable granular intracytoplasmic immunoreactivity for neuron specific enolase in approximately 70% of neoplastic cells, while chromogranin A and synaptophysin were negative, findings interpreted as supportive for neuroendocrine differentiation. Previous case reports of pheochromocytomas in avian species are rare compared with domestic mammals and they are generally considered slow-growing neoplasms with variable clinical manifestations typically related to the overproduction of catecholamines.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Avian, **Systems:** Endocrine

34: DISSEMINATED LISTERIA MONOCYTOGENES INFECTION PRESENTING AS SEVERE NECROTIZING GRANULOMATOUS PANCARDITIS IN A BANTAM CROSS CHICKEN

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Background:

Systemic bacterial infections in poultry often present with overlapping lesions. While Pullorum disease and fowl cholera are common differentials, *Listeria* is a less frequently identified but significant cause of fatal avian systemic disease capable of producing severe cardiac pathology.

Objective:

To characterize the pathological lesions of fatal *Listeria monocytogenes* septicemia, presenting primarily as severe pancarditis, in a juvenile chicken.

Methods:

A 5-month-old male Bantam cross chicken exhibiting lethargy and an upright "penguin-like" stance was submitted for postmortem examination. Tissues were evaluated using hematoxylin and eosin (H&E), Gram, Grocott’s Methenamine Silver (GMS), and Ziehl-Neelsen (ZN) stains. Bacterial culture and viral polymerase chain reaction (PCR) testing of the heart were performed.

Results:

Grossly, the pericardial sac was diffusely thickened and adhered to the epicardium. The myocardium was severely effaced by pale, firm tissue, greenish gelatinous exudate, and clotted blood, resembling a massive granuloma or neoplastic infiltrate. Histologically, the primary cardiac lesion was a severe, necrotizing, granulomatous pancarditis. Multisystemic septicemic spread resulted in periportal granulomatous hepatitis, multifocal splenic necrosis, and lymphohistiocytic perivascular encephalitis. Twort's Gram stain highlighted abundant Gram-positive bacilli within necrotic foci. GMS, ZN, *Salmonella* culture, and viral PCR were negative. Bacterial culture of the heart revealed heavy growth of *Listeria monocytogenes*.

Conclusions:

This case highlights an unusually severe presentation of listeriosis in a backyard chicken. The profound cardiac effacement mimicking neoplastic or granulomatous disease underscores the importance of including *Listeria* in differential diagnoses for poultry mortalities with striking cardiac pathology.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Avian, **Systems:** Cardiovascular

35: HISTOMORPHOLOGICAL CHARACTERIZATION OF SPONTANEOUS CILIATED GASTRIC LESIONS IN SIBERIAN HAMSTERS (PHODOPUS SUNGORUS)

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Background: During a histologic characterization study of a closed colony of naive Siberian hamsters (*Phodopus sungorus*), a previously unrecognized gastric lesion was identified.

Objective: This study aimed to describe the morphology, prevalence, and distribution of this spontaneous finding.

Methods: Grossly unremarkable stomachs from untreated hamsters (n=158; both sexes; age 3 weeks - 25 months) were fixed in 10% neutral buffered formalin, routinely processed, paraffin-embedded, sectioned, and stained with hematoxylin and eosin for brightfield evaluation.

Results: Ciliated gastric epithelial proliferations were identified in 111 of 158 (70%) hamsters. Lesions were consistently located at the junction of the keratinized stratified squamous mucosa and glandular mucosa, near the limiting ridge. Histologically, they exhibited a spectrum of architectural patterns, including simple tubular profiles, multilocular cystic structures, papillary cystic proliferations, or mixed patterns. These structures were lined by a well-differentiated simple columnar epithelium with prominent apical cilia. The epithelial cells had basal, round nuclei, eosinophilic cytoplasm, and rare mitotic figures. Lumina variably contained proteinaceous material, inflammatory cells, and/or cellular debris. Foci of non-ciliated club cell-like secretory cells were occasionally noted. Common additional findings included mixed inflammatory infiltrates in the adjacent stroma and alterations of the

limiting ridge/nonglandular mucosa, including hyperplasia/papillary change and hyperkeratosis.

Conclusions: We describe a highly prevalent, spontaneous, and novel ciliated gastric lesion in Siberian hamsters. The location and respiratory-like cellular phenotype suggest a developmental error from the embryonic foregut common to both the GI and lung. This finding establishes an important baseline lesion for this species in research settings.

Discipline: Anatomic pathology, **Topic:** Comparative pathology, **Species:** Rodents, **Systems:** Gastrointestinal

36: PATHOLOGICAL AND CLINICAL DESCRIPTION OF 17 ISOCUPRESSIC ACID–INDUCED ABORTIONS IN CATTLE

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Isocupressic acid (IA) is a resin acid found in certain conifers, especially *Pinus ponderosa*. Exposure to IA causes livestock abortion and detection of its metabolites in fetal fluids confirm exposure. A retrospective study was conducted to characterize the clinicopathologic features associated with this toxicosis in 17 bovine abortions from 12 submissions with detection of IA metabolites by LC/MS submitted to CAHFS (2012–2026) from eight counties across three states. Breeds were Charolais (23.5%), Angus (35.3%), mixed (29.4%), or unreported (11.8%). Most abortions occurred in the third (76.6%) or second (11.7%) trimester; gestational age was unknown in 11.7% of fetuses. Placentas were not submitted with any of the cases. Clinical information was limited, but 82.3% of cases had reported pine needle exposure of the dams and 23.5% had history of previous abortions. Gross lesions were absent in 47% of fetuses; remaining fetuses had mild hepatomegaly (29%) and subcutaneous neck/ventrum edema (17%). Moderate (70.7%) to severe (5.9%) autolysis was present in the fetuses. Histologically, the alveoli contained meconium/keratin (88.2%), occasionally with mild multifocal inflammation. Mild hepatocellular vacuolation and cholestasis occurred in 41.2% of fetuses. Standard abortion panel was negative or considered unrelated to abortion in all fetuses. Yet, 41% of fetuses showed mildly increased or decreased selenium concentrations. Overall, 82.3% of cases were diagnosed as pine needle abortion based on toxicology and exposure history. The source of the IA was not determined for 17.7% of cases, in which other IA containing genera as *Juniperus*, *Cupressus* and *Gutierrezia* were considered as differentials.

Discipline: Anatomic pathology, **Topic:** Toxicology, **Species:** Bovine, **Systems:** Reproductive

37: CRESCENTIC AND HEMORRHAGIC MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS IN AUSTRALIAN WHITE LAMBS – A SUSPECTED NOVEL INHERITED DISEASE

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Crescentic and hemorrhagic, membranoproliferative glomerulonephritis has been identified in Australian White breed lambs aged 2 to 10-weeks-old from seven properties in New South Wales, Australia. Lambs presented with lethargy, recumbency, neurological signs, respiratory distress, and rapid or sudden death. This condition is suspected to be an emerging, inherited congenital renal disease affecting Australian White lambs.

Postmortem examination of fourteen lambs at the Elizabeth Macarthur Agricultural Institute revealed renomegaly with miliary cortical hemorrhages, variable degrees of bilateral, non-obstructive hydronephrosis, pulmonary congestion and edema, and intracavity effusions. Histopathology consistently revealed severe, chronic, membranoproliferative glomerulonephritis with crescent formation, glomerular hemorrhage, Bowman's space dilation, glomerulosclerosis, interstitial fibrosis and mild lymphoplasmacytic interstitial nephritis. Ultrastructural examination of two kidney samples revealed mesangiolysis, absence of endothelial cells lining glomerular capillaries, and deposits of electron dense material within the glomerular lamina dens resembling immune complex or complement deposits. Ancillary testing excluded common bacterial etiologies, and toxicities including copper, lead, and arsenic.

Although the exact pathogenesis of this disease is unknown, it is suspected to be an inherited, congenital disease given the age distribution, breed specificity, and exclusion of common etiologies for glomerulopathies. Similar inherited glomerulopathies have been reported in some species including Finnish Landrace-mixed breed lambs, Brittany Spaniel dogs, Norwegian Yorkshire piglets and humans.

Further genetic investigation using SNP chip genotyping, Illumina sequencing, and Oxford Nanopore sequencing is underway to identify the likely causal variant. A shared region of homozygosity containing a candidate gene has been identified, with candidate SNPs and structural variants currently undergoing validation.

Discipline: Anatomic pathology, **Topic:** Genetic/hereditary disorders, **Species:** Small ruminants, **Systems:** Urinary

38: DISSEMINATED MYELOID ROUND CELL NEOPLASIA WITH MONOCYTIC DIFFERENTIATION IN A POT-BELLIED PIG

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A 4-year-old pot-bellied barrow was presented for necropsy after euthanasia due to acute progression of chronic hindlimb paraparesis to quadriparesis with urinary and fecal retention. On gross examination, there was extensive accumulation of extradural yellow-green material extending along the ventral vertebral canal from the brainstem to the lumbar spinal cord. Similar material effaced vertebral marrow and air cells of left mastoid process; and disseminated pale tan cortical micronodules were also present in both kidneys. Histologically, the yellow-green material was comprised of densely cellular sheets and packets of round cells that effaced vertebral myeloid components and extended into the extradural space to compress the overlying spinal cord, with severe axonal degeneration correlating to clinical neurologic deficits. Neoplastic cells had abundant, densely granular eosinophilic cytoplasm, paracentral oval nuclei with coarsely stippled chromatin and mild anisocytosis/anisokaryosis. Similar cells comprised the renal cortical micronodules. Immunohistochemically, neoplastic cells demonstrated strong cytoplasmic and membranous immunoreactivity for Iba1, weak CD117 labeling, and were negative for myeloperoxidase, lysozyme, CD3, CD20, chromogranin, synaptophysin, and toluidine blue staining, supporting a diagnosis of myeloid round cell neoplasia with monoblastic differentiation. Myeloid sarcomas (chloromas) are rare granulocytic neoplasms reported in swine, which share the striking gross and histologic features of the neoplasm in this animal. However, the strong Iba1 immunoreactivity and lack of myeloperoxidase expression in his case support monoblastic differentiation, representing an unusual variant of myeloid neoplasia. This case expands the spectrum of porcine myeloid neoplasms and highlights the importance of immunophenotyping in distinguishing monocytic from granulocytic myeloid sarcomas.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Porcine, **Systems:** Nervous

39: GRANULOMATOUS MURAL FOLLICULITIS ASSOCIATED WITH OVINE HERPESVIRUS-2 IN A NIGERIAN DWARF GOAT

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A 2-year-old, intact male Nigerian Dwarf goat was presented with a 2-month history of generalized alopecia with lichenification, scaling, and crusting that was unresponsive to dietary modification and treatment with ketoconazole, Mal-A-Ket® shampoo, Liquamycin® LA-200 and Multimin®. A 6-mm representative punch skin biopsy revealed that the follicular epithelium was frequently disrupted by nodular to ill-defined infiltrates of epithelioid macrophages, multinucleated giant cells, neutrophils, eosinophils, and nuclear debris. Inflammation involved multiple levels of affected follicles, predominantly the inner-to-mid portion of the follicular wall, with occasional full-thickness obstruction of the follicular epithelium. Affected follicular wall also contained spongiotic pustules, keratinocyte dissociation, acantholysis, and occasional apoptosis. No bacterial or fungal organisms were identified with special stains. After confirming the animal co-lived with sheep, scrolls from formalin-fixed paraffin-embedded blocks were submitted for ovine herpesvirus 2 (OvHV-2) polymerase chain reaction, yielding a positive result (Ct 23.6). These findings supported a diagnosis of OvHV-2–associated granulomatous mural folliculitis (GMF).

Three months after biopsy, the owner reported hair regrowth over previously affected areas with grossly normal skin. Granulomatous mural folliculitis is an uncommon and incompletely understood manifestation of malignant catarrhal fever that may occur as a localized cutaneous lesion or as part of systemic disease. Although the relationship between OvHV-2 and GMF remains unclear, follicular injury may result from direct viral effect or immune-mediated mechanisms. Histologic features together with molecular detection of the OvHV-2 support the diagnosis and help distinguish this conduction from important differentials, such as dermatophytosis and adverse cutaneous drug reaction.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Small ruminants, **Systems:** Integument

40: TONSILLAR ACANTHOLYTIC SQUAMOUS CELL CARCINOMA WITH MICROVASCULAR TUMOR CELL EMBOLI IN A DOG

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A 14-year-old neutered male Beagle dog presented with progressive dyspnea, respiratory stridor, tachypnea, and abdominal distension. Clinicopathologic findings were nonspecific, and imaging was unremarkable. Following rapid clinical deterioration, the dog was euthanized and submitted for necropsy. Gross findings included multifocal hemorrhages, a unilateral tonsillar mass, and a focal urinary bladder nodule in the mucosa. Histologically, the tonsillar mass was an infiltrative epithelial neoplasm with squamous differentiation and prominent, non mucin producing, pseudoglandular structures with acantholysis, consistent with Acantholytic Squamous Cell Carcinoma (ASCC). A concurrent high-grade Urothelial Carcinoma was identified in the urinary bladder. Numerous neoplastic cells were present within capillaries of the lung, heart, and adrenal glands, without discrete metastatic masses. To determine the origin of these neoplastic cells, immunohistochemistry was performed for IBA-1, pan-cytokeratin, vimentin, uroplakin III and cytokeratin 5 on sections of the tonsil, lung, adrenal gland and urinary bladder. Intravascular neoplastic cells shared immunoreactivity (Cytokeratin-5) with the tonsillar tumor, confirming a squamous epithelial origin and supporting hematogenous dissemination of tonsillar ASCC. This aggressive type of neoplasm remains rarely reported in veterinary literature, and this case highlights an unusual presentation of canine tonsillar ASCC with widespread intravascular tumor cell embolization, consistent with early metastatic stages, and underscores the value of immunohistochemistry in distinguishing multiple primary neoplasms.

Content based on forthcoming publication

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Canine, **Systems:** Systemic

41: ATYPICAL MYCOBACTERIAL DERMATITIS IN THREE RHESUS MACAQUES (MACACA MULATTA)

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Atypical mycobacteria (AM) are a ubiquitous group of environmental organisms that can cause varying clinical presentations ranging from skin lesions to deeper infection with or without systemic dissemination. In this work, we describe the clinical signs, gross pathology and histopathology of atypical mycobacterial dermatitis in three experimentally naive Indian origin rhesus macaques. The three animals presented to the clinic at Tulane National Biomedical Center (TNBRC) for persistent non healing, ulcerated skin wounds and abscesses. The three animals, histologically, had severe pyogranulomatous panniculitis and dermatitis with large numbers of multinucleated giant cells. Bacterial cultures of skin wounds/abscesses yielded *Mycobacteria sp*. in two out of the three animals. The abscess from the third animal yielded *Mycobacterium abscessus*. Acid fast staining of the skin sections revealed the presence of rare rod-shaped bacteria within the cytoplasm of macrophages. Also, macrophages contained abundant red cytoplasmic globules. Atypical mycobacterial dermatitis is rare in experimentally naïve, immunocompetent rhesus macaques.

Discipline: Anatomic pathology, **Topic:** Comparative pathology, **Species:** NHP, **Systems:** Integument

42: FIBROSING AND CALCIFIED THORACIC AORTIC ANEURYSM WITH PULMONARY INTERSTITIAL AND MEDIAL ARTERIAL FIBROSIS WITH EHV-5 INFECTION IN AN AGED HORSE WITH CHRONIC EIPH

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A 34-year-old Thoroughbred (ex-racehorse) gelding with a history of chronic kidney disease, mild mitral valvular degeneration, pituitary pars intermedia dysfunction (PPID), and degenerative vertebral osteoarthritis was euthanized and submitted for autopsy. At autopsy, the thoracic aorta was palpably hard and segmentally and severely dilated (aneurysm) from the aortic arch to the mid-thoracic region measuring approximately 20cm x 10cm, with a mildly eroded, undulant, yellow to red brown, intimal surface. Also, both lungs had multifocal, firm, white, interconnecting bands of fibrosis primarily centered around the vessels. Histology of the aortic wall revealed loss of endothelium, expansion of the intima by lymphocytes and plasma cells, and marked degeneration of the tunica media characterized by changes in tinctorial affinity due to elastic fiber loss, accumulation of amorphous extracellular matrix, collagen deposition, mineral deposition, and osseous metaplasia. Comparison with an age-matched control horse using Verhoeff, Masson's trichrome, and von Kossa stains highlighted the severity of the lesions. Histology of the lungs confirmed marked, multifocal interstitial, peribronchiolar, and perivascular fibrosis with abundant interstitial hemosiderophages, and the latter is consistent with Chronic Exercise Induced Pulmonary Hemorrhage (EIPH). In situ hybridization for equine herpes virus 5 (EHV-5) detected a rare but consistent signal in macrophages situated within areas of pulmonary fibrosis. These findings together indicate a process of thoracic aortic degeneration occurring concurrently with perivascular pulmonary fibrosis in addition to EHV-5 infection. We speculate a common pathogenesis of the lesions in the aorta and lung in which either EHV-5 or chronic EIPH may play a role.

Discipline: Anatomic pathology, **Topic:** Comparative pathology, **Species:** Equine, **Systems:** Cardiovascular

43: STATIC CYTOLOGY, WHOLE SLIDE CYTOLOGY, AND HISTOLOGY CORRELATES OF AN ORAL "HIGH-LOW" FIBROSARCOMA IN A GOLDEN RETRIEVER

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Background: Canine biologically high-grade/histologically low-grade oral fibrosarcoma ("high-low" FSA) presents a significant diagnostic challenge. These neoplasms are locally aggressive yet possess a misleading benign histologic appearance that mimics benign fibrous proliferations. Reports detailing the cytologic features of canine "high-low" FSA are remarkably scarce.

Objective: To describe the clinical, cytologic, and histopathologic correlation of an oral "high-low" FSA in a dog, emphasizing its unique cytological presentation.

Methods: A 7-year-old male neutered Golden Retriever presented with a poorly defined, progressive, firm swelling of the dorsal left muzzle and left maxillary gingiva, with radiographic periosteal lysis around tooth 204. Fine-needle aspiration (FNA) was performed and submitted for static cytology. The lesion progressed, and four months later FNA was repeated and submitted for whole slide cytology with an incisional biopsy.

Results: Static cytology and whole slide evaluation of the mass revealed paucicellular cytologic specimens composed of a monomorphic population of individualized, uniform, bland spindle cells with elongate nuclei and bipolar cytoplasmic tails (fibroblast morphology). Histopathology revealed a paucicellular infiltrative proliferation of spindle cells resembling fibroblasts with minimal atypia embedded in abundant collagenous matrix. The neoplastic cells exhibited minimal nuclear pleomorphism and low mitotic activity despite local tissue invasion.

Conclusion: To the authors' knowledge, this is the first dedicated report describing the cytological findings of a canine oral "high-low" FSA. Recognizing that cytologically bland, uniform spindle cell populations can represent highly aggressive oral malignancies is crucial for pathologists and clinicians. Awareness of this entity prevents underdiagnosis and ensures prompt, aggressive therapeutic intervention.

Discipline: Both, **Topic:** General pathology, **Species:** Canine, **Systems:** Gastrointestinal

44: PROLIFERATIVE GASTRITIS RESULTING IN PYLORIC STENOSIS IN A MIDDLE-AGED DUTCH WARMBLOOD HORSE

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A 16-year-old Dutch Warmblood gelding horse presented to the Cummings School Hospital for Large Animals for chronic gastric ulcerations and pyloric outflow

obstruction. A previous endoscopic biopsy of the pylorus showed necrosuppurative inflammation, followed by another biopsy 2 months later that showed marked epithelial proliferation. Both hyperplasia and epithelial neoplasia were included in the differential at that time. The horse was euthanized for progressive weight loss 2 months following the second biopsy despite medical management. On gross examination, circumscribing the pylorus is a circumferential, multinodular, white, friable, and multifocally ulcerated mucosal mass measuring 6 x 2 x 3 cm, leading to marked stenosis of the pyloric outflow tract. On histologic examination, the mucosa is composed of a marked proliferation of cuboidal to columnar epithelial cells forming budding tubules, acini, and papillary fronds on a fibrovascular stroma without invasion into the deeper gastric layers. Multifocally, tubules contain hypereosinophilic proteinaceous material and mucin. Anisocytosis and anisokaryosis are mild, and there are no mitotic figures in 2.37 mm^2. Using Alcian blue/PAS, the goblet cell differentiation is highlighted with PAS (consistent with gastric pits). The adjacent gastric mucosa is expanded by an increased number of lymphocytes, plasma cells, and eosinophils. This case presents clinical signs and the gross and histological findings associated with proliferative gastritis that leads to pyloric outflow obstruction in a horse. In this case, the proliferation of gastric pits is similar to that seen in chronic hypertrophic pyloric gastropathy in terrier dogs.

Discipline: Anatomic pathology, **Topic:** Comparative pathology, **Species:** Equine, **Systems:** Gastrointestinal

45: CLINICAL, MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL FEATURES OF A RENAL CELL CARCINOMA IN A SUMATRAN TIGER (PANTHERA TIGRIS SUMATRAE)

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Clinical history

An 18-year-old male Sumatran tiger (*Panthera tigris sumatrae*) from Barcelona Zoo presented with recurrent episodes of lethargy and hyporexia over approximately one year. Clinical evaluation revealed chronic renal disease, supported by hematological, biochemical, and urinalysis findings. The animal underwent repeated anesthetic procedures for reassessment of renal function and administration of fluid therapy. Local stem-cell treatments were also performed. Despite exhaustive supportive treatment, renal parameters progressively worsened, clinical signs became increasingly frequent, and the animal's quality of life declined, leading to euthanasia.

Macroscopic findings

Gross examination revealed severe pallor of the oral and ocular mucous membranes, poor body condition (BCS 1/5), and mild dehydration. A larger multilobulated, heterogeneous grey-green mass (6 × 5.5 cm) with central necrosis expanded the right kidney, while the left kidney exhibited mild pelvic dilation containing a white exudate and reddening of the parenchyma (pyelonephritis). Multifocal, well-demarcated, white to pale cream, nodules with central umbilication were also present throughout the spleen and liver.

Microscopic findings

The right kidney was replaced by an epithelial neoplasm of cuboidal cells forming ectatic tubules and solid nests, displaying cellular pleomorphism and low mitotic activity. Similar neoplastic proliferations were observed in the liver and spleen. Renal lesions included membranoproliferative glomerulonephritis, interstitial fibrosis, chronic pyelonephritis, and tubular mineralization.

Immunophenotyping

Neoplastic cells were positive for vimentin, CK7 and CD10, and negative for CD117 and COX2. Internal positive controls reacted appropriately with the exception of PAX8 that did not cross-react. This immunophenotype is supportive of papillary renal cell carcinoma.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Wildlife/Zoo, **Systems:** Urinary

46: THYROGLOBULIN AND CALCITONIN CO-EXPRESSION IN COMPACT (SOLID) THYROID CARCINOMAS IN TWO DOGS

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Background： Canine mixed medullary and follicular cell carcinoma (MMFCC) is a rare subtype of canine thyroid carcinoma (CTCs), with only one case reported to our knowledge. CTCs include follicular thyroid carcinomas (FTCs) and medullary (C-cell) thyroid carcinomas (MTCs). Solid thyroid carcinomas are difficult to distinguish between MTCs, FTCs and MMFCCs based on histomorphology alone. Since there is a difference in biologic behavior between FTCs and MTCs and those of MMFCCs are unknown, compact cell components should be characterized by immunohistochemistry.

Methods： To retrospectively evaluate the gross, histologic, and immunohistochemical features in two cases of canine thyroid carcinomas.

Results： Both dogs were neutered males characterized by swelling of the neck. Histologically, these tumors most closely resembled compact thyroid carcinoma. In Case 1, a large number of neoplastic cells were immunopositive for thyroglobulin, calcitonin, TTF-1, and synaptophysin and were immunonegative for chromogranin A. In Case 2, neoplastic cells immunopositive for calcitonin, TTF-1, synaptophysin and chromogranin A were intermixed with cells that were immunopositive for thyroglobulin and TTF-1. In some areas, the neoplastic cells were immunopositive for both thyroglobulin and calcitonin.

Conclusion： Both case 1 and case 2 were confirmed to be MMFCCs based on immunohistochemistry. One previous case report and these two cases suggest that MMFCCs may potentially be more common in neutered males. Since both cases showed co-positivity for thyroglobulin and calcitonin, they are considered tumors possessing characteristics of both types rather than collision tumors, a finding consistent with the previous study.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Canine, **Systems:** Endocrine

47: MALAKOPLAKIA ASSOCIATED WITH CHRONIC ENDOCRINE AND RENAL DISEASE IN A BEAGLE

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Introduction: Malakoplakia is an uncommon histiocytic inflammatory condition characterized by accumulation of macrophages with impaired intracellular degradation. It is most commonly associated with immune stimulation and recurrent bacterial infections, particularly *Escherichia coli*. In veterinary medicine, malakoplakia has been reported in only a handful of species and most frequently affects the urinary tract of female dogs. This report describes malakoplakia in a dog with multiple chronic systemic comorbidities.

Methods: A 14-year-old spayed female Beagle with poorly managed hyperadrenocorticism, hypothyroidism, chronic kidney disease, chronic malabsorptive intestinal disease, recurrent urinary tract infections, and chronic heart disease underwent complete diagnostic necropsy with histopathology, periodic acid-Schiff (PAS) staining, bacterial culture, and fluorescence in situ hybridization (FISH).

Results: Necropsy confirmed multiple chronic endocrine, renal, cardiac, and musculoskeletal comorbidities. The urinary bladder mucosa and renal medulla/pelvis were expanded by dense infiltrates of enlarged plasmacytoid to histiocytic cells containing abundant PAS-positive intracytoplasmic granules, consistent with malakoplakia. FISH of urinary bladder and kidney sections demonstrated discrete clusters of intracellular short rod-shaped bacteria co-localized with PAS-positive histiocytic infiltrates within the urinary bladder, supporting impaired intracellular bacterial degradation as the underlying pathogenesis. Additional superficial bacteria associated with ulcerated bladder mucosa were considered secondary colonization or postmortem contamination.

Conclusion: This case highlights malakoplakia involving the urinary bladder and kidneys in a dog with multiple chronic systemic diseases. Combined histochemical and FISH findings support a bacterial etiology consistent with the recognized pathogenesis of malakoplakia. Malakoplakia should be considered in dogs with recurrent urinary tract infections, particularly in the setting of concurrent immune dysfunction.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Canine, **Systems:** Urinary

48: COLLAGENOFIBROTIC GLOMERULOPATHY ACCOMPANIED BY SEVERE MULTISYSTEMIC MINERALIZATION IN A JUVENILE AUSTRALIAN SHEPHERD

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Collagenofibrotic glomerulopathy, or type III collagen glomerulopathy, is a rare glomerular disease defined by abnormal deposition of type III collagen within glomeruli, leading to progressive renal dysfunction. Documented cases in dogs are extremely rare. This case describes collagenofibrotic glomerulopathy confirmed by special staining and ultrastructural evaluation in a juvenile Australian Shepherd presenting with multisystemic mineralization.

A 17-week-old female Australian Shepherd underwent complete necropsy, including histopathology with special stains (periodic acid-Schiff, Jones methenamine silver, Masson's trichrome, Congo red, and Von Kossa) and renal consultation through the International Veterinary Renal Pathology Service. Transmission electron microscopy was also performed.

Histopathology revealed marked glomerular remodeling consistent with collagenofibrotic glomerulopathy, characterized by severe mesangial and capillary wall expansion with concurrent tubular degeneration and mineralization. Renal consultation further identified glomerulocystic atrophy and chronic tubulointerstitial fibrosis. Masson's trichrome stain confirmed collagen deposition, while Congo red staining was negative for amyloid. Transmission electron microscopy demonstrated bundles of type III collagen fibrils with characteristic cross-striations expanding the mesangium and capillary walls, confirming collagenofibrotic glomerulopathy. Widespread metastatic mineralization affected the kidneys, myocardium, gastrointestinal tract, skeletal muscle, diaphragm, thymus, and thyroid gland. Severe myocardial degeneration and necrosis were considered the probable cause of death.

This case documents collagenofibrotic glomerulopathy confirmed by histochemical and ultrastructural evaluation in a juvenile dog, expanding the limited veterinary literature on this rare glomerular disorder. Recognition of the morphologic and ultrastructural features is essential for distinguishing this condition from other causes of glomerular matrix expansion and may facilitate diagnosis of hereditary renal disease in young dogs.

Discipline: Anatomic pathology, **Topic:** Genetic/hereditary disorders, **Species:** Canine, **Systems:** Urinary

49: PRIMARY CENTRAL NERVOUS SYSTEM DIFFUSE LARGE B CELL LYMPHOMA IN A DOG

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A 10-year-old, female spayed, Australian Shepherd dog presented on emergency for abnormal mentation and pelvic limb weakness of approximately 3 days duration. Bloodwork and thoracic, abdominal, and spinal radiographs at the primary veterinarian the previous day were unremarkable. Intracranial disease was suspected based on abnormal mentation and postural reactions, as well as paresis with ataxia. An MRI showed a strongly contrast-enhancing, intraventricular mass apparently arising from the choroid plexus of the left lateral ventricle with surrounding severe vasogenic edema and a moderate right midline shift. A choroid plexus tumor was prioritized. Due to poor prognosis, the patient was euthanized and submitted for necropsy. Serial sectioning of the brain post-fixation revealed a focal, homogenous,

pale tan, left-sided mass extending from the approximately the rostral commissure to the diencephalon and mesencephalon junction. The mass protruded into the left lateral ventricle and was surrounded by a thin rim of red tissue. Microscopically, this mass consisted of large round cells and extensive necrosis surrounded by reactive astrocytes and microglia. The round cells showed features of atypia, including anisocytosis, anisokaryosis, and abnormal mitotic figures. Immunohistochemcial staining confirmed a Pax5+, CD3-, MUM1-, Iba1- phenotype, consistent with a B-lymphocyte lineage. The patient was diagnosed with a primary central nervous system (CNS) diffuse large B cell lymphoma based on the IHC results and lack of masses or abnormal round cells in any other tissue examined. The cervical spinal cord was grossly and microscopically unremarkable. This case describes a rare CNS neoplasm in a dog.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Canine, **Systems:** Nervous

50: AVIAN POLYOMAVIRUS INFECTION IN TWO PSITTACINE BIRDS

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A golden conure (GC) and a yellow-naped amazon (YA), both 2 months old, from a bird store, were submitted for necropsy following sudden death. Necropsy revealed hepatomegaly, splenomegaly, severe multifocal myocardial hemorrhage, and serous to hemorrhagic pericardial effusion. Histopathologically, there was hemorrhagic necrotizing hepatitis and splenitis with large (approximately 6-9 µm in diameter), glassy, pale, basophilic to amphophilic, intranuclear inclusion bodies frequently in the spleen and rarely in the liver. Transmission electron microscopy of the spleen (GC) identified intranuclear replication and assembling organelles with icosahedral viral particles measuring 40–45 nm, suggestive of Polyomavirus. Avian polyomavirus (APV) PCR on fresh liver (GC) was positive, and subsequent next-generation sequencing revealed 98.88% sequence identity (4975/4981 bp) to *Gammapolyomavirus avis* isolate CBNU_APyV_4 sequenced from *Myiopsitta monachus*. Both conventional PCR for *Chlamydia psittaci* and Psittacid alphaherpesvirus 1 (Pacheco's disease virus) yielded weak positive bands in the liver (GC) with (Sanger sequencing pending). PCR for Psittacine Beak and Feather Disease Virus was negative (GC). Further molecular diagnostics (YA) are pending. APV, aka Budgerigar fledgling disease virus, is a multisystemic disease of nestling budgerigars, characterized by hepatic necrosis, membranous glomerulopathy, multiorgan hemorrhage, and intranuclear inclusions. Clinical disease is most severe in young birds, whereas subclinical infection and lifelong viral carriage may occur in adult birds. Viral shedding occurs through feces, oral secretions, and feather dander, facilitating transmission in densely housed populations. This case highlights APV as an important differential for acute mortality in young psittacines and underscores the challenges of biosecurity and disease control in flocks.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Avian, **Systems:** Systemic

51: RESPIRATORY DISTRESS SYNDROME IN A PREMATURE CALF

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Respiratory distress syndrome (RDS) has been reported in premature infants and newborn mammals. In calves, RDS has been associated with prematurity due to surfactant deficiency and pulmonary immaturity. An approximately 7-month-gestation aborted calf was submitted to the Kansas State Veterinary Diagnostic Laboratory for examination. Grossly, the lung lobes were diffusely heavy, wet, and rubbery with multifocal hemorrhagic foci. Histopathologic evaluation of the lung revealed marked interstitial pneumonia, intrabronchiolar and intra-alveolar hyaline membrane, hemorrhage, atelectasis, and hyperplastic bronchus-associated lymphoid tissues. Ancillary testing demonstrated negative PCR results for *Ureaplasma diversum* in fresh lung tissue and negative results for *Leptospira* spp., bovine viral diarrhea virus type 1 and 2, infectious bovine rhinotracheitis virus, and *Neospora* spp. in pooled fetal tissues. Aerobic bacterial culture of the lung yielded abundant *Pantoea agglomerans*, moderate *Streptococcus pluranimalium*, and slight *Siccibacter* sp. growth, and no known abortogenic bacterial pathogens were observed. A trace mineral panel on the calf liver showed deficient copper and vitamin E levels. Ocular nitrate level was within normal range. In this present case, the clinical history and pulmonary lesions are suggestive of a respiratory distress syndrome. Variable factors leading to RDS in premature calves have been proposed. Given the ancillary tests results, we suggested the trace elements deficiencies (copper, vitamin E) may play a role in impaired maturation of surfactant system and the subsequent development of RDS. This case report highlights the importance of including trace element deficiencies as differential diagnoses in cases of pulmonary lesions compatible with RDS in premature calves.

Discipline: Anatomic pathology, **Topic:** General pathology, **Species:** Bovine, **Systems:** Respiratory

Posters: Clinical Pathology, Experimental Disease, Industrial and Toxicologic Pathology, and Natural Disease

1: DISSEMINATED MYCOBACTERIUM AVIUM INFECTION IN A DOG WITH PROGRESSIVE GASTROINTESTINAL DISEASE

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A 6-year-old spayed female Siberian Husky presented with a one-month history of progressive gastrointestinal disease characterized by lethargy, fever, anorexia, weight loss, abdominal pain, and diarrhea that began after killing a skunk. The patient had a history of killing small mammals, including rabbits and opossum. Initial treatment with broad-spectrum antimicrobials resulted in improvement, but the patient's symptoms returned and the onset of hematochezia prompted referral to The Ohio State University internal medicine service. A complete blood count identified a

moderate, non-regenerative, normocytic, normochromic anemia (HCT 26%) and leukocytosis (21,000/µL) characterized by mature neutrophilia and mild lymphopenia. Abdominal ultrasound revealed an enlarged spleen with a honeycomb pattern and mildly heterogeneous liver. Fine needle aspirates of spleen and liver were submitted for cytologic evaluation, which revealed marked macrophagic inflammation and numerous, negatively staining, intracellular rods consistent with mycobacteria. Bacterial culture subsequently confirmed Mycobacterium avium. Despite aggressive antimicrobial therapy, the patient was euthanized due to disease severity. Post-mortem evaluation demonstrated multifocal, severe, granulomatous hepatitis, splenitis, nephritis, and lymphadenitis, with perivascular granulomatous infiltrates in the lung. Acid-fast rod-shaped bacteria were identified.

Mycobacterium avium complex (MAC) is a group of intracellular bacteria with a lipid coat that provides the characteristic negative staining on cytology. While the disease is usually self-limiting in adult dogs, it is difficult to treat when disseminated because of its inherent resistance to beta-lactam antibiotics. This case demonstrates the utility of cytologic evaluation in guiding diagnosis and clinical management.

Discipline: Clinical pathology, **Topic:** Infectious disease, **Species:** Canine, **Systems:** Gastrointestinal

2: CLINICOPATHOLOGIC AND MOLECULAR CHARACTERIZATION OF RECURRENT OPPURTUNISTIC INFECTIOUS AGENTS: NEOCUCURBITARIA UNGIS-HOMINIS, NOCARDIOSIS AND HISTOPLASMOSIS IN A DOG WITH IMMUNE-MEDIATED THROMBOCYTOPENIA

Basant Ahmed, Kari Frankhouse, Janice Park, Stephanie Shemilt, Anastasia Parashis, Shane Sills, Christopher Premanandan, Jenessa Winston, Maxey Wellman, Hamideh Esmaeilzadeh
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An eight-year-old neutered male mixed-breed dog presented to The Ohio State University with a 1–2 cm firm, cutaneous mass on the cranial right mid-antebrachium and a history of immune-mediated thrombocytopenia managed with leflunomide and prednisone. Cytology revealed numerous fungal hyphae measuring 2–8 µm in diameter with acute-angled branching, septal constrictions, internal magenta granulation, and a swollen, beaded appearance. Occasional 4–10 µm round yeast-like structures were also observed, accompanied by marked pyogranulomatous inflammation. Interpretation was most consistent with phaehyphomycosis. Fungal culture yielded nonspecific growth. Further evaluation with 18S rRNA sequencing from cytology slides using an ITS3/ITS4 amplicon generated forward and reverse sequences with 100% and 99.65% identity to Neocucurbitaria unguis-hominis. Three months later, an abscess involving the left hindlimb was noted. Cytology showed marked pyogranulomatous inflammation and many filamentous bacteria. Bacterial culture was consistent with nocardiosis, which was treated with doxycycline combined with leflunomide and prednisone. Four months later, right hindlimb swelling was noted. Cytology and enzyme immunoassay tests were supportive of histoplasmosis, and itraconazole was prescribed. Three months afterward, a left tarsal necrotic wound was noticed, and histopathology showed marked, multifocal-to-coalescing pyogranulomatous inflammation with suspect intracellular fungal yeasts. Positive staining with GMS and PAS was supportive of histoplasmosis. Poor quality of life prompted euthanasia. This case highlights the importance of cytology,

histopathology, and molecular diagnostics in identifying opportunistic infections associated with immunosuppression. Neocucurbitaria unguis-hominis has been rarely reported in immunocompromised humans with keratitis. but to the authors' knowledge, this is the first report in dogs.

Discipline: Clinical pathology, **Topic:** Infectious disease, **Species:** Canine, **Systems:** Systemic

3: THE CYTOLOGICAL FEATURES OF AMYLOID-PRODUCING AMELOBLASTOMA OF FACIAL SKIN IN A CAT

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Background: Amyloid-producing ameloblastoma (APAB) is a rare odontogenic tumor in cats that typically arises from the gingiva. Occurrence in the facial skin has rarely been reported in cats.

Objective: To describe the cytologic features of facial APAB in a cat.

Methods: A 2-year-old spayed female mixed-breed cat presented with a 1 cm mass on the muzzle. Fine-needle aspiration cytology, histopathology, Congo red staining, and polarized light examination were performed.

Results: Cytology revealed many clusters of cohesive and occasionally individualized polygonal epithelial cells. They had moderate amounts of blue cytoplasm, indistinct cell borders, round to oval nuclei containing finely stippled chromatin and indistinct nucleoli. They showed moderate anisocytosis and anisokaryosis, and frequent multinucleation. Small to moderate amounts of amorphous pink extracellular material and irregular angular crystalline material were present with mild macrophagic inflammation and occasional multinucleated giant cells. Histologically, the mass was composed of odontogenic epithelial cells arranged in islands and branching cords with evident intercellular bridges and occasional keratinization. Abundant extracellular homogeneous eosinophilic material was associated with fibrosis and macrophage infiltration. The eosinophilic extracellular material stained positively with Congo red and demonstrated apple-green birefringence under polarized light, confirming amyloid. These findings supported a diagnosis of APAB.

Conclusions: This case represents, to the authors' knowledge, the first description of the cytologic features of facial APAB in a cat. APAB should be included as a differential diagnosis for feline facial cutaneous masses characterized cytologically by polygonal epithelial cells accompanied by extracellular amyloid and crystalline material.

Discipline: Clinical pathology, **Topic:** Oncology, **Species:** Feline, **Systems:** Integument

4: SCEDOSPORIUM SPP. FUNGAL CHOLECYSTITIS IN A DOG

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A 5-year-old, castrated male, mixed-breed dog was presented with a 3-day history of anorexia, vomiting, and lethargy. A complete blood count and blood smear evaluation identified findings consistent with inflammation and concurrent glucocorticoid response (neutrophilia with a left shift and toxic change, monocytosis, and lymphopenia). The serum chemistry showed increased ALT and ALP activity and hyperbilirubinemia, findings supportive of hepatocellular injury and cholestasis. Abdominal ultrasound identified gallbladder wall thickening and bile was collected. Cytologic evaluation of the bile identified a mixed bacterial population with concurrent neutrophilic inflammation. Bacterial culture yielded growth of *Enterococcus casseliflavus* and hemolytic *Escherichia coli*. The patient was treated with amoxicillin/clavulanate potassium and enrofloxacin and monitored with serial chemistry panels and ultrasound evaluations. Two-months later, moderate amounts of debris was identified in the gallbladder via ultrasound. Bile was collected and submitted for cytologic evaluation. Moderate numbers of bacterial cocci and thin rods were identified in the submitted bile along with fungal hyphae. The fungal hyphae were approximately 2 to 3-µm wide, septated, and displayed 90˚ branching. Bacterial culture identified moderate growth of *Enterobacter hormaechei* and *faceium*. Fungal culture identified growth of *Scedosporium spp.*, but could not be further speciated. Two-days later, a cholecystectomy was performed. Histologic evaluation of the gallbladder had moderate diffuse proliferative and lymphoplasmacytic cholecystitis with fibrosis and hemorrhage. Scedosporiosis is a fungal disease of increasing clinical relevance in both human and veterinary medicine. *Scedosporium spp*. was previously considered a ubiquitous soil saprophyte, but is now recognized as an opportunistic pathogen, particularly for immunocompromised patients.

Discipline: Clinical pathology, **Topic:** Infectious disease, **Species:** Canine, **Systems:** Hepatobiliary

5: PLASMA CELL NEOPLASIA WITH IGM BI-CLONAL GAMMOPATHY AND EXTREME CRYOGLOBULINEMIA

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A 10-year-old male castrated Golden Retriever presented to Mizzou on emergency for signs of lethargy, anorexia, vomiting, and exercise intolerance. His sampled blood was an abnormal, gel-like consistency at room temperature. A blood smear revealed very low numbers of plasma cells and abundant aggregates of basophilic, smooth material arranged in ill-defined lakes interpreted as cryoglobulin. Fine needle aspiration of a splenic mass revealed moderate numbers of plasma cells, suggesting neoplastic expansion, and extramedullary hematopoiesis. Liver aspirates contained low numbers of morphologically similar plasma cells. PARR results from the spleen and liver supported clonally rearranged immunoglobulin gene. Serum protein electrophoresis with immunofixation was consistent with an IgM bi-clonal gammopathy. Clinicopathologic findings in this case support probable plasma cell neoplasia with IgM bi-clonal gammopathy and associated extreme cryoglobulinemia. In veterinary medicine, IgM gammopathies have been

associated with multiple myeloma (MM), lymphoplasmacytic lymphoma/Waldenström macroglobulinemia (WM), B cell chronic lymphocytic leukemia, B cell lymphoma, and other myeloma related disorders. IgM M-proteins in WM and related disorders are associated with hyperviscosity syndrome, peripheral neuropathy, amyloidosis, cold agglutinin disease, and cryoglobulinemia. Cryoglobulins are immunoglobulins that precipitate at low temperatures and re-dissolve upon warming. Cryoglobulinemia is a rare phenomenon in animals and humans. Monoclonal cryoglobulinemia has been described in dogs and cats with MM and a dog with WM. IgM cryoglobulinemia has been reported in a dog with WM; to our knowledge, IgM cryoglobulinemia associated with MM or extramedullary plasmacytoma has not been previously reported.

Discipline: Clinical pathology, **Topic:** Oncology, **Species:** Canine, **Systems:** Hematopoietic

6: MALASSEZIA-ASSOCIATED ANAL SACCULITIS WITH ANAL SAC RUPTURE IN A DOG

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A 13-year-old spayed female Rhodesian Ridgeback was presented for evaluation of an acute episode of hematochezia and vomiting. Physical examination was unremarkable except for obesity (BCS 8/9) and a firm 3.5 cm left anal sac region mass. Fine-needle aspiration yielded a highly cellular sample with moderate blood contamination, keratin, cholesterol crystals, and nuclear streaming. Nucleated cells consisted predominantly of degenerate neutrophils, with fewer macrophages, mixed lymphocytes, occasional reactive fibroblasts, and rare mast cells. Numerous intra- and extracellular, 1.5 x 5.0 µm, ellipsoidal, bilobed yeasts consistent with Malassezia spp. were present. Two days later, the dog was re-presented with weakness, lethargy, and decreased appetite. Abdominal ultrasonography, complete blood count, and serum biochemistry revealed no significant changes. Supportive outpatient management was elected; however, the patient deteriorated further and was euthanized the following day because of progressive hind limb weakness and declining clinical status. At necropsy, the left anal sac was filled with black, pasty, malodorous material and was surrounded by fibrosis. Histologically, yeasts were present in the anal sac corneal layer. The connective tissues surrounding the anal sac were effaced by pyogranulomatous inflammation with similar yeasts and free keratin, consistent with anal sac rupture. Fungal culture of the anal sac wall yielded Malassezia pachydermatis, identified by MALDI-TOF. This case demonstrates yeast-associated anal sacculitis with anal sac rupture, supported by unusually high numbers of Malassezia yeasts within the anal sac lumen and within the surrounding pyogranulomatous inflammation, highlighting Malassezia as a clinically relevant opportunistic pathogen.

Discipline: Both, **Topic:** Infectious disease, **Species:** Canine, **Systems:** Integument

7: CANINE ONCO-SURVEILLANCE VIA CYTOLOGY: ASSESSING TUMOR PREVALENCE AND PREDICTORS OF MALIGNANCY IN A SMALL ANIMAL HOSPITAL

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Background: While fine-needle aspiration cytology (FNAC) is a vital tool, cytologically derived epidemiological data and multivariable risk factor assessments remain limited in the diagnosis of abnormal growths in canines.

Objectives: This study aimed to cytologically characterize spontaneous canine growths and utilize multivariable analysis to evaluate demographic and pathological factors associated with tumor malignancy.

Methods: FNACs from 702 canine growths presented to a small animal hospital (May 2018–May 2020) were processed. Neoplastic cases (*n*=414) were categorized and statistically analyzed using Python.

Results: Of 414 neoplastic cases, 83.3% were benign and 16.7% were malignant. Mesenchymal tumors were most frequent (48.8%), followed by round cell (29.7%) and epithelial tumors (21.5%). Labrador Retrievers (31.6%) and dogs older than 9 years (57.2%) showed the highest incidence. Lipoma was the most common diagnosis (36.9%). Lymphatic and splenic lesions accounted for a 70.3% crude malignancy rate. Subcutaneous and dermal growths (69.08%) were most prevalent, with the highest incidence on the flank and abdominal regions (38.8%). Geriatric dogs had significantly higher odds of malignancy (*P*=0.017) compared to adult dogs.

Conclusions: Canine neoplasms in the current study were predominantly benign subcutaneous lipomas, highly prevalent in older Labrador Retrievers. Advancing age, abdominal location, and lymph node and spleen involvement serve as crucial clinical indicators of malignant behavior, highlighting the continued utility of FNAC in canine oncology surveillance.

Discipline: Clinical pathology, **Topic:** Oncology, **Species:** Canine, **Systems:** Systemic

8: MAST CELL TUMOR OF THE TONGUE IN A HORSE WITH LYMPHOVASCULAR INVASION

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Background: Mast cell tumors (MCTs) are uncommon in horses and most frequently reported in the skin. Occurrences within the oral cavity, particularly those involving the tongue, are exceedingly rare and have been sparsely documented.

Case description: A 13-year-old Warmblood mare was presented to Purdue University Veterinary Hospital for evaluation of ulcerative lesions affecting the tongue and gingiva. Physical examination identified a 2.5 cm ulcer on the left tongue

surrounded by fibrous, whitish tissue. Cytological imprints were highly cellular and composed predominantly of well-differentiated mast cells, with a moderate eosinophilic infiltrate, consistent with mast cell tumor (MCT). Histologically, one fragment from the ulcer was composed of sheets of neoplastic round cells with distinct borders, a moderate amount of amphophilic cytoplasm, round to reniform nuclei, stippled chromatin, and one faint nucleolus. Moderate anisocytosis and anisokaryosis, and 5 mitotic figures in ten 400x HPF (2.37 mm^2) were observed. Rows of similar neoplastic round cells within the muscular connective tissue and lymphovascular invasion were also identified in the remaining fragments. Neoplastic cells stained positively with Toluidine blue and Giemsa, confirming MCT. Previously reported equine lingual MCTs have demonstrated benign biological behavior. In the present case, however, lymphovascular invasion suggested malignant potential. Interesting, although complete surgical excision was not achieved, aggressive debridement of the ulcerated lesion resulted in healing, with no gross evidence of recurrence 12 months after diagnosis. The relationship between the gingival lesions and the lingual MCT could not be determined.

Discipline: Clinical pathology, **Topic:** Oncology, **Species:** Equine, **Systems:** Gastrointestinal

9: CYTOLOGIC AND HISTOLOGIC FEATURES OF A KELOIDAL FIBROSARCOMA IN A DOG

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A 5.5-year-old female spayed American Pit Bull Terrier was presented for a wellness examination. A small mass on the right shoulder was identified. Cytologic evaluation revealed a moderately to highly cellular mesenchymal neoplasm composed of plump oval to spindle-shaped cells with wispy cytoplasmic tails and a single oval nucleus containing one to multiple, variably sized, round to angular nucleoli. Moderate to marked anisocytosis and anisokaryosis, and occasional mitotic figures were observed. The distinctive feature was moderate to abundant extracellular thick, glassy, eosinophilic matrix along with hyalinized collagen. Complete excision was performed, and histopathology revealed an unencapsulated, fairly well-circumscribed, infiltrative neoplasm expanding to the subcutis and mildly elevating the overlying epidermis and dermis. The neoplasm was composed of spindle to oval mesenchymal cells admixed with numerous dense, homogeneous, eosinophilic collagen bundles and light fibrovascular stroma. Mild to moderate anisocytosis and anisokaryosis were present, with three mitotic figures in ten high-power fields. Cytologic and histologic findings were consistent with keloidal fibrosarcoma. Polarized light microscopy and Masson’s trichrome staining were used to characterize the collagen-rich extracellular matrix. Vimentin immunohistochemistry supported mesenchymal origin, and the absence of smooth muscle actin immunoreactivity argued against myofibroblastic differentiation, supporting a fibroblastic origin. Keloidal fibrosarcoma is a rare canine variant of fibrosarcoma characterized by a fibroblastic spindle-cell population and abundant keloid-like hyalinized collagen. This case highlights the value of recognizing the distinctive

cytologic appearance of keloidal collagen and the diagnostic value of cytology–histology correlation in a rare collagen-rich soft tissue sarcoma of dogs.

Discipline: Clinical pathology, **Topic:** Oncology, **Species:** Canine, **Systems:** Integument

10: DISSIMINATED HISTIOCYTIC SARCOMA WITH BLOOD AND BONE MARROW INVOLVEMENT AND PROGRESSIVE HEMOPHAGOCYTIC ACTIVITY IN A BERNESE MOUNTAIN DOG

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A 7-year-old Bernese Mountain Dog presented with an intermittent cough, which led to the discovery of a pulmonary mass. Cytology of the mass and sternal and right iliac lymph nodes were consistent with disseminated histiocytic sarcoma. Four months after initiation of localized radiation therapy and systemic chemotherapy, the patient developed mild non-regenerative anemia (RBC count 5.2, RI: 5.5-8.5 M/µL) and marked thrombocytopenia (42, RI: 200-500 K/µL). Bone marrow aspiration with a core biopsy revealed metastatic infiltration of neoplastic histiocytes involving approximately 8% of all nucleated cells, with pronounced erythrophagia and leukophagia compared with the pulmonary lesion. Immunohistochemical staining (Iba-1 and CD11d) showed low to focally moderate numbers of cells with positive cytoplasmic and membranous immunolabeling, most consistent with hemophagocytic histiocytic sarcoma. One month later, a complete blood count revealed mild regenerative anemia (31%, RI:37-57%), marked thrombocytopenia (11, RI: 200-500 K/µL), and occasional large round cells with similar cytomorphology to the lung mass and bone marrow aspirates, with evidence of erythrophagocytic activity. Subsequently, the patient developed a fever. The combination of fever, bicytopenia, and erythrophagocytosis raised a concern for hemophagocytic syndrome (HPS). Additional laboratory investigation showed marked hyperferritinemia (6,490, RI:89-489 ng/mL), moderately increased C-reactive protein (69.6, RI:0.0-12.0 mg/L), and hyperfibrinogenemia (701, RI:100-384 mg/dL). However, the diagnostic criteria for HPS were not met, and the regenerative anemia was not supportive of this differential diagnosis. This case highlights the cytohistologic, and immunohistochemical features of disseminated hemophagocytic histiocytic sarcoma and underscores the need for further investigation into the pathogenesis of histiocytic diseases in dogs.

Discipline: Clinical pathology, **Topic:** Oncology, **Species:** Canine, **Systems:** Hematopoietic

11: SPONTANEOUS RECURRING CUTANEOUS NEOPLASM IN FLUORESCENT ZEBRA DANIO (DANIO RERIO)—DIAGNOSTIC CHALLENGE FOR CYTOLOGY AND HISTOPATHOLOGY CORRELATION

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Spontaneous cutaneous neoplasia in zebrafish is rare, and cytologic evaluation with histopathologic and immunohistochemical correlation is scarcely reported. A 1.75-year-old fluorescent-green Zebra Danio (*Danio rerio*) was presented for a 3 × 3 × 3 mm, pedunculated, black, dermal mass above the left eye. Impression smears and an excisional biopsy of the mass were performed.

Cytology revealed numerous tissue cells with a suspected biphasic appearance. Approximately 70% were intermediate-sized, fusiform, and associated with abundant bright eosinophilic extracellular matrix. They exhibited moderate pleomorphism, with frequent nuclear molding and occasional binucleation. Sparse, black-green pigmentation, suspicious for melanin, was observed in very few cells. Other tissue cells consisted of either uniform small cuboidal cells or monomorphic large polygonal cells, both forming large cohesive clusters, occasionally overlaid by small amounts of extracellular melanin. Melanophores with dendritic processes were scattered among neoplastic cells. Fusiform melanophoroma and sarcoma were the initial differential diagnoses.

Histologically, the hypodermis was expanded by an unencapsulated, densely cellular neoplasm, composed of polygonal cells arranged in solid packets within a delicate fibrovascular stroma, and further separated by streams of spindle cells. Melanophores were scattered throughout the fibrovascular stroma. Rare spindle cells contained melanin pigments. Immunohistochemistry revealed that the polygonal cells were immunolabeled for cytokeratin but lacked PNL2 immunoreactivity, with approximately 5% showing immunolabeling for Melan-A. The overall morphology and immunohistochemistry findings favored carcinoma over chromatophoroma.

This case highlights the diagnostic challenges of zebrafish cutaneous tumors and underscores the importance of correlating cytologic evaluation with histopathologic and immunohistochemical findings for a definitive diagnosis.

Discipline: Both, **Topic:** Oncology, **Species:** Aquatic, **Systems:** Integument

12: THEILERIA ORIENTALIS IN AN ADULT BEEF COW: AN EMERGING TICK-BORNE DISEASE

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An 8-year-old Aberdeen Angus cow from southern Ohio presented for stumbling and weight loss after calving 4 days prior to presentation. Several other cows on the same farm had recently aborted and several of those cows died. Physical examination revealed tachycardia, tachypnea, and pale, icteric mucous membranes. A CBC showed marked, regenerative anemia. A blood smear review revealed numerous small, irregularly shaped intraerythrocytic structures consistent with *Theileria orientalis*, which was confirmed by PCR. *Theileria orientalis* is a protozoal parasite that causes hemolytic anemia, pyrexia, icterus, malaise, ill thrift, sporadic abortions, and death in cattle of all ages. The parasite is transmitted by the Asian longhorned tick, *Haemaphysalis longicornis*. Infections can be economically devastating due to decreased milk production, reproductive losses, morbidity, and mortality. There currently is no effective treatment. The farmer also noted that many deer had been dying on multiple farms in the area, prompting screening for

additional infectious diseases. The cow was positive for Enzootic Hemorrhagic Disease based on PCR. Enzootic Hemorrhagic Disease is an orbivirus transmitted by biting midges, particularly *Culicoides sonorensis*, which were prevalent due to recent drought conditions. Enzootic Hemorrhagic Disease causes severe, often fatal disease in white-tailed deer, but typically causes subclinical infection in cattle. However, co-infection with *Theileria sp*. has the potential to cause serious clinical disease in cattle, especially as tick populations continue to spread across the United States. This case highlights the importance of monitoring infectious diseases that may have an increasing economic impact on the cattle industry.

Discipline: Clinical pathology, **Topic:** Infectious disease, **Species:** Bovine, **Systems:** Hematopoietic

13: BACTEREMIA IN AN ASIAN TUSKER (ELEPHAS MAXIMUS MAXIMUS): A CASE REPORT

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Bacteremia is the presence of viable bacteria in the bloodstream. This case highlights bacteremia in an Asian tusker. The Clinical Science Department, University of Peradeniya, was requested by the Department of Wildlife to examine a 20-year-old, male, Asian tusker with a history of gunshot wounds, three months ago, initially treated by wildlife veterinarians. Ten days before the examination, the animal developed an abnormal gait and reluctance to stand. When evaluated, the tusker was in lateral recumbency in an artificial muddy pond. The patient was alert, responsive, and able to move its trunk to grasp food offered. Clinical examination revealed a deep wound at the base of the left ear. Additionally, the right carpal joint was in a permanently flexed position, with notable uneven wear of the sole in both forelimbs. Given the elephant's posture, location, and clinical condition, CBC and serum chemistry were the only feasible diagnostic aids. Hematological evaluation revealed a normocytic, normochromic nonregenerative mild anemia characterized by a mildly decreased PCV. The leukogram was unremarkable. However, the peripheral blood smear examination revealed numerous rod-shaped bacteria extracellular and intracellular in heterophils. Based on morphology, *E.coli* and *Pseudomonas* were considered likely. BUN, Creatinine, Total protein, and ALT were evaluated in the serum chemistry panel. Creatinine was within the normal range, but trending toward the lower limit, indicating reduced muscle mass. A fourfold elevation in ALT indicated moderate hepatic injury. To our knowledge, the presence of intracellular bacteria on the blood smears of Asian elephants has not been reported previously.

Discipline: Clinical pathology, **Topic:** Infectious disease, **Species:** Wildlife/Zoo, **Systems:** Systemic

14: DIAGNOSIS OF PRESUMED PRIMARY IMMUNE-MEDIATED NEUTROPENIA IN A 1-YEAR-OLD SCHNAUZER MIX DOG

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A 1-year-old female spayed Schnauzer mix dog presented for a one-day duration of acute-onset lethargy and hyporexia to anorexia. CBC revealed a marked leukocytopenia characterized by marked neutropenia (<50/µL), and a mild, non-regenerative, minimally macrocytic (MCV 79.7 fl; RI 60.00 - 77.00 fl) and normochromic (MCHC 34.3 g/dl; RI 32.00 - 36.00 g/dL) anemia (PCV 27%). Bone marrow cytology showed marked hypercellularity with a compelling depletion of the myeloid cell line, represented by a M:E ratio of 0.15. The myeloid cell line displayed a significant left shift, and maturation was halted at myelocytes and metamyelocytes, which were predominantly eosinophilic, with a complete lack of band and segmented granulocytes. Mild to moderate lymphocytosis and plasmacytosis (23% and 10% of all nucleated cells, respectively) were also observed. Main differential diagnoses included selective immune-mediated destruction of neutrophils, and myelosuppression secondary to drug reaction or infectious diseases, particularly chronic tick-borne diseases. Additional PCR tests for anaplasmosis, ehrlichiosis, Bartonella, babesiosis, rickettsial diseases, and hemotropic Mycoplasma were negative. Repeat blood work seven days after initiation of immunosuppressive dose of prednisone showed a marked improvement of peripheral neutrophil count (7220/µL), which remained within reference interval through the following two months. Follow-up bloodwork is pending as the patient started tapering of prednisone dose. Primary immune-mediated neutropenia is an unusual disorder and often poses tremendous diagnostic challenges as no confirmatory test exists. This case demonstrates the importance of comprehensive clinicopathologic testing to investigate differential diagnoses and guide treatment decisions.

Discipline: Clinical pathology, **Topic:** General pathology, **Species:** Canine, **Systems:** Hematopoietic

15: RETROSPECTIVE ASSESSMENT OF LYMPHOCYTE SUBTYPE POPULATIONS IN FELINE LYMPHOCYTE-RICH EFFUSIONS AND CHALLENGES ASSOCIATED WITH EFFUSION IMMUNOCYTOCHEMISTRY

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Background:

Management of feline lymphocyte-rich effusions (LREs), including chylous effusions, can entail repeated centesis, which may negatively impact the patient's nutritional and immune status. Human literature reports a predominance of T-cells in chylous effusions, with selective loss of CD4+ lymphocytes in chyle compared to peripheral blood. A single analysis of feline chylous effusion was T-cell predominant and in proportion to peripheral blood.

Objective:

To retrospectively characterize lymphocyte subsets in feline LREs using immunocytochemistry.

Methods:

Four antibodies were validated for use in previously stained and coverslipped cytology samples. CD3, CD4, CD8, and CD20 antibodies were utilized to determine the proportions of T cells, T subtypes (T helper cells and cytotoxic T cells) and B cells, respectively, in archived cytospin preparations of feline LREs prepared with a tissue transfer technique.

Results:

40 samples were stained, however, 13 were eliminated due to problems with tissue transfer. There was no CD3 immunoreactivity in 44% of the remaining 27 samples, and convincing immunoreactivity for CD4 and CD8 was only observed in 7.4% of samples. The percentage of CD20+ lymphocytes ranged from 0-95%. B lymphocytes were the predominant cell type in 85% of samples.

Conclusions:

The inherent characteristics of biological fluids, along with preanalytical factors that could not be controlled for and analytical limitations, likely contributed to the erroneous results. This emphasizes the importance of interpreting immunocytochemistry with knowledge of context and understanding of specimen characteristics. Retrospective immunocytochemistry of feline LREs is not recommended.

Discipline: Clinical pathology, **Topic:** General pathology, **Species:** Feline, **Systems:** Systemic

16: ESTABLISHING URINE ANALYTE REFERENCE INTERVALS IN HEALTHY BOTTLENOSE DOLPHINS (TURSIOPS TRUNCATUS) UNDER MANAGED CARE

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Background: Common bottlenose dolphins (Tursiops truncatus) possess specialized renal morphological and physiological adaptations for maintaining water and electrolyte balance in marine environments. However, published reference intervals (RIs) for urine chemistry analytes in this species are limited. This study established RIs for both routine and advanced urinary analytes, including urine specific gravity, pH, osmolality, creatinine ratios, gamma-glutamyl transferase (GGT) index, and fractional excretion (FE) of electrolytes in common bottlenose dolphins in a controlled salinity environment.

Methods: Samples were collected from 63 clinically healthy dolphins under managed care via voluntary voiding or catheterization, with concurrent blood collection from the ventral peduncle or tail fluke. Urine and serum biochemical analytes were measured on neat urine and thrombin-coagulated serum, respectively, using an Olympus AU400E chemistry analyzer and manufacturer-supplied reagents. Creatinine ratios, GGT index, and fractional excretion values were calculated using previously described equations. Reference intervals were calculated using the

American Society for Veterinary Clinical Pathology guidelines.

Results: RIs were established for 32 urinary analytes and derived indices. There was a significant positive correlation between habitat salinity and urine chloride (adjusted p = 0.018, Spearman's rho = 0.359). Urine specific gravity (1.013 – 1.037) and osmolality (514 – 1759 mOsm/L) RIs overlapped with several domestic terrestrial mammals. Urine protein-creatinine ratio RI (0.08 – 2.02) was potentially higher than that of domestic carnivores.

Conclusions: These data provide a basis for interpreting urinary analytes in managed bottlenose dolphins and may aid investigations of health and osmoregulatory function in free-ranging populations exposed to hyposaline environments.

Discipline: Clinical pathology, **Topic:** Comparative pathology, **Species:** Aquatic, **Systems:** Urinary

17: COMPARATIVE CLINICAL, CYTOLOGIC, AND HISTOLOGIC FINDINGS OF CANINE ECHINOCOCCUS MULTILOCULARIS AND MESOCESTOIDES TETRAHYRIDIA INFECTIONS IN TWO DOGS

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A 4-year-old spayed female mixed-breed dog from Alberta, Canada presented with progressive abdominal distension. Abdominal radiographs, ultrasonography, and exploratory laparotomy identified multiple large, soft tissue-opaque hepatic cysts. Cytology revealed many degenerate neutrophils, macrophages, folded membranous parasitic structures, and numerous calcareous corpuscles without protoscolices. PCR of the cystic fluid confirmed *Echinococcus multilocularis*. Fecal flotation was negative for eggs, cysts, and oocysts. Histopathology demonstrated marked lymphohistiocytic inflammation with multifocal eosinophilic structures consistent with remnants of brood capsules, supporting hydatid disease. No evidence of neoplasia was identified.

A second case involved a 5-year-old spayed female Anatolian Shepherd imported from Turkey presenting with abdominal effusion, hepatic and splenic masses, and pulmonary nodules. Computed tomography demonstrated cavitated splenic masses, multifocal cystic hepatic masses, pleural and peritoneal cysts, pulmonary nodules, peritonitis, and pleural effusion. Cytology of hepatic aspirates revealed neutrophilic inflammation with rare fragmented parasitic structures containing homogeneous eosinophilic material and refractile calcareous corpuscles. PCR was positive for *Mesocestoides tetrahyridia*. Following euthanasia, necropsy revealed severe chronic fibrinous pleuritis and peritonitis with 2 L of turbid abdominal effusion containing numerous free-floating cestodes. Histopathology identified *Mesocestoides* larvae, multifocal pulmonary and hepatic granulomas, and concurrent hepatic and splenic

sarcomas with strong vimentin immunoreactivity and multifocal smooth muscle actin immunoreactivity, consistent with a myofibroblastic phenotype. Larvae were present within both neoplasms, raising the possibility of neoplastic transformation secondary to chronic inflammation. These cases demonstrate that aberrant cestode infections may closely mimic or accompany neoplasia or chronic granulomatous disease, emphasizing the diagnostic value of cytology, molecular diagnostics, and postmortem examination.

Discipline: Clinical pathology, **Topic:** Infectious disease, **Species:** Canine, **Systems:** Hepatobiliary

18: INTER-PATHOLOGIST VARIABILITY IN THE QUANTIFICATION OF ABNORMAL RED BLOOD CELL MORPHOLOGIES

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Background: Red blood cell (RBC) morphology assessment, a key component of blood film evaluation, is not evaluated on automated complete blood count analyzers, and can influence diagnosis and clinical management. There is limited information in veterinary literature around inter-pathologist variability in identification and quantification of RBC morphologies.

Objective: This study assesses interobserver agreement and interpretive variability in RBC morphology reporting among clinical pathologists.

Methods: Inter-pathologist variability was assessed using 4,585 digitally scanned canine blood films. Each film was independently reviewed by three clinical pathologists. Pathologists counted abnormal RBCs in ten 100x high power fields (hpf) and recorded the mean of each abnormal RBC morphology per hpf. Krippendorff's alpha (KA) statistic was used to evaluate reliability of counts; a KA of 0 indicates agreement no better than chance given the observed distribution of responses and a KA of 1 indicates perfect agreement. A KA of <0.67 is considered unreliable. The median of pairwise Spearman's correlation coefficients, ρ, was also calculated.

Results: All morphologies showed poor reliability and weak correlations. Reliability was highest for echinocytes (KA=0.35), polychromatophils (KA=0.26), ghost cells (KA=0.23), target cells (KA=0.16), spherocytes (KA=0.15), schistocytes (KA=0.13), acanthocytes (KA=0.12), and keratocytes (KA=0.10). All other alphas were <0.10. All Spearman's ρ values were positive, but exceeded 0.4 only for echinocytes and polychromatophils.

Conclusion: These findings indicate substantial variability in RBC morphology assessment between clinical pathologists. This variability may have implications for diagnostic interpretation, patient monitoring and clinical decision-making, highlighting the need for improved standardization and objective analytic approaches in veterinary hematologic evaluations.

Discipline: Clinical pathology, **Topic:** General pathology, **Species:** Canine, **Systems:** Hematopoietic

19: EVALUATION OF DIRECT ANTIGLOBULIN TESTING FOLLOWING ALLOGENEIC BLOOD TRANSFUSION IN 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 the 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 of conversion from DAT negative to DAT positive, in transfusion naïve dogs following allogeneic blood transfusion. To characterize the time interval between blood transfusion and DAT conversion. To inform clinicians regarding the reliability of the DAT for diagnosis of IMHA in previously transfused dogs.

Methods: Transfusion naïve dogs requiring blood transfusion have a pre-transfusion DAT and major crossmatch performed using polyspecific canine anti-globulin/anti-complement gel column technology (Alvedia). Patients with a positive pre-transfusion DAT, history of steroid administration, blood product transfusion, and suspected autoimmune disease are excluded. Samples are collected 24 hourly for DAT testing, depending on patient availability.

Preliminary results: In 49 patients, one positive DAT has been detected (T24), which was followed by further negative results (T36, T48). Post-transfusion DATs range from T24 to T1080 (45 days).

Conclusions: Preliminary results suggest allogenic blood transfusion does not cause sustained DAT seroconversion, and positive results should not be dismissed as the result of allogeneic blood transfusion in suspected IMHA patients.

Discipline: Clinical pathology, **Topic:** Immunology, **Species:** Canine, **Systems:** Hematopoietic

20: SPATIAL TRANSCRIPTOMIC ANALYSIS OF LOW-GRADE INTESTINAL T-CELL LYMPHOMA AND LYMPHOPLASMACYTIC INFLAMMATORY BOWEL DISEASE IN CATS

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Background: Low-Grade Intestinal T-Cell Lymphoma (LGITL) and Inflammatory Bowel Disease (IBD) are relevant entities in feline medicine with overlapping clinical, imaging, cytological, and even histopathological features. Unraveling molecular differences at the transcriptional level can bring relevant information to better understand both entities' physiopathology and identify candidate genes that allow for more accurate differentiation.

Objectives: To identify transcriptional differences in formalin-fixed paraffin-embedded (FFPE) samples of cats with LGITL or IBD using Spatial transcriptomics.

Methods: Jejunum and ileum FFPE samples from cats with histological diagnoses of LGITL and IBD were retrospectively evaluated; samples with PARR results in concordance with the histological diagnosis were subsequently selected. Three ileum samples of cats without intestinal diseases were collected as controls. Spatial transcriptomics was performed using the platform GeoMx (Bruker) and a commercial canine panel (Canine Cancer Atlas). Gene expression of CD3-positive and CD20-positive cells was assessed and compared between disease groups, and between tissue areas: superficial mucosa (SM), deep mucosa/submucosa (DM), and Peyer patches (PP) on ileum.

Results: Twelve samples (6 LGITL, 6 IBD) were analyzed. A total of 26 genes were differently expressed between T-cells of LGITL and IBD groups. Genes included PIK3CD, CLDN7, and BCL2. Variable gene expression between tissue areas was present, examples including APOA1 and ID1 on IBD (SM vs DM, CD3-cells) or increased TXNIP and CFD on LGITL (DM vs PP, CD20-cells).

Conclusion: Spatial transcriptomics can be applied to cat samples and allowed to identify differently expressed genes in LGITL and IBD, paving the way to identify potential biomarkers.

Discipline: Clinical pathology, **Topic:** Oncology, **Species:** Feline, **Systems:** Gastrointestinal

21: PROOF-OF-CONCEPT STUDY OF MICRORNA EXPRESSION IN INTESTINAL SAMPLES FROM CATS WITH LOW-GRADE INTESTINAL LYMPHOMA AND LYMPHOPLASMACYTIC INFLAMMATORY BOWEL DISEASE

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Background: Low-grade intestinal T-cell lymphoma (LGITL) and lymphoplasmacytic inflammatory bowel disease (IBD) present a significant challenge in veterinary clinical practice, as both share overlapping findings in clinical presentation, routine laboratory and imaging exams. Differences in microRNA (miRNA) expression may help identify minimally invasive biomarkers to distinguish IBD from LGITL.

Objectives: To identify differentially expressed miRNAs on formalin-fixed paraffin-embedded (FFPE) samples of cats with LGITL, IBD, and without intestinal disease.

Methods: Jejunum and ileum FFPE samples from cats with histological diagnosis of LGITL (n = 6) and IBD (n = 6), and PARR results in concordance with the histological diagnosis were chosen. Three ileum samples from cats without intestinal diseases were prospectively collected as controls. RNA was sequenced and, given the absence of a cat miRNA database, annotations were performed using canine (cfa), human (hsa), and murine (mmu) miRNA databases. MiRNAs were ranked by integrating the magnitude of difference between groups, p-value, overall expression level, and detection across samples.

Results: Of the 1,928 miRNAs detected, 477 were consistently detected in at least three samples. When comparing IBD with LGITL, highly expressed miRNA in IBD included NovelmiRNA-1671 and cfa-miR-361, the latter being downregulated in lymphoma in other species. Other miRNAs, such as cfa-miR-150, cfa-miR-363, and NovelmiRNA-1287, showed higher expression in LGITL. Interestingly, cfa-miR-150 has been found downregulated in aggressive B-and T-cell lymphoma, but studies on indolent lymphomas are lacking.

Conclusion: Candidate miRNAs differentially expressed between IBD and LGITL were identified and will be validated for further investigation as potential diagnostic biomarkers.

Discipline: Clinical pathology, **Topic:** Oncology, **Species:** Feline, **Systems:** Gastrointestinal

22: IDENTIFICATION OF LEISHMANIA SPP. AMASTIGOTES IN PLEURAL EFFUSION FROM A DOG

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A 10-year-old intact female mixed-breed dog was presented with lethargy, anorexia, weight-loss, hind limb lameness, and a one-year history of swelling of the right pelvic limb that had reportedly improved following empirical corticosteroid administration. Physical examination revealed mild dehydration, a body condition score of 3/9, and proprioceptive deficits. CBC and serum chemistry abnormalities included mild nonregenerative anemia, mildly increased ALT, and mild hypoalbuminemia. Thoracic radiographs demonstrated a lytic lesion affecting the T7 vertebral body, with mild extension to T6 (suspected neoplastic process/discospondylitis), in addition to pleural effusion. Following drainage of 500 mL of pleural fluid, repeat radiographs revealed hepatomegaly and possible right-sided cardiomegaly. The effusion was colorless and hazy, with a TNCC of 4,700 cells/µL, total protein of 3.4 g/dL, albumin of 1.4 g/dL, and triglycerides of 10 mg/dL. Nucleated cells consisted predominantly of small lymphocytes, with fewer macrophages and non-degenerate neutrophils. Numerous small, round-to-oval intra- and extracellular organisms with pale basophilic cytoplasm and an eccentric nucleus associated with a kinetoplast were observed, consistent with Leishmania sp. amastigotes; the diagnosis was subsequently confirmed by PCR. Although Leishmania spp. amastigotes were

identified in the effusion, a direct relationship between the lymphocyte-rich effusion and leishmaniasis could not be established, as concurrent vertebral lesions and possible cardiomegaly may also have contributed to effusion development. This report describes an uncommon presentation of canine leishmaniasis in which amastigotes were identified in pleural effusion, expanding the spectrum of clinical findings associated with this infection.

Discipline: Clinical pathology, **Topic:** Infectious disease, **Species:** Canine, **Systems:** Systemic

23: ELECTROPHORETIC URINE PROTEIN BANDING PATTERNS IN HEALTHY CATS AND CATS WITH KIDNEY DISEASE

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Background: Determining the cause of proteinuric renal disease in cats is a diagnostic challenge and often requires renal biopsy for definitive diagnosis. The use of urine electrophoretic banding patterns to determine origin of renal proteinuria is underexplored in cats.

Objective: Determine the urine protein banding patterns of clinically healthy cats and compare to cats with histopathology-confirmed kidney disease.

Methods: Biobanked urine supernatant was analyzed from 20 clinically healthy cats and 45 cats with histopathology-confirmed kidney disease: 13 stable chronic kidney disease (CKD) with tubulointerstitial (TI) damage; 20 immune complex-mediated glomerulonephritis (ICGN); 12 non-ICGN glomerular disease (amyloidosis, podocytopathy, focal segmental glomerulosclerosis). Urine proteins were separated with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Low (LMW, <36.5 kDa), intermediate (IMW, 36.5-66.3 kDa), high (HMW, 66.3-200 kDa), and very high (VHMW, >200 kDa) molecular weight bands were enumerated.

Results: Healthy cat urine contained protein bands at molecular weights consistent with albumin, cauxin, and occasionally uromodulin. The number of LMW and IMW bands were significantly greater in ICGN and non-ICGN glomerular disease than healthy cats and in ICGN cats compared to TI CKD cats. HMW and VHMW bands were significantly increased in ICGN and non-ICGN glomerular cats compared to healthy and TI CKD cats. ICGN cats had the highest median number of VHMW bands, and >2 VHMW bands were 100% sensitive and 78% specific for ICGN.

Conclusions: Urine SDS-PAGE differentiates cats with glomerular disease from

healthy cats and TI CKD cats and has modest to high sensitivity and specificity for identifying ICGN cats.

Discipline: Anatomic pathology, **Topic:** General pathology, **Species:** Feline, **Systems:** Urinary

24: MALDI-TOF MS TO DETECT MELANOMA CIRCULATING TUMOR CELLS IN DOGS: A PROOF-OF-CONCEPT ANALYSIS

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Canine oral melanoma (COM) is the most prevalent oral neoplasm in dogs, characterized by high metastatic potential. Blood-based liquid biopsy offers a novel approach to detect systemic dissemination via circulating tumor cells (CTCs). While Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) Mass Spectrometry (MS) is well-established for differentiating microbiological samples, its use for other sample types remains underexplored in oncology practice. This pilot study aims to demonstrate the capability of MALDI-TOF MS to distinguish the mass spectra of canine white blood cells (WBC) from COM cell lines. Blood samples were obtained from three healthy dogs, and WBC were isolated. A biological triplicate of a COM cell line (TLM-1) was used to define the melanoma spectrum. Ten technical replicates per sample were analyzed to obtain an average spectrum for each sample. Exploratory analysis demonstrated a clear difference between the groups. Principal component analysis revealed high variance in PC1 (92.8%), and hierarchical clustering showed similar robust separation. In total, 192 peaks were detected. Statistical analysis using the *limma* method identified 137 peaks with differential intensities (log2 fold-change> 2, FDR < 0.01), of which 100 showed higher intensity in melanoma cells. The significant differences in mass spectra between COM cells and WBC provide a strong proof of concept for the use of MALDI-TOF MS in liquid biopsies. To advance this diagnostic tool, future studies will focus on establishing the assay's limit of detection using serially diluted, CTC-spiked blood samples and on developing machine learning algorithms to classify CTCs presence from proteomic fingerprint spectra.

Discipline: Clinical pathology, **Topic:** Oncology, **Species:** Canine, **Systems:** Gastrointestinal

25: APPLICABILITY OF EXPLAINABLE AI IN CYTOPATHOLOGICAL EVALUATION OF CANINE LYMPHOMA

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Background: Explainable artificial intelligence (AI) can improve transparency in image-based diagnostic models, but its clinical utility remains underexplored in veterinary oncology and cytology. This study aimed to develop and evaluate explainable image-based classification models using cytological images from canine lymphoma and reactive lymphoid hyperplasia (RLH).

Methods: A total of 5,378 cytological images were obtained from 182 dogs, including 2,284 lymphoma images from 105 dogs and 3,094 RLH images from 77 dogs. Images were stratified by patient and divided into training, validation, and test subsets. Model robustness was assessed using 10-fold cross-validation. Binary classification models were developed using the ResNet-50 architecture. Clinical interpretability was evaluated using Gradient-weighted Class Activation Mapping (Grad-CAM).

Results: The model achieved strong image-level performance, with test accuracy, weighted precision, weighted recall, and weighted F1-score each reaching 0.945. The area under the receiver operating characteristic curve was 0.979, and the area under the precision-recall curve was 0.981. At the patient level, 18 of 20 dogs with lymphoma were correctly classified, and all 16 dogs with RLH were correctly classified, corresponding to a sensitivity of 0.900, specificity of 1.000, positive predictive value of 1.000, and negative predictive value of 0.889. Grad-CAM highlighted regions relevant to lymphoid cell morphology, providing visual explanations for model predictions.

Conclusions: These findings support the potential use of explainable AI for cytological classification of canine lymphoma and RLH in diagnostic decision support. Grad-CAM improved interpretability by identifying diagnostically relevant image regions, suggesting that explainable AI may provide both classification support and pathologically meaningful visual feedback.

Discipline: Clinical pathology, **Topic:** Methods/technology, **Species:** Canine, **Systems:** Hematopoietic

26: EVALUATION OF DEEP LEARNING ARTIFICIAL INTELLIGENCE ALGORITHM FOR IDENTIFICATION OF CANINE AND FELINE URINE SEDIMENT CASTS AND EPITHELIAL CELLS ON WHOLE SLIDE IMAGING PLATFORM

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Background: Urinalysis is an essential component of baseline patient diagnostic evaluation. Artificial intelligence (AI) may help provide rapid, reliable diagnostic support of urine sediment evaluation, particularly for identifying clinically important elements that may be overlooked at the point of care.
Objectives: (1) to assess agreement between a deep learning convolutional neural network, the Vetscan Imagyst® AI Urine Sediment algorithm v2.0, and expert clinical pathologists (CP) for semi-quantitative identification of casts and epithelial cells; and (2) to evaluate AI performance in detecting these objects in canine and feline urine

specimens.
Methods: Ninety-eight urine sediment wet-mount preparations were digitized as whole slide images and independently evaluated by three CP and the AI algorithm. Casts were classified as hyaline or non-hyaline, and epithelial cells as squamous or other epithelial. Objects were quantified per low-power field (casts) or high-power field (epithelial cells) for semi-quantitative partitioning. CP consensus served as the reference standard for calculating agreement, sensitivity, specificity, and accuracy.
Results: Inter-CP agreement across the four objects ranged from 80.6-94.9%. Agreement between CP consensus and AI was higher for hyaline casts (94.9%) and squamous cells (91.8%) than for non-hyaline casts (73.5%) and other epithelial cells (79.6%). The AI demonstrated higher accuracy, specificity, and sensitivity for squamous cells and hyaline casts (91.8-95.9%, 91.7-95.5%, 100%) compared to non-hyaline casts and other epithelial cells (79.6-83.7%, 77.9-91.1%, 52.6-85.7%).
Conclusions: The AI algorithm reliably identified common elements (hyaline casts, squamous cells). Further algorithm training/refinement may improve detection of less common objects (non-hyaline casts, other epithelial cells).

Discipline: Clinical pathology, **Topic:** Methods/technology, **Species:** Canine, **Systems:** Urinary

27: COOPERATIVE ONCOLOGY-PATHOLOGY CONSENSUS EFFORTS IN VETERINARY CANCER: FIFTEEN YEARS OF THE VCS-ACVP ONCOLOGY PATHOLOGY WORKING GROUP

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Naturally occurring cancers in companion animals provide a unique opportunity for advancing diagnosis, prognosis, therapeutic intervention, and translational cancer research; however, variability in tumor classification and grading has historically limited reproducibility and clinicopathologic correlations. The Oncology Pathology Working Group (OPWG), a joint initiative of the Veterinary Cancer Society (VCS) and American College of Veterinary Pathologists (ACVP), was established in 2011 to: (1) foster and promote interdisciplinary collaboration between veterinary oncologists and pathologists, and (2) generate consensus-based standards and best-practices guidelines through comprehensive, standardized, and rigorous review of the published literature. Over its first fifteen years, the OPWG, through its engaged and driven membership, has generated nine consensus-driven documents, including five scientific peer-reviewed publications, addressing histologic classification, grading, and diagnostic reporting for common malignancies; most recently primary nodal lymphomas in dogs. Additional OPWG consensus initiatives have focused on challenges in canine melanoma, mast cell tumors, soft tissue sarcoma, mammary tumors, prostate cancer, and immunocytochemistry. The OPWG is membership-driven and encourages open communication and member proposals to tackle challenges faced across the oncologic pathology landscape. As such, member-proposed surveys have also been conducted to include evaluating oncology and pathology resident cross-training needs and implementation barriers. Collectively,

these efforts reflect the OPWG's ongoing commitment to advancing standardized, evidence-based oncologic pathology through a multidisciplinary membership-driven cooperative model. Future work will build on this foundation to continue "*Putting veterinary cancer care on the right path*" by refining frameworks and addressing emerging needs in veterinary cancer diagnostics and reporting. For more information, visit https://vetcancersociety.org/membership/oncology-pathology-working-group/.

Discipline: Both, **Topic:** Oncology, **Species:** Canine, **Systems:** Other (Any/All)

28: VALIDATION OF A FECAL TAQMAN REAL-TIME PCR ASSAY FOR DETECTION OF HETEROBILHARZIA AMERICANA IN FORMALIN-FIXED PARAFFIN-EMBEDDED CANINE TISSUES

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Background: *Heterobilharzia americana* is an emerging trematode parasite that primarily infects dogs and causes granulomatous disease. Histopathologic diagnosis relies on identification of adult trematodes and/or eggs. However, these may be absent or difficult to distinguish from morphologically similar parasites. Molecular detection of *H. americana* in canine tissues has not been evaluated.

Objective: Evaluate the performance of a TaqMan real-time PCR assay, previously validated for fecal samples, for detection of *H. americana* DNA in archived formalin-fixed paraffin-embedded (FFPE) canine tissues.

Methods: Canine FFPE tissues containing trematode parasites and/or eggs morphologically consistent with *H. americana* were retrospectively identified. Fecal sedimentation results were reviewed when available. DNA extracted from FFPE tissue curls was analyzed using the validated fecal TaqMan real-time PCR assay. Analytical specificity was assessed using tissues containing non-target trematodes, and diagnostic specificity using tissues without histopathological evidence of *H. americana* infection.

Results: Forty-one FFPE tissues from 28 cases were evaluated. Fecal sedimentation was available for 17 cases, all positive for *H. americana* eggs. Tissue PCR detected *H. americana* DNA in all 41 tissues, yielding a diagnostic sensitivity of 100% (95% CI: 91.4 to 100%). All tissues (12) representing 6 non-target trematode species tested negative, yielding an analytical specificity of 100% (95% CI: 73.5 to 100%). All 15 negative control tissues tested negative, yielding a diagnostic specificity of 100% (95% CI: 78.2 to 100%).

Conclusion: The TaqMan real-time PCR assay demonstrated high diagnostic performance in FFPE tissues and may serve as a valuable adjunctive test for confirming *H. americana* infection.

Discipline: Anatomic pathology, **Topic:** Methods/technology, **Species:** Canine, **Systems:** Systemic

29: HAEMOPROTEUS SP. SIAMEX01 INFECTION IN TWO FLORIDA GRASSHOPPER SPARROWS (AMMODRAMUS SAVANNARUM FLORIDANUS)

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Haemoproteus sp. is an apicomplexan hemoparasite closely related to *Plasmodium* sp., the causative agent of avian malaria. *Haemoproteus* sp. infection is typically host-specific and associated with subclinical disease in avian species, though there have been reports of clinical disease in exotic species under managed care and these cases often result from infection with parasites present in native avian species. The objective of this study was to describe the cytologic, histologic, and ultrastructural findings of *Haemoproteus* sp. infection in two 17-day-old Florida grasshopper sparrows (*Ammodramus savannarum floridanus*) that died without premonitory clinical signs at a Florida zoological institution. Postmortem liver and kidney impressions revealed inflammation and structures suggestive of meronts and megaloschizonts. Histopathologically, these protozoal life stages were associated with tubular necrosis, interstitial fibrosis, and lymphoplasmacytic inflammation in the kidney and minimal granulomatous inflammation in the liver. Transmission electron microscopy is pending. Molecular testing of liver from one individual detected *Haemoproteus* sp. SIAMEX01. This *Haemoproteus* lineage has been reported in at least 18 native passerine species, predominately in the western United States. While these natural hosts develop chronic asymptomatic infections, this lineage has been associated with mortality in exotic avian species in zoological institutions, including lesser flamingos (*Phoeniconaias minor*) and Montezuma oropendolas (*Psarocolius montezuma*) in Texas and six exotic tanager, bird of paradise, cardinal, and oropendola species in California. This data adds to the literature that this lineage occurs in the Eastern U.S. and, to our knowledge, represents the first report of fatal *Haemoproteus* sp. infection in a U.S. native passerine.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Wildlife/Zoo, **Systems:** Systemic

30: HOW DID THIS ALL BEGIN? POSSIBLE ENTRY ROUTES FOR HPAI H5N1 IN GOATS, ALPACAS, AND SHEEP

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Based on previous studies demonstrating abundant influenza A virus receptors in bovine mammary tissue and similar receptor presence in the mammary glands of sheep, goats, alpacas, pigs, and humans, we investigated potential routes of avian influenza H5N1 clade 2.3.4.4b infection in small ruminants and camelids. The

emergence of highly pathogenic avian influenza (HPAI) H5N1 in dairy cattle in 2024, followed by spillover into goats, alpacas, and sheep, raised important questions about routes of infection, including whether respiratory or gastrointestinal exposure offers alternative portals of entry. Prior work showed widespread distribution of avian-type (SA α2,3-galactose) and mammalian-type (SA α2,6-galactose) receptors in mammary tissues, supporting susceptibility to viral replication.

In this study, lectin histochemistry was used to characterize influenza receptor distribution in the respiratory and gastrointestinal tracts of sheep, goats, and alpacas. Both receptor types were identified in conjunctival tissue and throughout respiratory and gastrointestinal tissues in all species examined. Viral binding assays using recombinant LPAI viruses demonstrated consistent viral attachment in respiratory tissues across species, whereas binding in gastrointestinal tissues was limited and less extensive.

These findings suggest that the respiratory tract is a more likely route of HPAI H5N1 entry in these species, while gastrointestinal infection appears less probable despite receptor presence. Understanding receptor distribution and viral attachment patterns provides critical insights for improving farm-level biosecurity, enhancing surveillance, and developing mitigation strategies to reduce HPAI H5N1 transmission and prevent further spillover events.
THE CONTENT OF THIS ABSTRACT IS BASED ON A FORTHCOMING ARTICLE

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Small ruminants, **Systems:** Respiratory

31: HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS (CLADE 2.3.4.4B) AND OTHER CONTRIBUTORS TO MORTALITY IN GULLS (FAMILY: LARIDAE) IN THE SOUTHEASTERN UNITED STATES

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Background:

Charadriiformes and Anseriformes are the principal natural reservoirs of avian influenza A viruses. Gulls (Charadriiformes, family Laridae) are widespread, abundant, and frequently occupy the human–wildlife interface, making them highly visible sentinels of ecosystem health. Despite their ecological importance and frequent detections, the clinicopathologic features of natural clade 2.3.4.4b highly pathogenic avian influenza (HPAI) virus infections in gulls and the contribution of concurrent disease processes to morbidity and mortality remain poorly understood.

Objective:

To characterize HPAI and the spectrum of comorbidities diagnosed in gulls submitted to the Southeastern Cooperative Wildlife Disease Study (SCWDS) since the 2021 HPAI virus introduction to North America.

Methods:

Demographic and diagnostic data were reviewed from gull cases from Florida and North Carolina submitted to SCWDS from 2022-2025.

Results:

Seventeen gulls representing five species were examined. The study population comprised 13 males, 3 females, and 1 individual of undetermined sex. Ten gulls were juveniles, 6 were adults, and 1 was of undetermined age. HPAI virus was detected in 8/17 gulls (47%); brain was most commonly affected and included neuronal necrosis and nonsuppurative encephalitis. The most common comorbidities identified in HPAI virus-detected gulls were emaciation (63%), aspergillosis (50%), and amyloidosis (50%).

Conclusions:

Compared with many susceptible species, HPAI virus-infected gulls appear to survive longer following infection, allowing for development of secondary infections and other concurrent disease processes that may contribute to morbidity and mortality. Characterizing these comorbidities is important for improving interpretation of HPAI virus-associated lesions and understanding the overall impact of HPAI in Charadriiform populations.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Wildlife/Zoo, **Systems:** Systemic

32: HISTOPATHOLOGIC CHARACTERIZATION OF SUSPECTED INFLAMMATORY BOWEL DISEASE IN PET RABBITS (ORYCTOLAGUS CUNICULUS DOMESTICUS): A RETROSPECTIVE REVIEW (2016–2026)

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Objective: To characterize the prevalence, demographics, and anatomical distribution of microscopic gastrointestinal (GI) lesions matching the Inflammatory Bowel Disease (IBD) case definition in pet rabbits (*Oryctolagus cuniculus domesticus*). Materials and **Methods:** Autopsy records of rabbits at UW-Madison Veterinary Care from 2016 to 2026 were searched for GI histologic findings. IBD was defined by previously described case report findings after excluding other etiologies (Farris et al., 2025). **Results:** Notable GI microscopic findings were described in 42 privately owned rabbits. GI-associated mortality/moribundity was reported in 21 animals attributed to hypomotility/stasis (7), mechanical obstructions (6), infectious enterotyphlitis (5), idiopathic inflammation (2), or neoplasia (1). Non-specific enteritis/typhlitis/colitis was identified in 18 rabbits without an attributed cause defined in the reports (18/42), and over half of these cases exhibited a thin body condition. The age distribution showed a bimodal pattern similar to IBD in people, peaking in young adults aged 1–3 years (7/18) and older patients >6 years (5/18).

Six of the 18 cases were GI-associated mortality/moribundity, while the remaining 12 cases had non-GI primary etiologies. Inflammatory cell infiltrations most frequently involved small intestine (15), followed by cecum (6) and colon (4). Mini Lops were the most commonly affected breed (5/18). **Conclusion:** Idiopathic inflammation in the digestive tract is a frequent finding in postmortem exams of pet rabbits. Further definition of histologic and clinical criteria of IBD can impact the medical management and diagnosis of these cases. Pathologists should include IBD as a differential for rabbits with non-specific GI signs or unexplained weight loss.

Discipline: Anatomic pathology, **Topic:** General pathology, **Species:** Rabbits, **Systems:** Gastrointestinal

33: CHARACTERISATION OF MELIOIDOSIS LESIONS (CAUSED BY BURKHOLDERIA PSEUDOMALLEI) IN 43 AVIAN AND MARINE MAMMAL CASES IN HONG KONG

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Research Rationale

Melioidosis is a disease caused by the bacterium *Burkholderia pseudomallei* that has a case mortality rate of up to 100%, especially with late diagnosis or delayed treatment. Melioidosis infections in veterinary species is understood mainly from infections in experimental animal models. Isolated case reports have been published in a variety of mammalian and avian species including two case series in captive terrestrial and marine mammals in Hong Kong.

We present a case series from the same oceanarium and zoo of two marine mammal taxa and birds, between 1989–2008, with an expanded histopathologic dataset, with objectives listed below:

Research Objectives

Qualitative characterization of histopathologic lesions, and documentation of the differences between cetaceans, pinnipeds, and birds.

Methodology

Retrospective observational study on archival histopathologic dataset of cetaceans, pinnipeds, and birds diagnosed with melioidosis by bacterial culture in Ocean Park Hong Kong, between 1989 – 2024. Lesions were independently graded by a boarded Veterinary Anatomic Pathologist and Resident, and compared between the three animal groups.

Results & Conclusion

- Overall there is severe, multisystemic, acute inflammation, most commonly manifesting as hepatitis, splenitis, and pneumonia.

- Bronchopneumonia is more common in birds; Interstitial pneumonia is more common in mammals, implying different routes of infection. Pinnipeds have bronchopneumonia more frequently than cetaceans.

- Only cetaceans have vasculitis, adrenalitis and Waterhouse-Friderichsen syndrome.

- Myocarditis and myositis are most frequently observed in pinnipeds.

- Hepatitis and splenitis are more necrotizing and fibrinous in cetaceans, compared to pinnipeds.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Wildlife/Zoo, **Systems:** Systemic

34: CLINICOPATHOLOGIC FEATURES, HORMONE RECEPTOR EXPRESSION, AND DNA METHYLATION OF MAMMARY CARCINOMA IN CALIFORNIA SEA LIONS (ZALOPHUS CALIFORNIANUS) FROM MANAGED CARE

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Background/Objective: Mammary carcinoma (MC) is relatively common in California sea lions (*Zalophus californianus*; CSL) in managed care; however, pathologic features of MC have not been well characterized. This study aimed to describe MC in CSLs including clinical presentation, pathologic features, hormone receptor expression, and global DNA methylation levels (DNAm).

Methods: Necropsy cases from female CSLs in managed care submitted to the Zoological Pathology Program between 1996-2026 were reviewed. MC tumors were classified and graded using canine mammary tumors schemes. Estrogen receptor-α (ER) and progesterone receptor (PR) gene expression was evaluated via RNAscope *in situ hybridization* (n=7). Tumor DNAm was evaluated via immunohistochemistry (n=10). Tumor hormone receptor expression and DNAm histochemical scores were compared to normal mammary gland from free-ranging CSLs (n=6 and n=11, respectively) via Welch's t-tests.

Results: MC was observed in greater than one-third of cases (12/30 cases). Affected CSL ages ranged from 25-34 years old, and clinical signs were often non-specific (10/12 cases). Histologic subtypes varied; most tumors were high-grade (7/12) with fewer intermediate- (3/12) and low- (2/14) grade tumors. MC had significantly decreased ER ($p=0.0427$) and PR ($p=0.0495$) expression as well as DNAm levels ($p=0.0141$) compared to normal mammary gland.

Conclusions: Findings support MC is an important cancer in older female CSLs in managed care. Significant decreases in ER and PR expression as well as DNAm levels indicates further investigation of hormonal and epigenetic mechanisms of carcinogenesis are warranted.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Wildlife/Zoo, **Systems:** Mammary

35: FELINE PANLEUKOPENIA VIRUS INFECTION IN AN ASIAN PALM CIVET

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Background:

A privately owned 12-week-old female intact Asian palm civet (*Paradoxurus hermaphroditus*) was presented to NC State's College of Veterinary Medicine for progressive lethargy, diarrhea, and anorexia. Initial bloodwork revealed leukopenia, neutropenia, severe hypoglycemia, and hypoalbuminemia, and a parvovirus SNAP immunoassay was positive. The patient developed rectal prolapse and subsequently died under anesthesia prior to surgery to repair the prolapse.

Methods:

Postmortem examination, histopathology, and PCR analysis were performed to further characterize the extent of this animal's disease.

Results:

Gross post-mortem evaluation revealed prolapse of the rectum and proximal colon and intussusception of the distal colon involving the colon, cecum, and ileum. The small intestines were diffusely distended by dark red, opaque, watery fluid. The thymus and lymph nodes were grossly inapparent, and the spleen was subjectively small. Histologic evaluation of the jejunum and colon found diffuse necrotizing enterocolitis with crypt necrosis and regeneration. The thymus and spleen had diffuse lymphoid atrophy, and the bone marrow was severely pancytopenic. To further characterize the causative parvovirus, a section of small intestine was submitted for PCR analysis, which was positive for feline panleukopenia virus (FPV).

Conclusions:

Histologic findings were strongly supportive of infection with a parvovirus, which targets rapidly dividing cells in the gastrointestinal tract and bone marrow. Viruses within the Parvoviridae family, including FPV and CPV, commonly affect carnivores worldwide. However, there are rare reports of infection with FPV in this species, and it is unclear where this animal acquired the infection.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Wildlife/Zoo, **Systems:** Gastrointestinal

36: PARASITE DISTRIBUTION AND LESION SEVERITY IN CAPTIVE CHELONIANS WITH TESTUDINE INTRANUCLEAR COCCIDIOSIS (TINC)

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Testudine intranuclear coccidiosis (TINC), caused by an Eimeria-like parasite, is an important cause of morbidity and mortality in captive tortoises and turtles. Despite its growing clinical significance and often fatal disease progression, the spectrum of microscopic lesions in affected chelonians and the tissue distribution of the causative agent are poorly characterized. To address these knowledge gaps, we evaluated >300 tissue samples from 30 tissue types collected during necropsy of chelonians

(n=23), including both Old and New World tortoises and Asian box turtles. All tissues were scored for inflammation using a semiquantitative microscopic evaluation system. To assess parasite distribution and abundance, RNAscope in situ hybridization (ISH) using a ZZ-probe complementary to a portion of the parasite 18S rRNA gene was performed. ISH slides were analyzed digitally with an open-source digital pathology software (QuPath) to quantify the number of ISH-positive parasites per unit area. Pathogen-associated lesions were present in nearly all tissue types examined, with pronounced epitheliotropism in all cases. The gastrointestinal, respiratory, and urinary tracts were most consistently affected, with prominent involvement of the oral and nasal cavities. There was a clear disparity in organism detection between ISH and routine light microscopy, with ISH detecting up to 10-fold more organisms. This study provides a systematic analysis of complete tissue sets from tortoises and turtles with TINC, highlights the significance of this systemic disease in captive chelonians, and emphasizes the poor sensitivity of organism detection by light microscopy alone. Ancillary diagnostics may be necessary for definitive diagnosis of this debilitating disease.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Wildlife/Zoo, **Systems:** Systemic

37: CAUSES OF MORTALITY AND HISTOPATHOLOGICAL FINDINGS IN BLACK-NECKED SWANS (CYGNUS MELANCORYPHUS) FROM A NATURE SANCTUARY IN VALDIVIA, CHILE (2012 TO 2022)

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Background: The Carlos Anwandter Nature Sanctuary is a critical waterfowl habitat located in Valdivia, Chile. While habitat degradation is recognized as the primary threat to the resident black-necked swan (*Cygnus melancoryphus*) population, comprehensive data regarding specific infectious, parasitic, and anthropogenic causes of mortality remain limited.

Objective: To determine the primary causes of death and characterize secondary gross and histopathological findings in black-necked swans over a ten-year period.

Methods: Standard diagnostic necropsy reports, hematoxylin and eosin (H&E)-stained histological slides, and formalin-fixed tissues from 110 black-necked swans submitted to the Universidad Austral de Chile between 2012 and 2022 were retrospectively reviewed. Selected tissue sections were evaluated using Perl's Prussian blue stain to characterize iron deposition.

Results: The primary causes of death were South American sea lion (*Otaria flavescens*) predation (38.18%) and trauma (33.63%), followed by enteritis (7.27%), American mink (*Neovison vison*) predation (2.73%), aspergillosis (2.73%), miscellaneous conditions (pneumonia, salpingitis, peritonitis, visceral gout; 2.73%), and undetermined causes (12.73%). Key histopathological findings included ventricular nematodiasis (79%; consistent with *Epomidiostomum vogelsangi*), renal

trematodiasis (55.5%; consistent with *Paratanaisia* sp.), and moderate-to-severe hepatic (56,3%) and mild renal (58,8%) hemosiderosis.

Conclusions: Sea lion predation and anthropogenic trauma (presumed powerline collisions) represent the leading direct causes of mortality in this avian population. Furthermore, the persistence of visceral hemosiderosis two decades after the severe environmental disaster occurred in 2004—historically linked to industrial effluent from a local cellulose mill—suggests that anthropogenic chemical pollution or ongoing dietary stressors remain unresolved, highlighting the vital need for continued toxicological monitoring.

Discipline: Anatomic pathology, **Topic:** General pathology, **Species:** Wildlife/Zoo, **Systems:** Systemic

38: HISTOLOGICAL PREVALENCE AND CHARACTERISTICS OF ENTERIC CAMALLANIDAE NEMATODES IN COLORADO PLAINS TOPMINNOW (FUNDULUS SCIADICUS)

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Background/Objective. Plains Topminnow (*Fundulus sciadicus*, PTM) populations have been in decline due to habitat loss, fragmentation, and competition with non-native species. There is limited information regarding the diseases of PTM and the potential role of parasitic infections in population decline. This retrospective study investigates the prevalence and features of Camallanid nematode infection in PTM.

Methods. One hundred and twenty PTM were collected between 2023 and 2025 from a South Platte Tributary in Colorado's Pawnee National Grasslands, Weld County. Fish were euthanized via buffered MS-222, fixed in 10% neutral buffered formalin, bisected on the midsagittal plane, embedded in paraffin, and stained with haematoxylin and eosin.

Results. Camallanid nematodes were found in 44 of 120 PTM (37%). Of these, 35 PTM (81%) had nematodes restricted to the lumen of the intestinal tract, while 8 PTM (19%) had nematode profiles within the wall (submucosa or muscularis) of the intestinal tract. Presence of nematodes was associated with mild to moderate mixed granulocytic and mononuclear submucosal inflammation and mild to marked intestinal distension.

Camallanid nematodes had an eosinophilic annulated cuticle, coelomyarian musculature, and orange-brown buccal capsule with two lateral valves with multiple ridges resembling teeth. Nematodes have a prominent muscular anterior and glandular posterior esophagus, intestinal tract lined by cuboidal uninuclear cells, and reproductive tract.

Conclusions. Camallanid nematodes are common in wild PTM. Enteric Camallanids are associated with a host inflammatory response and intestinal distension, suggesting that these parasites likely contribute to host morbidity.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Aquatic, **Systems:** Gastrointestinal

39: INTERDIGITAL KERATOLYTIC LESIONS AS A POSSIBLE PREDISPOSING CONDITION FOR TREPONEME-ASSOCIATED HOOF DISEASE (TAHD) IN ELK (CERVUS CANADENSIS)

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Background: Treponeme-associated hoof disease (TAHD) is an emerging disease that causes lameness in elk (*Cervus canadensis*). Some elk with and without TAHD display a keratolytic lesion similar to pitted keratolysis (PK) of humans; moisture is relevant to the development of PK and is suspected to be so for TAHD. This project's objectives were to document these keratolytic lesions and investigate their association with TAHD and geographic differences in occurrence.

Methods: Elk hooves submitted to the Washington Animal Disease Diagnostic Laboratory (WADDL) September 2022 – December 2025 (n = 105) were examined for TAHD and PK-like lesions using H&E and Warthin-Starry stained sections. Logistic regression analysis was used to assess for an association between TAHD and keratolysis and for differences in TAHD prevalence between samples from west and east of the Cascades.

Results: 37 elk and 45 hooves were diagnosed with TAHD, 36 elk and 59 hooves with keratolysis, and 12 elk and 12 hooves with both. Keratolysis was characterized by epidermal pallor and loss in pitting and/or plaque-like distributions with mixed bacterial invasion of the keratin. The statistical association between presence of TAHD and keratolysis was not significant, nor was the difference in geographical prevalence.

Conclusion: Concurrent affliction with TAHD and keratolysis was present in approximately one-third of elk with either condition. While no statistically significant relationship between keratolysis and TAHD, nor a strong geographic pattern of prevalence, were discovered, investigating the links between TAHD and lesion severity are warranted, as is assessing these relationships across a larger sample size.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Wildlife/Zoo, **Systems:** Integument

40: BEYOND THE COLD TUMOR: STEMNESS-ASSOCIATED TRANSCRIPTOMIC SIGNATURES SPATIALLY CORRELATE WITH IMMUNOSUPPRESSIVE MICROENVIRONMENTS IN CANINE GLIOMA

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Canine glioma (CG) is an aggressive, invariably fatal CNS tumor with limited therapeutic options. Recent clinical trials have highlighted immunotherapy as a promising novel treatment. However, CG are “cold” immunologically and exhibit enhanced cell plasticity/stemness pathways (EMP), which correlate with immune exhaustion and immunotherapy resistance in humans. Therefore, defining the immune landscape of CG in relation to EMP is critical to understanding mechanisms that may limit immunotherapy response in canine patients.

We analyzed 30 CG cases, including astrocytomas (n=10), oligodendrogliomas (n=10), and undefined gliomas (UG; n=10). Quantification and spatial distribution of T-cells (CD3, FOXP3), B-cells (PAX5, CD20), plasma cells (MUM1), and macrophages (IBA1, CD204) were assessed by immunohistochemistry (IHC) and correlated with stemness markers (SOX10, SOX2) in QuPath. Tumor phenotype and immune cell polarization were evaluated via spatial transcriptomics (Xenium v1) on tumor center and invasive front, employing the use of tissue microarrays.

On IHC, astrocytomas had more T-cells/mm² than oligodendrogliomas and UG; SOX10 expression was regionally associated with reduced $CD3^+$ T-cell infiltration. Spatial transcriptomics revealed that EMP gene upregulation correlated with exhausted T-cells (CTLA-4, PD-1) and immunosuppressive macrophages (TAMs). Oligodendrogliomas had a distinct stem-like transcriptional signature (*OLIG2, PDGFRA, SOX4, SOX10, MYC, MMP2, PRRX1*) (p-adj < 0.05) that correlated weakly with TAMs (Spearman ρ = 0.23). A weak correlation was also identified between *SNAI1*, *LAG3*, and TAMs across tumor subtypes (ρ = 0.2).

We conclude that upregulated stemness signatures in CG are linked to T-cell exhaustion and TAM enrichment. Importantly, our results emphasize oligodendrogliomas as a potentially distinct immunotherapeutic challenge.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Canine, **Systems:** Nervous

41: SERUM MICRORNA PROFILING IDENTIFIES CIRCULATING BIOMARKERS FOR CANINE HEMANGIOSARCOMA VALIDATED BY DIGITAL PCR

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Background: Reliable minimally invasive biomarkers for canine hemangiosarcoma (HSA) are lacking. This study identified circulating serum microRNAs (miRNAs) associated with HSA and evaluated their biological and technical validation through comparison with tumor tissue sequencing and digital PCR (dPCR).

Methods: Serum samples from 168 dogs enrolled in the Golden Retriever Lifetime Study were evaluated, and 39 samples with measurable RNA (23 HSA, 16 controls) underwent small RNA sequencing. Differential expression was analyzed using EdgeR with false discovery rate (FDR) correction. Serum miRNAs were compared with previously generated cardiac and splenic HSA tissue sequencing datasets. Candidate biomarkers were subsequently validated by dPCR and evaluated individually and in combination using receiver operating characteristic (ROC) analysis.

Results: Thirty-four circulating miRNAs were differentially expressed; ten of those demonstrated concordant dysregulation with HSA tissue. miR-224 was consistently upregulated in serum and both cardiac and splenic HSA tissues, whereas miR-30c was downregulated in serum and cardiac tissue. Additionally, miR-127, miR-381, miR-455, and miR-494 were upregulated in both serum and splenic HSA tissue, while miR-191 was downregulated in both sample types. dPCR confirmed overexpression of miR-224, miR-127, miR-381, and miR-494. Among individual biomarkers, miR-224 demonstrated the highest diagnostic performance (AUC = 0.869); a four-miRNA panel (miR-224, miR-127, miR-381, and miR-494) achieved an AUC of 0.872.

Conclusions: Serum miRNA sequencing identified circulating biomarkers that were independently supported by tumor tissue sequencing and dPCR validation. The consistent identification of miR-224, miR-127, miR-381, and miR-494 across multiple analytical platforms supports their potential as biologically relevant minimally invasive biomarkers for canine HSA.

Discipline: Clinical pathology, **Topic:** Oncology, **Species:** Canine, **Systems:** Hematopoietic

42: CHARACTERIZATION OF SIN NOMBRE HANTAVIRUS TISSUE TROPISM IN NATURALLY INFECTED DEER MICE USING RT-QPCR AND RNASCOPE IN SITU HYBRIDIZATION

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Background: The North American deer mouse (*Peromyscus maniculatus*) is the primary reservoir of Sin Nombre virus (SNV, *Orthohantavirus sinnombreense*), the etiologic agent of hantavirus cardiopulmonary syndrome in humans. The role of reproductive tissues in SNV maintenance and transmission in rodents remains unclear.

Objective: Characterize SNV tissue distribution in naturally infected deer mice, with emphasis on reproductive tissues.

Methods: Free-ranging *P. maniculatus* (n=169) were collected from an SNV-endemic site. Oral and urogenital swabs, testes or ovary, kidney, and pooled spleen-liver-lung were evaluated by SNV RT-qPCR. A subset with SNV-positive reproductive tissues underwent histopathology, RNAscope in situ hybridization (ISH, targeting positive sense SNV RNA), and SNV RNA strand specific RT-qPCR.

Results: Overall, 48.5% (n=82/169) of individuals were RT-qPCR positive in at least one specimen. Reproductive tissues were RT-qPCR positive in 32% (54/169) of mice. SNV strand-specific testing revealed higher genomic (negative sense) RNA loads compared to anti-genomic (positive sense) in reproductive tissues versus kidney and pooled spleen-liver-lung. ISH was performed on eight rodents (four RT-qPCR positive and four negative). SNV RNA signal was not detected in negative animals. Among positive animals, signal localized within lung, spleen, and kidney, with no signal detected in reproductive tissues.

Conclusions: SNV RNA was detected in multiple tissues by RT-qPCR and ISH. Absence of positive-sense ISH signal in reproductive tissues despite RT-qPCR positivity, combined with lower levels of positive-sense RNA by strand-specific PCR, suggests reproductive tissues differ from other organs in SNV replication and tissue localization. Analyses of epididymis, seminal vesicles, uterus, and vagina are in progress.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Wildlife/Zoo, **Systems:** Systemic

43: PRIMARY LARYNGEAL NEOPLASIA IN AN ASIAN SMALL-CLAWED OTTER (AONYX CINEREUS): A CASE REPORT

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Asian small-clawed otters (ASCOs, Aonyx cinereus) are semi-aquatic members of the family Mustelidae and considered a vulnerable species by the International Union for Conservation of Nature. A 7-year-old, intact female, Asian small-clawed otter under managed care was euthanized after a history of acute respiratory difficulty and identification of a mass obscuring the glottis. On gross and microscopic examination, there was a 0.6 cm diameter, raised, soft, pink mass that originated from the left arytenoid cartilage, occluded the tracheal lumen, and extended cranially to the epiglottis. Histologically, the neoplasm was composed of round to polygonal cells with abundant granular, PAS-positive, and diastase-resistant cytoplasm. Differentials considered include granular cell tumor (GCT), oncocytoma, rhabdomyoma, and plasma cell tumor. The morphologic features of GCTs, oncocytomas, and rhabdomyomas are similar on routine H&E staining. Moreover, GCTs exhibit variable immunohistochemical profiles given their non-uniform histogenesis. In this case, neoplastic cells exhibited cytoplasmic immunoreactivity for vimentin and NSE, and lacked immunoreactivity for Melan-A, MUM1, GFAP, and S100. Ultimately, electron microscopy provides the most diagnostic information based on ultrastructural characteristics. Across US zoos and aquaria from 2000 to 2020, malignant neoplasia was the second most common cause of natural death or euthanasia in ASCOs. To date, there are two reports of oral neoplasia in ASCOs due to sublingual/pharyngeal squamous cell carcinoma that resulted in mortality. To the authors' knowledge, this is the first report of primary laryngeal neoplasia in an ASCO, and highlights the importance of considering laryngeal neoplasia as a differential diagnosis in ASCOs experiencing respiratory distress.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Wildlife/Zoo, **Systems:** Respiratory

44: THE PATHOLOGY, HOST RANGE, AND GEOGRAPHIC DISTRIBUTION OF EMERGING ZOONOTIC THELAZIA CALLIPAEDA IN PENNSYLVANIA, USA

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Thelaziosis is a zoonotic orbital disease caused by a spirurid nematode of the genus *Thelazia.* Their definitive hosts include people and a variety of domestic and wild animals. Originating in Asia with subsequent spread to Europe, *Thelazia callipaeda* has been implicated in most cases of thelaziosis. In 2020 the first autochthonous case in the United States of America (USA) was reported in a domestic dog from New York; since then, the incidence of thelaziosis has expanded. The parasite's novel range and naive hosts raise concerns about the potential for clinical disease and spillover events. Our objectives were to investigate the host range of *T. callipaeda*, characterize associated histologic lesions in the definitive hosts, and provide insights into the pathogen's epidemiology and ecology in Pennsylvania, USA. During the 2025/2026 hunting season in Pennsylvania, USA, wildlife were opportunistically examined at check stations for evidence of infection with *Thelazia* spp. Infected hosts included 118 black bears, 13 coyotes, two fishers, and one red fox. Cases were predominantly located in northeastern Pennsylvania with occasional cases as far as the southwestern part of the state. The eyes from six of these animals were fixed in 10% NBF and routinely processed with H&E. Histologically, lesions consisted of mild to moderate regional to multifocal chronic ulcerative conjunctivitis and blepharitis with occasional hemorrhage. Our findings reflect the establishment and increased prevalence of *T. callipaeda* in non-endemic regions, further highlighting the necessity for continued research into the clinical significance and extent of disease in humans, domestic animals, and wildlife.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Wildlife/Zoo, **Systems:** Eye/ear

45: IN SILICO CHARACTERIZATION OF CANINE ORAL MELANOMA IDENTIFIES PLASMACYTOID DENDRITIC CELLS AS MARKERS OF IMMUNE-TRANSCRIPTOMIC PROFILES

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Canine oral melanoma (COM) is a highly immunogenic tumor in which complex interactions between neoplastic and immune cells significantly influence disease progression. Characterizing this tumor immune microenvironment (TIME) is critical for identifying prognostic biomarkers and therapeutic targets. To characterize the COM TIME, we integrated three public bulk RNA-sequencing datasets (PRJNA421895, PRJNA749900, PRJNA527141) and applied a canine-specific single-cell RNA-sequencing signature matrix (PRJNA936588) for immune cell deconvolution using CibersortX algorithm. Unsupervised K-means clustering based on the immune landscape revealed two distinct profiles characterized by 17 differentially abundant cell subpopulations. Differential expression and over-representation analyses were carried out using an FDR < 0.05. The hot cluster exhibited an immune-inflamed phenotype characterized by a higher abundance of antitumor cells, upregulated pathways for leukocyte activation and cell adhesion, and

high expression of immune checkpoints. Conversely, the cold cluster presented an immune desert phenotype, dominated by immunosuppressive populations, upregulated cell cycle pathways, and a sustained expression of melanocytic lineage genes. An in silico validation in an independent cohort (SUB14547907) highlighted the importance of plasmacytoid dendritic cells (pDCs). While their role in veterinary oncology remains underexplored, our analysis revealed two key findings. First, pDCs retained immune pathway enrichment across independent cohorts. Second, survival analysis demonstrated that higher pDC score is a significant predictor of poor prognosis (p=0.031). Our study demonstrates the pDCs as key markers to recover transcriptional profiles of hot and cold COM. Further studies are needed to validate these results using routine methods, such as immunohistochemistry and flow citometry.

Financial support: FAPESP 2024/01379-8 and 2026/01648-4

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Canine, **Systems:** Gastrointestinal

46: THE ROLE OF HEPATIC MIRNAS IN THE REGULATION OF IMMUNITY IN CATTLE AND HORSES WITH INFECTIOUS DISEASES

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Background: Infectious diseases cause economic and welfare losses in cattle and horses. The liver contributes to immunity through production of acute phase proteins, cytokines, complement components, and metabolic regulation. Hepatic microRNAs (miRNAs) may influence host responses to infection through post-transcriptional control of immune-related genes.

Objective: To identify hepatic miRNAs associated with infectious disease in cattle and horses and characterize their predicted immune-related functions.

Methods: Postmortem liver samples were collected from 61 cattle (42 with infectious and 19 non-infectious diseases) and 64 horses (26 with infectious and 38 non-infectious diseases). The samples were analyzed by small RNA sequencing. Differential miRNA expression was assessed using QIAGEN CLC Genomics Workbench. Putative targets were identified using miRWalk, TargetScan, and miRTarBase, followed by Gene Ontology enrichment analysis and network visualization.

Results: In cattle, 33 differentially expressed miRNAs (< 1.5-fold change) were identified, including 17 downregulated and 16 upregulated miRNAs. In horses, 49 differentially expressed miRNAs were identified, including 10 downregulated and 39 upregulated miRNAs. Predicted targets and enriched pathways in both species were linked to immune and inflammatory regulation. In cattle, enriched functions involved Toll-like receptor, JAK/STAT, MAPK, PI3K and cytokine signaling, NF-κB-associated pathways, phagosome maturation, and inflammatory cell recruitment. In horses, enriched pathways included NF-κB, TGF-β, PI3K/AKT, MAPK, and cytokine signaling.

Conclusion: Infectious disease was associated with altered hepatic miRNA expression in cattle and horses. Although different miRNAs were identified in each species, both datasets implicated pathways involved in innate and inflammation, supporting hepatic miRNAs as potential regulators of host responses to infection.

Discipline: Anatomic pathology, **Topic:** Immunology, **Species:** Equine, **Systems:** Systemic

47: VARIATION IN SUB-STRAIN PATHOGENESIS AND CONSPECIFIC TRANSMISSION OF HIGHLY PATHOGENIC AVIAN INFLUENZA (H5N1, CLADE 2.3.4.4B) IN DOMESTIC CATS

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Background: Transmission of H5N1 highly pathogenic avian influenza (HPAIV) remains poorly understood in domestic cats. The primary assumption is that cats acquire HPAIV by consuming contaminated food. However, transmission may occur between cats through social behavior. Mutations in viral genes associated with mammalian adaptation have been identified in isolates of H5N1 derived from B3.13 and D1 genotypes, while these mutations are absent in B3.1.

Objective: Our goal is to assess genotype differences in pathogenesis and transmission between mammalian and poultry adaptive H5N1.

Methods: Three groups of 5 cats were intranasally and orally infected with genotypes B3.1, B3.13, and D1.1. After 2 days, contact animals were introduced and allowed to free roam with infected animals. Viral shedding was monitored 3 times per week through the study. At end of study, or following euthanasia due to disease progression, animals were necropsied and tissues collected for virus isolation, PCR and sequencing, histopathology, and immunohistochemistry.

Results: We identified variable pathogenesis in cats infected with each genotype. Cats inoculated with B3.13 developed severe clinical disease necessitating euthanasia, while those infected with B3.1 developed mild clinical disease and recovered. Cats in the D1.1 group demonstrated mild to moderate clinical disease. Within the D1.1 group, one contact cat contracted H5N1 and shed of infective virus, confirming conspecific transmission of D1.1 in domestic cats.

Conclusion: The D1.1 genotype of H5N1 allows for conspecific transmission between cats with less severe disease than seen in genotype B3.13, suggesting viral adaptation to domestic cats.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Feline, **Systems:** Systemic

48: TEMPORAL REMODELING OF IRON ACQUISITION AND EXPORT TRANSCRIPTS IN LPS-STIMULATED BOVINE MACROPHAGES

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Background/**Objective:** Macrophage iron handling contributes to inflammatory hypoferremia and anemia of inflammation, but the timing of these responses is poorly characterized in cattle. We tested whether lipopolysaccharide (LPS) produces time-dependent changes in transcripts governing iron acquisition, processing, and export in bovine macrophages.

Methods: Monocyte-derived macrophages from five cattle were exposed to LPS or phosphate-buffered saline and sampled at 30 minutes and 6 hours. RNA sequencing generated 40 libraries across 20 cow-treatment-time combinations (mean 43.5 million read pairs/library; 82.7-88.7% mapping); the two libraries corresponding to each combination were collapsed before analysis. DESeq2 models accounted for cow and tested treatment-by-time interactions (FDR < 0.05; absolute $\log_2$ fold change >= 1).

Results: At 30 minutes, LPS induced a predominantly inflammatory response, including aconitate decarboxylase 1 (*ACOD1*; +1.7 $\log_2$ fold change), whereas the focal iron-handling genes were unchanged. At 6 hours, *TFRC*, encoding the transferrin receptor (+2.6), *STEAP3*, encoding an endosomal ferrireductase (+1.5), and *SLC11A2*/*DMT1*, encoding divalent metal transporter 1 (+2.5), were all increased. However, *SLC40A1*, encoding the iron exporter ferroportin, was decreased (-2.3) at 6 hours. Each iron gene showed a significant treatment-by-time interaction, and all five cattle changed concordantly. Genome-wide enrichment was dominated by inflammatory and innate-immune pathways.

Conclusions*:* LPS induced delayed remodeling of transcripts supporting transferrin/endosomal iron acquisition and ferroportin-mediated export within a broader inflammatory response. This pattern provides cellular context for inflammatory iron restriction but does not establish ferroportin protein loss, intracellular iron retention, or anemia of inflammation.

Discipline: Clinical pathology, **Topic:** Immunology, **Species:** Bovine, **Systems:** Systemic

49: SLIDE AGE VS. ANTIGENICITY: A MULTI-MARKER STUDY FOR COMPARATIVE PATHOLOGY LABORATORIES

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Background:

There are a wide range of environmental factors that can influence the antigenicity of stored formalin fixed, paraffin embedded (FFPE) slides. In experimental pathology laboratories or core facilities it is common to store unstained FFPE slides for archival purposes or control tissue slides for months to years.

Objective:

This study investigates the effects of slide age and storage conditions on tissue antigenicity for 10 separate antibodies in mouse tissues.

Methods:

The age of slides evaluated included fresh cut, stored for 24 hours, 1 week, 2 weeks, 1 month, 3 months, 6 months, 9 months, 1 year, and 2 years. The slide storage conditions assessed focused on temperature (room temperature vs 4°C) and paraffin dipped vs non-dipped.

Results

For analysis, immunoreactivity was scored semi-quantitatively by a board-certified veterinary pathologist and quantitative data was obtained using Qupath to identify the percent positive staining for each slide. 8 of the 10 markers analyzed showed insignificant antigenicity reduction across all slide ages and conditions, including CD4, Ki67, F4/80, CK, SMA, CD45r and vimentin. Collagen 4 immunoreactivity decreased from 25.26% positive staining at 7 days to 5.92% positive at 2 weeks. CD31 decreased from 22.03% positive staining at 1 year to 5.92% at 2 years. No significant differences in positive staining were observed between the paraffin dipped and non-paraffin dipped slides.

Conclusions:

Tissue section aging and decreased tissue antigenicity is antigen dependent and should be considered a confounding factor in immunohistochemistry data interpretation for research labs using pre-sectioned slides to ensure accurate staining results.

Discipline: Anatomic pathology, **Topic:** Comparative pathology, **Species:** Rodents, **Systems:** Systemic

50: PATHOLOGY AND LARVAL RETRIEVAL OF EXPERIMENTAL GUINEA WORM (DRACUNCULUS MEDINENSIS) INFECTION IN DOGS AND FERRETS

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Human and canine cases of Guinea worm (GW) persist in sub-Saharan Africa. To study GW development and pathology, 5 dogs and 6 ferrets were experimentally infected with third-stage larvae (L3). Inoculation was performed either via gastric tube or intraperitoneally. Animals were necropsied 10-13 months post-infection. Dogs: A skin blister was observed in 3/5 dogs. Female GWs were recovered from 4/5 dogs (1-3 per dog), total n=8, ranging from 49-63 cm. Females were in the subcutis of the extremities (5/8), and trunk (2/8), and 1 was within the abdominal cavity. First-stage larvae (L1) were retrieved from 3 gravid females (4,160-46,350 per dog), but only one had motile L1s (2% motility). Ferrets: 1/6 ferrets had blisters. Twelve female worms were recovered from 4/6 ferrets (1-6 per ferret; 6-100 cm). Female worms were within the subcutis or musculature of the extremities (5/12), trunk (3/12), cervical (2/12), and inguinal regions (2/12). Ten gravid females yielded numerous L1s (185,000-2,122,500 per ferret), and 9 of the adult female worms had from 0.39% to 88% motility of the L1s. One male GW was in the left perirenal adipose tissue of a ferret. Histologically, granulomatous and eosinophilic dermatitis surrounded L1s and adults, often enclosed by a fibrous capsule. Histologically, adult nematodes were morphologically consistent with Dracunculus. The GW distribution

found in the dogs closely mimicked natural infection. The ferrets developed a high nematode burden, making them an ideal model for retrieving viable L1, enabling life-cycle maintenance, and generation of biological samples to support diagnostic and therapeutic research efforts.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Non-rodent lab animals, **Systems:** Integument

51: ARSENIC RESISTANCE PROTEIN 2 REGULATES IL-17 INDUCTION AND PROMOTES TH17 MEDIATED PATHOLOGY DURING EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Background: Th17 cells are critical for host protection against extracellular bacterial and fungal infections. Although Th17 cells play these protective roles, they have also been implicated in the pathogenesis of autoimmune diseases like human multiple sclerosis (MS) and canine neurodegenerative disease. Our previous finding implicates nuclear cap-binding molecule arsenic resistance protein 2 (ARS2) in IL-17 production but not IFN-γ, by $CD4^+$ T cells from human PBMCs. However, whether ARS2 determined neuroinflammation was not assessed in that study.

Objective: To determine whether ARS2 is required for the generation and function of pathogenic Th17 cells during autoimmune neuroinflammation.

Methods: Experimental autoimmune encephalomyelitis (EAE) was induced in ARS2 deficient (IL-17Cre × $ARS2^{fl/fl}$) mice and their wild type counterparts (IL-17Cre × $ARS2^{+/+}$) by administering myelin antigen (MOG) subcutaneously on the left and right dorsal hind limb regions. Afterwards, Clinical scoring, spinal cord histopathology, flow cytometry of cells from the draining lymph node, spleen and CNS as well as MOG-specific cytokine restimulation were used to assess pathogenic Th17 cells frequency and functions.

Results: ARS2 deficient mice were resistant to EAE (reduced clinical severity and histopathology), and this resistance was associated with reduced generation of pathogenic Th17 cells in their draining lymph node, spleen, and CNS. MOG-specific restimulation demonstrated selective reduction of IL-17A but not IFN-γ, suggesting ARS2 loss specifically impairs Th17 during EAE.

Conclusion: Our study shows that ARS2 promotes Th17-mediated neuroinflammation by regulating pathogenic Th17 differentiation, suggesting it could be potentially targeted in IL-17 mediated neuroinflammation like multiple sclerosis and canine neurodegenerative diseases.

Discipline: Anatomic pathology, **Topic:** Immunology, **Species:** Rodents, **Systems:** Nervous

52: INTRAPERITONEAL INOCULATION YIELDS SUPERIOR PATHOLOGICAL OUTCOMES IN A MURINE MODEL OF SALMONELLA ABORTUSEQUI-INDUCED ABORTION

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Salmonella enterica serovar Abortusequi (*S.* Abortusequi) is an equid-restricted pathogen causing equine paratyphoid, which is primarily characterized by infectious abortion without overt maternal clinical signs. To investigate the pathogenesis of *S.* Abortusequi-induced abortion, this study aimed to establish a murine abortion model by comparing outcomes of different inoculation routes. Forty-eight pregnant ICR mice were inoculated perorally (PO; 2×10^3, 2×10^5, 2×10^7, or 2×10^9 CFUs), intraperitoneally (IP; 2×10^2, 2×10^3, 2×10^4, or 2×10^5 CFUs), or intravenously (IV; 2×10^2, 2×10^3, 2×10^5, 2×10^6, or 2×10^7 CFUs) on gestation day (GD) 14.5. Mice were monitored until GD 18.0 or the onset of abortion, at which point samples were harvested. While the PO group exhibited no abortions (0/16 dams), abortions occurred in both the IP (8/12 dams) and IV (7/20 dams) groups. Notably, IP inoculation induced abortion at lower doses without causing the systemic weight loss observed with high-dose IV inoculation, thereby more closely mimicking the clinical features in mares. In aborted dams, numerous bacteria were detected in the placenta. Histopathological evaluation of aborted placentas in both the IP and IV groups demonstrated diffuse narrowing of CD31-positive fetal vessels and trophoblastic septal thickening. Clusters of bacteria were localized primarily within the fetal vessels of the placental labyrinth zone. These findings suggest that intraperitoneal inoculation is the optimal route for establishing a murine abortion model to investigate the mechanisms of *S.* Abortusequi-induced equine abortion.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Equine, **Systems:** Reproductive

53: ENGINEERING A 4-1BB AGONIST FOR CANINE CANCER IMMUNOTHERAPY

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Background: First-generation 4-1BB/CD137 agonistic monoclonal antibodies showed potent antitumor activity but were limited by dose-dependent hepatotoxicity resulting from systemic 4-1BB activation. Although tumor-targeted 4-1BB immunotherapies are being developed in human oncology to restrict activation to the tumor microenvironment, comparable approaches for canine cancer remain limited.

Objective: To develop and evaluate a canine-specific 4-1BB agonist platform capable of activating canine T cells and supporting future translational immunotherapy studies in dogs.

Methods: Two canine-specific recombinant protein constructs were designed using the extracellular domain of canine 4-1BB ligand fused to canine IgG subclass D to promote 4-1BB signaling while minimizing unfavorable Fc-mediated antibody-dependent cellular cytotoxicity. The constructs differed in linker design to test enforced versus intrinsic multimerization for ligand trimerization and receptor agonism. Recombinant canine Fc-4-1BBL constructs were expressed and purified from stable Expi293F cell lines. Binding to canine 4-1BB and Fcγ receptors was assessed by flow cytometry. Functional activity was evaluated using a 4-1BB luciferase bioassay, canine T-cell proliferation assays, and CellTiter-Glo viability assays.

Results: Both constructs specifically bound canine 4-1BB-expressing Expi293 cells and activated canine T cells. 4-1BB expression kinetics on activated canine T cells were similar to reported human profiles. Both constructs also showed favorable Fc-binding profiles, demonstrated agonistic activity in luciferase bioassays, and enhanced canine T-cell proliferation. Transcriptional profiling is ongoing.

Conclusion: These findings support the feasibility of developing canine-specific 4-1BB agonists and provide a foundation for further preclinical evaluation in canine cancer models.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Canine, **Systems:** Other (Immune)

54: MICE WITH LOSS-OF-FUNCTION MUTATIONS IN THE GENES ENCODING THE METABOLIC PROTEINS FNIP1 AND FNIP2 DEVELOP SEVERE SYSTEMIC HISTIOCYTOSIS

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Folliculin-interacting proteins 1 and 2 (FNIP1, FNIP2) are binding partners of Folliculin (FLCN), a tumor suppressor that regulates mechanistic target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK) signaling, key pathways controlling cellular metabolism. While Fnip1-deficient mice develop B-cell deficiency, agammaglobulinemia, cardiomyopathy, and skeletal myopathy, Fnip2-deficient mice have a normal lifespan. To investigate the combined role of Fnip1 and Fnip2 in myeloid cells, we generated mice with myeloid-specific deletion of Fnip1 and Fnip2. All double-knockout mice developed progressive illness and required euthanasia by 6 months of age. At necropsy, affected mice had marked hepatosplenomegaly. Histologically, they exhibited severe systemic histiocytosis, characterized by widespread infiltrates of round cells involving the skin, bone marrow, lung, liver, spleen, lymph nodes, gastrointestinal tract, and adipose tissue. Prominent erythrophagocytosis was present in the spleen and liver of two mice. Two mice also had abundant intracytoplasmic eosinophilic hyaline droplets (lysozyme granules) within renal tubular epithelial cells. Immunohistochemically, the infiltrating cells were predominantly F4/80-positive macrophages with admixed CD138-positive plasma cells and mast cells. Flow cytometry similarly demonstrated increased $F4/80^{+}CD11b^{+}$ macrophage-lineage cells in bone marrow, spleen, and liver. Mice with myeloid cell lineage specific deletion of FLCN have previously been shown to develop systemic histiocytic inflammation in a TFE3-dependent manner by 12 months of age.

Together, these findings identify myeloid-specific Fnip1/Fnip2-deficient mice as a novel model of systemic histiocytosis, and suggest that disruption of the FLCN-FNIP signaling axis may contribute to the pathogenesis of human histiocytic disorders, such as Erdheim-Chester disease, a form of non-Langerhans' cell histiocytosis.

Discipline: Anatomic pathology, **Topic:** Comparative pathology, **Species:** Rodents, **Systems:** Hematopoietic

55: PRIMARY FELINE CUTANEOUS SQUAMOUS CELL CARCINOMA CELL CULTURES EXHIBIT PREDOMINANTLY CYTOPLASMIC LOCALIZATION OF CONNEXIN 31 AND CONNEXIN 43

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Background: Connexins are essential components of gap junctions that regulate epithelial homeostasis, differentiation, and intercellular communication. Connexin 31 (Cx31) and Connexin 43 (Cx43) are widely expressed in stratified epithelia and are key in maintaining gap junctional intercellular communication, with dysregulation frequently associated with epithelial carcinogenesis. Alterations in connexin expression and subcellular localization have been implicated in epithelial carcinogenesis in humans, but remain poorly characterized in feline cutaneous squamous cell carcinoma (cSCC), particularly in primary in vitro models.

Objective: To establish and immunophenotypically characterize primary feline cSCC cell cultures, with emphasis on the subcellular localization of connexin 31 (Cx31) and connexin 43 (Cx43).

Methods: Primary keratinocyte cultures were established from feline cSCC using the explant technique and maintained under keratinocyte-specific culture conditions. Immunofluorescence was performed using antibodies against pan-cytokeratin (AE1/AE3), Cx31, and Cx43. Fluorescence microscopy was used to assess epithelial phenotype and connexin localization.

Results: Primary neoplastic keratinocytes displayed strong AE1/AE3 immunoreactivity, confirming preservation of their epithelial phenotype in vitro. Both Cx31 and Cx43 exhibited predominantly cytoplasmic immunolabeling with limited membranous localization. This distribution pattern is consistent with altered connexin trafficking and gap junction dysregulation described in epithelial malignancies and may reflect impaired intercellular communication during feline cSCC progression.

Conclusion:

Primary feline cSCC cell cultures retained epithelial identity while exhibiting predominantly cytoplasmic localization of Cx31 and Cx43, supporting their potential as a biologically relevant in vitro model for investigating connexin trafficking and gap junction dysregulation during feline cutaneous carcinogenesis and as a valuable comparative oncology platform for studying epithelial tumor biology.

Discipline: Anatomic pathology, **Topic:** Oncology, **Species:** Feline, **Systems:** Integument

56: HISTOPATHOLOGIC EVALUATION OF EXPERIMENTAL OLIGOFRUCTOSE-INDUCED EQUINE LAMINITIS FOLLOWING CLOPIDOGREL ADMINISTRATION

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Background: Laminitis is a common, debilitating disease of horses, donkeys, and ponies. The oligofructose-induced laminitis model reliably produces acute developmental and early active-phase laminitis characterized by epidermal lamellar detachment, basement membrane disruption, dermal inflammation, and vascular microthrombus formation. Because platelet aggregation contributes to microvascular dysfunction, clopidogrel has been investigated as a potential therapeutic due to its inhibitory effects on platelet activation and thrombus formation.

Objective: To characterize the histopathologic lesions and compare morphometric and semiquantitative histologic outcomes in horses with experimental oligofructose-induced laminitis treated with clopidogrel or placebo.

Methods: Eleven horses were enrolled at the time of this abstract submission in a randomized, blinded study evaluating clopidogrel as a potential treatment for oligofructose-induced laminitis. Morphometric outcomes included secondary epidermal lamellar (SEL) length, width, and angle. Semiquantitative histologic grading assessed basement membrane integrity, dermal inflammation, vascular and microthrombotic lesions, and lamellar detachment.

Results: Histopathologic lesions consistent with acute oligofructose-induced laminitis were observed in both groups and included epidermal lamellar detachment, basement membrane disruption, dermal inflammation, vascular lesions, and microthrombi. No differences were identified between the clopidogrel- and placebo-treated horses for composite scores of basement membrane integrity, dermal inflammation, vascular and microthrombotic lesions, and lamellar detachment (Mann-Whitney $U = 21.5$, $p = 0.26$, rank-biserial $r = -0.43$).

Conclusion: Clopidogrel treatment was not associated with detectable differences in the histopathologic or morphometric outcomes evaluated. However, additional investigations incorporating clinical, physiologic, and molecular outcomes are needed to more fully assess its potential effects in laminitis.

Discipline: Anatomic pathology, **Topic:** General pathology, **Species:** Equine, **Systems:** Integument

57: CHARACTERIZATION OF PROLIFERATIVE LESIONS IN THE OVIDUCT OF THE LAYING HEN AS A MODEL FOR OVARIAN CANCER PRECURSORS

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Background: Serous tubal intraepithelial carcinomas (STICs) are fallopian tube-arising precursors to ovarian high grade serous carcinoma (HGSC), an important cause of cancer-related death in women. There is no established in vivo model to study STIC development and progression to HGSC, due to anatomic and physiologic differences between most mammalian species and humans. Laying hens spontaneously develop both adenocarcinomas and adenomas affecting the ovary, oviduct, or both, with advanced disease showing clinical similarities to human HGSC. We propose oviductal adenomas and hyperplasia in the hen as precursors to reproductive tract adenocarcinoma, analogous to STICs and HGSC, and we present foundational characterization of these lesions here.

Objective: Characterize the prevalence, localization, and immunohistochemical properties of oviduct hyperplasia, adenomas, and adenocarcinomas in a cohort of laying hens, and compare to human lesions.

Methods: Oviducts were examined histologically from 22 3-year-old laying single comb White Leghorn hens, and proliferative lesions (focal hyperplasia, adenomas, adenocarcinoma) were identified and stained for tumor-associated glycoprotein 72 (TAG-72). Human STICs and HGSC were stained for comparison.

Results: Proliferative lesions were predominantly found in the magnum and variably positive for TAG-72 (patchy surface and/or cytoplasmic labeling or none). Human STICs exhibited surface staining for TAG-72, while HGSC showed patchy surface and cytoplasmic labeling.

Conclusions: These findings provide baseline knowledge of localization of proliferative lesions in the laying hen oviduct. Immunohistochemical similarities to human STIC and HGSC support hens as a feasible model for study of early phases of HGSC development. Future directions include genetic analysis to determine clonality of lesions.

Discipline: Anatomic pathology, **Topic:** Comparative pathology, **Species:** Avian, **Systems:** Reproductive

58: EQUINE HERPESVIRUS 1 IMMEDIATE-EARLY PROMOTER-ASSOCIATED LONG NON-CODING RNA IS COEXPRESSED WITH ITS IE GENE, IN VIVO

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Background. Although herpesviruses encode numerous non-coding RNAs (ncRNAs), few have been identified in equine herpesvirus 1 (EHV-1) infections. Recently, our group demonstrated that a 998-nucleotide ncRNA antisense to the EHV-1 immediate-early (IE) gene promoter, named *IE pancRNA*, is expressed in a rat neuronal cell line, and functions as transactivator of the IE gene.

Objective. This study further demonstrates the *in vivo* expression of IE pancRNA, providing novel insights into its potential role during natural EHV-1 infection.

Methods. Rat neuronal cell line (RN33b-A68B2M) and BALB/c mice were infected with EHV-1 Ab4 strain *in vitro* and *in vivo*, respectively. Archived paraffin blocks from PCR-confirmed equine abortion cases were also examined.

Results. Histological analysis of tissues from EHV-1-infected mice and naturally-aborted equine fetuses revealed distinct intranuclear inclusion bodies and lymphocytic infiltration. RT-qPCR detected both IE pancRNA and IE mRNA in the lungs, brains, and trigeminal nerves of infected mice. RNA fluorescence *in situ* hybridization (RNA-FISH) demonstrated co-expression of both transcripts in EHV-1-infected RN33B-A68B2M cells, infected mice, and aborted equine fetuses. Notably, characteristic spherical structures formed by IE pancRNA were consistently observed within the nuclei in both *in vitro* and *in vivo* settings. Furthermore, combined RNA-FISH and immunofluorescence showed no distinct colocalization between IE pancRNA and chromatin modification domains.

Conclusions. These findings demonstrate that IE pancRNA and IE mRNA are co-expressed both *in vitro* and *in vivo* during active phase of EHV-1 infection. This supports the hypothesis that IE pancRNA may contribute to the promotion or maintenance of IE gene expression during productive infection.

Discipline: Anatomic pathology, **Topic:** Infectious disease, **Species:** Equine, **Systems:** Systemic

59: CROSS-MODEL AGILITY FOR DEEP LEARNING DETECTION OF HEPATOCELLULAR NECROSIS ACROSS VIRAL HEMORRHAGIC FEVERS

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Introduction: Viral hemorrhagic fevers caused by Ebola virus (EBOV) and Lassa virus (LASV) share pathological features, including multifocal liver necrosis with minimal local inflammatory responses. We investigated whether a U-Net model trained exclusively on EBOV-associated necrosis could detect LASV-associated necrosis, testing model generalizability across distinct viral diseases. Materials and **Methods:** A U-Net model trained to recognize necrosis exclusively on archived histopathology slides from livers of lethally EBOV-exposed non-human primates (NHPs) was used to segment hepatocellular necrosis on slides (n=12) from LASV-exposed animals. U-Net-generated predictions were compared to pathologist-generated ground truth masks; segmentation performance was evaluated for accuracy, sensitivity (Sn), specificity (Sp), positive predictive value (PPV), and

negative predictive value (NPV). **Results:** Model performance was precise and reliable in distinguishing LASV-associated necrosis, with 98% accuracy, 97% Sp, 90% PPV, and 96% NPV. Slightly lower Sn (65%) for LASV-associated necrosis was comparable to model Sn for EBOV-associated necrosis. **Conclusion:** Although trained only on livers from an NHP model of lethal EBOV infection, a U-Net model retained accuracy in segmentation of hepatocellular necrosis caused by a different virus. This study demonstrates the potential for deep learning models to identify pathologic features across viral infection models and explore common threads of pathogenesis. Funding: This research was supported by the Intramural Research Program of the National Institutes of Health (NIH) and with federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. 75N91019D00024.

Discipline: Anatomic pathology, **Topic:** Comparative pathology, **Species:** NHP, **Systems:** Hepatobiliary

60: HYDROXYSTEROID 17-BETA DEHYDROGENASE 7 IS REQUIRED FOR PROPER NEURAL DEVELOPMENT

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Background: Cholesterol biosynthesis disorders are a heterogenous suite of genetic diseases with potential life-threatening consequences for early development and a range of craniofacial and CNS clinical features. Cholesterol supplementation has limited effect on clinical progression; cholesterol precursor buildup secondary to cholesterol biosynthesis disruption may contribute to disease. *Hydroxysteroid 17-Beta Dehydrogenase 7* (*Hsd17b7*) encodes for a cholesterol biosynthesis enzyme. **Objectives:** Investigate consequences of *Hsd17b7* deletion on the CNS. **Methods:** Cre-Lox system is leveraged to delete *Hsd17b7* in *Wnt1* and *Emx1* cell lineages. Long-read DNA whole-genome sequencing confirmed integration of LoxP sites around *Hsd17b7* Exon 4. *Wnt1cre* embryonic timepoint (E)18.5 embryos were collected for skeletal preparation, imaged, and measured. Double-fluorescent cre reporter mT/mG was crossed into the *Wnt1* model and embryos were collected, imaged, and cleared for whole-mount CC3 IHC. Brains from postnatal timepoints (P) and embryos from *Emx1cre* and *Wnt1cre* mice were collected for histological examination. Antibodies against TUJ1 were applied to E14.5 *Emx1cre* cerebrums, TBR2 to E12.5 and E14.5 cerebrums, and CC3, PHH3, and PAX6 to E10.5, E12.5, and E14.5 cerebrums. **Conclusions:** *Wnt1cre/wt;Hsd17b7flox/wt* mice reveal cerebellar dysgenesis linked to changes in the midbrain-hindbrain boundary, hydrocephaly linked to malformation of the subcomissural organ, and craniofacial anomalies. *Emx1cre/wt;Hsd17b7flox/wt* mice have a hypoplastic forebrain. Discussion: Loss of *Hsd17b7* leads to early apoptosis of the *Wnt1* and *Emx1* cell lineage, compromising development of the cerebellum, craniofacial primordia, and cerebrum. Buildup cholesterol precursors are hypothesized to cause early loss of pertinent developmental cell lineages. We show *Hsd17b7* is required for proper CNS development.

Discipline: Anatomic pathology, **Topic:** Genetic/hereditary disorders, **Species:** Rodents, **Systems:** Nervous

61: HISTOPATHOLOGICAL AND IMMUNOLOGICAL CHARACTERIZATION OF STAPHYLOCOCCUS AUREUS-DRIVEN SKIN INFLAMMATION IN A NOVEL MURINE MODEL OF ATOPIC DERMATITIS

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Background: Atopic dermatitis (AD) is a chronic inflammatory skin disorder affecting 15%–20% of individuals in developed countries. More than 90% of patients with AD are colonized with *Staphylococcus aureus* on lesional skin. Although several invasive mouse models have been used to study *S. aureus*-induced skin inflammation, these models do not recapitulate the natural process of bacterial colonization. Therefore, we developed a novel topical colonization model that mimics natural *S. aureus* skin colonization to determine how *S. aureus* exacerbates skin inflammation.

Methods: Mouse ears were topically inoculated with the *S. aureus* USA300 strain once daily for 10 consecutive days, followed by histopathological and immunological analyses

Results: Histopathological examination of ear tissues from wild-type (WT) mice inoculated with *S. aureus* demonstrated marked epidermal hyperplasia with orthokeratotic hyperkeratosis and dense inflammatory cell infiltration. Flow cytometric analysis revealed increased numbers of IL-17A- and IFN-g-producing T lymphocytes following inoculation. In contrast, *Rag1*$^{-/-}$ mice, which lack mature T and B lymphocytes, exhibited only mild epidermal hyperplasia and minimal inflammatory cell infiltration. Although mice deficient in either IL-17A/F or IFN-g alone developed skin inflammation comparable to WT controls, combined deficiency of IL-17A/F and IFN-g markedly reduced ear swelling, indicating cooperative roles for these cytokines in driving skin inflammation.

Conclusion: These findings demonstrate that topical *S. aureus* colonization induces AD-like chronic skin pathology and identify T cell-derived IL-17A/F and IFN-γ as cooperative mediators of disease. This model provides a physiologically relevant platform for investigating host-pathogen interactions and therapeutic strategies for AD.

Discipline: Anatomic pathology, **Topic:** Immunology, **Species:** Rodents, **Systems:** Integument

62: DISTINGUISHING NORMAL AND ABNORMAL TISSUES IN NONCLINICAL TOXICITY STUDIES USING UNSUPERVISED FOUNDATION MODELS

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Nonclinical toxicity studies require labor-intensive review of large histopathology slide sets. Most pathology AI tools use supervised learning, requiring extensive

annotation and often focusing on predefined lesions, which limits scalability and generalizability. We aimed to validate an unsupervised out-of-distribution abnormality-triage foundation model for liver, kidney, and spleen whole slide images (WSI) from historical Sprague-Dawley rat toxicity studies. AIRA Matrix foundation models analyzed ≥100 metadata-free WSI per tissue spanning a broad range of findings and generated abnormality scores, binary normal/abnormal predictions, and localization heatmaps. Performance was compared against the originally reported diagnosis as ground truth and further assessed in a retrospective, semi-blinded review by three board-certified veterinary pathologists, who rated slide-level abnormality predictions as accept/reject and heatmap quality as good/fair/poor. Across tissues, slide-level triage was strong and broadly consistent, improving with lesion severity, and reaching near-complete acceptance for moderate-to-severe findings. Against ground truth, sensitivity/specificity were 89%/20% in liver, 88%/30% in kidney, and 91%/73% in spleen. In retrospective semi-blinded review, pathologists accepted 87% of liver, 85% of kidney, and 88% of spleen slide-level predictions. Heatmaps generally supported abnormality predictions but were less robust, with good-to-fair abnormality localization in 73%, 81%, and 58% WSI for liver, kidney, and spleen, respectively. Performance was best in moderate-to-severe lesions and showing greater variability in subtle findings and within-normal-limits slides. The models showed promising utility for triaging rat liver, kidney, and spleen slides and may help streamline nonclinical slide review. The findings also highlighted key training-data requirements, with early evidence of improved overall performance following quality-control optimization.

Discipline: Anatomic pathology, **Topic:** Methods/technology, **Species:** Rodents, **Systems:** Systemic

63: DEVELOPMENT OF A HISTOLOGIC SCORING SYSTEM ASSESSING CYCLOPHOSPHAMIDE-INDUCED INTESTINAL INJURY IN C57BL/6J MICE

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Background and **Objective:** While many enteric pathogens exploit intestinal barrier disruption during infection, the plasticity of the intestinal epithelium is often owed to its ability to reconstitute damaged cells as a result of a robust stem cell population. In contrast, damage to the intestinal epithelium is a frequent side-effect of chemotherapeutics targeting mitotically active cells, resulting in gastrointestinal distress and secondary bacterial infections chemotherapy patients. However, the specific mechanisms, beyond intestinal epithelial cell loss, regulating increased risk of secondary bacterial infections is not entirely clear. Given the critical importance of the intestinal epithelium and gut microbiota in regulating intestinal barrier function, we sought to histopathologically characterize the effects of antimicrobials and chemotherapeutics on the intestinal tract in mice.

Methods: Ceca from a mouse model of gastrointestinal barrier loss using streptomycin with or without cyclophosphamide, as well as a PBS control were submitted for histopathology and stained with H&E or alcian blue. A histopathologic scoring system was developed that assessed goblet cell numbers, morphology, and distribution, quality of the brush border, inflammation, mucosal edema, individual cell

death, and mitoses.

Results: Cyclophosphamide treatment resulted in significantly increased epithelial cell death at the mucosal surface in an antibiotic-independent manner. Moreover, while antibiotics slightly increased the number of goblet cells, antibiotics along with cyclophosphamide treatment resulted in significantly elevated goblet cells.

Conclusions: Cyclophosphamide administration results in significant increases in cecal goblet cell numbers and individual epithelial cell death. However, further studies are needed to ascertain if these changes are relevant to increased bacterial translocation.

Discipline: Anatomic pathology, **Topic:** Toxicology, **Species:** Rodents, **Systems:** Gastrointestinal

64: LOCAL AND SYSTEMIC IMMUNE RESPONSES TO CONTROLLED WOOD-SMOKE EXPOSURE IN PRE-WEANED DAIRY CALVES

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Fine particulate matter ($PM_{2.5}$) in wildfire smoke is associated with respiratory disease and systemic inflammation in calves, but the direct contribution of $PM_{2.5}$ remains unclear. We examined pulmonary and systemic immune responses to acute wood smoke exposure in dairy calves under controlled environmental conditions. Male Holstein calves (37 ± 2 d old) were randomly assigned to control (CON, not exposed; n=12) or wood smoke (WS; n=12) and individually exposed in enclosures for 6 hours. Four replicates of 6 calves each were used. Mean (±SD) $PM_{2.5}$ concentration was 238.44 ± 62.97 μg/m^3 in WS enclosures and 9.94 ± 4.50 μg/m^3 in CON enclosures. Blood was collected for hematology, granulocyte phagocytic activity, and acute phase protein concentrations, including plasma serum amyloid A, haptoglobin, and albumin. Post-mortem bronchoalveolar lavage fluid (BALF) was collected for leukocyte immunophenotyping. Trachea samples were collected, fixed, and stained for histology. Data were analyzed using mixed models with treatment as a fixed effect and replicate as a random effect. Results showed that WS calves had lower blood neutrophil counts than CON (P=0.02), without detectable differences in total leukocyte count. Phagocytosing granulocytes (%) were greater in WS (P=0.01). In BALF, granulocyte, CD68$^+$ macrophage, and natural killer cell proportions were greater in WS compared with CON (P≤0.05), whereas CD68$^+$CD163$^+$ macrophages did not differ. Tracheal mucosal neutrophil and eosinophil counts were greater in WS (P<0.04). Acute phase proteins did not differ. Wood smoke $PM_{2.5}$ induced localized pulmonary leukocyte responses without a systemic acute phase response.

Discipline: Both, **Topic:** Toxicology, **Species:** Bovine, **Systems:** Respiratory

65: HISTOPATHOLOGIC CHARACTERIZATION AND PERSISTENCE OF AN INTRA-ARTICULAR HYDROGEL IN RHESUS MACAQUES WITH NATURALLY OCCURRING OSTEOARTHRITIS

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Background:
Osteoarthritis (OA) is common in aging rhesus macaques and closely parallels naturally occurring human disease. Although intra-articular collagen-elastin hydrogel administration has been evaluated in other species, minimal information is known about its persistence and local histologic effects in rhesus macaques. To our knowledge, this is the first histologic evaluation of this material in this species.

Objective:
The objectives of this study were to characterize hydrogel localization, persistence, and local tissue responses following intra-articular administration.

Methods:
Four rhesus macaques with radiographically confirmed OA received one or more intra-articular injections prior to clinically scheduled necropsy. Animals were evaluated at necropsy 11, 28, 54, or 68 days post-injection. Synovial tissues from injected and contralateral non-injected joints (when available) were collected.

Results:
Gross examination revealed significant periarticular remodeling, osteophyte formation, and synovial fibrosis in affected joints, corresponding to radiographic severity. Histologic evaluation identified OA-associated lesions including synovial fibrosis, cartilage degeneration, and remodeling of periarticular tissues. Hematoxylin and eosin, Masson's trichrome, and periodic acid-Schiff stains demonstrated incorporation of the hydrogel into the synovial membrane by day 11 post-injection. Material remained identifiable through day 28 and appeared reduced in amount by day 54. Minimal inflammatory cellular infiltrates were observed in association with the material. Additional histochemical and immunohistochemical characterization is ongoing.

Conclusions:
These findings suggest that the hydrogel becomes incorporated into synovial tissues, persists for several weeks following administration, and is associated with minimal local inflammatory responses, supporting continued investigation as an intra-articular therapeutic for OA.

Discipline: Anatomic pathology, **Topic:** Comparative pathology, **Species:** NHP, **Systems:** Bone and joints

66: IMPACT OF TWO DIFFERENT BLOOD COLLECTION SITES AND METHODS ON CLINICAL PATHOLOGY PARAMETERS IN SPRAGUE DAWLEY RATS

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Background: Published data are lacking on the potential effects of current commonly used blood collection methods on clinical pathology parameters in rats, which could affect the design and interpretation of non-clinical safety studies.

Objective: To determine the impact of blood collection site and method in Sprague Dawley rats (alert collection via jugular vein vs. anesthetized terminal collection via abdominal aorta) on hematology, coagulation and clinical chemistry parameters. Additionally, the potential impact of sample volume and tube size was determined.

Methods: Samples for hematology (Advia 2120), coagulation (STA Compact MAX) and clinical chemistry (Cobas 6000) evaluation were collected and analyzed from two groups (16/sex/group) of 12-week-old Sprague Dawley rats via either alert venipuncture from the jugular vein or anesthetized (isoflurane) terminal collection from the abdominal aorta. To determine the potential impact of sample volume and tube size, samples of two different volumes were collected from the terminal collection group for hematology, coagulation, and clinical chemistry.

Results: Differences in clinical pathology parameters between collection methods that were of sufficient magnitude to affect data interpretation were observed in red blood cell mass, leukocytes, prothrombin time, fibrinogen, glucose, proteins, minerals and electrolytes. Differences in clinical pathology parameters between different sample volumes and tube sizes were generally small in magnitude and unlikely to affect data interpretation.

Conclusions: Collection method impacts numerous clinical pathology parameters in Sprague Dawley rats and should be considered when designing and interpreting data from non-clinical safety studies, especially in cases where multiple blood collection methods may be used.

Discipline: Clinical pathology, **Topic:** Toxicology, **Species:** Rodents, **Systems:** Systemic