



## 2025 Veterinary Student Platform and Poster Presentations

### Veterinary Student Platform Presentations

#### **A GENOMIC AND TRANSCRIPTOMIC ANALYSIS OF CANINE SMALL CELL B CELL LYMPHOMA**

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Canine small cell B cell lymphoma (BCL) is a heterogeneous and poorly characterized disease. These tumors account for ~12% of B cell tumors diagnosed by the CSU Clinical Hematopathology Lab, but little is known about their pathogenesis and their relevance as a human disease model. This project aims to characterize canine small BCL to improve diagnostics and treatments for dogs, while also evaluating its potential as a preclinical translational model for human BCL. We performed whole exome sequencing (n=125) and bulk RNA sequencing (n=121) on lymph node aspirates from dogs with small BCL, diagnosed by flow cytometry, to identify mutations and pathways involved in disease pathogenesis.

The majority of our samples fell into two k-means defined clusters by transcriptomic analysis, while a smaller subset of samples (n=12) clustered with canine large BCL samples. These two prominent clusters of small BCL (n=70 and 39), show different mutational profiles, clinical features, predominant breeds, and pathway enrichment patterns. Cluster 1 (n=70) is characterized by upregulation of NF- $\kappa$ B signaling, inflammatory responses, and B cell receptor signaling. Cluster 3 (n=39) is characterized by PI3K-AKT-mTOR signaling, higher rates of lymphocytosis, and high Ki67 positivity. Our data suggest that on a molecular level, there are at least two subsets of canine small BCL, potentially with different cells of origin and driven by different oncogenic signaling mechanisms. These data support the need for further classification of these different tumor subtypes given their potential differences in outcome, treatment response, and relevance as a pre-clinical translational model for human BCL.

#### **HOST ORIGIN INFLUENCES CORYNEBACTERIUM BOVIS CLINICAL PRESENTATION AND DETECTION IN ATHYMIC NUDE MICE**

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### **Background:**

In February 2021, the UC Davis sentinel mouse colony experienced an atypical outbreak of *Corynebacterium bovis* (*C. bovis*). Infected nude mice lacked skin lesions and evaded detection by PCR and culture for months, leading to unintended dissemination of *C. bovis*-positive mice to multiple vivaria.

### **Objectives:**

This study aimed to characterize the pathogenesis of this apathogenic, slow-growing *C. bovis* outbreak strain and identify genetic factors contributing to its atypical phenotype.

### **Methods:**

Athymic nude sentinel records from the outbreak period were reviewed for necropsy, histopathology, culture, and PCR findings. Culture results were used in a Fisher's Exact Test to evaluate whether co-colonization with *C. amycolatum* affected *C. bovis* growth. Whole genome sequencing of *C. bovis* isolates was performed to determine host origin. In situ hybridization (ISH) on tissue blocks was performed to identify bacterial presence and characterize bacterial distribution.

### **Results:**

The outbreak strain did not induce the hyperkeratosis and acanthosis typical of "classic" corynebacterium-associated scaling dermatitis, both grossly and histologically. Significantly fewer bacteria were detected by ISH in outbreak cases compared to classic cases ( $p = 0.0179$ ). Genomic analysis showed the outbreak strain clustered most closely with human-origin *C. bovis*. Co-colonization with *C. amycolatum* showed no significant impact on *C. bovis* growth ( $p = 0.0898$ ).

### **Conclusion:**

These results emphasize the variable pathogenicity of *C. bovis* strains and supports their anthroponozoonotic potential. Further work would be required to reevaluate *C. bovis* diagnostic methods and transmission pathways to protect the health of laboratory rodents and humans working with them.

## **THE ROLE OF NEURONAL DISHEVELED-2 (DVL2) IN PRION-INDUCED SYNAPTOTOXICITY IN MICE**

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### **Background**

The Wnt pathway is crucial for the development and function of synapses. Wnt dysregulation is implicated in synapse loss in neurodegenerative diseases, including Alzheimer's and Parkinson's disease, but remains poorly understood in prion disease. Disheveled-2 (Dvl-2), a key cytoplasmic mediator in the Wnt pathway, bridges membrane receptors Fzd3 and LRP5/6 and downstream signaling proteins essential for synaptic integrity. We have observed prion-induced synaptic hyperactivity and

significant loss of Dvl-2 in the brain during early stages of disease prior to neuroinflammation and synapse loss.

### **Objective**

To investigate the role of neuronal *Dvl2* in driving synapse loss in prion disease.

### **Methods**

*Dvl2<sup>ff</sup>* mice were crossed with *synapsin 1-Cre*(*Syn1-Cre<sup>+/+</sup>*) mice to deplete Dvl-2 from neurons. *Dvl2<sup>ff</sup> Syn1-Cre<sup>+</sup>*(*Cre<sup>+</sup>*) and *Cre<sup>-</sup>* mice were inoculated intracerebrally with mouse-adapted scrapie prions (22L strain), and brains were collected pre-terminally (70% incubation period).

### **Results**

Histopathological analyses revealed similar spongiform degeneration, prion aggregates (PrP<sup>Sc</sup>), and astrogliosis in the cerebral cortex and hippocampus of *Cre<sup>+</sup>* and *Cre<sup>-</sup>* prion-infected mice. Western blotting of the cerebral cortex revealed higher phosphorylation of NMDA and AMPA receptors in the *Cre<sup>-</sup>* mice, suggesting less neuronal hyperactivity in the Dvl-2-depleted mice. The *Cre<sup>-</sup>* mice also show increased p-PKA substrates and CaMKII $\beta$  levels as compared to the *Cre<sup>+</sup>* mice. There was higher SNAP25 observed in the *Cre<sup>-</sup>* mice compared to Dvl-2-depleted mice.

### **Conclusion**

These results suggest *Cre<sup>-</sup>* mice were in a more advanced disease state with increased neuronal hyperactivity. Overall, these findings suggest the lack of *Dvl2* may slow the onset of neuronal hyperactivity.

## **AI-DRIVEN ONCOPATHOLOGY: ENHANCING OBJECTIVITY AND EFFICIENCY IN CANINE ORAL MELANOMA ANALYSIS**

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Digital Slide Imaging (DSI) has revolutionized histopathological analysis, particularly through the integration of Artificial Intelligence technologies (AI). One of the most impactful developments in this field is the use of AI-based tools, such as HALO AI, to improve the objectivity and precision of digital histopathology.

In this study, we developed and tailored AI algorithms to perform objective assessments of malignant features in canine oral melanoma (COM), an aggressive cancer in which clinical behavior remains challenging to predict. Specifically, we focused on key proliferation markers such as mitotic figures (n=44) and Ki-67 expression (n=12), which are traditionally assessed through time consuming methods that are prone to high inter-observer variability. In addition, we explored CD163 (n=13), a marker of immunoinhibitory or M2 macrophages within our COM cohort.

Our AI models were designed to identify and classify nuclei, enabling automated quantification of mitotic figures. Additionally, we integrated HALO's IHC and Microglial modules with our customized nuclear segmentator algorithm to quantify Ki-67 and CD163 expression, respectively.

Mitotic counts obtained via HALO AI showed strong agreement with those recorded by the study pathologist and documented in original pathology reports. A positive correlation was observed between mitotic count and Ki-67 expression, while CD163 expression appeared independent of proliferation markers.

We conclude that AI tools such as HALO AI hold promise as accurate and efficient methods for quantifying mitotic figures and Ki-67 expression. These findings support the potential for AI-assisted image analysis to contribute to more standardized and reproducible assessments of proliferation markers in histopathology.

## **VALIDATION AND TEMPORAL ANALYSIS OF DIFFERENTIALLY EXPRESSED MICRORNAS IN HORSE SERUM FOLLOWING INTRAVENOUS LIPOPOLYSACCHARIDE ADMINISTRATION**

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**Background:** Sepsis remains one of the leading causes of mortality in horses and presents significant diagnostic challenges, particularly in its early stages. MicroRNAs (miRNAs), small non-coding RNA molecules that regulate gene expression, offer a promising avenue for early detection of sepsis before clinical signs become evident. However, limited research exists on the role of miRNAs in equine sepsis. Our preliminary analysis identified several miRNAs that were differentially expressed in horse serum in response to lipopolysaccharide (LPS) challenge through small RNA sequencing.

**Objective:** To validate and investigate temporal changes in previously identified differentially expressed miRNAs in horse serum following LPS infusion.

**Methods:** Eight healthy horses received intravenous LPS to induce systemic inflammation. EDTA plasma samples were collected at baseline and at 1, 6, 12, and 24 hours post-infusion. RNA was extracted using a column-based method, and absolute quantification of selected miRNAs was performed via digital PCR. Expression patterns were analyzed over time.

**Results:** MiR-145 and miR-126-3p showed significant upregulation following LPS infusion, peaking at early time points. Pathway enrichment analysis of miRNA target genes revealed the ErbB signaling pathway as the most enriched, suggesting its involvement in immune modulation during endotoxemia.

**Conclusions:** Circulating miRNAs exhibit dynamic expression changes in response to LPS-induced inflammation and may serve as biomarkers for the early detection of sepsis in horses. Further research is needed to validate these candidates in naturally septic cases. Ultimately, understanding miRNA dynamics in sepsis could contribute to faster and more accurate diagnostics, as well as improved outcomes, for equine patients.

## **INVESTIGATING THE ROLE OF SPRAYED SERUM-DERIVED MATERNAL ANTIBODIES IN POTENTIATING DISEASE IN INFECTIOUS BRONCHITIS VIRUS (IBV) CHALLENGED CHICKS**

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Infectious bronchitis virus (IBV) causes respiratory and urogenital disease in chickens. If layers are infected during the first weeks of life, in absence of maternal antibodies, they can develop “false layer syndrome” (FLS). IBV targets epithelial tissues and induces inflammation with subsequent fibrotic repair. Early inflammation of the developing oviduct leads to cystic formation and atrophy related dysfunction. Early administration of vaccines at day of age protects against field challenges; however, if maternal antibodies are not present at appropriate concentrations, developmental abnormalities may still occur. Maternal antibodies are not consistent within flocks; multiple breeder flocks contribute to egg laying flocks resulting in dissimilar antibody makeup. This study aims to investigate if spray delivered antibodies to day old chicks can provide local protection that prevents early infection, boosting maternal antibodies, and eliminating IBV related FLS. We utilized day-old chicks treated with serum alone or serum containing M41 (IBV) maternal antibodies and challenged with either M41 or CA 1737 IBV strains to assess viral pathogenesis and mechanisms of viral mediation. Necropsies were performed six- and twelve-days post challenge. We assessed gross pathology of tracheas and airsacs, collected tracheas for histomorphometry, cloacal swabs for viral load analysis, and serum for IBV antibody quantification. The results demonstrate that protection was conferred from both serums with and without antibodies in challenges using two IBV genotypes. The protection was non-specific, but significant comparing the non-treated and challenged group. This suggest the mechanism of protection is not specific viral neutralization but local mucosal barrier.

## **TRANSCRIPTOMIC PROFILING IN FELINE INTESTINAL SMALL CELL T CELL LYMPHOMA SUGGESTS A NATURAL KILLER-LIKE T CELL ORIGIN SIMILAR TO HUMAN REFRACTORY CELIAC DISEASE TYPE II**

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Small-cell intestinal T-cell lymphoma (SCL) is the most common cancer in cats and is hypothesized to arise from chronic inflammation (inflammatory bowel disease, IBD), paralleling human refractory celiac disease type II (RCD II). RCD II is an indolent malignancy marked by clonal T-cell expansion and JAK/STAT pathway mutations. In 60–80% of cases, RCD II progresses to enteropathy-associated T-cell lymphoma (EATL), an aggressive disease with a five-year survival of only 3–20% with treatment. We propose that feline SCL is a naturally occurring, translational model for human RCD II. To compare human and feline diseases, we investigated the transcriptome and STAT5B mutation frequency in 71 intestinal biopsies from 50 cats with IBD or SCL, as defined by histology (interpreted by 3 blinded pathologists) and clonality testing. Gene set enrichment analysis showed the top upregulated pathways in feline SCL involved T-

cell activation and differentiation, including enrichment for alpha-beta T-cell and NKT-like/cytotoxic signatures. These findings suggest the tumor cell of origin in SCL is an activated intraepithelial alpha-beta T-cell with NK-like features—similar to that proposed in human RCD II. Eight percent of IBD cases and eighty percent of SCL cases had the activating STAT5BN642H mutation detected via a droplet digital PCR assay. Given these transcriptomic, genetic, and pathological parallels, cats offer a valuable preclinical model to study the pathogenesis and treatment of intestinal intraepithelial T-cell lymphomas and human RCD II.

### **CD3+/CD20+ RETINAL LYMPHOMA AND UVEODERMATOLOGIC SYNDROME IN A DOG**

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A 12-year-old, male, neutered, mixed breed dog was presented to a veterinary ophthalmologist for a one-month history of left-sided ocular discharge. On ophthalmic examination, both eyes were diagnosed with panuveitis, with the right eye more mildly affected. The patient also had a history of depigmentation and ulceration of the nasal planum and perinasal skin. The entire left eye and a punch biopsy of perinasal skin were submitted for histopathology. Histologic examination of the eye revealed sheets of neoplastic round cells multifocally infiltrating the retina, within the subretinal space, and multifocally subtending the retinal pigment epithelium, with suspected vascular invasion. The neoplasm was morphologically consistent with intermediate-cell lymphoma. Immunohistochemistry showed strong positive reactivity to CD3 and CD20, and PCR for antigen receptor rearrangements was suggestive of a clonal T-cell receptor gene, supporting a T-cell phenotype with aberrant expression of CD20. Additional findings in the eye and perinasal skin included melanophage-rich histiocytic and lymphoplasmacytic panuveitis and lichenoid dermatitis, suggestive of concurrent uveodermatologic syndrome. Lymphoma is one of the most common ocular neoplasms in dogs, but predominant retinal involvement is rare (~1% of canine ocular lymphomas). Furthermore, retinal lymphomas in dogs typically fall under the subtype of diffuse large B-cell lymphoma. T-cell lymphomas with aberrant expression of B-cell antigens have been reported in dogs, including a case of retinal lymphoma.

### **LONG-LATENCY DOXORUBICIN TOXICITY IN A RAT MODEL: NON-CARDIAC LESIONS TO CONSIDER**

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**Background:** Doxorubicin (DOX) is a potent cancer chemotherapy; however associated toxicities limit clinical application. Rats are a good model for DOX toxicity studies, as similar tissues are damaged in humans given DOX treatment. Historically, in

animal models, high dose, short interval models have been used, with only cardiotoxicity studied.

**Objectives:** This study aims to provide a comprehensive assessment of non-cardiac lesions induced by DOX treatment in a long-latency rat model, which translate better for human medicine.

**Methods:** Sprague-Dawley rats 6-7 weeks old with a cumulative 6 mg/kg DOX dose administered IV with 2-week intervals were necropsied after death or after euthanasia (when fractional shortening reached  $\leq 30\%$ ). Blood was collected before all euthanasia. Kidneys, mammary masses, intestines, and testes were assessed for histopathologic lesions.

**Results:** Renal disease with a variety of histologic lesions was found in all rats given DOX. BUN and albumin were measured in a group of rats, and those values reflected the severity of the renal disease. Across three treatment cohorts, the mammary mass incidence in the DOX group was 62%, while that in the control was 11%. DOX-induced mammary tumors encompassed fibroadenomas, adenocarcinomas, and sarcomas, while the control group had only 1 fibroadenoma. Finally, enteritis, colitis, and testicular cell death were found in males.

**Conclusions:** Our study describes the range of non-cardiac toxicities from low-dose DOX treatment with a long-latency cardiomyopathy. Understanding the range of lesions possible with this DOX model allows for better evaluation of treatment success, translation to human medicine, and collaboration among researchers.

## **IMPACT OF BLOOD SAMPLING SITE ON HEMATOLOGIC PARAMETERS IN ASIAN COMMON TOADS WITH IMPLICATION FOR FIELD RESEARCH**

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Amphibians are one of the most threatened groups due to climate change and habitat loss, and veterinary medicine plays a key role in their conservation. This study was performed in collaboration between Iowa State University College of Veterinary Medicine and Omaha's Henry Doorly Zoo & Aquarium, an institution which contributes significantly to the captive breeding and release of several endangered amphibian species. The objective was to compare the accuracy and reliability of cardiac versus peripheral blood samples, to provide guidance regarding the most appropriate and least invasive sample technique for researchers in the field. Blood was obtained from 55 Asian Common Toads via cardiocentesis and the venipuncture of the medial saphenous vein to evaluate hematologic parameters. Packed cell volume, leukocyte estimate, and thrombocyte estimate were significantly increased in the medial saphenous samples when compared to cardiocentesis samples. All other parameters analyzed were not significantly different between the collection methods. Necropsy identifies a larger proportion of toads suffered from cardiac trauma related to cardiocentesis, indicating peripheral sampling as the safer and less invasive method for animals that would be released back into the field after collection. Based on these findings, the medial

saphenous vein appears to be the superior and recommended blood sampling site for improved hematologic evaluation and decreased patient morbidity.

## Veterinary Student Poster Presentations

### 1: SPLENDORE-HOEPLI PHENOMENON IN A 4-YEAR-OLD DOG WITH ACTINOMYCOSIS SECONDARY TO A MIGRATING FOREIGN BODY

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**Background:** A 4-year-old castrated male Labrador Retriever presented to the UIUC emergency service with a history of lethargy and elevated respiratory rate. Diagnostics revealed pleural effusion, a caudal thoracic soft tissue mass, and marked suppurative inflammation within the abdominal cavity. Based upon suspicion for septic infection of the thoracic and abdominal cavities, likely due to foreign material migration, surgery was performed. Samples of the mediastinum, omentum, spleen and lung were submitted for histopathologic examination.

**Results:** On histologic examination, there was chronic and fibrosing pyogranulomatous omentitis, mediastinitis, steatitis and pleuropneumonia with intralesional swarming bacterial colonies. Inflammatory infiltrates were centered around large basophilic clouds of bacteria that were occasionally encircled by a club-shaped eosinophilic rim consistent with the Splendore-Hoepli phenomenon. These findings were consistent with a severe disseminated bacterial infection. Bacterial culture revealed heavy growth of *Neisseria* and *Actinomyces hordevulneris*.

**Conclusion:** This case shows the clinical and histological lesions of a severe disseminated bacterial infection with the formation of Splendore-Hoepli material secondary to a suspected migrating foreign body. *Actinomyces* is a normal inhabitant of the mucous membranes in canines but can cause an opportunistic infection when inoculated into tissues after disruption of mucosal barriers by the migration of foreign material or penetrating wounds. Actinomycosis is common in young adult to-middle aged dogs, especially hunting and retriever breeds with outdoor access, and is often related to penetrating plant materials like grass awns. These plant awns become contaminated with *Actinomyces* after ingestion or inhalation and can then migrate to various sites throughout the body.

### 2: FATAL NECROTIZING FASCIITIS IN A DOG CAUSED BY ESCHERICHIA COLI

Gabrielle Acquaviva<sup>1</sup>, Fabio Del Piero, DVM, PhD, DACVP<sup>1</sup>, Ingeborg Langohr, DVM, MS, PhD, DACVP<sup>1,2</sup>, William Holl, DVM, Resident, Anatomic Pathology<sup>1</sup>

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**Background:**

Necrotizing fasciitis is a life-threatening bacterial infection where a disruption of the skin barrier, such as from trauma, surgical wounds, or injections, facilitates the invasion of cutaneous microflora into deeper skin layers. This process results in inflammation and necrosis of the hypodermis, fascia, and, in some cases, muscle and bone. Gram-positive bacteria, particularly *Streptococcus* species, are the typical primary pathogen, while gram-negative organisms are more often secondary invaders in mixed infections.

Death commonly occurs within hours to days from septic shock, disseminated intravascular coagulation or multiorgan failure.

**Case Description:**

A 3-year-old, female, spayed Golden Retriever dog was presented for acute onset of diarrhea and azotemia. Subcutaneous fluid therapy was initiated but the patient continued to decline and was found dead four days later. Postmortem examination revealed purple discoloration of the skin over the left forelimb and left lateral ventrum, with the hypodermis expanded by dark red, gelatinous fluid. Histopathology revealed marked neutrophilic infiltration and degeneration, edema, and hemorrhage involving the dermis, subcutis, and deep fascia, with numerous intra- and extracellular short, plump, gram-negative bacilli. Aerobic culture yielded pure, heavy growth of *Escherichia coli*, which tested negative for multiple virulence genes, including LT, STa, Stx1, Stx2a, CNF1, CNF2, *eaeA*, and F4/K88. The cause of death was septic shock, supported by multiorgan congestion and intravascular bacteria consistent with the cultured isolate.

**Conclusion:**

This case documents a rare presentation of necrotizing fasciitis in a canine patient, with *Escherichia coli* as the sole etiologic agent.

### **3: CENTRAL NERVOUS SYSTEM INFLAMMATION IN CATS – A RETROSPECTIVE STUDY**

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**Objective:** Characterize cases of inflammation in the central nervous system (CNS) in domestic cats submitted to the Animal Health Diagnostic Center (AHDC) from 2000 to 2024.

**Method:** The AHDC database was searched for specific terms, cases were reviewed, and data (signalment, diagnosis, histologic findings, cause, ancillary testing) recorded. When necessary, additional ancillary tests were employed.

**Results:** A total of 115 cases of CNS inflammation were identified. Age ranged from 1-month-old to 15-years-old, with 1-year-old cats most affected (16/115; 13.9%). Male (62/115; 53.9%) and Domestic Shorthair cats (71/115; 61.7%) were overrepresented. Etiology was dominated by viral causes (47/115; 40.9%), followed by protozoal (22/115; 19.1%), bacterial (20/115; 16.5%), fungal (6/115; 5.2%), parasitic (3/115; 2.6%), and penetrating trauma (1/115; 0.9%), with 21/115 (19.1%) cases having open etiology. The confirmed causative agents were feline infectious peritonitis virus (FIP) (42/115; 36.5%), *Toxoplasma gondii* (22/115; 19.1%), mixed bacteria (7/115; 6.1%), *Cryptococcus spp.* (3/115; 2.6%), *Cuterebra* (3/115; 2.6%), mixed fungi (3/115; 2.6%), intervertebral disc disease (1/115; 0.9%), feline panleukopenia virus (1/115; 0.9%), and rabies virus (1/115; 0.9%); 35/115 cases (30.4%) remained open. Encephalitis (49/115, 42.6%) and meningoencephalitis (41/115; 35.7%) were the most common diagnoses, with multifocal inflammatory pattern (65/115; 56.5%) most often reported. The inflammation was dominated by lymphoplasmacytic (33/115; 28.7%), followed by plasma cell-rich necrosuppurative (19/115; 16.5%).

**Conclusions:** CNS inflammation in cats is often associated with an infectious agent.

Viral infections (FIP) and protozoal organisms (*Toxoplasma gondii*) were the most common causes with a relatively high number of open diagnoses, supporting the need for enhanced testing.

#### **4: DIGITAL PATHOLOGY OF THE FEMORAL HEAD: USING A NOVEL RGB-TRICHROME STAIN FOR TISSUE QUANTIFICATION USING HALO®**

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**Background:** Accurate identification and quantification of hard tissues is essential for understanding tissue remodeling and disease progression, both in animal models of musculoskeletal disorders and in diagnostic orthopedic cases. There is no reliable method to accurately quantify mineralized versus unmineralized bone (osteoid vs. mature bone) in decalcified bone samples. A modified alcian-blue/picrosirius red stain (RGB-Trichrome) shows promise in addressing this need; however, its utility in non-human tissues has not yet been determined.

**Objective:** The objective of this study was to utilize HALO® Image Analysis Software to 1) compare the efficacy of a novel RGB-Trichrome stain vs. alcian blue-picrosirius red (ABPR) in quantifying mature bone, osteoid, cartilage, and marrow, and 2) to determine how RGB-Trichrome compares to Gomori's Trichrome staining in the quantification of fibrosis within the porcine femoral head.

**Methods:** This study retrospectively analyzed 9 partially ischemic decalcified femoral heads from a piglet model of Legg-Calvé-Perthes disease, using four stains: RGB-Trichrome, ABPR, Safranin-O and Gomori's Trichrome. Slides were then quantitatively assessed for total area and percent composition based on tissue type, with differentiation of marrow, mature bone, cartilage, osteoid, and fibrosis using HALO®.

**Results:** RGB-Trichrome was able to differentiate osteoid from mature bone within the epiphysis and performed similarly in determining the area of cartilage and marrow as compared to Safranin-O and ABPR. Gomori's Trichrome was more sensitive in identifying areas of fibrosis.

**Conclusion:** This study demonstrates the utility of RGB-Trichrome staining in the analysis of tissue composition of decalcified hard tissue samples, with potential for quantification of osteoid.

#### **5: THE ROLE OF NEURONAL DISHEVELED-2 (DVL2) IN PRION-INDUCED SYNAPTOTOXICITY IN MICE**

Caitlyn Anderson<sup>1</sup>, Mahsa Pourhamzeh<sup>2</sup>, Amanda Wilpitz<sup>2</sup>, Josephina Ronson<sup>2</sup>, Travis Murphy<sup>2</sup>, Christina Sigurdson<sup>1,2</sup>

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## Background

The Wnt pathway is crucial for the development and function of synapses. Wnt dysregulation is implicated in synapse loss in neurodegenerative diseases, including Alzheimer's and Parkinson's disease, but remains poorly understood in prion disease. Disheveled-2 (Dvl-2), a key cytoplasmic mediator in the Wnt pathway, bridges membrane receptors Fzd3 and LRP5/6 and downstream signaling proteins essential for synaptic integrity. We have observed prion-induced synaptic hyperactivity and significant loss of Dvl-2 in the brain during early stages of disease prior to neuroinflammation and synapse loss.

## Objective

To investigate the role of neuronal *Dvl2* in driving synapse loss in prion disease.

## Methods

*Dvl2<sup>ff</sup>* mice were crossed with *synapsin 1-Cre* (*Syn1-Cre<sup>+/+</sup>*) mice to deplete Dvl-2 from neurons. *Dvl2<sup>ff</sup> Syn1-Cre<sup>+</sup>* (*Cre<sup>+</sup>*) and *Cre<sup>-</sup>* mice were inoculated intracerebrally with mouse-adapted scrapie prions (22L strain), and brains were collected pre-terminally (70% incubation period).

## Results

Histopathological analyses revealed similar spongiform degeneration, prion aggregates (PrP<sup>Sc</sup>), and astrogliosis in the cerebral cortex and hippocampus of *Cre<sup>+</sup>* and *Cre<sup>-</sup>* prion-infected mice. Western blotting of the cerebral cortex revealed higher phosphorylation of NMDA and AMPA receptors in the *Cre<sup>-</sup>* mice, suggesting less neuronal hyperactivity in the Dvl-2-depleted mice. The *Cre<sup>-</sup>* mice also show increased p-PKA substrates and CaMKII $\beta$  levels as compared to the *Cre<sup>+</sup>* mice. There was higher SNAP25 observed in the *Cre<sup>-</sup>* mice compared to Dvl-2-depleted mice.

## Conclusion

These results suggest *Cre<sup>-</sup>* mice were in a more advanced disease state with increased neuronal hyperactivity. Overall, these findings suggest the lack of *Dvl2* may slow the onset of neuronal hyperactivity.

## 6: CUTANEOUS EPITHELIOTROPIC LYMPHOMA IN A LARGEMOUTH BASS

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### Signalment and history:

An adult largemouth bass (*Micropterus nigricans*) from a zoo was submitted for necropsy to the Louisiana Animal Disease Diagnostic Laboratory after being found dead on 4/3/2025. The fish had a history of depigmentation, waxing and waning appetite, and loss of body condition since January 2025.

### Postmortem findings:

Gross examination revealed multifocal flat areas of cutaneous depigmentation with

erythema. The gills were diffusely pale tan. The coelomic fat and spleen had numerous tan soft nodules measuring up to 0.4 cm in diameter and later identified as granulomas. Histologically, the epidermis and dermis were infiltrated by monomorphic small lymphocytes forming dense sheets and intraepidermal packets (Pautrier's microabscesses). Similar lymphocytes expanded the gill epithelium, lamellar troughs, gill arch, and gill filaments. PTAH stain confirmed segmental expansion of glomerular capillaries by fibrin thrombi.

#### **Conclusions:**

The histologic lesions in the skin and gills are characteristic for cutaneous epitheliotropic lymphoma. The suspected cause of mortality is sepsis with glomerular fibrin thromboses secondary to skin ulceration predisposing to bacterial infection despite having identified no infectious agents with special stains.

#### **Significance:**

Cutaneous epitheliotropic lymphoma is a rare neoplastic condition in fish with few confirmed etiologies. Anecdotal evidence of hematopoietic tumors in fish suggests commonality of chemical or retroviral origins. Chemical exposure is unlikely in this case, and further investigation into viral origin could be performed with transmission electron microscopy or viral culture. Spontaneous lymphoma remains a possibility in this case.

### **7: IMMUNOHISTOCHEMICAL CHARACTERIZATION OF SALIVARY PLEOMORPHIC ADENOMAS IN AGED RHESUS MACAQUES (*MACACA MULATTA*)**

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Pleomorphic adenomas (PA) are the most common salivary neoplasm in humans. We reviewed 30 years of historical necropsy records and found five cases of salivary PAs in rhesus macaques (*Macaca mulatta*). H&E slides were reviewed, and immunohistochemistry (IHC) was performed using CK7, GFAP, SMA, CKpan, KI67, and cytomegalovirus. Tumors were identified in four male and one female, ranging from 24 to 32 years of age. Cases were incidental findings on necropsy, with no clinical signs associated with tumors. The masses were up to 2x1 cm, round to oval, firm, mottled tan to grey with multifocal areas of necrosis, affecting both the parotid and submandibular salivary glands. Histologically, they were encapsulated, densely cellular and composed of tubules and acini (epithelial component) admixed with nests of polygonal cells (myoepithelial component) supported by variable hyalinized vascular stroma. Anisocytosis and anisokaryosis were moderate. Mitotic figures ranged from 1-3 in 2.37 mm<sup>2</sup>. There was no evidence of metastasis or vascular invasion in any of the cases. Pancytokeratin expression was diffuse throughout the tumor, while CK7 expression varied from sparse to patchy. SMA expression was weak within the myoepithelial component. Ki-67 expression was limited to up to 20% of neoplastic cells, mainly in the epithelial component. GFAP expression was variable between cases: 1/5 case expressed GFAP in myoepithelial cells, 3/5 was in the stroma only, and 1/5 was negative. All cases were negative for cytomegalovirus. This study presents five cases of salivary PAs in rhesus macaques identified over a span of 30 years.

## **8: A RARE CASE OF MEDIASTINAL HEMANGIOSARCOMA IN A GERMAN SHEPHERD**

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**An 8-year-old, female spayed German Shepherd dog was presented to the Purdue University Veterinary Hospital with a two-day history of lethargy, anorexia, and dyspnea. On presentation, 770 mLs of hemorrhagic effusion were obtained via thoracocentesis, and CT examination revealed multifocal cavitated masses within the thoracic cavity. The dog was euthanized due to poor prognosis and submitted for necropsy at the Willie M. Reed Animal Disease Diagnostic Laboratory. At necropsy, approximately 1,700 mLs of frank blood were in the pleural cavity. Within the mediastinum and adhered to the serosal surface of the pericardium near the apex were innumerable, variably-sized, dark red masses. The masses ranged up to 7 cm in diameter, and were homogeneously dark red and cystic, containing clotted blood on cut section. Histologic examination of the largest mediastinal mass revealed neoplastic spindled to epithelioid cells exhibiting a moderate amount of eosinophilic cytoplasm, oval nuclei with 0-1 nucleoli, and moderate anisocytosis and anisokaryosis with 1 mitotic figure per ten 400x HPFs (2.37 mm<sup>2</sup>). Multifocally, blood-filled channels contained partially organized fibrin thrombi. Neoplastic cells showed moderate to strong cytoplasmic immunoreactivity to CD31, confirming a diagnosis of hemangiosarcoma. No other organs contained neoplastic foci on gross or histologic examination, indicating that the mediastinum was the primary site of this neoplasm. This site of origin is considered rare for hemangiosarcoma.**

## **9: MULTICENTRIC B-CELL LYMPHOMA IN TWO AGED FEMALE GUINEA PIGS (CAVIA PORCELLUS)**

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**Background:** Lymphoma is one of the most common neoplasia in guinea pigs (*Cavia porcellus*), often presenting with peripheral lymphadenopathy and systemic involvement. This report describes a maternal-offspring pair of aged intact female guinea pigs from the same household with multicentric B-cell lymphoma.

**Signalment and History:** Two guinea pigs presented to Oklahoma State University Zoological Medicine Service for cervical and submandibular lymphadenopathy. Lymphoma was diagnosed with cytology from fine needle aspirates and ancillary immunocytochemistry (ICC) for Pax-5. Following diagnosis, the owner elected humane euthanasia and necropsy.

**Methods:** The guinea pigs were submitted to Oklahoma Animal Disease Diagnostic

Laboratory for necropsy and additional testing. Immunohistochemistry and ICC for anti-CD3 and anti-Pax5 antibodies were performed on ante-mortem cytologies and post-mortem lymph nodes at the Colorado State University Clinical Pathology Laboratory.

Results: Grossly, both guinea pigs exhibited enlarged cervical and submandibular lymph nodes, with one also showing mesenteric lymphadenopathy. Histopathology confirmed multicentric lymphoma involving lymph nodes, lungs, liver, spleen, adrenal glands, kidneys, heart, and brain. The majority of neoplastic lymphocytes have a nuclear immunolabeling for Pax5 consistent with a B-cell neoplasm. Additionally, uterine leiomyoma was incidentally identified in one animal, composed of well-differentiated smooth muscle cells with minimal atypia and rare mitotic figures.

Conclusion: These cases highlight the systemic nature of B-cell lymphoma in guinea pigs. Further research is warranted to investigate potential genetic or infectious etiologies of lymphoma in guinea pigs.

## **10: SPONTANEOUS ORAL SQUAMOUS CELL CARCINOMA WITH WIDESPREAD METASTASES IN AN ATLANTIC BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS*)**

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A 37-year-old Atlantic bottlenose dolphin (*Tursiops truncatus*) developed multifocal ulcerative plaques (tongue and sublingual), initially diagnosed as mucosal hyperplasia and dysplasia, which progressed over nine years. Broad-range PCR (FAP59/64, CP4/5, MY09/11) and metagenomic next-generation sequencing were negative for papillomavirus (at two time points), pan-herpesvirus, and poxvirus. In 2024, the lesions rapidly progressed, causing halitosis, oral pain, and anorexia. Autopsy revealed necrotic sublingual and tongue masses, firm white nodules in mediastinal lymph nodes, lungs, and the left kidney. Additionally, a hyperplastic plaque was present on the dermis of the dorsal fin. Histology showed a malignant epithelial neoplasm in the oral cavity with pulmonary and nodal metastases. The renal tumour was initially poorly differentiated, but deeper sections revealed keratinisation and intercellular bridges, confirming squamous morphology. Neoplastic cells in the primary and metastatic tumour immunolabelled with CK14. COX-2 immunolabelling was present in approximately 35% of the less differentiated neoplastic cells in the oral mass. No COX-2 labelling was observed in the metastatic lesions. Neoplastic epithelial cells (primary mass and metastases) did not immunolabel with uroplakin, CK5, or PAX8. The morphological diagnosis was primary oral squamous cell carcinoma with lymph node, pulmonary, and renal metastases. An incidental finding on the dorsal fin plaque was confirmed as lacaziosis (*Paracoccidioides ceti*) by histology and fungal PCR. This case illustrates malignant transformation of long-standing dysplastic lesions in the absence of detectable viral infection and highlights diagnostic constraints of current

immunohistochemical panels in marine mammals. Vigilant surveillance of chronic mucosal lesions in cetaceans under professional care is warranted.

## **11: ANGIOINVASIVE ASPERGILLUS FUMIGATUS INFECTION WITH RESPIRATORY AND CENTRAL NERVOUS SYSTEM INVOLVEMENT IN A GOAT KID**

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A 9-day-old mixed-breed male goat was submitted to the Mississippi State University College of Veterinary Medicine for postmortem examination following dystocia-related complications. Grossly, the lungs exhibited an accentuated lobular pattern with multifocal atelectasis. The ventral aspects of the left and right cranial lung lobes were consolidated, displaying multiple well-demarcated dark red foci. Numerous 1-5 mm in diameter, slightly raised, firm, pale tan nodules were scattered throughout the pleural surfaces of all lobes. Histologically, these nodules effaced the pulmonary parenchyma, disrupted large vessels, and consisted of pyogranulomatous inflammation, hemorrhage, necrotic debris, and numerous intralesional 4–8  $\mu$ m wide, septate, parallel-walled, acute-angled branching, argyrophilic fungal hyphae. Multifocally, similar hyphae and necrohemorrhagic debris markedly expanded the visceral pleura. In less affected regions, large pulmonary vessels were variably occluded by fibrin thrombi. Focal pyogranulomatous meningitis, centered on a meningeal vessel over the telencephalon, also contained fungal hyphae. Omphalophlebitis was present, but fungal elements were not observed in the umbilicus or other tissues. Panfungal PCR on lung tissue identified *Aspergillus fumigatus* with 100 percent sequence identity. These findings support the occurrence of primary fungal pleuropneumonia shortly after birth, with hematogenous spread to the meninges. Although an intrauterine fungal infection near term remains a possibility, the absence of fungal omphalophlebitis in the kid and the lack of systemic disease in the doe suggest that this route of infection is less likely. This case highlights the importance of considering fungal infections, particularly *Aspergillus fumigatus*, as a differential diagnosis of pleuropneumonia and disseminated infections in neonatal goats.

## **12: MICROPTERUS NIGRICANS ADOMAVIRUS-1 IN MELANOPHORE PROLIFERATIONS IN LARGEMOUTH BASS (MICROPTERUS NIGRICANS)**

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*Adomaviridae* is a recently proposed family of circular double-stranded DNA viruses, which cause proliferative or necrotizing diseases in an increasing variety of fish. *Micropterus nigricans* adomavirus-1 (MnA-1) and *Micropterus dolomieu* adomavirus-1 (MdA-1) cause black-pigmented cutaneous lesions, characterized by increased melanophores, in largemouth and smallmouth bass, respectively. Recently, MnA-1 was associated with malignant melanophoromas in largemouth bass. The study objectives were to describe MnA-1 associated lesions genetically and cellularly and to identify

correlations between hyperpigmented lesions and malignant melanophoroma, hypothesizing that non-neoplastic cutaneous melanocytic lesions can transform to malignant melanophoromas. Affected skin was sampled from seven aquarium-housed largemouth bass and assessed using histology, immunohistochemistry, PCR, sequencing, and transmission electron microscopy (TEM). Histologic examination revealed melanophore proliferations within the dermis and epidermis in 100% (7/7) of fish, with progression to malignant melanophoroma in 14% (1/7) of fish. The fish with neoplastic transformation had a recent history of local trauma. Chronic inflammation composed of lymphocytes and few eosinophilic granular cells was identified in 43% (3/7) cases, including the melanophoroma. Melanophores were immunoreactive for PNL2 in 50% (1/2) of cases and immunonegative for MelanA in one case. MnA-1 was detected in 100% (7/7) of lesions using PCR and Sanger sequencing. Smallmouth bass adenoviruses, MdA-1 and MdA-2, were not detected. TEM revealed no virions in melanocytes or epidermal cells (6/6), potentially due to late-stage infection or low virion numbers. Study findings support hyperpigmented skin lesions are associated with MdA-1 infection, and chronic lesions can progress to malignant melanophoromas, potentially with inciting factors such as trauma.

### **13: ENDOCARDITIS IN A KEMP'S RIDLEY SEA TURTLE (LEPIDOCHELYS KEMPII)**

Amy Bodine<sup>1</sup>, Kaylin McNulty<sup>1,2</sup>, Zachary Lott<sup>1</sup>, Alexandra Emelianchik<sup>1,2</sup>, Debra Moore<sup>1,2</sup>, Ryanne Murray<sup>1,2</sup>, Beth Peterman<sup>1,2</sup>, Stephen Reichley<sup>1,2</sup>, Mark Lawrence<sup>1,2</sup>

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A female adult Kemp's Ridley Sea Turtle was caught on hook and line on 7/25/24. The hook was surgically removed on the same day. In the following weeks, she battled anorexia, weight loss, and infection until being found dead at the bottom of the enclosure on 9/17/24.

On postmortem examination, the surgical incision was grossly normal. The subcutaneous tissues underlying this area contained a hematoma. The left shoulder joint contained fibrin. The coelomic cavity contained a marked amount of malodorous, tan to red fluid (~1.5 to 2Ls). The left liver lobe and stomach were multifocally adhered to the coelomic lining. The pericardial sac contained 107mLs of straw-colored cloudy, gelatinous fluid. The atrioventricular valve was markedly nodular with a granular surface.

On histopathologic examination, the atrioventricular valve was diffusely covered in eosinophilic amorphous material with myriad intralesional cocci bacteria. The hematoma also contained abundant intralesional bacteria. Lesions supportive of sepsis included multi-organ thrombi, hepatic piecemeal necrosis, splenic lymphocytolysis, and renal tubule necrosis. Few spirorchiid ova were found throughout the tissues including the skin, lung, liver, kidney, spleen, stomach, intestine, pancreas, and thyroid. There were no spirorchiid ova present within the valvular lesion.

Antemortem blood cultures and postmortem cultures of the lung, liver, spleen, and intestine reveal growth of antibiotic-resistant *Enterococcus faecalis*.

To the authors' knowledge, this is the first case report of *Enterococcus faecalis* causing

endocarditis in a sea turtle. This bacterium is considered an opportunistic pathogen and has been documented in cases of osteomyelitis and septicemia in sea turtles.

#### **14: THE ROLE OF C-MYC IN THE REGULATION OF THE INTEGRATED STRESS RESPONSE AND LIPOGENESIS IN MEIBOCYTES**

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The Integrated Stress Response (ISR) is a complex signaling pathway that serves as a key regulator of cell survival, while also promoting apoptosis through downstream effectors when prolonged or severe stress cannot be resolved. The ISR's role in malignancies has been investigated due to its complex role in cell survival and death; however, the role of the ISR has not been evaluated in ocular adnexal sebaceous carcinoma (SebCA), an invasive neoplasm of the human Meibomian gland (MG). Though uncommon, mortality rates of up to 40% have been reported in SebCA. The determinants of SebCA tumorigenesis are still unknown; however, upregulation and copy number gain of the c-MYC locus has been documented. Our objective was to interrogate the role of MYC in modulating the ISR in meibocytes. Human MG epithelial cells (HMGECs) and three primary human SebCA cell lines were subject to both pharmacologic and genetic modulation of MYC and fatty acid synthase (FASN). Cytotoxicity, proliferation, apoptosis, and protein and gene expression were assessed. Eyelids of tamoxifen-inducible MYC-overexpressing mice were harvested for histology, immunohistochemistry, immunoblotting, and qPCR. MYC-inhibited HMGECs exhibited dose-dependent decreases in proliferation with increased apoptosis, and MYC-overexpressing HMGECs and both high-MYC expressing SebCA cells and murine MGs exhibited reduced rates of apoptosis and increased sebaceous differentiation characterized by cytoplasmic expansion and upregulated FASN expression. Downregulation of MYC was demonstrated in all FASN-inhibited cells. These results may suggest that MYC upregulation may suppress the ISR by promoting lipogenesis, processes which may serve as apoptosis evasion mechanisms for neoplastic meibocytes.

#### **15: PUTATIVE RADIATION-INDUCED SQUAMOUS CELL CARCINOMA OF THE ANAL SAC IN AN ADULT LABRADOR RETRIEVER**

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Thirty-five months status post right anal saccullectomy and 18 fractions of radiation therapy (RT) for a histopathologically-confirmed apocrine gland anal sac adenocarcinoma (AGASACA), a 13.64-year-old, F/S Labrador retriever was presented for a history of ribbon-like stool and tenesmus. An approximately 4.5cm diameter, firm, left anal sac mass was palpated rectally. Fine needle aspiration with cytopathology of the mass was non-diagnostic. A left anal saccullectomy was performed, and the excised tissue was formalin-fixed and routinely processed for evaluation of H&E-stained sections. Histopathologic examination revealed an invasive neoplasm of the perianal dermis and subcutis with the effacement of the anal sac architecture. Neoplastic cells

were arranged in infiltrative nests supported by a variably dense fibrovascular stroma which was multifocally expanded by extensive fibrillar extracellular matrix (desmoplasia). Neoplastic cells demonstrated poorly distinct cytoplasmic margins with a moderate volume of brightly eosinophilic cytoplasm. Multifocally, concentric rings of squamous epithelial cells enclosed aggregates of anucleate keratin debris (keratin pearls). Anisocytosis and anisokaryosis were moderate, and there was moderate mitotic activity (16 mitotic figures/2.37mm<sup>2</sup>). Squamous cell carcinoma (SCC) of the left anal sac was diagnosed histologically. AGASACA's are common malignancies in dogs that comprise 17% of perianal tumors. However, SCC arising from the anal sac is exceptionally uncommon, with only a few case reports in the literature. Due to the history of contralateral AGASACA, treated with regional RT, and the rare nature of SCC in this region, a putative link between therapeutic radiation exposure and the development of the second malignancy is proposed.

## 16: IN SITU HYBRIDIZATION FOR DETECTION OF ADENOVIRUSES IN CERVIDS

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**Background:** Of the six recognized adenovirus genera, only *Barthadenovirus* (formerly *Atadenovirus*) and *Mastadenovirus* include viruses known to infect cervids. *Odocoileus adenovirus 1* (OdAdV-1; *Barthadenovirus cervi*), is the most pathogenic, causing adenoviral hemorrhagic disease characterized by endothelial tropism and systemic vascular damage. Mastadenoviruses are also reported, rarely causing tracheitis and bronchopneumonia with apparent tropism to respiratory epithelium.

Diagnosis of OdAdV-1 via histopathology can be challenging, as characteristic intranuclear inclusion bodies (INIBs) are often sparse and indistinct, increasing the risk of underdiagnosis. Similarly, lack of recognition and specific testing methods may contribute to underdiagnosis of mastadenovirus infections.

**Objectives:** Application of RNAscope® in situ hybridization (ISH) to confirm the presence of *Mastadenovirus* within histologic lesions, and to illustrate its potential as an ancillary assay for improving visualization of OdAdV-1-associated lesions.

**Methods:** Formalin-fixed, paraffin-embedded lung tissues from a PCR-confirmed OdAdV-1 case and a separate case with novel mastadenovirus infection were processed for ISH. RNAscope® assays were performed utilizing custom-designed probes, targeting a viral DNA polymerase gene, and following manufacturer's protocol (Advanced Cell Diagnostics, ACD).

**Results/Conclusions:** RNAscope® ISH successfully detected robust OdAdV-1 signal in pulmonary endothelium and *Mastadenovirus* signal in infected respiratory epithelium, with no cross-reactivity observed between probes. These findings support ISH as a valuable adjunct when histopathology alone is inconclusive and highlight its potential to

enhance routine detection and increase diagnostic sensitivity for both established and emerging adenoviral pathogens in cervid populations.

### **17: SEBACEOUS TUMORS IN TEXAS KANGAROO RATS**

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Five adult male Texas kangaroo rats (*Dipodomys elator*) within the same breeding colony under zoo-managed care presented with masses arising from the dorsal scent gland, a sexually dimorphic modified sebaceous gland that functions in intraspecies communication of sexual receptivity. Masses were raised, pale tan to dark brown, multilobulated, and alopecic with variable ulceration. On histologic evaluation, tumors were diagnosed as sebaceous gland hyperplasia (n=1), sebaceous adenoma (n=2), and sebaceous carcinoma (n=2). Masses were composed of moderately well-demarcated, expansile (hyperplasia) to compressive (adenoma), and non-encapsulated sebaceous epithelial proliferations. In adenomas, the neoplasm was composed of well-differentiated lobules with peripheral basaloid epithelial cells that differentiated into sebocytes with a centralized duct. In carcinomas, arising within more differentiated lobules, some regions were regionally distorted with loss of cellular differentiation and stromal invasion of small clusters of foamy neoplastic epithelial cells with mitotic figures observed within sebocytes. The underlying cause of hyperplastic and neoplastic sebaceous tumors in this population is unknown. Seasonality in the months of April to June correlated to the spring peak of dorsal gland development and breeding. In combination with sex predilection, a hormonal influence, similar to testosterone driven hepatoid gland proliferation in domestic dogs, was postulated. Lack of a shared genetic ancestry decreased the suspicion of a potential genetic cause. Future investigation of possible environmental, hormonal, or viral etiologies is warranted for best management practices in breeding colonies under human care. Ultimately, these populations are intended for reintroduction to boost vulnerable and declining free-ranging populations of this iconic Texas species.

### **18: AI-DRIVEN ONCOPATHOLOGY: ENHANCING OBJECTIVITY AND EFFICIENCY IN CANINE ORAL MELANOMA ANALYSIS**

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Digital Slide Imaging (DSI) has revolutionized histopathological analysis, particularly through the integration of Artificial Intelligence technologies (AI). One of the most

impactful developments in this field is the use of AI-based tools, such as HALO AI, to improve the objectivity and precision of digital histopathology.

In this study, we developed and tailored AI algorithms to perform objective assessments of malignant features in canine oral melanoma (COM), an aggressive cancer in which clinical behavior remains challenging to predict. Specifically, we focused on key proliferation markers such as mitotic figures (n=44) and Ki-67 expression (n=12), which are traditionally assessed through time consuming methods that are prone to high inter-observer variability. In addition, we explored CD163 (n=13), a marker of immunoinhibitory or M2 macrophages within our COM cohort.

Our AI models were designed to identify and classify nuclei, enabling automated quantification of mitotic figures. Additionally, we integrated HALO's IHC and Microglial modules with our customized nuclear segmentator algorithm to quantify Ki-67 and CD163 expression, respectively.

Mitotic counts obtained via HALO AI showed strong agreement with those recorded by the study pathologist and documented in original pathology reports. A positive correlation was observed between mitotic count and Ki-67 expression, while CD163 expression appeared independent of proliferation markers.

We conclude that AI tools such as HALO AI hold promise as accurate and efficient methods for quantifying mitotic figures and Ki-67 expression. These findings support the potential for AI-assisted image analysis to contribute to more standardized and reproducible assessments of proliferation markers in histopathology.

## **19: HEMANGIOSARCOMA IN A CAPTIVE CHIMPANZEE (PAN TROGLODYTES): A CASE REPORT**

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To our knowledge, we report the first documented case of hemangiosarcoma (HSA) arising from the sex skin in a 31-year-old female chimpanzee (*Pan troglodytes*), which presented with a rapidly enlarging and hemorrhagic perineal mass. Grossly, the mass was multiloculated and filled with dark red fluid. Histologically, it consisted of infiltrative, cavernous vascular channels and solid sheets of neoplastic endothelial cells exhibiting marked anisocytosis, anisokaryosis, and elevated mitotic activity. Metastatic spread was confirmed to the regional lymph nodes. Immunohistochemistry revealed diffuse strong positivity for CD31, ERG, and Factor VIII, and sporadic D2-40 immunolabeling, which supports the diagnosis of hemangiosarcoma. This case expands the anatomic and species spectrum of HSA in NHPs, emphasizing the importance of including this tumor in the differential diagnoses of vascular masses in primates. It also highlights the utility of immunohistochemical profiling in confirming tumor origin. Due to its rarity, further case documentation is needed to improve understanding of the biological behavior, prognosis, and diagnostic approaches for HSA in non-human primates.

## **20: RED WOLVES IN SHEEP'S CLOTHING: UNMASKING THE EFFECTS OF AGING BEHIND TESTICULAR DEGENERATION**

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Age-related changes in seminiferous tubular morphology and function have been documented in humans and dogs. However, these shifts have not been extensively studied in non-domestic canids. Red wolves (*Canis rufus*) are critically endangered, facing population bottlenecks that lead to inbreeding depression which reduces genetic diversity, adversely impacts the maximum reproductive age, and impairs sperm quality. Investigating these age-related changes in wolves is crucial for developing effective conservation approaches. We hypothesized that age will negatively correlate with area, diameter, and density of seminiferous tubules and positively correlate with tubular degeneration. Red wolf testicular tissue samples (n=31) were collected from various institutions at necropsy or castration. Sections from testes were processed and stained with hematoxylin and eosin (H&E). Forty random measurements of seminiferous tubule diameters and areas were quantified using QuPath software as a proxy for the quality of spermatogenesis. Tubules were scored for degeneration by evaluating the basement membrane, germ cells, and the presence of degenerative cells. Tubular density was estimated by overlaying a square on sections and counting tubules of which more than half were within the square. Paired t-tests and ANOVAs were conducted to assess the correlations between age, degeneration, diameter, density, and area of seminiferous tubules. Statistical analysis revealed that tubule area and diameter increased between mature and geriatric wolves, accompanied by increased degeneration, while a significant increase in area was observed between prepubertal and mature wolves. This study provides evidence of histological changes associated with aging in red wolves contributing to informed and effective breeding management strategies.

## **21: TRANSCRIPTOMIC PROFILING IN FELINE INTESTINAL SMALL CELL T CELL LYMPHOMA SUGGESTS A NATURAL KILLER-LIKE T CELL ORIGIN SIMILAR TO HUMAN REFRACTORY CELIAC DISEASE TYPE II**

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Small-cell intestinal T-cell lymphoma (SCL) is the most common cancer in cats and is hypothesized to arise from chronic inflammation (inflammatory bowel disease, IBD), paralleling human refractory celiac disease type II (RCD II). RCD II is an indolent malignancy marked by clonal T-cell expansion and JAK/STAT pathway mutations. In 60–80% of cases, RCD II progresses to enteropathy-associated T-cell lymphoma (EATL), an aggressive disease with a five-year survival of only 3–20% with treatment. We propose that feline SCL is a naturally occurring, translational model for human RCD II. To compare human and feline diseases, we investigated the transcriptome and

STAT5B mutation frequency in 71 intestinal biopsies from 50 cats with IBD or SCL, as defined by histology (interpreted by 3 blinded pathologists) and clonality testing. Gene set enrichment analysis showed the top upregulated pathways in feline SCL involved T-cell activation and differentiation, including enrichment for alpha-beta T-cell and NKT-like/cytotoxic signatures. These findings suggest the tumor cell of origin in SCL is an activated intraepithelial alpha-beta T-cell with NK-like features—similar to that proposed in human RCD II. Eight percent of IBD cases and eighty percent of SCL cases had the activating STAT5BN642H mutation detected via a droplet digital PCR assay. Given these transcriptomic, genetic, and pathological parallels, cats offer a valuable preclinical model to study the pathogenesis and treatment of intestinal intraepithelial T-cell lymphomas and human RCD II.

## **22: DEVELOPMENT OF A PROTOCOL FOR MANUAL LEUKOCYTE COUNTS OF FORMALIN-FIXED BLOOD FROM BIRDS AND REPTILES**

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Complete blood counts are essential diagnostics for assessing the health status of birds and reptiles. However, cell lysis and morphologic changes can affect manual leukocyte counts within hours to a few days even with appropriate sample storage. In several research and clinical scenarios, it may not be feasible to refrigerate or process samples for manual counts promptly, and although methods to extend blood sample stability have been reported in fish, no such methods have been established for other exotic species. To address this issue, we sought to develop leukocyte count protocols that utilize formalin fixation to extend whole blood stability in birds and reptiles. Using clinically-derived blood samples, leukocyte differentials and hemocytometer counts with Eosin Y and Natt-Herricks stains were performed to establish reference values for each patient. Aliquots of whole blood were added to 10% neutral buffered formalin, and hemocytometer counts were repeated with the formalin-fixed blood on days 0, 2, 7, and 28 post-collection. In general, methods utilizing Eosin Y had higher leukocyte count agreement with reference methods compared to those utilizing Natt-Herricks solution. Agreement analyses (n = 14) suggest leukocyte counts of formalin-fixed blood stored at room temperature are clinically similar (within a total allowable error of 20%) to fresh counts up to at least 48 hours post-collection using the Eosin Y technique. Future work will include a greater sample size to improve confidence in mean bias and variation coefficients for each method and will evaluate potential benefits of corrective formulas.

## **23: HISTOLOGIC ANALYSIS OF FLASH AND CONVENTIONAL IRRADIATION OF THE LUNG TISSUE OF MICE**

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**Background:** FLASH radiotherapy is a novel cancer treatment method that delivers radiation dose over an ultrashort period of time. This technique targets tumor tissue while reducing the radiation toxicity to surrounding healthy tissue.

**Objective:** Evaluate the effects of dose fractionation to lung tissue of mice by FLASH radiotherapy and use equitoxic conventional radiotherapy dosing for comparison.

**Methods:** Cohort 1 (n=40) tested single and hypo-fractionated dose delivery and cohort 2 (n=40) tested conventional fractionations. Histopathology was performed, and lung tissue stained with hematoxylin and eosin and Masson's trichrome. QuPath was used to quantify collagen in the lungs through thresholding (n=37), and statistical significance was found using ANOVA and post hoc testing. Scireq flexiVent was used for pulmonary function testing (n=14).

**Results:** FLASH 6 fraction had significantly higher collagen levels than the control ( $p = 0.005$ ). Two-way ANOVA assessed effects of radiation type and fraction number and found significance in radiation type ( $p = 0.04$ ), with higher collagen levels in FLASH groups compared to conventional groups. There was no significant interaction between radiation type and fraction number ( $p = 0.53$ ).

**Conclusions:** This project found FLASH radiotherapy exhibited a trend towards higher collagen deposition to the lung compared to conventional radiotherapy for 6 fractions of 6.5 gray but was not significantly different. On pulmonary function testing, mice that received conventional radiotherapy demonstrated a trend toward lower total lung capacity and compliance but were not significantly different. Additionally, bronchiolitis obliterans was found to be a common sequela to lung irradiation in mice.

## **24: A CASE OF RAPIDLY PROGRESSIVE, CD18-NEGATIVE, ABBERANT LARGE B-CELL LYMPHOMA IN A CAT**

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Lymphoma is a common feline neoplasia that exhibits diverse clinical presentations, biologic behaviors, and immunophenotypic profiles. This is a case of a two-year-old, spayed female, retroviral negative, domestic short hair cat that presented for evaluation of red discoloration of the left iris. Upon examination, generalized peripheral lymphadenomegaly was observed. Fine needle aspirates were obtained and cytologic evaluation showed expansions of intermediate to large lymphocytes in multiple lymph nodes, spleen, and liver, consistent with lymphoma. Flow cytometry was performed on tissue from a lymph node aspirate and a homogenous population of CD18-, CD4-, CD8-, CD5-, and CD21- cells was identified. PCR for antigen receptor rearrangements (PARR) was performed on the same sample, revealing a clonally rearranged immunoglobulin gene. Fourteen days post-diagnosis, cytology of a skin mass revealed large lymphocytes similar to those observed in the lymph nodes, spleen, and liver. At this time, atypical blastic cells were also observed in the peripheral blood. Despite treatment with oral prednisolone and L-Asparaginase, the patient experienced progressive systemic disease and was euthanized 18 days post-diagnosis. Postmortem examination identified extensive infiltration of nearly every examined organ by large neoplastic round cells, including bone marrow and central nervous system tissues.

Immunohistochemical stains were performed. Neoplastic cells were CD20+, CD3-, MUM1-, and CD18-. These findings are consistent with a diagnosis of stage V, CD18-, large B-cell lymphoma. This case illustrates the value of employing a multimodal diagnostic approach to identify atypical forms of lymphoma and suggests loss of CD18 expression may correlate with clinically aggressive behavior.

## **25: THE PIGLETS THAT TOOK OUR BREATH AWAY**

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Four Yorkshire piglets, 1 gilt and 3 barrows, aged 2-3 months were found dead at a production facility with no known history. Gross examination revealed that all four piglets had moderate to marked lymphadenopathy in the inguinal, mesenteric, tracheobronchial, and submandibular lymph nodes. All piglets had marked interstitial pneumonia, with 2/4 piglets exhibiting marked bronchopneumonia. Cavitory effusion with small to marked amounts of fibrin was present in all piglets, varying from single cavity to tri-cavitory effusion. In one piglet, fibrin was noted on the surface of the abdominal organs, pleura, and within the pericardial sac. Histopathology identified lung lesions consistent with marked interstitial pneumonia with alveolar septa infiltrated by macrophages, neutrophils, and lymphocytes. In addition, sections contained deeply basophilic chromatin aggregates. Two of the four piglets also demonstrated severe necrotizing bronchopneumonia with fibrin membranes and large colonies of cocci bacteria consistent with a secondary bacterial infection. Lymph node sections demonstrated marked paracortical hyperplasia with moderate to marked lymphocyte apoptosis and multifocal necrotizing lymphadenitis. Ancillary PCR testing of fresh lung tissue were positive for Porcine Circovirus-2 (PCV-2) and Porcine Reproductive and Respiratory Syndrome (PRRS) virus, specifically the North American strain. Bacterial culture of the spleen identified *Streptococcus suis* (3/4) and *Pasturella multocida* (2/4). PCV-2 and PRRS virus are two of the most economically devastating viruses affecting the swine industry. In this case series, the concurrent infection of PCV-2 and PRRS virus led to marked immune suppression, thereby leading to severe secondary bacterial infections and ultimately death in these animals.

## **26: OLFACTORY GANGLIONEUROBLASTOMA IN A DOG**

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An 8-year-old FS Wire Fox Terrier presented for new-onset seizures, as well as progressive lethargy, inappetence, and right forelimb lameness. Three weeks prior, the patient had been evaluated for moderate general lameness and lethargy and treated with doxycycline upon discovery of a lesion suspicious for a tick bite and newly positive Anaplasma serology. Despite treatment, the patient developed progressive neurologic signs including episodes characterized by tremors, heavy drooling, panting, ataxia, and disorientation. On neurologic examination, clinicians noted dull mentation with a slight left head tilt and slight rightward bias. An MRI of the patient's brain revealed a large, right-sided mass centered on and causing destruction of the cribriform plate, with

extension into the nasal cavity and cranial vault. Significant vasogenic cerebral edema and transtentorial herniation were present secondary to the mass. These findings were consistent with neoplasia, with differentials including olfactory neuroblastoma (esthesioneuroblastoma), carcinoma, or less likely, round cell neoplasia or hemangiosarcoma. The owners elected humane euthanasia. Necropsy identified a 5.6 x 1.5 x 1.0 cm gray, soft, irregular mass extending from the caudal right nasal cavity to the right frontal lobe. Histopathologic examination revealed infiltrative, densely cellular neoplasm composed of polygonal-to-elongate cells arranged in anastomosing trabeculae, sheets, tubular and neuroblastic rosettes amongst a moderate amount of fibrovascular and neurofibrillary stroma. Multifocally, there are disorganized aggregates of ganglion cells with neuronal process representing a ganglioneuromatous component, confirming the diagnosis of olfactory ganglioneuroblastoma. To the authors' knowledge, only two other such cases of olfactory ganglioneuroblastoma have been reported in dogs.

## **27: EVALUATION OF METASTATIC CANINE APOCRINE GLAND ANAL SAC ADENOCARCINOMAS WITH IMMUNOHISTOCHEMICAL ASSAYS COMMONLY APPLIED TO NEUROENDOCRINE NEOPLASMS AND GRIMELIUS STAIN**

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### **Background:**

Canine apocrine gland anal sac adenocarcinomas (AGASACAs) and neuroendocrine neoplasms (NENs) share similar histomorphologic features. Histologic diagnosis of primary AGASACA is straightforward with knowledge of the neoplasm arising from the anal sac. It can be challenging, however, to differentiate a metastatic AGASACA from a NEN histologically without knowledge of a primary neoplasm arising from the anal sac.

### **Objectives:**

To evaluate how canine AGASACAs label with immunohistochemical assays commonly applied to NENs and to evaluate if canine AGASACAs possess Grimelius-positive (argyrophilic) granules.

### **Method:**

Tissue microarrays were assembled using formalin-fixed paraffin-embedded (FFPE) blocks from 23 metastatic AGASACAs where tissue from the primary and metastatic neoplasms was available. The following immunohistochemical assays were applied: protein gene product 9.5 (PGP9.5), neuron-specific enolase (NSE), synaptophysin, chromogranin A, insulinoma-associated protein 1 (INSM1), microtubule-associated protein 2 (MAP2), cluster of differentiation 56 (CD56), cytokeratin, and vimentin. Grimelius staining was also performed.

### **Results:**

All neoplasms immunolabeled with cytokeratin & chromogranin A. None of the neoplasms immunolabeled with PGP9.5, INSM1, or Vimentin. Patchy immunolabeling of neoplastic cells was noted in a proportion of neoplasms with MAP2 (62%), NSE (44%), synaptophysin (19%), and CD56 (15%). None of the neoplasms possessed argyrophilic granules.

### **Conclusions:**

The immunohistochemical profiles of AGASACAs and NENs share similarities; however, important differences exist. AGASACAs and NENs cannot be differentiated with chromogranin A, NSE, synaptophysin, MAP2, or CD56. AGASACAs are PGP9.5-negative, cytokeratin-positive, and lack argyrophilic granules. In contrast, the vast majority of NENs are expected to be PGP9.5-positive, cytokeratin-negative, and possess argyrophilic granules.

## **28: FIRST REPORT OF METASTATIC SQUAMOUS CELL CARCINOMA AND TYMPANOKERATOMA OF THE MIDDLE EAR IN A CAT PRESENTING WITH NEUROLOGICAL SIGNS**

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Ross University School of Veterinary Medicine, Basseterre, Saint Kitts and Nevis

### **Background:**

Squamous cell carcinoma (SCC) is a common malignant epithelial tumor in domestic animals, particularly cats. Tympanokeratomas are rare epidermal cysts of the middle ear, occasionally reported in humans and dogs but rarely in cats. They can be congenital or acquired (post-inflammatory). The coexistence of both lesions has not been previously documented in animals.

### **Materials and Methods:**

A 7-year-old neutered female domestic shorthair cat with a history of surgically managed left auricular SCC was euthanized due to progressive vestibular disease. A postmortem examination with histopathological assessment of collected tissues were conducted according to standard procedures.

### **Results:**

The left middle ear contained a cystic structure filled with a large amount of keratin. The cyst was lined by non-atypical stratified keratinizing squamous epithelium with a granular layer and small numbers of sebaceous glands, consistent with tympanokeratoma. No evidence of SCC was seen in the pinna remnant; however, infiltrative squamous cell carcinoma was identified in the regional lymph node, auricularis muscle, and parotid salivary gland.

### **Conclusion:**

This case represents the first documented coexistence of SCC and tympanokeratoma in a cat. The vestibular signs were likely due to mass effect from the tympanokeratoma within the middle ear, along with secondary inflammation extending into the inner ear. Additionally, the presence of metastatic SCC in adjacent lymph nodes suggests that the primary auricular tumor had already metastasized prior to surgical excision. Middle ear lesions may be clinically silent and are frequently overlooked, as examination of the tympanic bullae is not routinely performed in standard necropsy protocols.

## **29: CYTOLOGIC FEATURES OF BARIUM PERITONITIS IN A DOG**

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A 2.5-year-old spayed female Labrador Retriever underwent an exploratory laparotomy after a radiographic barium series revealed an intestinal obstruction. During surgery, multiple intestinal perforations secondary to a linear foreign body were identified, with concurrent leakage of barium into the peritoneal cavity. Postoperatively, the patient was referred to the University of Illinois Veterinary Teaching Hospital for hospitalization. Abdominal ultrasound revealed peritoneal effusion, and fluid analysis revealed a protein-rich, highly cellular exudate, cytologically characterized by the presence of 86% mildly degenerate neutrophils and 14% vacuolated macrophages both containing intracellular, refractile, granular-to-rod-shaped material, suspected to represent phagocytosed barium based on the clinical context. Relevant CBC and chemistry findings during hospitalization included a persistent inflammatory leukogram, progressive thrombocytopenia and panhypoproteinemia. Seven days after initial presentation to ER Service, the patient's clinical condition deteriorated, prompting an exploratory laparotomy. Intraoperatively, dehiscence of the jejunal enterotomy sites, multifocal severe intestinal adhesions, and a moderate amount of purulent free fluid were identified. Cytologic evaluation of the fluid revealed septic suppurative inflammation. The patient was eventually euthanized due to postoperative hypotension which was nonresponsive to therapy. Intraperitoneal barium represents a life-threatening condition due to complications such as adhesions, granulomas and severe peritonitis. Early diagnosis and treatment are critical, as prognosis is often guarded to poor. The aim of this report is to characterize the cytologic and clinicopathologic features of barium-induced peritonitis, thereby facilitating recognition by clinicians and contributing to a better understanding of its prognostic implications in cases of gastrointestinal perforation.

## **30: PROTEIN EXPRESSION FIBROBLAST GROWTH FACTOR-21 (FGF-21), A FUNCTIONAL MARKER OF THE FAT LIVER AXIS, IN FELINE HEPATIC LIPIDOSIS AND OTHER DISEASES OF FELINE METABOLIC STRESS**

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**Background:** Feline hepatic lipidosis (FHL) is a devastating nutritional disease in cats caused primarily by a negative energy balance, where an excessive storage of triglycerides leads to liver dysfunction. Fibroblast Growth Factor-21(FGF21), produced at very low levels in health, is predominantly liver-induced and regulates liver fat metabolism, protecting the liver from lipotoxicity through modulating glucose homeostasis and enhancing insulin sensitivity.

**Objectives:** The study aimed to assess the correlation between the degree of hepatic steatosis and hepatic expression of FGF21 in cats with hepatic lipidosis and other diseases of metabolic stress, expecting the highest FGF21 expression in FHL.

**Materials and Methods:** 34 biopsies (2020 – 2024) of feline hepatic diseases were classified based on the major disease. 5 normal, immediately post-euthanasia controls were utilized from research cats on other studies. FGF21 immunohistochemistry was performed and scanned with paired H&E slides. Lipid quantification and IHC staining density and intensity were investigated using HALO digital pathology analysis software. Data was analyzed with GraphPad Prism 10.

**Results:** Of the 34 feline biopsies, including those with hepatic co-morbidities, 11 (32.4%) had steatosis, 8 (23.5%) had neutrophilic cholangitis, 3 (8.8%) had lymphocytic cholangitis, 2 (5.9%) had lymphoma, 2 (5.9%) were equivocal for lymphoma v. lymphocytic cholangitis, 2 (5.9%) had reactive hepatitis, 2 (5.9%) had fibrocystic hepatic lesions, 1 (2.9%) had mast cell neoplasia, 1 (2.9%) had extrahepatic biliary obstruction, and 1 (2.9%) had a congenital portosystemic shunt.

**Conclusion:** FGF21 mRNA expression is needed to determine if hepatic FGF21 production acts in an endocrine or autocrine/paracrine fashion.

### **31: INFILTRATIVE CHEMODECTOMA WITH VENTRICULAR INVASION IN A GERMAN SHEPHERD DOG**

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**Chemodectomas are typically benign, heart-based neuroendocrine tumors with low metastatic potential. In dogs, they are commonly found near the aortic body and rarely exhibit myocardial invasion. A 3-year-old neutered male German Shepherd dog, Drake, presented in respiratory distress with hypoglycemia, pleural effusion, and pulmonary consolidation. Gross necropsy revealed 835 mL of red-tinged thoracic fluid and a firm, white infiltrative mass encompassing approximately 20% of the atria and 75% of the myocardium, with concurrent pericardial effusion. Histologically, the neoplasm was a non-encapsulated, densely cellular mass composed of polygonal cells arranged in bands and small packets with high mitotic activity (22 mitoses/2.37 mm<sup>2</sup>). Notably, the neoplasm infiltrated and effaced the myocardium of the atria and ventricles and extended along the pericardium, epicardium, and endocardium. Immunohistochemistry revealed diffuse, strong cytoplasmic immunoreactivity for synaptophysin, consistent with a neuroendocrine origin; however, chromogranin A, calcitonin, and neuron-specific enolase were negative. While these IHCs may supplement the identification of neuroendocrine tumors, they lack specificity for chemodectomas, and the expression across cases varies.**

**To our knowledge, this is a unique case of chemodectoma exhibiting extensive myocardial invasion. These findings emphasize the need for comprehensive diagnostic evaluation, including histopathology and immunophenotyping, as well as contributing to the evolving understanding of chemodectoma biology in veterinary medicine.**

### **32: BEWARE THE SNEAKY PLANTS: WHAT LIVESTOCK SHOULD NEVER NIBBLE - A PRELIMINARY SURVEY OF TOXIC FLORA AFFECTING LIVESTOCK IN ST.KITTS**

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#### **Introduction**

Toxic plants are a persistent but underreported cause of morbidity and mortality in livestock, particularly in tropical island environments where biodiversity is high and veterinary surveillance is limited. In the Caribbean, there is a distinct lack of published data cataloging toxic flora and their clinical implications in food animals.

#### **Materials and Methods**

17 farms across all nine parishes of St. Kitts were visited between June 2023 and March 2024 to document toxic plants encountered by livestock. Plants were identified visually using regional botanical guides, geotagged, photographed, and assessed for proximity to grazing areas. Farmers were interviewed using a standardized questionnaire covering plant recognition, livestock illness or death, and pasture management. Identified plants were categorized by toxic compounds and associated clinical or pathologic findings from the literature.

#### **Results**

The most frequently identified toxic plants included *Nerium oleander* (oleander), and *Ricinus communis* (castor bean). Oleander exposure is associated with myocardial necrosis, hydropericardium, pulmonary congestion and gastrointestinal hemorrhage; castor bean ingestion leads to hemorrhagic enteritis, hepatic necrosis, and renal tubular injury. These plants were found within or adjacent to grazing areas in over 60% of cases.

#### **Conclusion**

This study represents the first systematic documentation of toxic plants affecting livestock on St. Kitts. Findings confirm that hazardous species are common in grazing areas, with several producing lesions consistent with known toxic mechanisms. While not all plants had confirmed pathologic outcomes, many contain compounds with established mammalian toxicity. These results reinforce the need for increased diagnostic awareness and preventive measures in tropical livestock systems.

### **33: A RARE CASE OF RIGHT ATRIAL HEMANGIOSARCOMA IN A CAT**

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A 13.5-year-old, neutered male, domestic longhaired cat presented for evaluation of anorexia, lethargy, and shallow, rapid breathing. On physical examination, the cat was hypothermic, tachypneic, bradycardic, and had muffled heart and lung sounds. Point of care ultrasound showed pericardial and pleural effusion. Thoracocentesis yielded 200mL of transudative effusion. Echocardiography demonstrated a right atrial mass

accompanied by moderate to severe pericardial effusion, mild pleural effusion, and evidence of cardiac tamponade with right atrial diastolic collapse. Due to the poor prognosis, humane euthanasia was elected. Necropsy identified a  $1.8 \times 1.6 \times 1.5$  cm, variably well-demarcated, irregular, firm, and mottled dark red and gray mass at the tip of the right auricle, along with hemorrhagic pleural and pericardial effusion, pulmonary congestion and edema, and mild hepatomegaly. Gross pathologic findings were suggestive of a right atrial neoplasm with secondary congestive heart failure likely secondary to cardiac tamponade. Histopathology of the mass revealed a malignant neoplasm composed of spindle cells forming irregular vascular spaces and capillary-like structures supported by minimal collagenous stroma. On immunohistochemistry, neoplastic cells exhibited moderate cytoplasmic immunoreactivity for CD31, confirming a diagnosis of hemangiosarcoma. This report describes a rare case of right atrial hemangiosarcoma with secondary right-sided congestive heart failure from cardiac tamponade in a cat, diagnosed through clinical signs, echocardiography, histopathology, and immunohistochemistry. While hemangiosarcoma is the most common primary cardiac neoplasm in dogs, to the authors' knowledge, only a single documented case of right atrial hemangiosarcoma in cats has been previously reported.

### **34: OSTEOSARCOMA EXTRACELLULAR VESICLES TARGET RESIDENT LUNG MACROPHAGES AND PROMOTE METASTASIS**

Laurel Haines<sup>1</sup>, Alex Baeckler<sup>1</sup>, Sophi Schofield<sup>2</sup>, Eric Palmer<sup>1</sup>, Kathryn Cronise<sup>1</sup>, Daniel Regan<sup>1</sup>

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**Background.** Osteosarcoma, the most common primary bone cancer in dogs and humans, has a high propensity to metastasize to the lungs leading to survival rates of under 25%. Therapeutic options have not improved in over four decades underscoring the need to better understand mechanisms driving lung metastasis. Other cancers have been shown to “prime” distant organs via extracellular vesicle (EV) signaling to promote metastasis. However, the role of EVs in osteosarcoma lung metastasis is poorly understood.

**Objective.** We aimed to identify the cellular targets osteosarcoma EVs and their role in mediating metastasis.

**Methods.** To localize EVs within lung cells, we optimized a fluorescent EV labeling approach and leveraged spectral flow cytometry. To determine how osteosarcoma EVs impact lung metastasis, we used cytokine analyses, RNA sequencing, and intravital imaging in mouse models and primary human lung macrophages. Finally, to translate our findings to the clinic, we assessed metastatic changes in bronchoalveolar lavage fluid (BALF) using single cell RNA sequencing in dogs with naturally occurring osteosarcoma.

**Results.** We found that osteosarcoma EVs preferentially accumulate in interstitial and alveolar macrophages and elicit distinct pro-tumorigenic phenotypes in these cells. In mice, osteosarcoma EVs promoted tumor cell seeding and pro-tumorigenic immunological changes in the lungs. Finally, dogs with osteosarcoma displayed unique airway macrophage phenotypes that could serve as biomarkers of disease progression

in BALF.

**Conclusions.** Our findings suggest osteosarcoma EVs are early drivers of lung metastasis and elicit tumor-promoting changes in lung macrophages. These pre-metastatic changes could serve as novel therapeutic targets for metastatic osteosarcoma.

### **35: INVESTIGATING THE ROLE OF SPRAYED SERUM-DERIVED MATERNAL ANTIBODIES IN POTENTIATING DISEASE IN INFECTIOUS BRONCHITIS VIRUS (IBV) CHALLENGED CHICKS**

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Infectious bronchitis virus (IBV) causes respiratory and urogenital disease in chickens. If layers are infected during the first weeks of life, in absence of maternal antibodies, they can develop “false layer syndrome” (FLS). IBV targets epithelial tissues and induces inflammation with subsequent fibrotic repair. Early inflammation of the developing oviduct leads to cystic formation and atrophy related dysfunction. Early administration of vaccines at day of age protects against field challenges; however, if maternal antibodies are not present at appropriate concentrations, developmental abnormalities may still occur. Maternal antibodies are not consistent within flocks; multiple breeder flocks contribute to egg laying flocks resulting in dissimilar antibody makeup. This study aims to investigate if spray delivered antibodies to day old chicks can provide local protection that prevents early infection, boosting maternal antibodies, and eliminating IBV related FLS. We utilized day-old chicks treated with serum alone or serum containing M41 (IBV) maternal antibodies and challenged with either M41 or CA 1737 IBV strains to assess viral pathogenesis and mechanisms of viral mediation. Necropsies were performed six- and twelve-days post challenge. We assessed gross pathology of tracheas and airsacs, collected tracheas for histomorphometry, cloacal swabs for viral load analysis, and serum for IBV antibody quantification. The results demonstrate that protection was conferred from both serums with and without antibodies in challenges using two IBV genotypes. The protection was non-specific, but significant comparing the non-treated and challenged group. This suggest the mechanism of protection is not specific viral neutralization but local mucosal barrier.

### **36: METHOD VALIDATION OF TWO HUMAN HBA1C ASSAYS FOR DOGS**

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Glycated hemoglobin (HbA1c) is the current gold standard test for diagnosing and managing both prediabetes and diabetes in humans, and it reflects average serum glucose levels over 2-3 months. Validated HbA1c assays for veterinary species are not readily available in the USA, and there is limited data on their applicability in companion animals. The goals of this study were to determine: (1) if canine blood products can be

artificially glycosylated, stored, and used as reliable control materials for HbA1c assays, (2) the limit of detection, linearity, and precision of two HbA1c assays, and (3) if HbA1c measurements are comparable to fructosamine measurements. We determined that: (1) washed and artificially glycosylated canine blood products can be used to generate two levels of stable, standardized, HbA1c control and (2) the limit of detection, linearity, and precision of the high-throughput (Beckman) and point-of-care (A1CNow+) tests are excellent with canine samples and comparable to that of human samples with all CV  $\leq$  0.05 and  $R^2 \geq 0.95$ . (3) Method comparison and reference value studies are in progress and will be available shortly. In summary, preliminary data indicates the Beckman HbA1c and A1CNow® assays are appropriate (but not interchangeable) for measuring HbA1c in dogs and may be useful diagnostic tests for companion animal medicine.

### **37: VISCERAL LEISHMANIOSIS IN A DOG LIVING IN MICHIGAN FOR 3 YEARS**

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A 7-year-old female spayed Spanish Greyhound adopted at 4 years of age, received medical care at local veterinary practices and Michigan State University, College of Veterinary Medicine (MSU-CVM). The dog had a 1-year history of vomiting, diarrhea, and anorexia that improved with a hydrolyzed protein diet and steroid treatment. Recently, proteinuria was detected on a urinalysis performed to rule out a urinary tract infection for nocturia at MSU-CVM. Abnormalities noted during a follow-up visit included: patchy alopecia, hyperproteinemia consisting of hypoalbuminemia with hyperglobulinemia, increased liver enzyme activities, and persistent marked proteinuria. Additional questioning of the owners elucidated that the dog was originally from Spain. Hepatic and splenic fine needle biopsy aspirates were collected with ultrasound guidance for submission to the MSU Veterinary Diagnostic Laboratory; numerous intracellular 2-4  $\mu$ m ovoid protozoal organisms with a nucleus and kinetoplast supportive for *Leishmania* spp. amastigotes, were detected on cytologic examination. A high serologic titer against *Leishmania infantum* spp. and PCR analyses (genus and species) confirmed a visceral leishmaniosis diagnosis. The dog showed significant clinical improvement following initiation of allopurinol and a course of miltefosine treatments. *Leishmania infantum* is a zoonotic parasite transmitted horizontally by sandflies or vertically from dam to offspring that is endemic in tropical and subtropical regions. Sporadic veterinary cases in non-endemic regions are typically linked to international travel or local vertical transmission from infected dams. This case underscores the importance of obtaining a complete lifelong history including source of adoption to facilitate consideration of all potentially pertinent infectious diseases.

### **38: THE VIRULENCE FACTOR TCDB IS REQUIRED FOR FULL BURKHOLDERIA MALLEI PATHOGENESIS IN A MOUSE MODEL OF AEROSOL INFECTION**

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**Background:** *Burkholderia mallei* (*Bm*) is the causative agent of glanders, a severe and highly fatal disease to animals and humans. *Bm* is difficult to treat and lacks a vaccine, necessitating the development of novel countermeasures. The Tc-like toxin, TcdB, is required for *Burkholderia* intracellular fitness and multinucleated giant cell formation *in vitro* but its biological role during acute glanders infection *in vivo* is unknown.

**Objective:** This study aims to evaluate TcdB's contribution to *Bm* virulence and explore its role during *in vivo* infection to determine if it could be a target for vaccine/therapeutic development.

**Methods:** Mice were infected intratracheally with varying doses of wild-type (WT) or *tcdB*-mutant *Bm* strains using a Microsprayer® device and euthanized 3 days later to collect lungs and spleens for histopathological, immunohistochemical, and molecular analyses.

**Results & Conclusions:** Substantially less bacteria were detected in the lungs and spleens of mice infected with the *Bm tcdB*-mutants, demonstrating impaired ability to colonize and/or disseminate within the host. The lungs of the mice infected with the mutants also had fewer, smaller, and/or poorly defined abscesses with rare multinucleated giant cells. The spleens of these mice had mild inflammation, compared to the moderate to severe inflammation seen in WT *Bm*-infected spleens, characterized by increased numbers of neutrophils within the red pulp and microabscesses. The significant reduction in pathology and bacterial colonization of target tissues during infection with *tcdB*-mutant strains confirms that TcdB is essential for full *Bm* virulence, strongly suggesting that TcdB could serve as a target for countermeasure development.

### 39: BABESIA 'COCO' IN A SPLENECTOMIZED DOG

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*Babesia* 'coco' is a large form *Babesia* spp. occurring in asplenic or immunosuppressed dogs in North America and is indistinguishable from *B. canis* via light microscopy, with PCR required to differentiate them. It is uncertain if dogs are the primary reservoir for *B. 'coco'* or if large numbers of dogs are exposed to *B. 'coco'* and only immunosuppressed or asplenic dogs then develop persistent infections and clinical disease. This case characterizes *B. 'coco'* in a 4-year-old, intact male Cocker Spaniel that presented for routine recheck of suspected primary immune-mediated hemolytic anemia. This anemia was recurring and variably regenerative anemia with occasional spherocytosis, positive saline agglutination tests, negative Coombs' test, negative comprehensive PCR for *Rickettsia* spp. and Apicomplexa, and hyperbilirubinemia. Although initially well managed on immunosuppressive therapy, the anemia became refractory to medical management and a splenectomy was performed 3 months prior. At this visit, the CBC revealed a mild normocytic, hypochromic, non-regenerative anemia. Wright-Giemsa stained blood smear revealed low to moderate numbers of erythrocytes containing one to three large protozoal piroplasms. Repeat PCR confirmed babesiosis secondary to *B. 'coco'*. This case highlights this poorly documented large-form *Babesia* spp. in North America and the importance of clinical history in considering differentials and selecting or suggesting further diagnostic testing.

#### **40: DISSEMINATED HISTIOCYTIC SARCOMA WITH HEMOCOELOM IN A BEADED DRAGON**

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A 3-year-old female intact central bearded dragon (*Pogona vitticeps*) presented with labored breathing, anorexia, and lethargy. Notable findings during physical examination included dehydration, an abnormal heart rate, labored breathing, and muscle wasting. Palpation revealed a distended, soft, fluctuant coelom and multiple coelomic masses correlating with ovarian follicles. Top clinical considerations included multiorgan dysfunction and neoplasia, and thus euthanasia was elected. Postmortem examination revealed a hemocoelom, hepatic lipidosis, and numerous poorly-defined tan nodules adhered to the coelomic viscera and vasculature including the aorta. Histologic examination of the nodules corresponded with sheets of pleomorphic, round to spindle, occasionally multinucleated cells infiltrating many tissues including the aorta. GMS and Fite's modified acid-fast stains revealed no organisms. Pancytokeratin, vimentin, CD18, myoglobin, Pax5, CD3, and Iba1 immunohistochemistry (IHC) was performed, with only pancytokeratin, CD3, PAX 5, pancytokeratin and myoglobin reacting appropriately with internal bearded dragon controls. Of the performed IHCs, neoplastic cells only demonstrated moderate to strong cytoplasmic Iba1 immunoreactivity. Transmission electron microscopy was attempted, but cellular detail was limited by autolysis. Based on gross appearance, cellular morphology, and the immunoreactivity pattern, a histiocytic sarcoma was diagnosed with aortic invasion and subsequent hemocoelom. Histiocytic sarcomas are rarely reported and confirmed with immunohistochemistry in bearded dragons, as immunohistochemistry does not always react appropriately with reptilian tissue. This case is particularly interesting due to its vascular association and subsequent hemocoelom, which is classically associated with aortic aneurysm in bearded dragons. Histiocytic sarcoma should be a differential for disseminated coelomic nodules and rarely hemocoelom in bearded dragons.

#### **41: UNUSUAL TUMOR EFFACING THE EYE - COULD IT BE PLEOMORPHIC IRIDOCILIARY ADENOCARCINOMA?**

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Pleomorphic iridociliary adenocarcinomas (PIA) are tumors that arise from the ciliary body and are characterized by an invasive growth pattern, loss of normal differentiation, and increased metastatic potential. PIAs are reported in human literature; a similar tumor has been reported in dogs. Histopathologic diagnosis is difficult, as the tumor may resemble other malignant tumors such as amelanotic melanoma or metastatic mammary carcinoma. This study identifies immunohistochemical labeling patterns of PIA and methods of differentiation between other intraocular neoplasms.

The COPLOW (Comparative Ocular Pathology Laboratory of Wisconsin) database was searched for canine PIA cases and 56 cases were identified between 2005-2023.

Twenty-six globes diagnosed with PIA were immunohistochemically labeled. Positive labeling included (12/26) cytokeratin [AE1/AE3], (24/26) NSE, (9/26) S-100, (16/26) desmin, (24/26) E-cadherin, (16/26) N-cadherin, (0/26) TTF1, (26/26) vimentin, (6/26) ER, (8/26) PR, and PAS often showed prominent basement membranes (18/26). The significance of positive ER and PR labeling in neoplasms previously identified histologically as PIAs is unclear, but these may represent metastatic mammary carcinomas. In the human literature, some other tumors, such as melanomas, are reported to express ER and PR, and additional immunohistochemical labeling is pending to rule out this possibility in these cases. Non-neoplastic ciliary body epithelium does not express ER or PR, but the possibility of a PIA developing this expression pattern also cannot be completely excluded. Based on these IHC findings, we propose that a diagnosis of PIA should be avoided without histology and immunohistochemistry, and that other intraocular tumors should first be ruled out.

#### **42: THE 'KING' IN COLORS: A VISUAL NECROPSY GUIDE**

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Texas Tech University School of Veterinary Medicine's purpose is to serve the veterinary educational and service needs of rural and regional communities: highlighted by intense in-house and field necropsy teachings to veterinary students.

The necessary and essential dissection techniques demonstrated by pathologists are time consuming, with possible inter-pathologist variations. The goal of this project was to develop a video of a standardized necropsy, a postmortem dissection guide and dissected organ models. A complete dog necropsy was videotaped with narration and includes organ dissections and sampling procedures. A written necropsy guide based on Dr. King's 'The Necropsy Book', was generated by adding necropsy photos side by side to the existing drawings. Additional detailed photo sequences illustrate proper tissue dissections and organ sampling methods for formalin fixation and ancillary test. An Artec 3D scanner was used to capture geometry and textures of dog kidney, heart and skull/brain and unique polylactic acid plastic models of these scanned organs were designed, each with either a dissection annotation or made to be disassembled and reassembled, demonstrating specific cuts. Multiple heart models were made to illustrate different steps of the dissection process. These materials provide effective standardized teaching tools for veterinary students and allow self-paced learning, supplemented with long lasting reusable models. This work will improve pre-lab teaching and learning, while reducing extensive direct faculty involvement and cadaver use for demonstrations. Students will be proficiently readied at the time of their first necropsy, optimizing the postmortem exam experience.

#### **43: UNDER PRESSURE: COMPRESSIVE MYELOPATHY IN THREE SOUTH AMERICAN CAMELIDS**

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While compressive myelopathy is a well-established cause of neurologic dysfunction in canines, it is less commonly recognized in South American camelids (SACs). In this case series, three SACs – two aged llamas and one young alpaca – presented with varying combinations of cervical dysfunction, recumbency, and progressive neurologic impairment. Postmortem examinations revealed spinal cord compression in all cases. One aged llama exhibited chronic intervertebral disc degeneration and herniation at C3-C6, resulting in ventral canal distortion and narrowing. The second llama showed marked vertebral ankylosis and dorsal canal narrowing from C7-T1. The young alpaca, who presented acutely recumbent, had partial herniation of the intervertebral discs at C2-C3 and C3-C4, causing minimal narrowing of the spinal canal. The alpaca also exhibited extensive subdural hemorrhage and malacia, suggesting ischemic injury as a secondary consequence of mechanical deformation. In all 3 SACs, histopathology consistently revealed lesions indicative of chronic spinal cord injury, including multifocal white matter degeneration, axonal swelling, and gliosis. In one aged llama, GFAP immunohistochemistry confirmed astroglial scarring in areas of chronic compression. Although the clinical presentations were varied, all three cases emphasized the potential for chronic vertebral or disc disease to produce significant neurologic compromise in SACs. These findings support the need for greater clinical awareness of compressive myelopathies in SACs and suggest that intervertebral disc degeneration and spinal remodeling may be underdiagnosed contributors to neurologic decline. Postmortem examination, particularly with spinal cord histopathology, remains a valuable diagnostic tool for characterizing the nature and chronicity of these lesions in camelids.

#### **44: INTRACRANIAL TREMATODE METACERCARIAE IN FATHEAD MINNOWS (*PIMEPHALES PROMELAS*)**

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##### **Background**

Fathead minnows (*Pimephales promelas*) are a common model in aquatic toxicology. This report details findings from histological examination of the fish from a University of Glasgow facility following increased mortalities linked to transient water quality decline.

##### **Objective**

To describe the histological presentation of intracranial trematode infestation, identify the likely causative species, and review behavioral consequences in affected fish.

##### **Methods**

Formalin-fixed specimens were decalcified (72–96 hours), dehydrated in ethanol and tertiary butyl alcohol, embedded in paraffin, and sectioned parasagittally at 15 nm. Standard haematoxylin and eosin staining was performed.

##### **Results**

Histology revealed severe, focally extensive malacia in the brain and spinal cord, with numerous intralesional parasites and Gitter cell accumulation. Parasites measured 65–150 µm, lacked a coelomic cavity, and had a cuticle, digestive tract, and sucker—features consistent with trematode metacercariae. All fish exhibited severe, diffuse

lamellar epithelial hyperplasia in the gills.

### **Conclusion**

The histological evaluation of these samples revealed severe lamellar epithelial hyperplasia in the gills. This is consistent with the previous episode of declining water quality, and it is

the most likely cause of the mortalities noted. However, superimposed to this, there was severe intracranial trematode infestation. Such infestation has previously been reported in fathead fish and may have predisposed to the mortalities observed. The causative species for this striking presentation is suspected to be *Ornithodiplostomum ptychocheilus*. This parasite has been documented to alter the piscine intermediate host behaviour (i.e. lack of shoaling and swimming close to the surface), and is an example of parasite-induced trophic transmission (PITT).

### **45: GIANT CELL TUMOR OF BONE IN THE MANDIBLE OF A GOAT: A CASE REPORT AND LITERATURE REVIEW**

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A 6-year-old, intact female, mixed-breed goat was presented to the University of Tennessee Veterinary Medical Center for a rostral mandibular mass. Radiographs showed bilateral osteolysis of the rostral mandible. Due to the poor prognosis, the goat was euthanized and submitted for necropsy. Grossly, a pink-tan, firm, intraosseous mass measuring 3 x 2 x 2 cm displaced the left mandibular second, third, and fourth incisors ventrally and extended caudally into the diastema.

Histologically, the mandibular bone was effaced by numerous multinucleated cells containing 2-12 nuclei and uninucleate, fibroblast-like spindle cells evenly distributed within fine fibrous stroma. Cells displayed low malignancy criteria with low nuclear pleomorphism and a mitotic count of less than 1 in 2.37mm<sup>2</sup>. Tumor-produced osteoid was not evident. There was multifocal hemorrhage and aggregates of hemosiderin-laden macrophages. Based on these histologic features, the lesion was suggestive of either a giant cell tumor of bone (GCTB) or central giant cell granuloma (CGCG). Similar masses have been previously described in the mandible of goats and designated as a giant cell tumor of bone.

Distinguishing between CGCG and GCTB remains controversial. In humans, CGCG and GCTB have similar clinical, radiological, and histological characteristics with a H3F3A mutation indicative of a diagnosis of GCTB rather than CGCG. There is a paucity of these cases in the veterinary literature, with only two previous such case reports in goats, one of which performed molecular analysis and could not confirm a H3F3A mutation. Further investigation is needed on giant cell-rich lesions of bone in veterinary species.

#### **46: CYTOLOGIC AND HISTOLOGIC ANALYSIS OF METASTATIC MALIGNANT TRICHOEPITHELIOMA IN A DOG**

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A 5-year-old male neutered standard poodle presented for evaluation of a 4.2 cm x 2.8 cm firm, ulcerated, subdermal mass in the left caudolateral inguinal area. Initial abdominal CT and punch biopsy of the mass was most consistent with a mast cell tumor. Tumor staging was performed, and fine needle aspirates of the mildly enlarged left medial iliac lymph node (MILN) revealed frequent clusters of variably shaped cells with moderate amounts of blue cytoplasm consistent with metastatic neoplasia with carcinoma and malignant melanoma considered as top differentials. Surgery to remove the left inguinal mass and the superficial inguinal lymph nodes was performed to derive a definitive diagnosis. Histopathology of the left inguinal mass showed an infiltrative and poorly demarcated neoplasm comprised of basilar polygonal cells forming keratin filled cystic structures with abrupt transition from basal cells to keratinized squamous cells consistent with a trichoepithelioma. Histopathology of the left inguinal lymph node revealed metastatic spread and vascular invasion by similar appearing basilar epithelial cells. The dog presented a month later for abdominal CT for radiation therapy planning, and multiple additional lymph nodes were found to be enlarged. Fine needle aspiration of right MILN revealed probable malignant trichoepithelioma metastasis due to the presence of aggregates of keratinized anucleate squamous epithelial cells and rare small clusters of poorly preserved basilar epithelial cells. This case highlights comprehensive cytologic, histopathologic, and diagnostic imaging analysis of an uncommon metastatic malignant trichoepithelioma in a dog.

#### **47: CITICOLINE SAFELY ATTENUATES HYPOXEMIC ACUTE RESPIRATORY FAILURE AND SYSTEMIC INFLAMMATION IN HOSPITALIZED COVID-19 PATIENTS**

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**Background.** Hypoxemic acute respiratory failure (HARF) is a common sequela of pulmonary infection. Aside from supplemental O<sub>2</sub>, effective therapeutics for HARF are lacking. We showed previously that early or late post-infection administration of CDP-choline/citicoline (an intermediate in *de novo* phospholipid synthesis) to mice inoculated with influenza A/WSN/33 (H1N1) and SARS CoV-2 resulted in attenuation of HARF and reduced pulmonary inflammation.

**Hypothesis.** Addition of citicoline to current standard of care management will result in attenuation of HARF in hospitalized COVID-19 patients.

**Methods.** Our hypothesis was tested in “SCARLET” (Supplemental Citicoline Administration to Reduce Lung injury Efficacy Trial), an NIH-funded single-site, double-

blinded, placebo-controlled, and block-randomized Phase 1/2 dose-ranging, safety, and efficacy trial of i.v. citicoline (0.5-5 mg/kg every 12 hours for 5 days) in adults hospitalized for SARS CoV-2-associated HARF.

**Results.** 9 placebo-treated and 24 citicoline-treated adults were enrolled. Unlike placebo treatment, administration of citicoline significantly improved oxygenation ( $S_aO_2$ :  $F_iO_2$  ratio) relative to Day 1 on Days 2-8. Citicoline also significantly reduced plasma C-reactive protein, ferritin, fibrinogen, and RAGE levels across this timespan. There was no citicoline dose effect. Treatment had no impact on plasma electrolytes, indices of renal and hepatic function, hematologic parameters, or clotting function.

**Conclusions and Relevance:** i.v. citicoline rapidly and safely improves oxygenation and attenuates systemic inflammation beyond standard of care in patients with SARS CoV-2-associated HARF. However, larger trials are needed to definitively show clinical benefit for citicoline in this population or in patients with HARF from other causes.

#### **48: SINONASAL SQUAMOUS CELL CARCINOMA IN FREE-RANGING WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS)**

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Neoplasia is uncommonly reported in free-ranging cervids and there is scant literature reporting squamous cell carcinomas (SCC) affecting the head and face of cervids. In domestic mammals, SCCs are among the most prevalent of malignant epithelial neoplasms, exhibiting aggressive growth and local invasion. Risk factors reported across different species include old age, ultraviolet light exposure, environmental contaminants, and papillomavirus infection. We report **four suspected or confirmed cases of sinonasal SCC in free-ranging white-tailed deer** and compare these tumors to SCC in domestic animals and similar neoplasms of the head reported in cervids. Gross dissection of the head and light microscopy (H&E) were performed. In all cases, the neoplastic mass was cavitated and ulcerated, protruding from the frontal sinus and obliterating the surrounding bones of the frontal and nasal sinuses. Neoplastic squamous epithelial cells formed coalescing islands and cords, occasionally forming keratin pearls, and separated by desmoplastic stroma. Mitotic activity was variable and there were large regions of necrosis and associated inflammation. Findings were consistent with a highly invasive SCC of the sinonasal cavity. In cervids, published reports of SCC of the head are limited to rare individual case reports, but their gross appearance and biologic behavior appear similar to sinonasal SCCs in horses. These sinonasal SCCs appear grossly similar to other cranial tumors in white-tailed deer, such as cutaneous fibroma, osteochondroma and antleroma, but exhibit more aggressive local invasion, resulting in significant morbidity. Further investigation to elucidate the etiopathogenesis of this tumor is being pursued.

#### **49: UNUSUAL GRANULOMATOUS AND EOSINOPHILIC PNEUMONIA IN A HARBOR SEAL (PHOCA VITULINA)**

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An 8-year-old female harbor seal (*Phoca vitulina*) in managed care presented with respiratory signs and was diagnosed radiographically with suspected granulomatous pneumonia. Although initially improving with empirical antibiotic and antifungal treatment, the patient later declined and humane euthanasia was elected. At necropsy, all lung lobes contained multiple round to irregularly shaped, firm, pale tan nodules with tortuous vessels extending along the pleural surface. The remainder of the pulmonary parenchyma was diffusely mottled dark pink to dark red. Histologically, two distinct processes were observed. The nodules were consistent with chronic granulomas containing rare intra-histiocytic fragments of fungal hyphae. In the rest of the lungs, alveoli were diffusely filled with mucus and abundant foamy histiocytes and eosinophils. Similar inflammation extended into the interstitium and the lumen of terminal bronchioles, but larger airways were spared. No evidence of infectious agents was seen. Ancillary diagnostics included aerobic culture, fungal PCR, special stains (PAS, Ziehl-Neelson, GMS, Gram), immunohistochemistry for canine distemper virus (CDV), and serology for CDV and phocine distemper virus. All tests were negative and did not reveal a definitive cause of the pneumonia. It is possible that these lesions represent a resolving bacterial or viral infection and prior treatment hindered detection of the inciting agent. Given the presence of eosinophils, an atypical hypersensitivity reaction was also considered. Although an inciting cause was not definitively identified, this case emphasizes the importance of monitoring disease in marine mammals that can serve as sentinel species for zoonotic and/or emerging pathogens.

#### **50: AN ASSESSMENT OF THE PROTECTIVE ABILITY OF SUPPLEMENTAL BLF ON GUT EPITHELIAL INTEGRITY IN AN AVIAN COCCIDIOSIS VACCINE CHALLENGE STUDY**

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Coccidiosis threatens the economic viability of the poultry industry. Caused by pathogenic *Eimeria* species, the significant intestinal damage of coccidiosis leads to poor performance and death. While current control measures rely on anticoccidial drugs and vaccines, continued emergence of drug resistance, increasing public pressure for a 'no antibiotics ever' industry, and the high cost of vaccine development necessitate alternative strategies. Bovine lactoferrin (bLF), an iron-binding whey glycoprotein with antimicrobial and immunomodulatory properties, is a worthy candidate, but its potential role in mitigating avian coccidiosis is understudied. To evaluate its ability to enhance the immune response and reduce the severity of intestinal lesions, chicks were examined post vaccine inoculation (10X dose) after 5 days of bLF supplementation [bLF-supplemented vaccine-challenged] or PBS [unchallenged healthy controls]. Positive

controls [vaccine-challenged, only] received no supplement. Following euthanasia, intestinal lesions were scored and parasite load will be histologically assessed using Hematoxylin & Eosin. Markers of gut integrity and inflammation (tight junction proteins; IFN- $\gamma$  and IL-10, respectively) will be examined using immunohistochemistry. We hypothesize that compared with positive controls, bLF-supplementation will result in fewer lesions, lower numbers of encysted coccidia, and a higher expression of tight junction proteins. Preliminary gross scores suggest bLF has a modest protective effect. Group 3 has the highest, Group 2 the lowest and Group 1, intermediate. While data analysis is ongoing, these findings support its potential to enhance poultry health, and promote sustainable agriculture. Statistical analysis will be performed using SAS with significance set at  $p < 0.05$ .

### **51: A RETROSPECTIVE STUDY OF CAUSES OF MORTALITY IN SQUIRREL SPECIES (*SCIURUS* SP.) FROM WASHINGTON AND IDAHO, USA**

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Between 2004 and 2024, necropsy was performed at the Washington Animal Disease Diagnostic Laboratory on 93 squirrels of four species from Washington and Idaho states, USA. The species were 32 western gray (*Sciurus griseus*), 29 eastern gray (*Sciurus carolinensis*), 29 fox (*Sciurus niger*), and 3 red (*Sciurus vulgaris*) squirrels. Causes of mortality or reasons for euthanasia were infectious disease (29/93, 31%), traumatic injury (13/93, 14%), parasitism (11/93, 12%), toxin exposure (4/93, 4%), metabolic disease (2/93, 2%), and hand-rearing complications (1/93, 1%). Tularemia, caused by *Francisella tularensis*, was the most commonly diagnosed infectious disease (11/29). Other infectious etiologic agents were: *Carnobacteriaceae*, *Clostridium perfringens*, *Klebsiella pneumoniae*, *Streptobacillus moniliformis*, rotavirus, and west nile virus. Among the cases of mortality caused by parasitism, nine individuals had microscopic lesions consistent with visceral or neural larva migrans, one individual was infected with *Echinococcus multilocularis*, and one had severe infestation of *Notoedres* mites. Identified toxins were fentanyl and anticoagulant rodenticide. Metabolic diseases were caused by hepatic lipidosis and emaciation. As synanthropic species, squirrels are often involved in wildlife-human interactions, and necropsy can be a simple and cost-effective tool in assessing population health and public health risks. This study provides a better understanding of pathogens circulating in tree squirrel species. Continued monitoring and expansion on the dataset can provide further insight on disease trends in individual species.

### **52: FELINE REFERENCE MIRNOME ESTABLISHMENT AND MIRNA EXPRESSION IN PITUITARY SOMATOTROPH ADENOMAS USING DDPCR**

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MicroRNAs (miRNAs) are small, non-coding RNAs responsible for post-transcriptional gene modification, and changes in expression have been linked to various neoplasias. Pituitary somatotroph adenomas are the most frequently reported pituitary tumors in cats and can result in an increased production of growth hormone, leading to hormone alterations and insulin-resistant diabetes mellitus. There is limited literature on next-generation sequencing (NGS) or using ddPCR for feline samples, and there are no available feline-specific miRNA primers. As miRNA is highly conserved across species, this study is evaluating the use of miRNA sequencing library data from other species to determine if the miRNA expression data can yield relevant feline data. The miRNA were extracted from formalin-fixed, paraffin-embedded tissues of cats who had undergone removal of the pituitary gland (n=10) due to adenomas and of patients undergoing necropsy for reasons other than pituitary disease (n=5). The miRNA content was quantified and NGS was performed on samples containing >19ng/μL miRNA (pituitary adenoma=8, control=5), utilizing the Illumina platform to the entire Illumina library due to the lack of an available feline-specific miRNA transcriptome. A total of 30,655 miRNAs were evaluated, with 3,778 miRNAs showing expression. Several candidate miRNA reference genes (n=2,224) are identified along with potential targets showing differential expression (n=440), with homologous miRNA sequences from 149 species. The information gained in this study will inform the use of translational miRNA data in felines, raising the potential for utilizing established NGS library data and ddPCR primers from other species for diagnostic and prognostic indicators in cats.

### **53: DISSEMINATED MYCOBACTERIOSIS IN A CAT: A RARE CASE OF SEPTIC EFFUSION**

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Mycobacteria are acid-fast, rod-shaped intracellular bacteria that cause infections in animals and humans, particularly in immunosuppressed individuals. In cats, infections typically present as cutaneous disease with draining tracts, nodules, ulceration, and regional lymphadenopathy. Although disseminated infection is rare, *Mycobacterium avium*–intracellulare complex (MAC) is most frequently implicated.

A 7-year-old spayed female domestic shorthair cat was presented to the University of Florida College of Veterinary Medicine with a six-month history of progressive weight loss and hyporexia, along with acute lethargy. The cat had stable chronic kidney disease and had been receiving cyclosporin (25 mg every other day) for atopic dermatitis for 2–3 years. Abdominal ultrasound revealed a 2.5 cm cranial abdominal mass, multifocal lymphadenomegaly, peritoneal effusion, and bilateral chronic nephropathy.

Wright–Giemsa-stained aspirates from the jejunal lymph node and cranial abdominal mass revealed numerous non-staining rods, consistent with mycobacteria, within macrophages and free in the background. Both sites also had evidence of lymphoid tissue. The cranial abdominal mass additionally contained well-staining rods with neutrophilic inflammation. Cytopathology of the peritoneal effusion showed

predominantly degenerate neutrophils with intra- and extracellular, well-staining rods of variable length and rare non-staining rods. Ziehl–Neelsen staining confirmed acid-fast organisms, and PCR identified *Mycobacterium avium* (99.5% similarity). Histopathology revealed marked enteritis, mesenteric and pancreaticoduodenal lymphadenitis, and pancreatic extension with intralesional acid-fast, Gram-positive rods.

This case highlights a rare presentation of mycobacteria-associated ascites with the first reported mixed bacterial co-infection, likely secondary to gastrointestinal leakage. Long-term cyclosporin treatment likely predisposed this otherwise immunocompetent cat to disseminated MAC infection.

#### **54: LYMPHOCYTIC CHOLANGITIS IN A CAT WITH CONCURRENT HYPERTROPHIC CARDIOMYOPATHY AND HEPATIC COPPER ACCUMULATION: A DIAGNOSTIC CHALLENGE**

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#### **Background**

Lymphocytic cholangitis (LC) is a chronic, immune-mediated hepatobiliary disease in cats characterized histologically by lymphocytic infiltration of the portal tracts and bile ducts. This case illustrates the diagnostic limitations of cytology and the value of postmortem examination when antemortem diagnostics prove inconclusive.

#### **Case Summary**

An 11-year-old spayed female Ragdoll cat was evaluated for marked icterus and decreased appetite. Serum biochemistry revealed severe hyperbilirubinemia and markedly elevated liver enzymes. Abdominal ultrasound identified hepatic cyst-like structures, raising concern for a polycystic liver disease related to a ductal plate malformation. Cholecystocentesis and liver fine-needle aspirates revealed moderate neutrophilic and lymphoplasmacytic inflammation, cytoplasmic copper accumulation, mild hepatocellular lipid accumulation, and cholestasis. Bile cultures were negative, making neutrophilic cholangitis less likely. Liver biopsy was recommended for definitive diagnosis.

During sedation for the ultrasound, the cat exhibited atrial and ventricular arrhythmias. Therefore, an echocardiogram was performed which identified concentric left ventricular hypertrophy with a suspected atrial thrombus, consistent with hypertrophic cardiomyopathy. Due to anesthetic risk, the liver biopsy was not pursued. After failing to respond to antibiotics and liver support, humane euthanasia was elected.

Postmortem histopathology revealed moderate to marked lymphocytic portal inflammation, bile duct targeting, bridging portal fibrosis, lipogranulomas formation, and panlobular copper accumulation (rhodanine score 5/5). These findings supported a diagnosis of lymphocytic cholangitis.

#### **Conclusion**

This case highlights the complexity of diagnosing LC without histopathology, particularly in the presence of comorbidities. It emphasizes the importance of postmortem evaluation in clarifying the diagnosis and illustrates how overlapping disease processes can complicate clinical interpretation.

#### **55: SINUSITIS-ASSOCIATED PERIOPHTHALMITIS AND SEPSIS ASSOCIATED WITH MIXED PSEUDOMONAS AERUGINOSA AND E. COLI INFECTION IN A ZOO-HOUSED HAMERKOP (SCOPUS UMBRETTA)**

Rebeca Machado<sup>1,2</sup>, Mariano Carossino<sup>1,2</sup>, Ingeborg M. Langohr<sup>1,2</sup>, Fabio del Piero<sup>1,2</sup>

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##### **Background:**

*Pseudomonas aeruginosa* and *Escherichia coli* are well-documented opportunistic pathogens in birds, particularly associated with respiratory infections, enteritis, and septicemia. While these pathogens have been described in zoo and wildlife species, progression from sinusitis to periophthalmitis and sepsis in a Hamerkop (*Scopus umbretta*) has not been previously reported.

##### **Case Summary:**

A 23-year-old female Hamerkop presented with left periorbital swelling, was diagnosed with conjunctivitis, and treated with oral enrofloxacin and topical neomycin-polymyxin-bacitracin. The swelling persisted for four days, so glaucoma was suspected, and meloxicam was added to the treatment plan. The bird was found dead in its enclosure three days later and submitted to the Louisiana Animal Disease Diagnostic Laboratory (LADDL) for postmortem examination. The left orbit was filled with caseous exudate extending into the nasal cavity and displacing the globe. Histopathology revealed chronic sinusitis with periophthalmitis characterized by granulomatous and heterophilic inflammation containing mixed bacterial colonies. Bacteriological culture of the exudate yielded moderate growth of *E. coli* and *P. aeruginosa*. Additional alterations included multifocal necrotizing hepatitis (indicative of sepsis) and splenic amyloidosis.

##### **Conclusion:**

This case highlights an unusual presentation of upper respiratory infection associated with mixed *P. aeruginosa* and *E. coli* infection in a zoo-housed Hamerkop that progressed to periophthalmitis and sepsis. It highlights the diagnostic value of postmortem examination in identifying severe sequelae of sinusitis in avian species.

#### **56: EQUINE PARVOVIRUS-ASSOCIATED MASSIVE HEPATOCELLULAR NECROSIS AND LOSS WITH CIRRHOSIS IN STANDARD BRED FILLY**

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A 3-year-old Standardbred filly was submitted for postmortem examination following acute clinical decompensation. The filly presented with lethargy, neurologic signs, and coagulopathy. Clinicopathologic testing revealed marked hyperbilirubinemia (24.4

mg/dL), severe elevations in hepatic enzymes (AST, GGT, ALP, GLDH), and hyperammonemia (297 µg/dL), findings consistent with hepatic failure and encephalopathy.

Grossly, the most striking lesion was a diffusely pale, friable, and mildly enlarged liver (1.28% body weight), accompanied by moderate icterus and serosanguineous effusion in both thoracic and abdominal cavities. Histologically, the liver exhibited diffuse massive to submassive hepatocellular degeneration, loss, and necrosis with stromal collapse, most pronounced in centrilobular and midzonal regions. Remaining periportal hepatocytes were cytomegalic, polyploid, and multinucleated, suggestive of syncytial formation. Bridging portal fibrosis and regenerative nodules were also present, confirmed by reticulin and Masson's trichrome stains. Alzheimer type II astrocytes were identified in the brain, consistent with hepatic encephalopathy. PCR confirmed equine parvovirus-hepatitis (EqPV-H); cultures were negative. An incidental renal *Klossiella equi* infection was also noted.

While EqPV-H is classically associated with acute hepatic necrosis, this case displayed both acute and chronic hepatic changes, including hepatocellular loss, bridging fibrosis, and nodular regeneration. The chronicity observed in such a young horse broadens the known histologic spectrum of parvoviral hepatitis and underscores the importance of integrating histopathology, special stains, and molecular testing when evaluating equine liver disease.

## **57: IMPACT OF IN UTERO METFORMIN EXPOSURE ON NEUROINFLAMMATORY MARKERS IN THE RHESUS MACAQUE MODEL**

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Gestational hyperglycemia is associated with health complications for mothers and offspring. Pharmaceutical management during pregnancy includes metformin which improves maternal outcomes but may have unanticipated impacts on fetal development due to its ability to cross the placental and blood-brain barrier. The developing hypothalamus is highly responsive to changes during gestation. *In utero* dysregulation results in altered neuroendocrine development and long-term metabolic consequences. This study aims to characterize the effects of maternal metformin treatment on fetal neuroinflammation (FNI) and hypothalamic development using a non-human primate (NHP) model. We hypothesized that an unhealthy maternal diet causes an increase in FNI, and that metformin treatment can rescue this effect.

Fetal NHP brains were collected at gestational day (G) 145 via Cesarean section from dams assigned to one of four groups: CHOW/vehicle, CHOW/metformin (MET), Western-style diet (WSD)/vehicle, or WSD/MET. Fixed hypothalami were sectioned on a microtome and analyzed using dual-labeled fluorescent immunohistochemistry for allograft inflammatory marker 1 (Iba-1) and glial fibrillary acidic protein (GFAP) to assess immune cell activation. Results indicated no significant changes in the expression levels of Iba-1 or GFAP in the mediobasal hypothalamus (MBH), arcuate nucleus (ARH) or posterior hypothalamic area (PHA) for any groups. Increased fetal brain weight resulted in decreased Iba-1 expression in the MBH and ARH. Iba-1 expression and GFAP expression were positively correlated for all groups. Microglial

activation analysis found that maternal WSD exposure alone causes an increase in total microglia count in the ARH of fetal offspring, but metformin treatment was able to blunt this effect.

### **58: HIGHLY PATHOGENIC AVIAN INFLUENZA (H5N1) IN A DOMESTIC CAT ASSOCIATED WITH BACKYARD POULTRY OUTBREAK IN OKLAHOMA**

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**Introduction:** Highly Pathogenic Avian Influenza (HPAI) is a growing concern in many animal species, including domestic cats. Feline infections are commonly associated with the consumption of unpasteurized milk or raw/undercooked meat from infected animals. Here we report HPAI in a cat that had direct contact with backyard poultry, underscoring an alternative route of transmission.

**Signalment and History:** An adult intact female cat from a property with HPAI-positive backyard poultry (chickens, ducks, geese, peafowl) was submitted to Oklahoma Animal Disease Diagnostic Laboratory (OADDL) for necropsy and HPAI testing. The cat had free access to poultry enclosures. Clinical signs included lateral recumbency, continuous seizures, and diminished awareness. Several other cats on the property also died with neurologic signs and one aborted prior to death.

**Methods:** A full necropsy was performed at OADDL with appropriate biosafety procedures. Tracheal samples were tested for influenza virus using PCR, following the National Animal Health Laboratory Network guidelines. Routine histopathology was conducted, and immunohistochemistry was performed using a polyclonal antibody specific for influenza virus antigen.

**Results:** PCR testing of the trachea was positive for H5N1 (H5 clade 2.3.4.4b) strain of influenza virus. Histopathology demonstrated multifocal, marked hemorrhages in the cerebral cortex along with interstitial pneumonia, pulmonary edema, and fibrin in the lungs. Immunohistochemistry detected influenza virus antigens in the brain, lung, heart and pancreas.

**Conclusion:** To the best of our knowledge, this is the first reported case of HPAI in a domestic cat associated with direct exposure to backyard poultry.

### **59: ESOPHAGEAL DUPLICATION CYST IN A DOMESTIC RABBIT (ORYCTOLAGUS CUNICULUS)**

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An 8-month-old mixed-breed buck was presented for necropsy following acute collapse, increased respiratory effort, and diarrhea. Postmortem exam revealed a large, cystic

mass arising from the submucosal and muscular layers of the esophagus causing caudal deviation of the lungs. The cyst contained approximately 15-20 milliliters of semi-opaque, white mucoid fluid and histologically was composed of a layer of disorganized skeletal muscle lined by keratinizing stratified squamous epithelium resembling esophageal mucosa. Based on these findings, the mass was diagnosed as an esophageal duplication cyst. Additional findings included chronic bronchopneumonia and chronic interstitial pneumonia presumed to be secondary to aspiration as a complication of the cyst's location. Duplication cysts are rarely reported within human and veterinary literature, but have been reported in dogs, horses, cats, and primates. Duplication cysts are most commonly described in humans as benign embryologic defects and are usually incidental findings. Clinical signs of gastrointestinal duplication cysts are most often observed in adolescents and include dysphagia, food impaction, emesis, dyspnea, and cough, similar to the antemortem signs in this rabbit. To the author's knowledge, this is the second described case of an alimentary duplication cyst in a rabbit. Although rare, esophageal duplication cysts should be considered as a differential for esophageal masses in rabbits.

## **60: HALICEPHALOBUS GINGIVALIS-INDUCED MENINGOENCEPHALOMYELITIS IN A HORSE**

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### **Background**

*Halicephalobus gingivalis* is a free-living, saprophytic rhabditid nematode capable of opportunistic infection in equines, often targeting the brain, kidneys, lymph nodes, spinal cord, and adrenal glands. While its life cycle is poorly understood, entry is thought to occur via mucous membranes or wounds, followed by parthenogenesis, and dissemination throughout tissues. Infections are rare, often diagnosed postmortem, and carry a grave prognosis.

### **Objectives**

Identify the cause of acute neurologic signs in a 24-year-old Quarter Horse gelding.

### **Methods**

Diagnostics included a physical exam, CBC, chemistry panel, cerebrospinal fluid (CSF) analysis, and PCR for equine herpesvirus-1. Lack of full recovery from anesthesia led to humane euthanasia and submission for necropsy.

### **Results**

The horse presented recumbent with a history of severe ataxia, head tilt, circling, bilateral gluteal muscle atrophy, polydipsia, and urinary incontinence. CSF analysis showed neutrophilic pleocytosis, elevated protein, and was negative for EHV-1. Bloodwork was unremarkable. Aside from a small nodule in the pituitary gland, the brain was grossly normal. Histopathology revealed severe, multifocal, histiocytic and lymphoplasmacytic meningoencephalomyelitis and hypophysitis with numerous intralesional rhabditiform nematodes morphologically consistent with *Halicephalobus gingivalis* in the brain, cervical spinal cord, and pituitary gland.

### **Conclusions**

This case demonstrates *Halicephalobus gingivalis* infection as the underlying cause of acute neurologic signs in a horse. Identification of intralesional nematodes in the brain, spinal cord, and pituitary gland highlights the parasite's affinity for neural tissue and supports its inclusion as a differential diagnosis in horses with neurologic disease, particularly when CSF cytology shows neutrophilic inflammation.

#### **61: PRESUMED THYMIC CARCINOSARCOMA IN A GERMAN SHEPHERD DOG**

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A 10-year-old male neutered German Shepherd presented on emergency to Veterinary Specialty Center for significant weight loss and abnormal breathing. Thoracic radiographs, thoracic ultrasound, and CT scan revealed a cranial mediastinal mass (17cm x 12cm). On cytologic evaluation, a population of moderately atypical, spindloid to polygonal cells arranged primarily in groups with indistinct cellular borders was present. Neoplasia was suspected, but neither epithelial nor mesenchymal origin could be confirmed; given the location, a spindloid thymoma was offered as a differential diagnosis. Excisional biopsy of the mass revealed a malignant neoplasm of undetermined origin with positive immunoreactivity to cytokeratin, vimentin, and PAS along with negative staining for S100, factor VIII, and thyroglobulin. The neoplastic cells also displayed positive cytoplasmic labeling for WT-1, which was of undetermined significance. Based on these results and the location of the mass, both biphasic mesothelioma and thymic carcinosarcoma remain differential diagnoses with thymic carcinosarcoma being considered more likely due to the presence of PAS-positive granules within the neoplastic cells. This case demonstrates a challenging diagnosis of a cranial mediastinal mass. Excisional biopsy with immunohistochemistry led to a suspected diagnosis of thymic carcinosarcoma. Thymic carcinosarcomas (or thymic sarcomatoid carcinomas as described in human medicine) are incredibly rare neoplasms in humans and animals, with only a few cases reported in veterinary literature. These tumors pose a diagnostic challenge given the variable appearance of the neoplastic cells on both cytologic and histologic examination. In such cases, immunostaining is important for exclusion of more common neoplasms of the cranial mediastinum.

#### **62: HEPATIC TREMATODIASIS IN EXOTIC HOOFSTOCK: RETROSPECTIVE INSIGHTS FROM 13 CASES**

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Hepatic trematodiasis mediated by *Fasciola* and *Fascioloides spp.* affects ungulates worldwide. While cervids act as definitive tolerant hosts and cattle often harbor subclinical infections, small ruminants and camelids typically have higher mortality. Data on captive exotic hoofstock remain scarce to date.

Between 2018 and 2024, postmortem samples from 12 antelopes (common eland, kudu, wildebeest, blackbuck) and one exotic bovid (n=13) were submitted to the Alabama State Diagnostic Laboratory (5 complete necropsies, 8 tissue submissions). All cases originated from a single property, or related source. Reported signs included weight loss, self-isolating behavior, and acute mortality, with most cases occurring in spring and early fall.

Gross lesions included poor body condition, perihepatic hemorrhage with black fluke exhaust tracts and, less commonly, fibrinous peritonitis and pericarditis. Livers were frequently nodular and fibrotic with hemorrhagic and/or pigmented cysts; adult flukes were grossly evident in three animals. Histopathology of the liver revealed fibrosing hepatitis, necrosis, abundant fluke eggs and pigment. Pulmonary migration was also documented (7/13, 54%). *Clostridia spp.* fluorescent antibody staining on formalin-fixed paraffin-embedded liver sections was negative for all cases, except for *C. septicum* detected in one animal. Five animals had concurrent gastrointestinal nematodes and coccidia.

These findings reveal that captive antelopes are susceptible to hepatic trematodiasis, characterized by heavy parasite burden, chronic active migration tracts, liver necrosis and hemorrhage, and frequent pulmonary migration. This presentation appears intermediate between cattle and small ruminants yet distinct from cervids. This data offers insight to some challenges presented by housing exotic hoofstock in the United States.

### **63: HISTOMORPHOLOGICAL CHARACTERIZATION OF PARASITES IN PENNSYLVANIAN BIG BROWN BATS (*EPTESICUS FUSCUS*)**

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**Background:** The conservation of post-epidemic white nose syndrome bat populations requires an understanding of ongoing pathogenic threats and their associated health consequences. Yet, little is known about the diversity of pathogens affecting hibernating bats, particularly as it relates to parasites. While parasitological examination of guano may identify intestinal-associated parasite life stages, histomorphological characterization of host-parasite interactions can help differentiate between pathogenic and incidental organisms. This may inform the diagnostician of their clinical importance, particularly in hosts as small as bats where gross recognition, isolation, and identification of parasites is challenging.

**Objective:** The primary aim of this study was to characterize common parasites of big brown bats (*Eptesicus fuscus*) seen via histopathological examination of bats submitted to a diagnostic pathology service. All bats had skin, viscera including intestines, and heads sectioned for routine histopathology.

**Results:** The most common parasites included intestinal cestodes (n=10; 32%) and trematodes (n=5; 16%), and nematode larvae within the lymphatics and/or subcutis of

the head (n=7; 23%). In fewer cases there were mites on the skin/wing (n=1; 3%), intestinal protozoa (n=1; 3%), and renal protozoa (n=1; 3%). All bats with visible parasites had other significant and presumably unrelated causes of morbidity and mortality.

**Conclusions:** This study highlights the prevalence of helminths commonly found in big brown bats in North America that may be encountered during routine histopathological evaluation. Future aims include molecular characterization of the nematodes as well as determining a potential relationship between parasitism and individual bat health as well as that of broader bat populations.

#### **64: DON'T LET IT SLIDE: A COMPARISON OF FOUR FIXATION METHODS FOR OPTIMAL CANINE AND FELINE BILE CYTOLOGY**

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**Background:** Bile viscosity and composition makes adequate glass slide fixation difficult. The need for reliable preparation is heightened by limited sample size, collection difficulty, and cost. Three suggested alternatives to air-drying are: methanol, acetone, and CytoPrep™. However, documentation of comparative fixation procedures is limited.

**Objective:** Determine which fixation method best preserves bile and if this alters content.

**Methods:** Smears were prepared from 10µL of surplus bile submitted for cytological evaluation from forty-eight veterinary patients. Slides were air-dried or pre-fixed with acetone, methanol, or CytoPrep™ solution before automated modified aqueous Wright-Giemsa staining, cover-slipping, and scanning. Virtual slide images were opened in Adobe Photoshop and cropped to a standardized size. The amount of material fixed on the slides was quantified using the Color Range tool, applying a preset fuzziness setting. The percent slide coverage was calculated by comparing the selected area to the unselected background. Air-dried and pre-fixed slides were then blinded and evaluated microscopically by a single investigator (SAN).

**Results:** Compared to air-dried slides, methanol showed more bile retention ( $p<0.001$ ) than CytoPrep™ ( $p=0.0004$ ) or acetone ( $p>0.9999$ ). Cytologically, methanol-pre-fixed slides subjectively contained significantly more overall material, mucus, epithelial cells, crystals, microliths, and bile pigment. The described bacterial morphology also differed occasionally. Crystal, inflammatory cell, and bacterial abundance exhibited no significant differences between methods. However, insufficient bile in one pair of air-dried slides hindered visualization of bacteria that was otherwise observed in the preparations pre-fixed with methanol.

**Conclusions:** A five-minute methanol submersion prior to staining best preserves bile and cytological content.

## **65: INTEGRATING BILE CYTOLOGY, LACTATE, AND MOLECULAR MARKERS IN THE EVALUATION OF CANINE AND FELINE HEPATOBILIARY DISORDERS**

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**Background:** Bile's harsh environment hinders cell preservation and bacterial culture, limiting documentation on the reliability of veterinary cytology, culture, and PCR. The translational validity of cytology for disease etiology or prognosis, and bile lactate's role as an inflammatory biomarker, remain unclear.

**Objective:** To evaluate the clinical utility of bile microenvironment analysis in veterinary patients with hepatobiliary disorders.

**Methods:** A retrospective study using surplus bile from 42 dogs and 29 cats submitted for cytology. Samples were classified as inflammatory, neoplastic, or non-specified, and by outcome (survival vs. non-survival) based on full clinical data. DNA was extracted for pan-bacterial, -fungal, and -Apicomplexa PCR, followed by Sanger sequencing. Lactate concentration was measured via LactatePlus™.

**Results:** Bile properties (e.g., viscosity) limited lactate measurements, reducing sample size and hindering statistical analysis. In cats, acellular bile correlated with higher survivability ( $p=0.025$ ). Inflammatory conditions often showed acellular bile ( $p=0.037$ ) or only epithelial cells ( $p=0.027$ ). Low lymphocyte count in dogs ( $p=0.044$ ) and high serum creatinine kinase (CK) in cats ( $p=0.025$ ) were negative prognostic markers. PCR identified 28 pan-bacterial, 34 pan-fungal, and 34 Apicomplexa-positive samples; bacterial culture found 16 positives. Sequencing is underway to identify pathogens and rule out contamination. Median bile lactate was  $<0.3$  mmol/L in dogs and  $0.8$  mmol/L in cats.

**Conclusions:** Inflammatory disease in cats may present with cytologically unremarkable bile, a positive prognostic indicator. Canine lymphopenia and high feline CK are negative prognostic markers. Pathogen detection should combine cytology, culture, and PCR. LactatePlus™ measures bile lactate, but results are sample-dependent.

## **66: CLINICOPATHOLOGIC STUDY OF FELINE OCULAR CHORIOCILARY MELANOMAS (ATYPICAL PRIMARY OCULAR MELANOMAS) - A RETROSPECTIVE STUDY**

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Feline diffuse iris melanomas (FDIMs) are the most common intraocular tumors in cats. A rarer primary ocular melanocytic tumor in cats (previously named "atypical primary ocular melanoma") with distinctive morphologic features from FDIMs has been previously reported. We aim to further characterize the clinicopathologic features of these melanomas and propose naming them feline choriociliary melanomas (FCMs). 87 cases submitted to COPLOW from 1996-2025 were included in this study. Histologic

slides, reports, and submission forms were evaluated. No sex or breed predispositions were found. The mean age of affected cats was 12.4 +/- 3.0 years. Common clinical findings included glaucoma and a pigmented intraocular mass predominantly affecting the globe's posterior segment. Histologically, tumors mostly presented as a heavily pigmented expansile mass affecting the ciliary body (81/87 cases) that infiltrated the choroid (85/87), retinal pigment epithelium (83/87), retina/subretinal space (72/87), and iris (58/87). Tumors consisted of heavily pigmented polygonal cells with oval nuclei, prominent nucleoli and low cellular atypia with occasional multinucleation. Mitotic figures were observed in 34/87 tumors, with a mean mitotic count of 4 in 10 2.37mm<sup>2</sup> fields (range 1-24). Necrosis was present in 81/87 of tumors. Glaucoma was confirmed histologically in 30/87 cases and attributed to neoplastic invasion of the iridocorneal angle.

These rarer feline ocular melanomas present as a localized mass with bland cellular features, affecting the ciliary body, choroid and retina, that should be differentiated from FDIMs. We propose a new nomenclature - feline choriociliary melanoma - to more precisely capture its distinctive characteristics.

## **67: CLINICOPATHOLOGIC FEATURES OF FELINE CEREBRAL INTRAVASCULAR LYMPHOMA**

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**Background:** Intravascular lymphoma (IVL) is a rare and aggressive neoplasm in which neoplastic lymphocytes are confined to the lumen of small vessels. Multiple organs can be affected, frequently the central nervous system. Reports in dogs are common and rare in cats.

**Objective:** Characterize six cases of feline cerebral IVL.

**Methods:** Retrospective searches were performed at four diagnostic laboratories. Formalin fixed, paraffin-embedded (FFPE) tissue from six cases were evaluated histologically coupled with immunohistochemistry (IHC).

**Results:** Six domestic shorthair cats ranging in age from 8 to 27 years were studied. Four cases were spayed females, with one castrated male and one intact male. Clinical signs included progressive weakness (6/6), altered mentation (5/6), ataxia (4/6), head tilt (2/6), and seizures (2/6), suggesting multifocal brain disease. Two cases had mass-like lesions noted on neuroimaging. Two animals had soft, red foci throughout the brain. In all cases, neoplastic lymphocytes occluded blood vessels of the brain with extraneural involvement in two cases. Neoplastic lymphocytes were round and large with a scant to moderate amount of cytoplasm, marked anisocytosis and anisokaryosis, variable mitoses, and were associated with thrombi that occluded the vascular lumina

and led to regional tissue infarction in 4/6 cases. Neoplastic lymphocytes in all cases were immunolabeled with CD3, with B lymphocyte and macrophage markers consistently negative.

**Conclusions:** IVL is an extremely rare and aggressive disease in cats. Similar to the dog, it can lead to CNS thrombosis and infarction. Based on IHC, all six cases were T-cell in origin.

## **68: VALIDATION OF A FLOW CYTOMETRY IMMUNOPHENOTYPING PANEL FOR EQUINE HEMIC NEOPLASIA**

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**Background:** Flow cytometry immunophenotyping is a clinical tool to classify hemic neoplasms, but validated diagnostic tests for horses are sparse. A readily available diagnostic test could improve classification, prognostication, treatment planning, and monitoring in horses. **Objective:** Validate a flow cytometry panel for equine hemic neoplasia by optimizing sample preparation and antibody concentrations. **Methods:** Whole blood from 10 healthy horses was collected by jugular venipuncture. Erythrocyte lysis time and diluent buffer additives for cell preservation were evaluated. Antibody concentrations for CD5, CD4, CD8, CD21, PanB, CD11b, and CD14 were optimized using antibody titration, stain index calculations, and user visualization. Antibody specificity was performed by assessing expected cell proportions. **Results:** A 10-minute erythrocyte lysis at room temperature sufficiently differentiated residual erythrocytes from lymphocytes via FSC vs. SSC. Cell preservation improved by adding 10% fetal bovine serum to PBS versus PBS alone. Optimal antibody dilutions were: CD4 1:10, CD8 1:10, CD5 1:100, PanB 1:10, CD21 1:15, CD14 1:10, CD11b 5 uL. Antibodies for lymphoid markers appeared specific based on the proportion of CD5 T cells (72 – 83 %) compared to the sum of CD4 plus CD8 T cells (66 – 82%). Total B cells, a sum of CD21+ and PanB+ events (13 – 32 %), aligned with CD5- lymphocytes (17 – 28 %). Myeloid markers (CD11b, CD14) showed unacceptable non-specific binding. **Conclusions:** Lymphoid markers appear to reflect expected proportions of subtypes in circulation. Future research includes confirmation of CD marker specificity via cell sorting, microscopic evaluation, and further troubleshooting of myeloid markers.

## **69: PARVOVIRAL ENTERITIS IN NORTH AMERICAN RACCOONS (*PROCYON LOTOR*)**

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**Background:**

Parvoviral enteritis causes morbidity and mortality in young raccoons (*Procyon lotor*) in North America. While histopathologic similarities with domestic species are noted, detailed characterization remains limited.

#### Objectives:

To evaluate gross, histologic, and viral antigen distribution and quantity in cases of parvoviral enteritis in North American raccoons.

#### Methods:

Retrospective evaluation of gross and histologic alterations was performed in n=16 cases (2016-2025). The cohort included 4 females (25%) and 12 males (75%), with a median age of 3.5 months (range: 6 weeks-2 years). Parvovirus infection was confirmed via immunohistochemistry (14/16), fluorescent antibody exam (1/16) or electron microscopy and PCR (1/16). Clinical records and intestinal bacterial cultures were available for 7/16 animals. Whole genome sequencing was performed on 1 case.

#### Results:

Dehydration enophthalmos was observed in 7/16 animals. Necrotizing enteritis was present in all cases, with 10 (62.5%) exhibiting concurrent colitis. Enteritis scores (0–4) had a median of 4 (IQR: 4–4), 15/16 (93.75%) were scored as severe. Lymphoid depletion (median of 3 IQR: 3–3.87), primarily affected spleen and mesenteric lymph nodes in 12/16 cases. Viral antigen (median of 3.5 IQR:2-4), was most prominent in crypt and colonic glandular epithelium. All cultured intestinal samples yielded heavy growth of commensal enteric bacteria (*Escherichia coli*, *Streptococcus canis*), suggesting opportunistic growth. Sequencing revealed >99% homology to Canine Parvovirus 2 (CPV\_86\_2c).

#### Conclusion:

Parvoviral enteritis in raccoons is characterized by severe intestinal lesions and viral antigen distribution that resembles infection in other domestic species with implications for wildlife rehabilitation, vaccination strategy, and CPV-2 ecology.

## **70: GROSS AND HISTOPATHOLOGIC LESIONS OF SYNANTHROPIC RODENTS OF INTERIOR ALASKA AND UTILITY IN GUIDANCE OF DISEASE SURVEILLANCE**

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Emerging zoonotic diseases are of the utmost importance as they pertain to human and animal health. Small mammals, especially rodents, vector many of these diseases. In Alaska, voles are the predominant rodent species; red-backed voles, meadow voles, and tundra voles are all commonly found in close contact with both people and domestic animals in Interior Alaska. Despite this, little is known about their capacity to vector many zoonotic diseases. The objective of this study is to evaluate voles for the presence of zoonotic diseases and associated gross and histopathological lesions. 50 voles were trapped from across Interior Alaska from areas at the domestic animal - wildlife interface, including sled dog kennels, farms, and wetlands. All voles were humanely euthanized, and necropsies were performed. Upon necropsy, 30/50 voles showed gross lesions including small multifocal white lesions on the liver (5/50), moderately sized tan lesions with undulating borders on the lungs (2/50), subcutaneous abscesses (2/50), and a mass within the abdominal cavity (1/50). Sections of all major organs were examined histologically, and fresh tissues and serum were collected for ancillary diagnostic testing. Parasitism was prevalent, and included ectoparasitism with

fleas, hepatic trematodes, pulmonary nematodes, and intestinal and hepatic cestodes. Ancillary diagnostic tests will be performed to survey for presence of highly pathogenic avian influenza, SARS cov-2, *Leptospira*, and *Bartonella* and results will be correlated with gross and histopathological findings. These efforts will help detail the vole's role as a vector of zoonotic diseases in the Alaska landscape.

## **71: THE PATHOLOGY OF EASTERN EQUINE ENCEPHALITIS IN FREE-RANGING AVIFAUNA IN THE EASTERN UNITED STATES**

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Eastern equine encephalitis virus (EEEV) is a mosquito-borne alphavirus that can cause neurological disease in avian and mammalian species. While passerines are considered subclinical virus-amplifying hosts, disease manifestations in other wild birds remain less well documented. To investigate the spectrum of EEEV-induced lesions in wild avifauna, we reviewed cases submitted to wildlife diagnostic laboratories confirmed as EEEV-positive by virus isolation and/or PCR. Identified cases comprise wild turkeys (WITU; n=4) and raptors (n=6) including the barn owl (ABOW), great horned owl (GHOW), Cooper's hawk (COHA), red-shouldered hawk (RSHA), osprey (OSPR), and bald eagle (BAEA). Three WITU showed lymphoplasmacytic meningoencephalitis, while one had suspected brain inflammation based on perivascular cuffing despite autolysis. Visceral lesions were also common in WITU and included interstitial nephritis (n=2), myocarditis (n=1), and splenic necrosis (n=1), while two also had avian pox. All four WITU had at least one extraneural (dermal or visceral), EEEV-induced lesion. In contrast, raptors were more likely to show isolated CNS involvement without visceral lesions. Classic EEEV-associated meningoencephalitis was observed in ABOW, COHA, and BAEA, and included lymphoplasmacytic and heterophilic inflammation, neuronal necrosis, and gliosis. RSHA had myocarditis without brain lesions. EEEV was detected in OSPR and GHOW (by PCR and virus isolation) but tissue autolysis and limited availability precluded histopathology. These findings highlight species-specific variation in lesion patterns, with WITU showing more systemic involvement and raptors exhibiting primarily neurotropic disease. Recognizing key histologic lesion patterns across differing avian species may improve diagnostic accuracy and thus strengthen EEEV surveillance efforts in free-ranging avian populations.

## **72: DEVELOPING AN AI IMAGE ANALYSIS MODEL TO QUANTIFY PULMONARY VASCULAR AND STROMAL CHANGES IN CATS WITH HYPERTROPHIC CARDIOMYOPATHY**

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Artificial intelligence (AI) models provide pathologists with new opportunities to quickly gather data on quantifiable disease changes. In humans, chronic hypertrophic cardiomyopathy (HCM) is associated with progression to pulmonary hypertension and pulmonary interstitial changes including increased collagen deposition and venous remodeling. While pulmonary hypertension is not often clinically recognized in feline HCM, potential pulmonary vascular and interstitial remodeling analogous to that in human disease provides new diagnostic opportunities and the possibility of an animal model for human HCM. We hypothesize that cats with clinical HCM will have interstitial pulmonary vascular and stromal remodeling secondary to chronic left-sided congestive heart failure. Our specific aims are to 1) develop an AI-based image analysis algorithm that will quantify pulmonary interstitial collagen and assess vascular remodeling and, 2) quantify pulmonary vascular and interstitial changes in cats with clinical HCM as compared to non-HCM cats. We predict that the presence of pulmonary interstitial collagen deposition and vascular remodeling will positively correlate with severity of clinical HCM as determined by clinician-provided medical history. By furthering our understanding of the pathogenesis of HCM, we could potentially inform clinicians on treatment approaches and utilize this species as an animal model.

### **73: IMPACT OF BLOOD SAMPLING SITE ON HEMATOLOGIC PARAMETERS IN ASIAN COMMON TOADS WITH IMPLICATION FOR FIELD RESEARCH**

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Amphibians are one of the most threatened groups due to climate change and habitat loss, and veterinary medicine plays a key role in their conservation. This study was performed in collaboration between Iowa State University College of Veterinary Medicine and Omaha's Henry Doorly Zoo & Aquarium, an institution which contributes significantly to the captive breeding and release of several endangered amphibian species. The objective was to compare the accuracy and reliability of cardiac versus peripheral blood samples, to provide guidance regarding the most appropriate and least invasive sample technique for researchers in the field. Blood was obtained from 55 Asian Common Toads via cardiocentesis and the venipuncture of the medial saphenous vein to evaluate hematologic parameters. Packed cell volume, leukocyte estimate, and thrombocyte estimate were significantly increased in the medial saphenous samples when compared to cardiocentesis samples. All other parameters analyzed were not significantly different between the collection methods. Necropsy identifies a larger proportion of toads suffered from cardiac trauma related to cardiocentesis, indicating peripheral sampling as the safer and less invasive method for animals that would be released back into the field after collection. Based on these findings, the medial saphenous vein appears to be the superior and recommended blood sampling site for improved hematologic evaluation and decreased patient morbidity.

#### **74: RENAL DYSPLASIA IN A 6-MONTH-OLD MALE LABRADOR RETRIEVER DOG.**

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A 6-month-old male intact Labrador Retriever was euthanized and submitted for necropsy after chronic weight loss, lethargy, anorexia, vomiting, and biochemistry abnormalities consistent with renal dysfunction. At post-mortem, kidneys were of normal size but misshapen, pale-tan and nodular. Microscopic findings included disorganized parenchyma with cortical structures located within the medulla, small immature glomeruli, occasionally hypertrophic glomeruli with dilated Bowman's spaces, persistent primitive mesenchyme, and metanephric ducts lined by tall pseudostratified columnar epithelium. Renal tubules showed mild to moderate dilatation and occasional protein casts. Small areas of interstitial nephritis as well as tubular nephrocalcinosis were noted. Additionally, there was pulmonary congestion and edema and abundant frothy fluid within the trachea and bronchi. Findings were interpreted as consistent with those of renal dysplasia, a congenital abnormality involving altered differentiation and abnormal development of the kidney. Although the pathogenesis of renal dysplasia is unclear, the altered differentiation of renal tissue and persistence of immature structures results in progressive juvenile-onset chronic kidney disease. Renal dysplasia typically presents with small kidneys and results in earlier fatality if the lesions are bilateral. Renal dysplasia is recognized as a familial inherited condition in specific breeds like Lhasa Apso and Shih Tzu dogs, but it is observed sporadically in many other breeds in which inherited or environmental etiologies are suspected. Renal dysplasia has rarely been reported in Black Labrador Retrievers but should be included in the differential diagnosis for Labrador puppies presented with clinical signs consistent with progressive renal disease.

## **75: UNDIFFERENTIATED PLEOMORPHIC SARCOMA IN A BABOON (PAPIO SPP.)**

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Spontaneous tumors of soft tissue are uncommon in non-human primates (NHP). A seven-year-old male baboon was euthanized due to a firmly adherent mass on the dorsal aspect of the tail base, resulting in malalignment of the tail vertebra. Histopathology revealed a subcutaneous non-encapsulated mass with marked cellular pleomorphism. The tumor had was composed of a predominant population of spindle cells and scattered polygonal to multinucleated cells supported by varying amounts of fibrovascular stroma. There was no metastasis nor involvement of the tail bones based on radiologic and histologic examination. The tumor had diffuse strong immunolabeling with vimentin, with few immunopositive immunolabeling for CD68, desmin, and muscle-specific actin; and negative for CD31. Complex histological and immunolabeling patterns point to mixed cell origin, supporting a diagnosis of undifferentiated pleomorphic sarcoma. Undifferentiated pleomorphic sarcomas are typically diagnosed by exclusion, which presents a diagnostic challenge.

## **76: EMERGING EVIDENCE OF MYCOPLASMA BOVIS AS A MULTI-HOST PATHOGEN IN NORTH AMERICA.**

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*Mycoplasma bovis* is a commonly recognized pathogen of cattle that contributes to the bovine respiratory disease complex and causes polyarthritis in feedlot calves and mastitis in dairy cattle. Recent detections in bison (*Bison bison*), free-ranging pronghorn (*Antilocapra americana*), and in isolated cases of mule deer (*Odocoileus hemionus*) demonstrate a broader host range. However, the pathogenesis of *M. bovis* in wildlife remains poorly understood. We present evidence that *M. bovis* is an emerging multi-host pathogen in North America, with documented spillover across the wildlife-livestock interface. We describe gross and histologic lesions in affected individuals with *M. bovis* and compare species-specific patterns of disease. Grossly, fibrinous pleuropneumonia was observed in all three wildlife species. Lung lesions in pronghorn were most acute, characterized by severe, fibrinosuppurative pneumonia with caseonecrotic foci in bronchi and bronchioles. Bison often present with chronic lung lesions, including lung abscesses and occasionally with necrotizing pharyngitis. A documented case of *M. bovis* in a mule deer revealed coalescing foci of acute coagulative necrosis and septal necrosis of the alveoli. These cases highlight the severity of pulmonary mycoplasmosis in wildlife and the potential for high mortality. The emergence of *M. bovis* as a multi-host

pathogen highlights the need for further investigation into the pathogenesis and the impacts on wildlife health to inform disease management at the wildlife-livestock interface.

## **77: SPURIOUS EQUINE BIOCHEMISTRY RESULTS DUE TO SODIUM IODIDE SAMPLE CONTAMINATION**

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<sup>1</sup>University of Missouri College of Veterinary Medicine, <sup>2</sup>University of Missouri Veterinary Medical Diagnostic Laboratory, Columbia, MO, USA

A 7-year-old Trakehner mare was presented to the University of Missouri Veterinary Health Center, Equine Medicine Service for the complaint of sinus fluid and drainage. CBC and plasma biochemistry results were unremarkable at presentation. Guttural pouch mycosis was diagnosed via endoscopy and cytologic evaluation. Treatment included endoscopic application of topical clotrimazole and IV administration of flunixin meglumine, potassium penicillin G, gentamicin, and sodium iodide. Blood submitted for repeat hematologic and biochemical assessment on day 8 of hospitalization revealed surprising results. Most notably, chloride concentration (159 mEq/L, RI: 96-105) exceeded that of sodium (148 mEq/L, RI 132-141). A negative anion gap was present (-25, RI 8-15), and proteins, minerals, glucose, urea, and creatinine were half or less their original concentrations. These findings were compatible with sodium iodide and saline contamination of the specimen. The clinician confirmed the sample was drawn from the IV catheter line where medications were being administered. Halide administration can result in artifactual hyperchloremia because of ion selective electrode (ISE) inability to distinguish chloride from other elements with similar properties (bromide, iodide). Pseudohyperchloremia is a well-recognized phenomenon in patients receiving potassium bromide therapy. This case highlights the risk of similar interference in samples contaminated with sodium iodide.

## **78: SEVERE INTERSTITIAL PNEUMONIA AND MULTINODULAR FIBROSIS WITH SYNCYTIAL CELL FORMATION IN A JUVENILE DONKEY**

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**Background:** A 12-month-old jack donkey "Nacho" was submitted for necropsy to the University of Illinois Veterinary Diagnostic Lab in fair nutritional condition with minimal decomposition, after dying of progressive respiratory disease.

**Methods:** A diagnostic necropsy was performed. Tissues were fixed in formalin and routinely processed before (H&E) staining. An equine viral respiratory panel was tested using a real-time PCR.

**Results:** The lungs were poorly collapsed, firm, and rubbery, with disseminated, tan, soft miliary nodules and 1cm wide, fibrous foci at random. Histopathologic examination of the lungs revealed suppurative bronchiolitis with epithelial hyperplasia and necrosis in the terminal bronchioles with the formation of syncytial cells. There was also type II pneumocyte hyperplasia, mild alveolar fibrosis, and areas of diffuse alveolar damage.

The stomach and colon showed mild, chronic inflammation, and the kidneys contained mild tubular hyaline casts. The PCR panel was negative for Influenza A virus, equine arteritis virus, and equine herpesviruses 1 and 4. Aerobic culture of the lung tissue yielded no significant bacterial growth.

**Conclusion:** The combination of severe interstitial pneumonia and syncytial cell formation, in the absence of a bacterial cause or common equine viruses, is similar to reports of infection with an asinine-specific gammaherpesvirus, such as AsHV-4 or AsHV-5. While uncommon, this condition is similar to the presentation of equine herpesvirus 5. Further specific testing for asinine herpesvirus is being pursued.

## **79: CHARACTERIZATION OF GLOMERULAR AND RENAL LESIONS IN CHAMELEONS**

Evelyn Sullivan, Sarah Linn-Peirano

University of Tennessee College of Veterinary Medicine, Knoxville, TN, USA

**Background:** Histopathologic evaluation of reptile kidneys frequently identifies degenerative changes to the tubulointerstitial compartment, including urate tophi (gout) deposition, mineralization, tubular degeneration, fibrosis, or infectious agents. However, documentation of glomerular pathology is uncommon.

**Objectives:** 1) To identify glomerular disease in chameleons and determine potential pathogenesises for glomerular pathology. 2) To further document renal lesions in routine chameleon necropsy cases.

**Methods:** Necropsy records from chameleons submitted to the University of Tennessee College of Veterinary Medicine Anatomic Pathology Service between 2004 and 2024 were obtained and 27 cases met inclusion criteria (kidneys present for histologic evaluation). H&E, PAS, and Masson's Trichrome stains were evaluated for all cases. Glomerular lipidosis and glomerular melanin were quantified as a percentage based on number of glomeruli affected versus unaffected. Renal gout was determined based on presence of urate tophi and quantified based on the percentage of kidney affected.

**Results:** 7 species of chameleon were included in the study with an age range of 6 to 48 months. There were 8 female and 13 male chameleons with 6 unspecified. Renal disease was considered the primary cause of death in 25.9% of cases. Glomerular lesions were identified in 1+ glomeruli in at least 67% of cases and included: mesangial foamy macrophages (lipidosis), lipid emboli, melanin, mineral, and sclerosis. Glomerular lipidosis was associated with yolk coelomitis in 2 cases. Renal urate deposition was present in 37% of cases.

**Conclusion:** Glomerular lesions are common in chameleons. These changes should be regularly evaluated and described to further determine their clinical significance.

## **80: ACTINOBACILLUS PLEUROPNEUMONIAE ANTIGEN INDUCES IMMUNE-MEDIATED GLOMERULONEPHROPATHY IN PIGS AND MICE**

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Azabu university, Sagamihara, Japan

### **Background**

*Actinobacillus pleuropneumoniae* (App) infection is implicated in mesangial proliferative glomerulonephritis (MPGN) in pigs, which morphologically resembles human IgA nephropathy. Although infection may contribute to the pathogenesis of such glomerulopathies, the mechanisms remain unclear. This study investigated App antigens that react with antibodies deposited in the glomeruli and established a murine model of App antigen-induced nephropathy.

### **Methods**

Kidneys from App-infected pigs were examined histologically and by immunofluorescence. Antibodies extracted from porcine glomeruli against App lysates were analyzed by Western blotting, while reactive antigens were analyzed by mass spectrometry. DDY mice were inoculated intraperitoneally with inactivated App multiple times and underwent necropsy after euthanasia. Kidneys and lymphoid organs, including the spleen, lymph nodes, and peritoneal fat-associated lymphoid clusters (FALCs), were histologically analyzed.

### **Results**

The pigs exhibited MPGN, with IgG, IgA, and IgM deposition in the glomerular mesangial region. Antibodies extracted from the glomeruli reacted with the soluble App antigens, which were approximately 80–90 kDa long, on Western blotting. The reacted proteins were identified on mass spectrometry as RTX-III A and RTX-II A. The mice exhibited glomerular enlargement, along with deposition of C3, IgM, and IgA in the mesangial region. A high degree of plasma cell aggregation accompanied by Mott cells was observed in FALCs.

### **Conclusion**

The study findings indicate that specific App antigens induce glomerulonephropathy in pigs and mice. Furthermore, the FALCs may serve as antibody production sites. This cross-species induction supports the role of pathogen antigens in immune-mediated glomerular disease.

## **81: TOLL-LIKE RECEPTOR AGONISTS INDUCE CANINE OSTEOSARCOMA CELL DEATH**

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Osteosarcoma is a highly aggressive primary bone tumor in dogs, with a median survival time of 10-12 months despite standard-of-care treatment consisting of surgery and chemotherapy. Most patients die from metastatic disease, and few improvements in

treatment have been made in decades. Bacteria-based immunotherapies have shown some promise in shrinking other tumor types by initiating an anti-tumor immune response to prolong patient survival. Bacteria express conserved microbial molecules that are recognized by innate immune receptors such as toll-like receptors (TLRs). TLR agonists may also enhance anti-tumor immunity and tumor regression. We hypothesized that TLR2 agonists (lipoteichoic acid from *Staphylococcus aureus* (LTA-SA)), TLR3 agonists (polyinosinic-polycytidylic acid (PolyIC) and NexaVant (NVT)), TLR4 agonists (lipopolysaccharide (LPS)), TLR7/8 agonists (imiquimod (IMQ) and resiquimod (R848)), and TLR9 agonists (ODN 2395) would kill osteosarcoma cells *in vitro*. Canine osteosarcoma cells were incubated with each agonist and viability was assessed with a biochemical cell viability assay and a live/dead microscopy assay. PolyIC, NVT, and LPS induced osteosarcoma cell death. IMQ had a dose-dependent effect on osteosarcoma cell viability. No effect was observed with TLR2 or TLR9 agonism. These findings suggest a role for TLR3, 4, and 7/8 in mediating osteosarcoma cell death, uncovering a potential new therapeutic target for canine osteosarcoma.

## **82: A GENOMIC AND TRANSCRIPTOMIC ANALYSIS OF CANINE SMALL CELL B CELL LYMPHOMA**

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Canine small cell B cell lymphoma (BCL) is a heterogeneous and poorly characterized disease. These tumors account for ~12% of B cell tumors diagnosed by the CSU Clinical Hematopathology Lab, but little is known about their pathogenesis and their relevance as a human disease model. This project aims to characterize canine small BCL to improve diagnostics and treatments for dogs, while also evaluating its potential as a preclinical translational model for human BCL. We performed whole exome sequencing (n=125) and bulk RNA sequencing (n=121) on lymph node aspirates from dogs with small BCL, diagnosed by flow cytometry, to identify mutations and pathways involved in disease pathogenesis.

The majority of our samples fell into two k-means defined clusters by transcriptomic analysis, while a smaller subset of samples (n=12) clustered with canine large BCL samples. These two prominent clusters of small BCL (n=70 and 39), show different mutational profiles, clinical features, predominant breeds, and pathway enrichment patterns. Cluster 1 (n=70) is characterized by upregulation of NF- $\kappa$ B signaling, inflammatory responses, and B cell receptor signaling. Cluster 3 (n=39) is characterized by PI3K-AKT-mTOR signaling, higher rates of lymphocytosis, and high Ki67 positivity. Our data suggest that on a molecular level, there are at least two subsets of canine small BCL, potentially with different cells of origin and driven by different oncogenic signaling mechanisms. These data support the need for further classification of these different tumor subtypes given their potential differences in outcome, treatment response, and relevance as a pre-clinical translational model for human BCL.

### **83: BPV-2 DETECTED IN MALIGNANT FIBROPAPILLOMA OF AN ANGUS BULL**

Brooke Turley, Kristen Shekelle, Bridget Savitske, Shannon Reed, Brian Porter  
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A four-year-old Angus bull presented for progressive weight loss of five months' duration. Physical examination revealed poor body condition, ptyalism, and an ulcerated mass along the lingual aspect of the left mandible. Due to poor prognosis for complete surgical excision, humane euthanasia was elected. At necropsy, the oropharyngeal mass was large, multilobulated, and firm, with dorsal and lateral extension into the soft palate, left caudal maxilla, and left mandibular ramus. Multiple exophytic, firm, irregular papillary masses were present in the rumen, the largest arising from the ventral aspect of the left longitudinal pillar. On cut section, both oropharyngeal and ruminal masses were firm and nodular, containing pockets of white to pale-yellow viscous fluid. Histologic evaluation of both masses supported a diagnosis of fibropapilloma. In the oropharyngeal mass, neoplastic spindle cells infiltrated the underlying salivary gland tissue and exhibited marked cellular atypia. PCR amplification and Sanger sequencing of episomal L1 DNA from frozen oropharyngeal tissue was analyzed by BLAST using the NCBI database and reported 100% identity with L1 of Bovine Papillomavirus Type 2 (BPV-2).

Although BPV-2 is frequently reported in bovine ruminal fibropapillomas, its presence in an invasive oropharyngeal fibropapilloma appears novel. Members of *Xipapillomavirus* such as BPV-4 are known to contribute to the malignant progression of alimentary papillomas into squamous cell carcinomas in conjunction with bracken fern ingestion. However, *Deltapapillomaviruses* like BPV-2 have not been reported in malignant oral fibropapillomas. This case raises the possibility that *Deltapapillomaviruses* have the potential to contribute to malignant transformation of mesenchymal oral papillomas.

### **84: VALIDATION AND TEMPORAL ANALYSIS OF DIFFERENTIALLY EXPRESSED MICRORNAS IN HORSE SERUM FOLLOWING INTRAVENOUS LIPOPOLYSACCHARIDE ADMINISTRATION**

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**Background:** Sepsis remains one of the leading causes of mortality in horses and presents significant diagnostic challenges, particularly in its early stages. MicroRNAs (miRNAs), small non-coding RNA molecules that regulate gene expression, offer a promising avenue for early detection of sepsis before clinical signs become evident. However, limited research exists on the role of miRNAs in equine sepsis. Our preliminary analysis identified several miRNAs that were differentially expressed in horse serum in response to lipopolysaccharide (LPS) challenge through small RNA sequencing.

**Objective:** To validate and investigate temporal changes in previously identified

differentially expressed miRNAs in horse serum following LPS infusion.

**Methods:** Eight healthy horses received intravenous LPS to induce systemic inflammation. EDTA plasma samples were collected at baseline and at 1, 6, 12, and 24 hours post-infusion. RNA was extracted using a column-based method, and absolute quantification of selected miRNAs was performed via digital PCR. Expression patterns were analyzed over time.

**Results:** MiR-145 and miR-126-3p showed significant upregulation following LPS infusion, peaking at early time points. Pathway enrichment analysis of miRNA target genes revealed the ErbB signaling pathway as the most enriched, suggesting its involvement in immune modulation during endotoxemia.

**Conclusions:** Circulating miRNAs exhibit dynamic expression changes in response to LPS-induced inflammation and may serve as biomarkers for the early detection of sepsis in horses. Further research is needed to validate these candidates in naturally septic cases. Ultimately, understanding miRNA dynamics in sepsis could contribute to faster and more accurate diagnostics, as well as improved outcomes, for equine patients.

## **85: FUNGAL ENCEPHALITIS IN A NEONATAL CALF WITH MORPHOLOGY AND ANGIOINVASIVENESS SUGGESTIVE OF IN-UTERO ZYGOMYCETES INFECTION**

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A 7-day old Hereford heifer was presented to the Colorado State University Livestock Medicine service with progressive neurological signs. The calf briefly stood after a normal birth and was unable to nurse. Euthanasia was elected due to poor prognosis and worsening central neurological signs and the calf was presented for postmortem examination. On gross examination, multifocal areas of petechiae were present on the surface of the cerebrum. On cut section, increased amounts of cerebrospinal fluid oozed from a dilated lateral ventricle and multifocal regions of cavitation and malacia with hemorrhage were observed throughout the cerebrum and midbrain, most severe at the ventral aspect. Histopathologically, extensive necrosis was found within the cerebrum and midbrain, surrounded by pyogranulomatous inflammation. Within areas of necrosis were numerous, multifocally angioinvasive fungal hyphae with nonparallel walls, bulbous projections, irregular septation, and acute angle branching which resembled a saprophytic fungal species such as *Mucor* or *Rhizopus*. Additional findings included moderate acute interstitial pneumonia with edema and a patent urachus. While fungal PCR or culture is needed for definitive diagnosis and is pending, fungal organisms in this case most closely resemble Zygomycetes. Reports exist of fungal pneumonia or gastrointestinal fungal infection in the dam leading to mycotic metritis and subsequent encephalitis or pneumonia in the neonate. For example, there are many reports of *Mortierella wolfii* causing fatal fungal disease in neonates. The fungal morphology, presence of angioinvasion, and the young age of this calf at the time of presentation suggest that this may represent in-utero transmission.

## **86: MYXOID MYOFIBROBLASTIC FIBROSARCOMA OF THE CALCANEAL TENDON IN A DOG**

Hannah Vander Ploeg, Wes Baumgartner

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**Background:** A recurrent soft tissue sarcoma from the distal calcaneal tendon of a 9 year old spayed mixed dog was examined. The mass infiltrated the tendon and sheath, extending into the subcutis. Cells were arranged in storiform fascicles in a finely collagenous and edematous stroma, occurring as solid nodules and finely villous arrays. There were spindle cells with fine pink fibrillar cytoplasm and large ovoid nuclei with fine chromatin and a large central nucleolus. Mitoses were 1 in 2.37mm squared. Many cells were multinucleated and pleomorphic, with intracytoplasmic vacuoles and tangled fibrillar pink cytoplasm.

**Methods/Results:** Additional immunohistochemical (IHC) and special stains: Neoplastic cell positive reactivity for: Vimentin, Pancytokeratin, Smooth muscle actin, S100, Collagen IV, Alcian blue pH2.5. Minimal Alcian blue positive matrix.

Neoplastic cells lacked reactivity to: CD18, Iba1, GFAP, Desmin, MelanA, PNL2, Cytokeratins 5&14, Periaxin, P63, CNPase, and nerve growth factor.

**Conclusions:** The top differentials for a tendon sheath neoplasm with giant cells are histiocytic sarcoma, and giant cell tumor of the tendon sheath (GCT). Our data rule out histiocytic sarcoma, and the descriptions of GCT in dogs are few – though our findings are similar to a report which had limited IHC data. The presence of many multinucleated giant cells with large cytoplasmic vacuoles that contain alcian-blue positive mucin (pseudolipoblasts) is unusual, and are characteristic of myxofibrosarcomas. Strong actin positivity is not consistent with myxofibrosarcoma, but is consistent with myofibroblast lineage. Staining also suggests a possible nerve sheath origin; further staining and ultramicroscopy are being pursued.

## **87: LONG-LATENCY DOXORUBICIN TOXICITY IN A RAT MODEL: NON-CARDIAC LESIONS TO CONSIDER**

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**Background:** Doxorubicin (DOX) is a potent cancer chemotherapy; however associated toxicities limit clinical application. Rats are a good model for DOX toxicity studies, as similar tissues are damaged in humans given DOX treatment. Historically, in animal models, high dose, short interval models have been used, with only cardiotoxicity studied.

**Objectives:** This study aims to provide a comprehensive assessment of non-cardiac lesions induced by DOX treatment in a long-latency rat model, which translate better for human medicine.

**Methods:** Sprague-Dawley rats 6-7 weeks old with a cumulative 6 mg/kg DOX dose administered IV with 2-week intervals were necropsied after death or after euthanasia

(when fractional shortening reached  $\leq 30\%$ ). Blood was collected before all euthanasia. Kidneys, mammary masses, intestines, and testes were assessed for histopathologic lesions.

**Results:** Renal disease with a variety of histologic lesions was found in all rats given DOX. BUN and albumin were measured in a group of rats, and those values reflected the severity of the renal disease. Across three treatment cohorts, the mammary mass incidence in the DOX group was 62%, while that in the control was 11%. DOX-induced mammary tumors encompassed fibroadenomas, adenocarcinomas, and sarcomas, while the control group had only 1 fibroadenoma. Finally, enteritis, colitis, and testicular cell death were found in males.

**Conclusions:** Our study describes the range of non-cardiac toxicities from low-dose DOX treatment with a long-latency cardiomyopathy. Understanding the range of lesions possible with this DOX model allows for better evaluation of treatment success, translation to human medicine, and collaboration among researchers.

## **88: CD3+/CD20+ RETINAL LYMPHOMA AND UVEODERMATOLOGIC SYNDROME IN A DOG**

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**A 12-year-old, male, neutered, mixed breed dog was presented to a veterinary ophthalmologist for a one-month history of left-sided ocular discharge. On ophthalmic examination, both eyes were diagnosed with panuveitis, with the right eye more mildly affected. The patient also had a history of depigmentation and ulceration of the nasal planum and perinasal skin. The entire left eye and a punch biopsy of perinasal skin were submitted for histopathology. Histologic examination of the eye revealed sheets of neoplastic round cells multifocally infiltrating the retina, within the subretinal space, and multifocally subtending the retinal pigment epithelium, with suspected vascular invasion. The neoplasm was morphologically consistent with intermediate-cell lymphoma.**

**Immunohistochemistry showed strong positive reactivity to CD3 and CD20, and PCR for antigen receptor rearrangements was suggestive of a clonal T-cell receptor gene, supporting a T-cell phenotype with aberrant expression of CD20. Additional findings in the eye and perinasal skin included melanophage-rich histiocytic and lymphoplasmacytic panuveitis and lichenoid dermatitis, suggestive of concurrent uveodermatologic syndrome. Lymphoma is one of the most common ocular neoplasms in dogs, but predominant retinal involvement is rare (~1% of canine ocular lymphomas). Furthermore, retinal lymphomas in dogs typically fall under the subtype of diffuse large B-cell lymphoma. T-cell lymphomas with aberrant expression of B-cell antigens have been reported in dogs, including a case of retinal lymphoma.**

## **89: HOST ORIGIN INFLUENCES CORYNEBACTERIUM BOVIS CLINICAL PRESENTATION AND DETECTION IN ATHYMIC NUDE MICE**

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### **Background:**

In February 2021, the UC Davis sentinel mouse colony experienced an atypical outbreak of *Corynebacterium bovis* (*C. bovis*). Infected nude mice lacked skin lesions and evaded detection by PCR and culture for months, leading to unintended dissemination of *C. bovis*-positive mice to multiple vivaria.

### **Objectives:**

This study aimed to characterize the pathogenesis of this apathogenic, slow-growing *C. bovis* outbreak strain and identify genetic factors contributing to its atypical phenotype.

### **Methods:**

Athymic nude sentinel records from the outbreak period were reviewed for necropsy, histopathology, culture, and PCR findings. Culture results were used in a Fisher's Exact Test to evaluate whether co-colonization with *C. amycolatum* affected *C. bovis* growth. Whole genome sequencing of *C. bovis* isolates was performed to determine host origin. In situ hybridization (ISH) on tissue blocks was performed to identify bacterial presence and characterize bacterial distribution.

### **Results:**

The outbreak strain did not induce the hyperkeratosis and acanthosis typical of "classic" corynebacterium-associated scaling dermatitis, both grossly and histologically. Significantly fewer bacteria were detected by ISH in outbreak cases compared to classic cases ( $p = 0.0179$ ). Genomic analysis showed the outbreak strain clustered most closely with human-origin *C. bovis*. Co-colonization with *C. amycolatum* showed no significant impact on *C. bovis* growth ( $p = 0.0898$ ).

### **Conclusion:**

These results emphasize the variable pathogenicity of *C. bovis* strains and supports their anthroponozoonotic potential. Further work would be required to reevaluate *C. bovis* diagnostic methods and transmission pathways to protect the health of laboratory rodents and humans working with them.

## **90: HISTOPATHOLOGIC CHARACTERIZATION OF CLINICALLY DIAGNOSED PULMONARY ABSCESES IN DOGS AND CATS: A RETROSPECTIVE STUDY**

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Pulmonary abscesses in dogs and cats are rarely described and, as a result, etiology and pathogenesis remain poorly understood. Histopathological characterization may help elucidate these processes. The aim of this study was to characterize the histopathologic features of pulmonary abscesses. 27 cases of definitively diagnosed or suspected pulmonary abscesses were identified from Penn Vet medical records based on computed tomography, surgical, microbiological, and pathology reports. Histology slides were reviewed by a board-certified veterinary pathologist. Features assessed included distribution and severity of inflammation, necrosis, hemorrhage, fibrosis, fibrin, and granulation tissue within bronchi, bronchioles, alveolar septa and spaces, vessels, and pleura. Abscesses were defined histologically as well-demarcated necrosis with tissue destruction and predominantly neutrophilic infiltrates, surrounded by fibrosis or granulation tissue. 20/26 cases had one or more histological abscesses, pyogranulomas, or foci of encapsulated necrosis. 2/26 cases had abscesses in the adjacent tissue (mediastinum or lymph node). 3/26 cases showed necrosis, hemorrhage, and neutrophilic inflammation without discrete encapsulation. One case had severe bronchiectasis. 23/26 cases had moderate to severe, predominantly neutrophilic inflammation within the bronchi and bronchioles. In 8 of these, airway rupture extended inflammation into adjacent alveolar parenchyma. Intralesional bacteria were identified in 10, foreign material in 2 cases, respectively. 2 feline cases showed evidence of bronchiectasis and chronic lower airway disease. The pattern of inflammation suggests inhalation or aspiration and local extension of disease as the pathogenesis in most cases of pulmonary abscess in dogs and cats.

## **91: IDENTIFYING MILK PROTEIN BIOMARKERS FOR PUP HEALTH AND SURVIVAL IN SOUTH AMERICAN FUR SEALS (*ARCTOCEPHALUS AUSTRALIS*)**

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### **Background:**

Otariids (fur seals and sea lions) are a group of pinnipeds that present a unique opportunity to study immune development, as the predominant cause for neonatal mortality amongst otariid pups is infectious disease. Milk—especially colostrum—is critical to neonatal health for its role in providing maternally derived passive immunity, notably through the transfer of immune proteins. There is, however, no comprehensive milk proteome for any otariid species, or pinnipeds in general.

### **Objective:**

In this study, we aimed to 1) characterize the milk proteome and 2) identify protein biomarkers associated with pup survival in South American fur seals (*Arctocephalus australis*), one of the most widely distributed otariid species in the southern hemisphere.

**Methods:**

We sampled milk from South American fur seals (n=72) to undergo proteomic analysis by liquid chromatography-mass spectrometry (LC-MS/MS). Survival data from the corresponding pups was also collected until they reached 8-10 weeks of age.

**Results:**

We identified 356 unique proteins with 50 proteins common across all samples. Unsurprisingly, the most consistently abundant proteins were nutritional proteins, such as beta-lactoglobulin and caseins; however, significant heterogeneity was also observed across individual protein profiles. Specifically, within immune proteins, we identified those with direct immune function, such as complement, lysozyme C, IgG, and IgA. Additionally, we identified several acute phase proteins, such as fibrinogen, haptoglobin, and alpha-1-antitrypsin, providing a window into the health status of the mother.

**Conclusion:**

This work presents a unique opportunity to assess maternal health, passive immunity, and pup survival in marine mammals.

**92: UNMASKING SUGAR GLIDER PATHOLOGIES: A 20-YEAR CASE SERIES FROM THE KANSAS STATE VETERINARY DIAGNOSTIC LABORATORY**

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Sugar gliders (*Petaurus breviceps*) are increasingly popular household pets. However, current literature suggests deficiencies in characterizing their pathological spectrum. This study summarizes cases submitted to the Kansas State Veterinary Diagnostic Laboratory from 2005-2025. Out of 100 cases reviewed, 66 were identified as significant after excluding cases that tested negative for bacteria and parasites. Cases were then categorized and contextualized within existing literature. Seven categories of lesions were identified: neoplasia, infectious, metabolic, dental, respiratory, hepatopathies, and miscellaneous systemic pathologies. Of these cases, two are previously unreported. To the authors' knowledge, sebaceous carcinoma and disseminated histiosarcoma with metastasis to the lung have not been identified in sugar gliders. Other notable findings include high incidence of bacterial abscesses and periodontal disease. This case series summarizes a broad range of pathologies to guide the diagnostic approach of both clinicians and pathologists alike. It also emphasizes the importance of comprehensive necropsy and histopathologic evaluation. These insights are invaluable to the growing body of exotic mammal literature as well as to future investigative efforts.