



2024 ACVP/ASVCP Annual Meeting Accepted Veterinary Student Abstracts – Platform and Poster

Sunday, November 17, 2024

12:15 PM – 12:21 PM

PROFILING BROWN ADIPOSE TISSUE IN PRIMATES IN RESPONSE TO ADRENERGIC STIMULATION

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Brown adipose tissue (BAT) stimulation is a promising treatment option for cardiometabolic disorders such as diabetes and obesity. Beta (B)2 and B3 adrenergic receptor (AR) agonists, such as mirabegron (an oral B3AR agonist), have been proposed as a way to stimulate BAT. We aimed to measure the histological characteristics of BAT pre- and post-mirabegron exposure in 9 female cynomolgus macaques (*Macaca fascicularis*), 4 of whom received placebo and 5 of whom received daily Mirabegron (2mg/kg) for 4 weeks. Repeated biopsies of left and right supraclavicular BAT depots were obtained pre- and post-exposure respectively. We hypothesized that BAT and white adipose tissue (WAT) would have higher levels of AR in less obese individuals. Tissue was stained for B3AR and B2AR and image analysis was completed with Visiopharm software (Denmark). We found that within individuals, B3AR and B2AR density were comparable and highly associated with each other (R-values>0.85). Between individuals, there was high variability in AR staining with more than a 2-fold range of values reported. Mirabegron induced expected increases in heart rate, blood pressure and neutrophilia but did not alter body composition, metabolic markers, or WAT fat cell size. The lack of biological effect was surprising, as mirabegron treatment did increase both B2AR (p<0.05) and B3AR (p=0.10) expression in BAT. In all cases, BAT showed compositional heterogeneity with significant WAT intermingled with BAT and equivalent B2/3AR staining. These data are a first in the evaluation of BAT expression and dynamic changes in situ in a relevant monkey model of human obesity.

Sunday, November 17, 2024

12:21 PM – 12:27 PM

AN INVESTIGATION OF ALKALINE PHOSPHATASE IN SITU HYBRIDIZATION FOR THE DIAGNOSIS OF OSTEOSARCOMA IN DOGS

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A microscopic diagnosis of osteosarcoma (OSA) requires the identification of tumor associated osteoid amongst neoplastic mesenchymal cells. This is a diagnostic challenge with small samples and tumors with minimal osteoid production. We hypothesized that alkaline phosphatase (ALP) in situ hybridization (ISH) detecting ALP RNA within poorly osteoid producing OSAs would aid in diagnosis. This experiment aimed to test the utility of ALP ISH for identifying OSA in dogs and distinguish it from other canine mesenchymal tumors (e.g. chondrosarcoma, fibrosarcoma, hemangiosarcoma, and histiocytic sarcoma). Cases were selected from the Washington Animal Disease Diagnostic Laboratory archives (CoreOne, VADDs), and categorized by tumor type by histopathology. OSAs

were further categorized by productivity (non-productive vs. osteoid producing—minimal, moderate, marked). Formalin-fixed paraffin embedded samples and the RNAscope® assay were prepared and performed following manufacturer's protocol. OSA sections of moderate and marked osteoid productivity displayed abundant ALP expression in neoplastic osteoblasts, while minimally productive OSAs were scant to abundant, though always had a positive detection. Nonproductive OSAs displayed variability in ALP expression, prompting the need to perform additional immunohistochemical staining (CD204, CD31). Scant traces of ALP expression were detected in lacunar cells of chondroblastic OSAs. ALP expression was appreciable in chondrosarcoma samples, while no ALP expression was seen in fibrosarcomas, hemangiosarcomas, or histiocytic sarcomas. Decalcified tissue samples and regions of necrosis and autolysis lacked ALP expression. From this retrospective study, ISH demonstrates great promise as a diagnostic tool for diagnosing OSAs in challenging cases. However, further investigation is needed to distinguish chondroblastic OSAs from chondrosarcomas.

Sunday, November 17, 2024

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INACTIVATION OF LATS1 AND LATS2 IN MATURE MOUSE WT1-POSITIVE RENAL CELLS LEADS TO CRESCENTIC GLOMERULONEPHRITIS

Laurence Banville, Laureline Charrier, Julie Brind'Amour, Guillaume St-Jean, Alexandre Boyer
Université de Montréal, Faculty of Veterinary Medicine, Saint-Hyacinthe, QC, Canada

Glomerulonephritis (GN) refers to a group of immune-mediated pathologies characterized by inflammation of the kidney's filtering structure, the glomerulus. Among various GN types, crescentic glomerulonephritis (CGN) has the poorest prognosis. Despite advancements in understanding CGN etiology, the intracellular signaling pathways involved in crescent formation remain unidentified. The Hippo pathway, a signaling pathway known for its role in cellular proliferation and differentiation, has recently been implicated in nephropathies, such as diabetic nephropathy and cystic kidney disease. However, no study has investigated its role in CGN. Here, we investigated the roles of the Hippo pathway's main kinases, large tumor suppressor kinases 1 and 2 (LATS1 and LATS2), in *Wt1*-positive renal cells (podocytes, and parietal epithelial cells PEC-A1 and PEC-A2). Using the *Wt1*^{CreERT2} strain (*Lats1*^{flox/flox}; *Lats2*^{flox/flox}; *Wt1*^{CreERT2/+}) we found that the conditional inactivation of *Lats1/2* in mature kidney *Wt1*-positive renal cells following tamoxifen-injection led to progressive glomerular crescent formation, glomerular tuft atrophy associated with cell apoptosis, macrophage recruitment around affected glomeruli, presence of hyaline casts in renal tubule, and some tubular degeneration. These phenotypic changes were accompanied by increased serum creatinine levels associated with renal failure resulting in mice death 12 days post-*Lats1/2* inactivation. Furthermore, tracing studies and Ki67 expression suggested that proliferating crescent cells originate from *Wt1*-positive cells. Together, these findings highlight the crucial role of Hippo signaling in maintaining glomeruli integrity and provide a new model for studying CGN in humans and domestic animals.

Sunday, November 17, 2024

12:33 PM – 12:39 PM

RETROSPECTIVE CLINICAL, GROSS, AND HISTOLOGIC CHARACTERIZATION OF FELINE EXOCRINE PANCREATIC NEOPLASMS

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Exocrine pancreatic adenocarcinoma (EPAC) is an uncommon, aggressive neoplasm in cats, but comprehensive data on clinical, gross, and histologic features are lacking. The aim of this study was 1) to evaluate a feline EPAC cohort for shared trends in clinical, gross, and histologic features and 2) define reproducible feline EPAC histologic sub-typing criteria. Retrospective evaluation of medical

records, gross reports, and histopathology slides for 67 candidate cases was performed. Lesions were reclassified as EPAC (n=29), benign pancreatic neoplasms/pre-neoplastic lesions (n=11), ampullary carcinoma (n=7), biliary carcinoma (n=7), carcinoma of unknown origin (n=3), pancreatitis/pancreatodochitis (n=3), nodular hyperplasia (n=3), islet cell tumor (n=2), lymphoma (n=1), and hepatocellular carcinoma (n=1). EPAC histologic morphology was highly variable: at least six subtypes were identified, including acinar (n=17), cystic/papillary (n=7), anaplastic (n=2), ductal (n=1), adenosquamous (n=1), and intermediate (n=1). Common clinical signs were non-specific, including anorexia (n=9/29) and lethargy (n=6/29). The average age of EPAC cats was 12.6 YO, with an equal sex distribution and overrepresentation of DSH (n=21/29). Metastasis was common (n=26/29) with the liver most frequently involved (n=17/29); cystic/papillary subtype metastasis was less common (n=5/7). Few cats had abdominal effusion (n=9/29) and paraneoplastic alopecia (n=3/29). Concurrent pancreatic lesions included pancreatitis (n=18/29), nodular hyperplasia (n=16/29), and islet amyloidosis (n=11/29). The EPAC acinar subtype predominance correlates with previous literature. Feline EPAC histologic phenotype varied widely, making sub-typing challenging. Previously uncharacterized benign neoplastic/pre-neoplastic lesions were identified that resemble human pre-neoplastic lesions. Further immunohistologic characterization of feline EPAC subtypes and investigation into benign neoplasm/pre-neoplastic lesion pathogenesis is warranted.

Sunday, November 17, 2024

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SNAKE-ASSOCIATED SARCOCYSTIS PANTHEROPHISI N. SP. INFECTION IN THREE FOALS WITH SEVERE COMBINED IMMUNODEFICIENCY

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Three foals, bred for severe combined immunodeficiency (SCID) for immunology research at Washington State University spontaneously developed clinical illness and were euthanized between 2-3 months of age. At necropsy, all three had severe chronic multifocal to coalescing necrotizing hepatitis and fibrosis with intracytoplasmic protozoa in hepatocytes. One foal had multisystemic spread of the protozoa, with the most severe infection in the liver and lungs, and the other two also had adenoviral associated broncho-interstitial pneumonia. PCR and sequencing of the liver using universal Sarcocystis sp. primers for a segment of the 18S ribosomal RNA gene produced a 100% match for Sarcocystis pantherophisi n.sp., currently only isolated from the Eastern rat snake, which is believed to be the definitive host. Related species with snakes as definitive hosts typically use rodents as intermediate hosts. While speculative, exposure was presumably through bedding or feed, and the protozoal parasite was likely able to cause disease in these three foals due to their SCID status, leaving them unable to mount an effective adaptive immune response.

Sunday, November 17, 2024

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CHARACTERIZING NEUROMA FORMATION AFTER TAIL DOCKING IN SHEEP

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BACKGROUND

Tail docking is a routine procedure performed in sheep that is known to cause both short- and long-term pain. Studies have found evidence of neuromas, characterized by abnormal proliferations of irregularly distributed nerve bundles, swirling patterns among axons, and fibrosis, at the site of docking in sheep tails. However, neuroma formation after a rubber ring is applied in the first days of

life, a common method of tail docking, has not been described.

OBJECTIVE

This study seeks to describe the histological characteristics of neuromas formed in sheep tails docked using a rubber ring at 1-2 days of age, as compared to age-matched controls.

METHODS

Eighteen sheep (8 docked at 1-2 days of age, 10 undocked controls) were euthanized at 7.8 ± 0.2 months of age (mean $\pm$ SD) and their tails collected and decalcified for histology. FFPE sections were stained with H&E and trichrome, and immunohistochemistry was performed using stains for s100 and neurofilament heavy chain (NFH). Each tissue sample was evaluated for abnormalities characteristic of neuromas, receiving a severity score ranging from 0 (absent) to 4 (severe).

RESULTS

Neuroma-like proliferations were found in all docked tails, ranging from mild to severe. These proliferations were particularly prominent in the periosteal region surrounding the vertebrae. Neuromas stained positively for both s100 and NFH, and trichrome staining showed that neural proliferation was accompanied by Schwann cell proliferation and fibrosis.

CONCLUSION

This study provides evidence that tail docking with a rubber ring in sheep causes neuromas, a known source of chronic pain.

Sunday, November 17, 2024

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ASSOCIATION OF SERUM REDOX STATUS WITH PROGNOSTIC FACTORS IN CANINE MAST CELL TUMORS: A PILOT STUDY

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¹Aristotle University of Thessaloniki, Thessaloniki, Greece, ²Aristotle University of Thessaloniki, Thessaloniki, Greece, ³University of the Aegean, Mytilene, Greece, ⁴University of Murcia, Murcia, Spain, ⁵International Hellenic University, Thessaloniki, Greece

Background: Mast cell tumors (MCT) are common skin neoplasms in dogs. Prognostic indicators include histopathologic grade, clinical stage, metastatic status, high Ki-67 index, elevated AgNOR index, c-kit mutations, and recurrence after surgery. Dogs with MCT show higher oxidative stress, with serum redox status linked to prognostic factors in canine lymphoma and mammary tumors.

Objectives: This study aimed to assess the correlation between established prognostic factors and serum redox status in dogs with MCT.

Materials and Methods: Dogs with MCT, without concurrent systemic diseases, were studied. Staging was evaluated based on cytology of regional lymph nodes and ultrasound-guided liver and spleen aspirates. Histologic grading and immunohistochemical staining for Ki-67 and KIT patterns were performed on excised tumors. Dogs were categorized by Patnaik grading (1-3), Kiupel grading (low/high), metastatic status, Ki-67 positive nuclei per cm² (>23 or ≤ 23), and KIT pattern (I, II-III). Serum redox status before surgery was evaluated measuring Paraoxonase-1, CUPRAC, d-ROMs, and oxy-adsorbent levels. ANOVA and independent t-tests were used to detect differences in mean values among groups.

Results: Thirty-nine dogs with MCT were evaluated, with six having subcutaneous MCTs. Paraoxonase-1 activity was significantly lower in Patnaik grade 3 ($p=0.003$) and Kiupel high-grade ($p=0.022$) MCTs. No significant differences were found in CUPRAC, d-ROMs, or oxy-adsorbent levels across different prognostic groups.

Conclusion: This pilot study found a significant correlation between histologic grading and Paraoxonase-1 activity suggesting a potential role of Paraoxonase-1 as prognostic biomarker in canine MCTs. Further studies with larger populations are ongoing.

Sunday, November 17, 2024

12:57 PM – 01:03 PM

ZOONOTIC-ASSOCIATED YERSINIA PESTIS IN A DOMESTIC CAT

George Abernathy, Rachael Gruenwald

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A five-year-old castrated male domestic feline (*Felis catus*) was diagnosed with *Yersinia pestis* (plague) after it was suspected of infecting its owner. The cat was presented to the referring veterinarian for a cervical abscess which was treated with antibiotics and surgical drains. The owner was subsequently hospitalized and diagnosed with *Y. pestis* by the CDC. Seven days after presentation the cat died and was submitted to the Oregon Veterinary Diagnostic Laboratory for necropsy. On postmortem examination, a 10 x 10 cm region of cutaneous swelling was present in the ventral cervical region containing the drains. Severe necrosis and multiple abscesses were found in the subcutaneous tissues and skeletal muscle. Cervical lymph nodes were not identified grossly. All lung lobes contained dozens of abscesses, and the spleen was markedly enlarged. Tissues collected for histopathology were routinely processed and stained with hematoxylin-eosin. Gram staining was performed on select tissues. Severe necrosuppurative inflammation containing myriad gram-positive coccobacilli was present in tissues from the cervical region and lung. Cervical lymph nodes contained suppurative inflammation. Severe lymphoid hyperplasia and plasma cell differentiation was present in the spleen; necrosis was not noted. Bacterial culture of the cat's spleen at the CDC isolated *Yersinia pestis*. The diagnosis of plague in the owner is Oregon's first human case in more than 8 years. Infection likely came through contact with the cat. This case is a reminder that *Yersinia pestis* remains a serious zoonotic disease in regions of the western United States.

Sunday, November 17, 2024

01:03 PM – 01:09 PM

NEUROPATHOLOGIC ANALYSIS OF IL-15-ENGINEERED NK CELL THERAPY IN A MURINE GLIOBLASTOMA MODEL

Samantha Hicks^{1,2}, Mayra Shanley¹, Sunil Acharya¹, Katy Rezvani¹, Natalie Fowlkes¹

¹University of Texas MD Anderson Cancer Center, Houston, TX, USA, ²Texas A&M University College of Veterinary Medicine and Biomedical Sciences, College Station, TX, USA

Background: Glioblastoma (GBM) is an aggressive astrocytoma and the most common primary brain tumor in adult humans. GBM induces immunosuppression in the tumor microenvironment (TME), limiting the efficacy of many immunotherapies. Adoptive cell transfer is one strategy for overcoming the lack of effective anti-tumor immune responses in the TME by administering effector immune cells engineered for improved tumor killing. Natural killer (NK) cells inherently resist many tumor defense mechanisms and have bolstered cytotoxic properties when armed with pro-inflammatory cytokines. However, some cytokines have been linked to significant toxicities.

Objective: We aimed to characterize neuropathology associated with reduced survival times after intratumoral injection of IL-15-engineered NK cells in a murine GBM model.

Methods: NSG mice were cerebrally engrafted with GSC20 patient-derived GBM stem cells and treated with vehicle, non-transduced (NT) NK cells, IL-15 NK cells or IL-21 NK cells (n=4 per group). Multiplex immunofluorescence staining was performed on brain sections (Akoya Biosciences Opal kit). Slides were scanned using Leica Versa 8 and biomarkers evaluated using HALO v3.6.4. Data was analyzed using GraphPad Prism 10.0.3.

Results: NK cells and microglia were increased in the IL-15 NK group. Spatial analysis showed microglia in close proximity to NK cells (100µm), suggesting recruitment. IL-15R expression increased in astrocytes and microglia, consistent with microglia-astrocyte IL-15-mediated inflammatory crosstalk.

Conclusions: IL-15-engineered NK cells caused overactivation of microglia and astrocytes leading to neurotoxicity and decreased survival times in mice. Improved understanding of mechanisms of

neurotoxicity may help guide development of novel immunotherapies with improved safety profiles in the future.

Sunday, November 17, 2024

01:09 PM – 01:15 PM

IDENTIFYING A MYCOPLASMA SP. AS THE CAUSAL AGENT OF SYNOVITIS IN BIG BROWN BATS (EPTESICUS FUSCUS)

Katherine Morucci¹, Madeline Vile², Justin Brown³, Stephanie Stronsick⁴, Jeffrey Lorch⁵, Gregory Turner⁶, Kevin Niedringhaus²

¹University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA, USA, ²Wildlife Futures Program, University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA, USA,

³Pennsylvania State University, University Park, PA, USA, ⁴Pennsylvania Bat Conservation and Rehabilitation, Mertztown, PA, USA, ⁵United States Geological Survey, Madison, WI, USA,

⁶Pennsylvania Game Commission, Harrisburg, PA, USA

Background:

Despite their important role in ecosystem health, little is known about infectious diseases in bats, aside from lyssaviruses and white nose syndrome. Big brown bats (*Eptesicus fuscus*) are increasingly observed with swollen joints in wildlife clinics and do not respond well to treatment, hindering successful release. The cause is not definitively known, but both an unnamed *Mycoplasma* species as well as a poxvirus, have been implicated.

Objective & Methods:

To determine the cause of synovitis in big brown bats, 25 big brown bats including 11 with enlarged joints and 14 “controls” with grossly normal joints were grossly and histologically examined and tested for both *Mycoplasma* spp. and *eptesipoxvirus* by PCR.

Results:

Mycoplasma was detected in 5/11 (45.5%) of bats with grossly enlarged and/or histologically inflamed joints. None of the bats with normal joints were PCR-positive for *Mycoplasma* ($p=0.0087$). Sequencing of the intergenic spacer region of *Mycoplasma* revealed that all five were identical to one another, shared poor similarity to other *Mycoplasma* species in Genbank (78% similarity to *M. iguana*) but were 100% identical to a *Mycoplasma* previously detected in big brown bats with polyarthritis. Microscopically the lesion consisted of neutrophilic synovitis, periosteomyelitis, fasciitis, and dermatitis similar to *Mycoplasma* infections in other species. Poxvirus testing is ongoing.

Conclusions:

This study is the first to concomitantly test for both suspected causative agents of this poorly understood syndrome in both clinical and unaffected big brown bats. Our data suggests that *Mycoplasma* is likely to be associated with swollen joints in this species.

1: ESTABLISHMENT OF UPDATED HEMATOLOGY AND BIOCHEMISTRY REFERENCE INTERVALS FOR NEW ZEALAND WHITE RABBITS

Megan Aalto, Lindsey Burton, Jackeline Cruz, Charlie Payne, Katie Sikes, Kelly Santangelo
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The New Zealand White (NZW) rabbit plays a pivotal role in research; they are docile, easy to breed, and inexpensive. They are commonly utilized in systemic toxicity studies for biocompatibility testing of novel medical devices. For this, hematology and biochemistry blood values are used to confirm safety. However, reference intervals (RIs) for NZW rabbits have not been recently updated and there is little data to understand sex and age-related effects. The goal of this study was to retrospectively

generate hematology and biochemistry RIs and apply these to evaluate the health of aging male and female NZW rabbits. To determine RIs, blood samples from rabbits utilized in our laboratory from 2023 (n=64; 10-24mo old; female) were analyzed for hematology and biochemistry panels; this retrospective data was input into Reference Value Advisor 2.1. Non-parametric RIs were determined using a bootstrap method. The potential influence of age and sex on parameters were then tested in a prospective manner. Male (n=3) and female (n=3) blood samples were taken in monthly intervals from 2-12mo of age. Parameters were qualitatively evaluated for fit within RIs and statistical analysis was performed using GraphPad Prism v10 to determine age and sex related differences ($p<0.05$). Sex related differences were identified in 1/24 hematology and 4/23 biochemistry parameters; age-related changes were recognized in 7/24 hematology and 13/23 biochemistry parameters. Values generally stabilized between 5-9mo of age. Overall, the establishment of RIs for NZW rabbits will provide enhanced standards to determine the well-being of rabbits utilized in research and clinically.

2: ZOONOTIC-ASSOCIATED YERSINIA PESTIS IN A DOMESTIC CAT

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3: INTRAOCULAR MELANOCYTIC NEOPLASIA IN DOGS: BENIGN OR MALIGNANT – DO WE REALLY KNOW?

Camila Amrein Almira, Brittany Rasche
Kansas State University, Manhattan, KS, USA

Melanocytic tumors are common intraocular neoplasms in dogs that usually arise from the anterior uvea, with malignant melanomas described as being less common than melanocytomas. In the veterinary literature, a mitotic count of >4 per 10 high power fields (hpf) or >2 per single hpf has been considered the most important indicator of malignancy. However, most of the tumors meeting this criterion don't appear to metastasize. Within the caseload of the Kansas State Veterinary Diagnostic Laboratory (KSVDL) in the past year, we retrospectively identified 4 cases of intraocular melanocytic neoplasia in dogs which would be considered malignant, as well as a noteworthy case which would be considered benign based on these criteria. One of the four malignant cases with a mitotic count of 5 per 10 hpf was incompletely excised and exhibited local recurrence in the orbit with a greatly increased mitotic count of 64 per 10 hpf. The other three malignant cases varied from infiltration of only the anterior uvea to diffuse infiltration of the uvea with corneoscleral and periocular invasion.

Notably, one markedly enlarged enucleated globe was diffusely effaced by neoplastic melanocytes with extensive invasion of the periocular connective tissues but had a mitotic count of only 2 per 10 hpf, classifying it as benign despite its aggressive biological behavior. This spectrum of case presentations of canine intraocular melanocytic neoplasia raises concern that mitotic count may not be the best indicator of malignancy for these neoplasms and prompts further investigation.

4: OUTBREAK OF AVIAN POLYOMAVIRUS

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Signalment & history:

Five psittacines, ranging in age from 9 weeks to 17 weeks, were submitted for necropsy between 7/5/2023 and 2/5/2024 to the Louisiana Animal Disease Diagnostic Laboratory (LSU Diagnostics). Common presenting complaints included acute lethargy (2/5), and decreased appetite (2/5). Two birds were found dead. Four were submitted from the same pet store, and one was submitted by a patron of the store.

Postmortem findings:

Lesions consisted of hepatomegaly (5/5) and splenic (3/5) and hepatocellular necrosis (5/5) with intranuclear glassy pale basophilic viral inclusion bodies within hepatocytes (5/5), as well as in renal glomerular mesangial cells or tubular epithelial cells (4/5), splenic periarteriolar sheath cells (4/5), and epithelial cells of the crop (4/5) and vent (2/5). PCR of the liver confirmed avian polyomavirus infection in all birds.

Conclusions:

Sudden death in young psittacines with hepatomegaly and intranuclear glassy basophilic hepatocellular inclusions is suggestive of avian polyomavirus infection, which was confirmed by PCR.

Significance:

Avian polyomavirus infection is a deadly disease of juvenile psittacines which poses a threat to aviculturists and pet owners. Mortality rates in avian nurseries can exceed 90%, and subclinically infected adults are capable of cloacal viral shedding for 4-8 weeks, perpetuating infection in juveniles and immunocompromised adults. This case series exemplifies the importance of adequate biosecurity and thorough diagnostic screening, including PCR of both cloacal swabs and blood samples. Failure in either of these areas can lead to virus spread among producers and client-owned animals, as seen in this instance.

5: INACTIVATION OF LATS1 AND LATS2 IN MATURE MOUSE WT1-POSITIVE RENAL CELLS LEADS TO CRESCENTIC GLOMERULONEPHRITIS

Laurence Banville, Laureline Charrier, Julie Brind'Amour, Guillaume St-Jean, Alexandre Boyer
Université de Montréal, Faculty of Veterinary Medicine, Saint-Hyacinthe, QC, Canada

Glomerulonephritis (GN) refers to a group of immune-mediated pathologies characterized by inflammation of the kidney's filtering structure, the glomerulus. Among various GN types, crescentic glomerulonephritis (CGN) has the poorest prognosis. Despite advancements in understanding CGN etiology, the intracellular signaling pathways involved in crescent formation remain unidentified. The Hippo pathway, a signaling pathway known for its role in cellular proliferation and differentiation, has recently been implicated in nephropathies, such as diabetic nephropathy and cystic kidney disease. However, no study has investigated its role in CGN. Here, we investigated the roles of the Hippo pathway's main kinases, large tumor suppressor kinases 1 and 2 (LATS1 and LATS2), in *Wt1*-positive renal cells (podocytes, and parietal epithelial cells PEC-A1 and PEC-A2). Using the

Wt1^{CreERT2} strain (*Lats1*^{flox/flox}; *Lats2*^{flox/flox}; *Wt1*^{CreERT2/+}) we found that the conditional inactivation of *Lats1/2* in mature kidney *Wt1*-positive renal cells following tamoxifen-injection led to progressive glomerular crescent formation, glomerular tuft atrophy associated with cell apoptosis, macrophage recruitment around affected glomeruli, presence of hyaline casts in renal tubule, and some tubular degeneration. These phenotypic changes were accompanied by increased serum creatinine levels associated with renal failure resulting in mice death 12 days post-*Lats1/2* inactivation. Furthermore, tracing studies and Ki67 expression suggested that proliferating crescent cells originate from *Wt1*-positive cells. Together, these findings highlight the crucial role of Hippo signaling in maintaining glomeruli integrity and provide a new model for studying CGN in humans and domestic animals.

7: HISTOLOGICAL DIAGNOSIS OF SPONTANEOUS TUMORS AND THEIR CHARACTERIZATION IN LONG-EVANS RATS: A RETROSPECTIVE STUDY FROM 2006-2024

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¹University of Wisconsin-Madison, School of Veterinary Medicine, Madison, WI, USA, ²Johns Hopkins University, School of Medicine, Baltimore, MD, USA

Long-Evans (LE) rats have been preferred models in behavioral research since 1922. Compared to Sprague-Dawley (SD), Wistar, and Fischer 344 (F344) rats, which are widely used and reported on in toxicologic pathology literature, publications on LE are limited. This retrospective study aims to characterize 66 tumors in 44 male and 5 female LE rats from studies at Johns Hopkins School of Medicine between 2006-2024. All cases represent CRL:LE rats submitted for pathology of resected lesions, unexpected death, or humane euthanasia. Inclusion criteria included mass (or plaque-like) lesions in skin and/or internal organs; 27 biopsies and 22 necropsies. A strong male bias (90%) reflects available submission data. Neoplasm frequency was high (94%) compared to other LE studies, however, animals reaching study endpoints were not submitted. Mesenchymal tumors were most frequent (73%), and 9/20 sarcomas were poorly differentiated or mixed. Mammary fibroadenomas, the most common tumor in SD, Wistar and F344 studies, were underrepresented here, possibly due to the small number of females. Epithelial neoplasms occurred at a similar frequency (19%) to SD, F344, and Wistar rats. No renal tumors were identified, in contrast to other LE studies. Two brain tumors (astrocytoma, medulloblastoma) were identified. Brain tumors are not common in LE but have been reported in SD or F344. Additionally, a cutaneous T cell lymphoma, a rare neoplasm in rats, was diagnosed. Limitations of this study include non-cross-sectional data and variability over 22 years. However, understanding LE tumor types and distribution can improve clinical interventions and research outcomes.

9: INVESTIGATING THE ASSOCIATION BETWEEN PRETERM BIRTH AND THE DEVELOPMENT OF VOCAL FOLDS IN LAMB LARYNGES

Gaibrielle Bressler, Abigail Cox

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The development of laryngeal vocal folds (VFs) plays a crucial role in voice production. Voice abnormalities in humans often stem from premature birth, yet few studies have explored how prematurity affects laryngeal health and voice outcomes. This study aims to characterize the preterm lamb larynx for future translational studies, specifically pertaining to preterm infants and school-age children speech pathology. Microscopic examination focused on 3 lamb larynges: term, preterm, and extreme preterm. Hematoxylin and eosin (H&E) stains evaluated VF lamina propria (LP) and epithelia width, while alcian blue (AB) stains assessed glycosaminoglycan and matrix protein levels. All developmental stages exhibited typical connective tissue components in the LP. In preterm and extreme preterm VFs, fibroblasts, collagen, elastin, and vessels were evenly distributed throughout the LP, contrasting with term VFs showing greater spatial differences, with higher fibroblast and

vessel densities in superficial versus deeper LP layers. AB staining revealed that the deeper LP layers in term VFs had more alcianophilic glycosaminoglycans than superficial layers, whereas preterm and extreme preterm VFs showed more uniform glycosaminoglycan distribution. These findings indicate significant LP reorganization in fetal lamb VFs during later gestational stages. Future studies should further investigate these developmental changes to understand their implications for human premature birth and associated voice pathology.

10: ABERRANT FLUKE MIGRATION IN TWO NEW WORLD CAMELIDS

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Two cases of aberrant fluke migration, one in a 1-year-old female alpaca and one in a 10-year-old female llama, were presented to MSU-CVM Food Animal Emergency Services and subsequently underwent post-mortem examinations. Upon gross examination, common findings included poor body conditions, bicavitary effusions, adhesions of the liver to the diaphragm, and adhesions of the lungs to either the pericardial sac or rib cage. Within the liver of the alpaca were cystic cavitations containing ~3cm long dorsoventrally flattened trematodes (*Fasciola magna*), wherein the liver of the llama contained multifocal, variably sized trematodes (*Fasciola magna* and *Fasciola hepatica*). Histopathologic findings were similar and included extensive regions of pulmonary and hepatic fibrosis interspersed with iron porphyrin pigment, trematode eggs, and mixed chronic inflammation. Additionally, biliary hyperplasia and hepatic vascular disruption by either hemorrhage or thrombi were appreciated. These cases represent uncommon instances of aberrant pulmonary fluke migration in two new world camelids (NWCs). NWCs serve as definitive and dead-end hosts for *Fasciola hepatica* and *Fasciola magna*, respectively, with snails being the intermediate host for both trematode species. Infection with *F. magna* is rare in the llama; however, *F. hepatica* commonly causes disease in NWCs. Infection typically manifests clinically as anorexia, anemia, weakness, recumbency, or peracute death. Diagnosis and treatment are limited, and prevention strategies focus on interrupting the fluke lifecycle by eliminating snails in the environment.

11: BACTERIAL SEPTICEMIA ASSOCIATED WITH SALMONELLA GROUP D1 IN A FRENCH BULLDOG PUPPY

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An 11-week-old, intact female French Bulldog presented with a one-week history of diarrhea. During hospitalization, the patient developed fever, leukocytosis, anorexia, tremors, and circling. Despite two weeks of intensive medical management, the puppy's condition deteriorated, and she was submitted to the Missouri Veterinary Medical Diagnostic Laboratory for postmortem examination. The puppy was purchased from a breeder and housed with two adult dogs, both clinically normal. Both the breeder and owner reported feeding raw food diets to their dogs. Necropsy findings were unremarkable, with no gross abnormalities observed in the brain. Histopathological examination revealed severe suppurative meningitis, mild to moderate chronic lymphoplasmacytic enteritis, and mild diffuse fibrinosuppurative and histiocytic interstitial pneumonia, indicative of bacterial septicemia. Bacterial culture of the brain and lung identified the causative agent as *Salmonella enterica* subsp. *enterica*, group D1, further classified as serovar Dublin. The isolation of *S. enterica* Dublin from dogs is rare, with few cases reported in the literature. Given that *S. enterica* Dublin infection is often linked to raw cattle products, the patient's exposure to raw food is considered the most likely source of infection. The proposed pathogenesis involves bacterial translocation from the intestine to the bloodstream, eventually reaching the brain. As *S. enterica* Dublin is zoonotic, this case underscores the potential risks of feeding raw food diets to pets and their handlers. Salmonellosis

should be considered a differential diagnosis in puppies with systemic illness and neurological signs, particularly when there is a history of raw food diet exposure.

12: NOVEL CONGENITAL MALIGNANT PERIPHERAL NERVE SHEATH TUMOR IN A CANINE

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An 11-week-old, 10.6 kg, female intact, Leonberger dog presented to Colorado State University for a mass on the left ventrolateral thorax that was present since birth. Microscopically, the 4cm x 5cm mass consisted of a highly invasive, densely cellular, non-encapsulated spindle-shaped perineural cell population arranged in bundles and concentric whorls (pseudo-onion bulb formations) supported by prominent microvasculature and a collagenous stroma. Neoplastic cells had indistinct borders, scant eosinophilic cytoplasm, and oval nuclei with finely stippled chromatin. Infrequently, the cells contained brown to black, granular pigment. Anisocytosis and anisokaryosis were mild to moderate and mitoses were 9 in ten high powered (400x) fields. Central fibers of pseudo-onion bulb formations exhibited strong positivity for glial fibrillary acidic protein (GFAP) and weak positivity for S100. Pigmented cells and scattered non-pigmented cells displayed strong cytoplasmic immunoreactivity for Melan A. The Ki-67 proliferative index was 9.7%. These findings are diagnostic for a congenital malignant peripheral nerve sheath tumor (MPNST) with hybrid features of neurofibroma and perineurioma. To date, congenital cases of MPNST have only been described in humans where they are locally aggressive with a high likelihood of recurrence and the potential for metastasis. There was no evidence of metastasis at the time of surgery and the patient was subsequently lost to follow-up.

14: CLEAR CELL ADNEXAL CARCINOMA IN A TONKINESE CAT

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Clear cell adnexal carcinoma is a rare, primary, undifferentiated adnexal neoplasm of the deep dermis and subcutis whose major diagnostic feature is the presence of clear cells. In veterinary medicine, clear cell adnexal carcinoma is considered a disease of dogs and is not reported in any other species. A 13-year-old spayed female Tonkinese cat presented with a small mass near the left mammary chain. On histopathology, the mass was a 7 x 4 mm, densely cellular, well-demarcated, encapsulated, multilobulated, subcutaneous nodule. Lobules were divided by a fibrovascular stroma and composed of nests of polygonal cells with occasional peripheral palisading. Some nests contained central areas of necrosis. Neoplastic cells had distinct borders and abundant, finely vacuolated, pale eosinophilic cytoplasm. Anisokaryosis was marked and there was occasional multinucleation and mitotic activity. 100% of neoplastic cells were positive for MNF116 (pancytokeratin) and cytokeratins 8/18 by immunohistochemistry (IHC), and 30% were positive for vimentin. Neoplastic cells were diffusely negative for Melan-A, S-100, and cytokeratins 5/6. The foamy intracytoplasmic material was negative on Oil red O, PAS (with and without diastase), mucicarmine, and Alcian blue histochemical stains. The histologic features of the neoplastic population and co-expression of pancytokeratin and vimentin were consistent with a diagnosis of clear cell adnexal carcinoma. Although the mass was incompletely excised, clinical follow-up showed no evidence of recurrence or metastasis. To our knowledge, this is the first report of clear cell adnexal carcinoma in any species other than humans and dogs.

15: ASSESSING MEGAKARYOCYTIC AND CONNECTIVE TISSUE CHARACTERISTICS IN HEALTHY AND FIBROTIC CANINE BONE MARROW

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Canine myelofibrosis is a poorly understood disease caused by abnormalities in blood cell production which results in normal marrow elements becoming replaced with fibrous tissue. Dysplastic megakaryocytes regulate fibrosis of the bone marrow and are an important diagnostic feature for this disease. This study aimed to optimize CD61 immunohistochemistry (IHC), a common megakaryocyte marker in human medicine, to visualize dysplastic megakaryocytes, quantify the megakaryocytes, and characterize connective tissue deposition within the marrow. Using the Ohio State University archive, we obtained formalin-fixed, paraffin-embedded normal and myelofibrotic canine bone marrow samples. Histochemistry for H&E, Masson's trichrome, and Gordon & Sweet's reticulin, along with IHC for CD61, was conducted on normal (n=27) and myelofibrotic (n=9) samples. Samples were blindly graded for fibrosis using The World Health Organization (WHO) bone marrow fibrosis grading scale. Results showed the antibody had positive expression of CD61 on megakaryocytes and we found significance in myelofibrosis samples having an increased number of dysplastic megakaryocytes (p= 0.001). For fibrosis scoring, we found in normal cases (n=27), 85.2% demonstrated grade 0 fibrosis and 14.8% were grade 1. Of myelofibrosis cases (n=9), 33.3% were grade 2 fibrosis, and 55.6% were grade 3; a single case had grade 0 fibrosis. This study confirms CD61 expression by canine megakaryocytes, and increased megakaryocyte atypia in canine myelofibrosis. Connective tissue stains also demonstrated that a subset of normal marrow may actually exhibit early (grade 1) fibrosis.

16: ENDOPARASITISM AND SUPPURATIVE MENINGIOENCEPHALITIS IN AN AMERICAN BLACK BEAR

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A free ranging, adult, female, American Black Bear (*Ursus Americanus*) was submitted to the University of Maine Veterinary Diagnostic Laboratory in July, 2024 by Maine's Department of Inland Fisheries and Wildlife. The animal was dispatched by the regional Game Warden after it was observed exhibiting severe neurological symptoms. Neurological signs included a lack of fear of people, hypermetria, head pressing, and ataxia. External examination revealed a negative energy balance, pale mucous membranes, and marked hemopneumothorax, consistent with a single gunshot wound. Internally, an empty gastrointestinal tract, nematodes within the lungs and trachea, and severe suppurative meningoencephalitis and ventriculitis were observed. Cytology performed on the ventricular exudate showed neutrophils and macrophages occasionally with few intracytoplasmic, small, Gram-positive cocci. Sterile swabs from the brain were plated for bacterial culture and nematodes gathered from the tracheal lumen were preserved in ethanol. Bacterial culture and subsequent 16S rRNA sequencing identified *Streptococcus gallinaceus* from the purulent brain exudate. For nematode identification, four gene targets were amplified, DNA sequences were run through the NCBI Blast database, and the samples were aligned and compared using Geneious Prime software showing 100% homology with *Crenosoma* sp., including matches to *C. mephitidis* and, weakly, for *C. vulpis*. *Crenosoma* sp. has previously been identified in black bears from the northeast U.S.; however, the origin of *Streptococcus gallinaceus* remains unknown.

17: THE SHEEP WITH A BROKEN HEART

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A 2-year-old Southdown ewe (*Ovis aries*) presented in lateral recumbency with open mouth breathing, harsh lung sounds, diminished heart sounds, and clinical marked hypoxia. Jugular pulses were normal, and rumen contractions were decreased. Thoracic ultrasound revealed concern for pulmonary edema with multifocal ground-glass appearance. Thoracic radiographs showed a

bronchointerstitial pattern. Initial treatment included Banamine, Ampicillin and flow-by oxygen, followed by administration of Draxxin without clinical improvement. The sheep subsequently died. Gross necropsy revealed several significant cardiac findings, including: 1. the right and left ventricular walls were roughly equal in width, 1.2 cm and 1.4 cm, respectively; 2. the pulmonary artery was absent, and both ventricles emptied into a single set of semilunar valves and a single common trunk that bifurcated 5 cm distally into the main pulmonary artery and aorta, consistent with type 1 truncus arteriosus; 3. an approximately 5x2x2 cm coalescing aggregate of proliferative, cauliflower-like masses covered the majority of the tricuspid valve. On cut-section, the masses were slightly friable with gritty material and mottled yellow to red, consistent with vegetative endocarditis. Other significant gross findings include a focal infarct in the right kidney and mottled dark red to tan liver, consistent with chronic passive congestion. Histopathology of the tricuspid valve confirmed vegetative endocarditis with numerous coccoid bacterial colonies. Preliminary aerobic bacterial culture identified *Staphylococcus aureus*. Truncus arteriosus is an exceedingly rare congenital cardiac defect that results in mixing of oxygenated and de-oxygenated blood, thus resulting in the marked hypoxia and right ventricular hypertrophy noted in this case.

18: CHRONIC HEPATITIS IN HORSES WITH EQUINE HEPACIVIRUS INFECTION

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Background: Equine hepacivirus (EqHV) is closely related to hepatitis C virus (HCV), which causes persistent infection and chronic hepatitis in people. EqHV causes subclinical hepatitis during acute resolving infection, however, there is limited information on hepatitis associated with chronic infection. Objectives: We report 26 clinical cases of chronic hepatitis in horses infected with EqHV. Study design: Mixed retrospective and prospective case series. Methods: Horses presented with the following inclusion criteria: 1) chronic hepatitis, defined as at least one month duration of elevated serum liver biomarkers and/or elevated serum liver biomarkers with findings of chronicity on liver histopathology, such as fibrosis; 2) serum or liver EqHV RT-qPCR positive; and 3) liver histopathology performed. Liver biopsies were independently reviewed for 17 individual features. Results: Twenty-six horses met inclusion criteria. Two horses had acute resolving infections and bacterial cholangiohepatitis. Eight horses died within 6 months and persistent infection could not be verified. Sixteen horses had persistent hepaciviral infection of 36 months follow-up. These 16 horses were median 16 (range, 6-20) years old, 6 mares and 10 geldings, and all light breeds. Median duration of documented hepatitis was 18 (5-120) months with median duration of documented EqHV viremia of 12 (6.6-42) months. The predominant histopathologic findings were lymphocytic inflammation and nodules, bridging and dissecting fibrosis, and individual hepatocyte necrosis. Main limitations: The definitive cause of hepatitis in these horses cannot be determined. Conclusions: The similarities between these cases and HCV suggest it is likely that EqHV causes chronic hepatitis and liver failure in horses.

19: HISTOLOGIC CHARACTERIZATION OF PULMONARY LESIONS IN FEEDLOT CATTLE MORTALITIES

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This project sought to characterize the subtype and chronicity of pulmonary lesions in feedlot calf pneumonias, to improve our understanding of this economically significant disease. Field necropsies (n=178) were performed in commercial Kansas feedlots (2022-2023). Affected lung tissues were examined grossly and photographed, and two representative samples from each animal were collected and routinely processed for histologic evaluation. Thirty-six cases were excluded due to

severe postmortem histologic autolysis.

Each sample was classified histopathologically, and results from the two samples combined to generate a case-level diagnosis of bronchopneumonia (BP), interstitial pneumonia (IP) or bronchopneumonia with interstitial pneumonia (BIP). A case diagnosis of BP indicated BP was found in one or more samples with no evidence of IP; IP was based on IP in one or more samples with no BP; BIP was based on finding both BP and IP in samples from a case. The remaining cases were grouped as other/non-pneumonia. For each case, pneumonia subtypes were then categorized as acute or chronic based on histologic features; cases with any evidence of chronicity were classified as chronic. The frequency of case diagnosis (n=142): BP (57/142, 40%), BIP (36/142, 25%), IP (33/142, 23%), and other /non-pneumonia (16/142, 11%). Most BP cases were acute (38/57, 67%), while IP and BIP were more commonly chronic (21/33, 64%; 24/36, 66%, respectively). This work illustrates IP is a frequent histopathologic finding in feedlot mortalities either alone or in conjunction with BP, and cases with an IP component tended to have chronic pathology.

20: GLOMERULONEPHROPATHY IN COLORADO PLAINS TOPMINNOW (*FUNDULUS SCIADICUS*)

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The Plains Topminnow (*Fundulus sciadicus*, PTM) is a native cyprinid fish with a distribution that extends into northeastern Colorado. Regional declines in PTM populations have been attributed to climate change, habitat loss and destruction, and competition with nonnative species.

Glomerulonephropathy was identified incidentally in a population of PTM collected by Colorado Parks and Wildlife. The purpose of this study was to characterize this finding to better inform conservation. Thirteen fish were evaluated by histopathology. Major features included the myxosporidians in renal tissue (77%) and sometimes specifically in glomeruli (39%), mesangial hypercellularity (92%), attenuation (69%) and necrosis (54%) of tubular epithelium with luminal debris (31%), tubular regeneration (92%), and tubular mucus production (69%). Understanding the impact of natural diseases in native plains fishes will be important to their conservation and management and should be considered in light of other ecological threats.

21: DETECTION OF MYCOPLASMA BOVIS IN FORMALIN-FIXED PARAFFIN EMBEDDED SPECIMENS USING REAL-TIME PCR

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Background: Respiratory disease in cattle due to *Mycoplasma bovis* (*M. bovis*) can lead to irreversible lung damage and mortality and has a substantial economic impact on the cattle industry. Clinical signs of infection are non-specific and various tests are available for diagnosis, including real-time polymerase chain reaction (qPCR) using fresh tissues. Postmortem samples are commonly stored as formalin-fixed, paraffin embedded (FFPE) tissues. The use of qPCR in FFPE sections of lungs for the detection of *M. bovis* has not been well studied.

Objective: Determine the feasibility of using qPCR for the detection of *M. bovis* in FFPE lung tissue.

Methods: Forty-three archived bovine cases that underwent postmortem examination between 2013 to 2019 were selected, including 10 cases with positive *M. bovis* culture results, and 33 cases with a diagnosis of bronchopneumonia and unknown *M. bovis* status due to lack of testing. Hematoxylin and

eosin staining, immunohistochemistry (IHC), and qPCR were conducted on all samples.

Results: qPCR detected *M. bovis* in FFPE lung tissue in 24 cases (55.8%). *M. bovis* was detected by qPCR in all 10 samples with positive mycoplasma culture. Of qPCR positive cases, 15 had histological lesions compatible with *M. bovis* infection and 13 had positive immunolabeling for *M. bovis* by IHC.

Conclusion: qPCR can detect *M. bovis* in FFPE lung tissue in historical samples and appears more sensitive than IHC. FFPE tissues can provide a well-preserved, readily available source for detection of *M. bovis* and aid in definitive postmortem diagnosis and future research utilizing archived tissues.

22: ASSOCIATION OF SERUM REDOX STATUS WITH PROGNOSTIC FACTORS IN CANINE MAST CELL TUMORS: A PILOT STUDY

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Background: Mast cell tumors (MCT) are common skin neoplasms in dogs. Prognostic indicators include histopathologic grade, clinical stage, metastatic status, high Ki-67 index, elevated AgNOR index, c-kit mutations, and recurrence after surgery. Dogs with MCT show higher oxidative stress, with serum redox status linked to prognostic factors in canine lymphoma and mammary tumors.

Objectives: This study aimed to assess the correlation between established prognostic factors and serum redox status in dogs with MCT.

Materials and Methods: Dogs with MCT, without concurrent systemic diseases, were studied. Staging was evaluated based on cytology of regional lymph nodes and ultrasound-guided liver and spleen aspirates. Histologic grading and immunohistochemical staining for Ki-67 and KIT patterns were performed on excised tumors. Dogs were categorized by Patnaik grading (1-3), Kiupel grading (low/high), metastatic status, Ki-67 positive nuclei per cm² (>23 or ≤23), and KIT pattern (I, II-III). Serum redox status before surgery was evaluated measuring Paraoxonase-1, CUPRAC, d-ROMs, and oxy-adsorbent levels. ANOVA and independent t-tests were used to detect differences in mean values among groups.

Results: Thirty-nine dogs with MCT were evaluated, with six having subcutaneous MCTs. Paraoxonase-1 activity was significantly lower in Patnaik grade 3 ($p=0.003$) and Kiupel high-grade ($p=0.022$) MCTs. No significant differences were found in CUPRAC, d-ROMs, or oxy-adsorbent levels across different prognostic groups.

Conclusion: This pilot study found a significant correlation between histologic grading and Paraoxonase-1 activity suggesting a potential role of Paraoxonase-1 as prognostic biomarker in canine MCTs. Further studies with larger populations are ongoing.

23: DUODENAL EXTRASKELETAL CHONDROBLASTIC OSTEOSARCOMA IN A BOSTON TERRIER

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An 8-year-old female spayed Boston Terrier was referred to MS State University College of Veterinary Medicine for surgical removal of a duodenal mass. Incisional biopsies performed by the referring DVM were consistent with a spindle cell neoplasm, likely a gastrointestinal stromal cell tumor. Abdominal imaging performed at MSU-CVM revealed decreased serosal detail in the mid cranial abdomen and multiple pinpoint mineral opaque foci superimposed over the ventral small intestines. A 4 x 3 x 2.5 cm, firm, intramural, white to tan duodenal mass and mesenteric lymph node were surgically excised via resection and anastomosis and submitted for histopathological examination. On

histologic examination of the duodenal mass, the duodenum was transmurally and markedly expanded by an infiltrative mass composed of haphazardly arranged spindle cells supported by slightly myxomatous stroma, but within multiple sections, cells produced and became embedded within large amounts of chondroid interspersed with multiple foci of variably mineralized osteoid. Low amounts of osteoid were also scattered amongst streams of spindle cells. The mesenteric lymph node was partially effaced by a similar neoplastic population. Based on these findings, the duodenal mass was diagnosed as an extraskeletal chondroblastic osteosarcoma (OSA). Extraskeletal OSA most commonly occurs in the mammary glands but has been reported in other locations, rarely including the gastrointestinal tract. OSA of any location has a poor prognosis with a high incidence of early distant metastasis, and median survival times for extraskeletal locations, particularly of visceral origin, are reported in days rather than months.

24: IDENTIFICATION OF A HERPESVIRUS AS A CAUSATIVE AGENT OF ULCERATIVE VULVAR DERMATITIS IN CAPTIVE POLAR BEARS (*URSUS MARITIMUS*) USING NEXT-GENERATION SEQUENCING

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Ulcerative vulvar dermatitis (vulvitis) is frequently observed in captive polar bears (*Ursus maritimus*) in U.S. zoos. Understanding its etiology is essential for developing effective treatments and improving animal welfare. This study histologically examined vulvar skin biopsy samples from nine captive polar bears, revealing acantholytic cells, along with ulcerated epithelium, inflammation, and dermal abscesses, suggestive of a possible viral etiology. We hypothesized that herpesvirus is a causative agent of vulvitis in captive polar bears. Genomic DNA (gDNA) was extracted from frozen affected vulvar tissue samples and submitted for next-generation sequencing (NGS). The 300 base pair reads were assembled and compared with known sequences using the NCBI Virus BLAST database. Sequences from three gammaherpesviruses were identified: Herpesvirus *ursus* isolate 25482 (phocid gammaherpesvirus 3 [PhoHV3]), otarine gammaherpesvirus 4, and human gammaherpesvirus 4, suggesting the presence of a novel virus. To further validate this, gDNA was extracted from formalin-fixed paraffin-embedded vulvar tissue samples from the remaining five polar bears. Primers targeting the viral DNA polymerase gene were designed using published sequences from the three identified gammaherpesviruses for polymerase chain reaction (PCR). Following PCR and TA cloning, nanopore sequencing will be performed to identify sequence similarity to the published sequences. In summary, a novel gammaherpesvirus may be associated with vulvitis in captive polar bears. Future research will validate this association using in situ hybridization and provide crucial information to support treatment development.

25: NONINFLAMMATORY ALOPECIA OF WILD FREE-RANGING NORTHERN RACCONS (*PROCYON LOTOR*) IN NOVA SCOTIA, CANADA

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Noninflammatory alopecia is relatively common in domestic animals, especially dogs, but is rarely reported and poorly characterized in wildlife. In raccoons, alopecia is commonly associated with sarcoptic mange, which is highly pruritic and characterized by dermatitis and crusting. Four severely alopecic, free-ranging northern raccoons (*Procyon lotor*) were reported in early spring from Cape Breton, Nova Scotia, Canada. A skin biopsy was obtained from one of these raccoons, a female, at a wildlife rehabilitation center, and approximately one month later that animal and another, a male, were received for complete necropsy examination. Corresponding skin samples were collected from two normally haired raccoons as controls. Both animals were identified as young (first-year) adults. On gross evaluation cases exhibited marked, generalized alopecia of the body, with sparse hair present on the limbs, face, and ventrum, and numerous comedones in higher densities on and around the head. Histologically, the biopsy and necropsy samples exhibited similar lesions: most hair follicles were in kenogen phase, characterized by a trichilemmal keratin-lined follicular lumen and a lack of hair fibers, with very rare anagen follicles on the ventrum of one individual. Follicular infundibula were markedly dilated and hyperkeratotic. The surface epithelium was of normal thickness and exhibited normal to slightly thickened, basket-weave keratinization. There was no epidermal or dermal inflammation, no evidence of an underlying infectious etiology, and endocrine tissues were within normal limits. These raccoons have similarities to known hereditary causes of alopecia in domestic animals such as Alopecia X which suggests a possible genetic etiology.

26: A PATHOLOGICAL STUDY OF OCULAR BIOPSIES SUBMITTED TO THE ROYAL VETERINARY COLLEGE DIAGNOSTIC LABORATORY (RVCOPATH)

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Background: Ocular diseases require prompt treatment as they pose a significant risk to animal welfare. Chronic glaucoma and primary uveal tract tumors are the leading causes of enucleation in dogs and cats, respectively. Glaucoma can be classified as primary, with mainly a genetic component, or secondary due to the effects of other ocular or systemic diseases. In dogs, the most common primary intraocular tumor is canine anterior uveal melanocytoma followed by iridociliary neoplasia. Meibomian gland adenoma is the most common tumor affecting the eyelid in dogs. In cats, feline diffuse iris melanoma is the most common primary intraocular tumor with lymphoma being the most common extraocular neoplasm to affect the globe. Ocular biopsies may also aid in diagnosing particular systemic diseases.

Objective: This study aimed to classify all ocular biopsies submitted to the Royal Veterinary College (RVC) Diagnostic Laboratory to create an ocular pathology database, RVCOPATH (RVC Ocular Pathology) for further research.

Results: Of the 917 surgical biopsies, 224 were classified as glaucoma, 45 primary glaucoma and 179 secondary glaucoma, 337 were classified as neoplastic, 134 intraocular and 203 extraocular, 188 were classified as inflammatory, 13 were classified as infectious cause, 111 trauma, and 44 miscellaneous causes. More unusual cases include intraocular metastasis of a pulmonary carcinoma in a cat and a urothelial carcinoma in a dog.

Conclusion: The RVCOPATH database is under construction and already contains a significant number of pathological entities representing the most classic ophthalmologic conditions that can be diagnosed based on gross and histopathological assessment.

27: MPOX INFECTION OF STROMAL CELLS AND MACROPHAGES OF MACAQUE WITH ENDOMETRIOSIS

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The mpox outbreak of 2022-2023 represented a new global health challenge and recognition of mpox as a sexually transmitted disease. The majority of cases were reported in men who have sex with men (MSM), but women are also susceptible, especially during pregnancy. We evaluated the reproductive tracts of a subset of macaques from a large rechallenge study of mpox infection with virus from the 2022 outbreak and identified intraabdominal mpox replication associated with endometriosis. Mpox virus (MPXV) was found not only in skin, but in the cervix, the uterus, and periovarian endometriotic lesions of the affected macaque. Mpox replication preferentially targeted vimentin-positive poorly differentiated endometriotic stromal tissue and infiltrating macrophages in the reproductive tract. Mpox tropism for stromal cells and macrophages has broad implications for mpox pathogenesis and associated clinical syndromes. In addition, women with endometriosis may be at heightened risk for adverse outcomes associated with mpox infection. The rhesus macaque provides rare insight into this disease and the potential complications of mpox infection in the context of genitourinary tract disease.

28: SILICOSIS IN A PREGNANT MARE: A CASE REPORT

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Background: Silicosis is common in Northern California, including Monterey County where this horse resided. Cristobalite is the predominant form of silicate that causes silicosis in horses.

Methods: A 7-year-old, pregnant mixed breed mare, presented for necropsy following a month-long history of respiratory difficulty with no response to treatment efforts.

Results: Necropsy revealed a 25-35cm diameter multinodular, firm, mass that surrounded the adventitia of the distal trachea. The pericardial sac contained 2.0 liters of clotted blood, and the cranioventral portions of the lungs were effaced by hard to firm pink to tan, irregular nodules. Mediastinal and hepatic lymph nodes were firm, enlarged, ranging from 5-20 cm and frequently coalesced. Microscopically, there were extensive areas of fibrosis and mild to moderate multifocal lymphocytic and histiocytic inflammation, along with intralesional crystals. No other significant lesions were present.

Conclusions: With severe, chronic fibrosing and granulomatous bronchopneumonia with intra-histiocytic crystals, coupled with a clinical history of labored breathing, residing in areas with high levels of silicate in the environment, silicosis was suspected. Osteoporosis which is common in silicosis cases was not present. Using Transmission Electron Microscopy- energy-dispersive X-ray spectroscopy the intralysosomal complex aggregate of electron dense crystals provided high peaks of silicon and oxygen, the components of Silicon dioxide (SiO₂), confirming the diagnosis of silicosis. It is important to understand the conditions for silicosis in both humans and animals in order to prevent future cases as this condition is not reversible.

29: PROFILING BROWN ADIPOSE TISSUE IN PRIMATES IN RESPONSE TO ADRENERGIC STIMULATION

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Brown adipose tissue (BAT) stimulation is a promising treatment option for cardiometabolic disorders such as diabetes and obesity. Beta (B)2 and B3 adrenergic receptor (AR) agonists, such as mirabegron (an oral B3AR agonist), have been proposed as a way to stimulate BAT. We aimed to measure the histological characteristics of BAT pre- and post-mirabegron exposure in 9 female cynomolgus macaques (*Macaca fascicularis*), 4 of whom received placebo and 5 of whom received daily Mirabegron (2mg/kg) for 4 weeks. Repeated biopsies of left and right supraclavicular BAT depots were obtained pre- and post-exposure respectively. We hypothesized that BAT and white adipose tissue (WAT) would have higher levels of AR in less obese individuals. Tissue was stained for B3AR and B2AR and image analysis was completed with Visiopharm software (Denmark). We found that within individuals, B3AR and B2AR density were comparable and highly associated with each other (R-values>0.85). Between individuals, there was high variability in AR staining with more than a 2-fold range of values reported. Mirabegron induced expected increases in heart rate, blood pressure and neutrophilia but did not alter body composition, metabolic markers, or WAT fat cell size. The lack of biological effect was surprising, as mirabegron treatment did increase both B2AR (p<0.05) and B3AR (p=0.10) expression in BAT. In all cases, BAT showed compositional heterogeneity with significant WAT intermingled with BAT and equivalent B2/3AR staining. These data are a first in the evaluation of BAT expression and dynamic changes in situ in a relevant monkey model of human obesity.

30: PANCYTOPENIA SECONDARY TO PHENOBARBITAL TREATMENT IN A DOG

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An 18-month-old female, spayed German Shepherd with a 6-week history of seizure management using phenobarbital was referred to Lloyd Veterinary Hospital at Iowa State University for marked neutropenia, anorexia and lethargy of three days duration. A complete blood count (CBC) revealed pancytopenia characterized by a severe neutropenia, marked thrombocytopenia, and mild non-regenerative anemia. Bone marrow aspiration and biopsy were performed. On cytology, the bone marrow was hypercellular with a high M:E ratio (6:1) with a left shift in the myeloid series to band neutrophils and metamyelocytes. Rare segmented neutrophils were scattered throughout. Other findings included megakaryocytic hyperplasia with evidence of dysplastic changes, a blast percentage of up to 10% and rare neutrophagocytosis by macrophages. Histopathology confirmed ineffective myeloid hyperplasia with left-shifted myeloid maturation and showed marked selective erythroid hypoplasia with an elevated presence of blast cells. The pancytopenia along with dysplastic changes, left-shifted maturation, and elevated blasts were concerning for a myelodysplasia. Given the recent introduction of phenobarbital, drug-induced dysmyelopoiesis was considered the likely cause. Phenobarbital therapy was discontinued and, over the following two weeks, the patient's CBC trended towards normal limits. Secondary dysmyelopoiesis is generally characterized by multilineage ineffective hematopoiesis. Other features may include neutrophagocytosis and dysmegakaryopoiesis, which are present in this case. The patient's history indicates the high blast percentage observed in this case likely reflects the timing of sampling rather than a neoplastic cause. This distinction is important to consider in cases of drug-induced dysmyelopoiesis with an increased blast percentage.

31: PATHOLOGICAL CHARACTERIZATION OF SYSTEMIC AA AMYLOIDOSIS SECONDARY TO BUMBLEFOOT IN CHINESE GEESE (ANSER CYGNOIDES)

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Background and Objective: AA amyloidosis frequently occurs secondary to bumblefoot in waterfowl, including Chinese geese. However, information about lesion distribution and the degree of amyloid deposition has been limited.

Material and Methods: Histopathological evaluation and immunohistochemistry using anti-human serum amyloid A (SAA) antibody were conducted on 32 Chinese geese. The degree of amyloid deposition was scored on a scale of 0 to 5. LC-MS/MS was performed on amyloid deposits collected by laser microdissection. The MS/MS data were collated with theoretical fragmentation patterns of peptide sequences in the NCBI database. Based on gene analysis, the protein sequence of Chinese geese SAA was confirmed.

Results: All cases showed amyloid deposition that was positively correlated with the severity of ulcerative pododermatitis. The amyloid was positive for Congo red, with green birefringence under polarized light. Immunohistochemically, the amyloid was positive for SAA. The predilection sites of amyloid deposition were liver (27/32, 84.4%), lungs (26/32, 81.3%), and heart (22/32, 68.8%). The degree of amyloid deposition varied among tissues, and nervous tissues were less commonly involved. On LC-MS/MS analysis, high levels of SAA were detected. Gene analysis showed the presence of heterozygous nucleotide substitutions in some cases.

Conclusions: Our study characterized the pathological findings and proteomic profile of systemic AA amyloidosis in Chinese geese. According to some reports, species-specific amyloid deposition patterns are possible in avian species, such as the nervous tissues in flamingos. Further investigation of amyloidogenic regions will clarify the pathogenesis of amyloidosis in Chinese geese.

32: NEUROPATHOLOGIC ANALYSIS OF IL-15-ENGINEERED NK CELL THERAPY IN A MURINE GLIOBLASTOMA MODEL

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Background: Glioblastoma (GBM) is an aggressive astrocytoma and the most common primary brain tumor in adult humans. GBM induces immunosuppression in the tumor microenvironment (TME), limiting the efficacy of many immunotherapies. Adoptive cell transfer is one strategy for overcoming the lack of effective anti-tumor immune responses in the TME by administering effector immune cells engineered for improved tumor killing. Natural killer (NK) cells inherently resist many tumor defense mechanisms and have bolstered cytotoxic properties when armed with pro-inflammatory cytokines. However, some cytokines have been linked to significant toxicities.

Objective: We aimed to characterize neuropathology associated with reduced survival times after intratumoral injection of IL-15-engineered NK cells in a murine GBM model.

Methods: NSG mice were cerebrally engrafted with GSC20 patient-derived GBM stem cells and treated with vehicle, non-transduced (NT) NK cells, IL-15 NK cells or IL-21 NK cells (n=4 per group). Multiplex immunofluorescence staining was performed on brain sections (Akoya Biosciences Opal kit). Slides were scanned using Leica Versa 8 and biomarkers evaluated using HALO v3.6.4. Data was analyzed using GraphPad Prism 10.0.3.

Results: NK cells and microglia were increased in the IL-15 NK group. Spatial analysis showed

microglia in close proximity to NK cells (100µm), suggesting recruitment. IL-15R expression increased in astrocytes and microglia, consistent with microglia-astrocyte IL-15-mediated inflammatory crosstalk.

Conclusions: IL-15-engineered NK cells caused overactivation of microglia and astrocytes leading to neurotoxicity and decreased survival times in mice. Improved understanding of mechanisms of neurotoxicity may help guide development of novel immunotherapies with improved safety profiles in the future.

33: BILATERAL PYELONEPHRITIS AND SUSPECTED UREMIC ENCEPHALOPATHY IN A NEW-WORLD CAMELID

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Pyelonephritis may manifest as acute or chronic renal failure and is typically a consequence of lower urinary tract infections. Cases of pyelonephritis in camelids are underreported, with only rare, isolated publications afflicting camels, with *Escherichia coli* and *Staphylococcus lugdunensis* as underlying etiologies. Clinical signs vary with chronicity, but commonly include lumbar pain, poly/oliguria, urinary incontinence, renal azotemia and, potentially, uremia. Uremic encephalopathy is poorly documented in veterinary species; characterized by varying neurologic signs including lethargy, recumbency, ataxia, tremors, and blindness; and histologically supported by Alzheimer type II astrocytes and white matter spongiform change.

An 18-year-old intact female llama was presented to the AULATH for urinary incontinence, ataxia, progressive hindlimb paraparesis, muscle atrophy, and weight loss. CBC and chemistry profiles showed a non-regenerative anemia, neutrophilia, and elevated creatinine. Euthanasia was elected after a 6-day hospitalization and postmortem examination revealed severe bilateral renomegaly with multifocal pockets of suppurative and hemorrhagic exudate within the medulla and cortex. Histologically, the inflammation was centered on ducts and tubules and accompanied by myriad intralesional bacteria. Bacterial culture of the kidneys returned heavy growth of *E.coli*. Evaluation of the nervous system revealed significant astrocytic swelling throughout, comparable to Alzheimer type II astrocytes. Necropsy findings, clinical presentation, and serum chemistry profile support a diagnosis of ascending bilateral pyelonephritis, likely due to chronic cystitis, with suspected uremic encephalopathy. This case exemplifies a common renal disease affecting a species that has been largely unrepresented, as well as a rare and severe sequela of chronic renal dysfunction.

34: MICRORNA DIFFERENTIAL EXPRESSION ANALYSIS IN PLACENTAL TISSUE FROM CASES OF MISCARRIAGE IN BRAZIL

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Miscarriage is a public health problem with multiple causes, including infections. *Ureaplasma parvum* is a commensal bacterium of the urogenital tract that, when dysregulated, has been identified as a potential cause of miscarriage in women. MicroRNAs regulate gene expression; their dysregulation has also been associated with miscarriage; they can be used as prognostic markers of pregnancy. We hypothesized that miRNAs are differentially expressed in the placenta of women with normal delivery versus subjects who suffered a miscarriage and the colonization of *U. parvum*. Placental samples from normal deliveries (N=10) and miscarriage cases (N=9), subgrouped based on *U. parvum* infection, collected at a maternal and child referral center in northeastern Brazil were subjected to RNA extraction and qPCR for microRNA differential expression analysis of miR-23a, miR-494-3p, and miR-146a-5p. miR-23a was upregulated in patients with normal delivery despite the

presence of *U. parvum* compared to patients who had miscarriage in addition to *U. parvum* infection. There were no significant differences in the expression of miR-494-3p and miR-146a-5p between groups. MicroRNA expression differences between these groups could be used as prognostic markers for pregnancy in women. Studies in larger cohorts and serum samples are needed and may contribute to a better understanding of the miscarriage process.

35: POSTMORTEM ONSET AND PROGRESSION OF RIGOR MORTIS AND CHANGES IN BODY TEMPERATURE IN CALVES AND DOGS

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After death, the body temperature will adjust to the environmental temperature and the skeletal muscles will enter rigor mortis. Studying the timing of these changes can help estimate the postmortem interval which could be of central importance in cruelty investigations. This project studied the changes in body temperature and the onset/resolution of rigor mortis in calves and dogs after death. Thirty calves and 24 dogs were freshly euthanized for unrelated reasons to this project and kept at 24°C for 5 days. During this time, body temperatures were read every 2 - 4h, using a rectal/meat thermometer for the rectal temperature; an ear thermometer for ear canal temperatures and a no-contact thermometer read temperatures of the outer ear, forehead, nose, front paw pad and perianal skin. Concurrently, rigor mortis was evaluated on the jaw, neck, front limb, hind limb and tail. In dogs and calves, the rectal temperature decreased to equate the environmental temperature within 22-25h and 10-12h respectively; and the ear thermometer was unable to read body temperature (<32°C) after 10 and 4 hours respectively. All the no-contact measurements decreased to the environmental temperature within 6h, with the perianal skin temperature decreasing the slowest. Rigor mortis started in the jaw, progressed to the neck, hindlimb, then forelimb and was at its maximum after 6h. The tail never entered full rigor in calves and occurred last in some dogs. Rigor reversed in the exact opposite order and regressed the most by 30h in dogs, and 50h in calves.

36: PROGNOSIS AND IMMUNOHISTOCHEMICAL CHARACTERIZATION OF CANINE PLEOMORPHIC IRIDOCILIARY ADENOCARCINOMA

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Iridociliary epithelial tumors are the second most common ocular neoplasms in dogs. Metastasis is rare, but has been reported in a more malignant, pleomorphic variant. Canine pleomorphic iridociliary adenocarcinomas (PIA) are metastatic tumors that arise from the ciliary body and are characterized by an invasive growth pattern and loss of normal differentiation. Globes affected with PIA often have chronic glaucoma, and proliferation of tumor cells may be linked to ciliary body ablation (CBA). The Comparative Ocular Pathology Laboratory of Wisconsin (COPLOW) database was searched and fifty-six PIA cases were identified between 2005-2023. Submitting clinicians were contacted and follow-up data regarding a history of glaucoma, CBA, systemic conditions, clinical evidence of metastasis, and time to euthanasia were obtained. Thirty-five males and twenty-one females were identified across thirty-three breeds. The average age at diagnosis was 11.1 years with life expectancy averaging 8.8 months following enucleation. Nine cases were identified with a history of CBA prior to development of PIA, and ten cases were identified with evidence of metastatic disease.

Based on available follow-up data, ten canine globes diagnosed with PIA were chosen to be immunohistochemically labeled for cytokeratin [AE1/AE3], NSE, S100, E-cadherin, N-cadherin, desmin, vimentin, TTF1, and HER2, as well as Periodic acid-Schiff (PAS). The goal of this study is to

aid in diagnosis of PIA by evaluating immunophenotype, estimating prognosis following enucleation, and gathering information from medical history to better understand this rare disease.

37: OSTEOBLASTIC OSTEOSARCOMA IN A BIG BROWN BAT (*EPTESICUS FUSCUS*)

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An adult, intact male, big brown bat (*Eptesicus fuscus*) from a captive colony presented for necropsy with a pedunculated, 3 mm in diameter, firm mass attached to the right 5th proximal phalanx and an additional 1.6 cm in diameter firm mass in the right axillary region. The bat had previously been diagnosed with a sarcoma on the right 5th phalanx via histopathology 3 years prior, after suffering a bite wound in the same area. Following initial excision, the digital mass recurred within 2 years. After the axillary mass appeared the following year, the patient developed restricted mobility of the right forelimb and was humanely euthanized. Necropsy showed a large, tan, hard, spherical mass arising from the axillary region, deviating the thorax laterally, and firmly fixed to the humerus, scapula, and ribs. Histologically, the mass was densely cellular, unencapsulated, poorly demarcated, and was composed of spindle-shaped cells arranged in bundles and streams with abundant lacy, irregular trabeculae composed of tumor osteoid. The patient was diagnosed with osteoblastic osteosarcoma. There are few reports of tumors in bats, even though they comprise 22.4% of all classified mammalian species. To our knowledge, this is the first report of osteosarcoma in a chiropteran.

38: HISTOPATHOLOGICAL AND IMMUNOLOGICAL STUDY OF INFLAMMATORY RESPONSES IN WILD-TYPE CHIKUNGUNYA VIRUS INFECTION IN MAURITIAN AND INDONESIAN MACAQUES

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Chikungunya virus (CHIKV) is a mosquito-borne Alphavirus causing widespread human epidemics and is considered a priority pathogen by the WHO. CHIKV causes acute febrile illness and severe polyarthralgia in humans and nonhuman primates serve as excellent models for understanding CHIKV pathogenesis. Studies examining the immunological response to CHIKV infection in lymphoid organs are limited.

Using histopathology and immunohistochemistry, characterise CHIKV infection in the spleen, liver, brachial lymph node and peripheral joints of macaques.

Five Mauritian and five Indonesian macaques were inoculated with wild-type (La-Reunion) isolate of CHIKV and tissues examined following necropsy at 14-15 days post-infection. Tissues from infected macaques (and 2 uninfected controls) were examined histopathologically and lesions semi-quantitatively scored. The same tissues were immunohistochemically labelled using antibodies against CD3, CD68, CD8, MPO, IL-6, DC-SIGN, IFN- α and IFN- γ and semi-quantitatively scored.

The most significant findings were identified in the spleen of infected macaques where there was marked hyperplasia of periarteriolar lymphoid sheaths, as well as marked hyperplasia of lymphoid follicles, highlighted by CD3, in comparison to uninfected animals. Additionally, CD68 and MPO highlighted marked diffuse expansion and hyperplasia of medullary sinusoids, which contained increased numbers of reticuloendothelial cells, resulting in overall splenomegaly. CD68 and MPO labelling were increased in Mauritian in comparison to Indonesian macaques.

Within the spleen, the inflammatory response was typical of acute viraemia. Based on overall findings in various tissues, the macaque is a good animal model to study the acute inflammatory responses of CHIKV and early indications suggest differences between macaques of different geographical origins.

39: FLOW CYTOMETRIC ANALYSIS OF LYMPHOCYTE-RICH EFFUSION AIDING IN THE DIAGNOSIS OF A CANINE THYMOMA

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A 9-year-old, NM, German Shepherd was presented for evaluation of worsening cough and pleural effusion. A complete blood count and serum chemistry profile revealed no significant abnormalities except for a mild lymphopenia (0.64 K/uL; RI 1.0-4.8). Computed tomography revealed a cranial mediastinal mass with compression into the cranial vena cava. Analysis of the pleural effusion revealed a protein-rich transudate with a predominance of small and intermediate well-differentiated lymphocytes and mild mesothelial reactivity. Fine needle aspirates of the mass showed mixed leukocytes with a majority population of small, well-differentiated lymphocytes, as well as mast cells and epithelial cells. Flow cytometry of the pleural effusion revealed a minor population of small CD4+CD8+CD3- T cells, which supported the diagnosis of thymoma. The dog was ultimately euthanized due to owner concern regarding a diagnosis of neoplasia, and histopathology confirmed the mass to be a type B2 thymoma upon postmortem examination. This case highlights the use of flow cytometry on pleural effusion as an additional diagnostic modality when working up mediastinal masses in small animals. Pleural effusion sample collection via thoracocentesis would serve as a less invasive and therapeutic procedure to aid in future diagnoses, especially if cytology of fine-needle aspirates of the mass are inconclusive or when alternative applications of flow cytometry, such as on peripheral blood, cannot be performed. To this authors' knowledge, this is the first report of utilizing flow cytometry of an effusion to aid in the diagnosis of canine thymoma.

40: SINGLE-CELL TRANSCRIPTOMIC ANALYSIS REVEALS GUT EPITHELIAL ADAPTATIONS FOLLOWING BARIATRIC SURGERY

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Background: The intestinal epithelium is a complex cellular interface that plays a central role in both the pathogenesis and treatment of diet-induced metabolic disease. Obesogenic diets perturb epithelial structure and function, contributing to the widespread development of obesity and diabetes mellitus. Conversely, bariatric surgery induces gut adaptations that promote long-term metabolic improvements in humans and animal models. However, the mechanistic basis of these pathologic and therapeutic adaptations remains underdefined.

Objective: Profile cell type-specific transcriptomic changes within the intestinal epithelium upon treatment of diet-induced obesity in a mouse model of bariatric surgery.

Methods: Adult mice were maintained on either a low-fat diet (LFD) or a high-fat diet (HFD) for 16 weeks. HFD-fed mice then underwent either vertical sleeve gastrectomy (VSG), a common type of bariatric surgery, or a sham procedure; LFD-fed mice were sham-operated. Following recovery and metabolic phenotyping, intestinal epithelial samples were collected for single-cell transcriptomic analysis.

Results: Upon development of diet-induced obesity, HFD-fed mice treated with VSG demonstrated

significantly decreased adiposity and improved glycemic regulation compared to HFD sham-operated animals. Across individual cell types, single-cell transcriptomic analysis revealed perturbations in gene expression between HFD- vs. LFD-sham mice that were rescued by bariatric surgery. Notably, VSG ameliorated HFD-induced alterations in genes related to nutrient digestion among intestinal villi in addition to genes relevant for cellular metabolism within the intestinal crypt niche.

Conclusions: A single-cell transcriptomic approach provides a high-resolution perspective into the cellular and molecular mechanisms underlying gut epithelial adaptations to dietary and surgical interventions.

41: CANINE PERITONEAL LARVAL CESTODIASIS WITH SARCOMA DEVELOPMENT

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A presumed healthy 5-year-old, female spayed Anatolian Shepherd dog adopted from Turkey presented for a teaching ultrasound. A splenic mass, multiple abdominal cysts, and peritoneal effusion were incidentally found. The presumptive diagnosis was peritoneal cestodiasis. Despite antiparasitic treatment, the patient developed lethargy, hyporexia, tachypnea, dyspnea, and progressive peritoneal effusion. Euthanasia and necropsy were performed. The main gross findings were peritoneal and pleural effusion with a large amount of tan, turbid fluid, fibrin and free-floating, pale tan, 6 cm-long tetrathyridia. Multiple, variably sized nodules with cysts containing tetrathyridia were throughout the serosal surfaces, spleen, liver and lungs. Two large, firm, pale tan, multilobular masses were in the spleen and liver. Histologically, the nodules consisted of parasitic granulomas with intralesional tetrathyridia, which presented calcareous corpuscles and lacked a digestive tract. The hepatic and splenic masses were characterized by interweaving streams of neoplastic spindle cells, consistent with a soft tissue sarcoma. The neoplastic cells presented strong positive immunolabeling for vimentin and smooth muscle actin indicating a mesenchymal origin with myofibroblastic phenotype. Interestingly, the tetrathyridia were multifocally within the neoplastic sections, suggesting a possible neoplastic transformation related to the chronic parasitic inflammatory response. PCR and next generation sequencing of the parasites identified the specimens as *Mesocoestoides* sp. confirming the diagnosis of canine peritoneal and pleural larval cestodiasis. A chronic inflammatory response to *Mesocoestoides* sp. tetrathyridia could have played a role in oncogenesis. To the best of our knowledge, this is the first report of *Mesocoestoides* sp. parasitism associated to a soft tissue sarcoma.

42: PREVALENCE AND CAUSES OF THE LEUKEMOID REACTION IN DOGS AND CATS

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The leukemoid reaction, characterized by extreme neutrophilic leucocytosis, is an uncommon clinical finding in companion animals. Currently, there are few studies that evaluate the leukemoid reaction in both dogs and cats. This study aims to determine the prevalence and the most common aetiologies of the leukemoid reaction in dogs and cats at the Royal (Dick) School of Veterinary Studies (R(D)SVS) Hospital for Small Animals (HfSA). Haematology results from canine and feline patients in the ProVet Database, from November 2018 to March 2024, were evaluated to identify the presence of a neutrophil count of $>50,000/\mu\text{L}$ (leukemoid reaction). For these animals, ProVet was also used to establish underlying causes of this change. The search identified 32 patients (23 dogs and 9 cats) with a leukemoid reaction on at least once blood test, resulting in a prevalence of 0.10% for dogs and

0.12% for cats. Causes, in order of frequency, include inflammation/infection, neoplasia, immune-mediated disease, and other pathology for dogs, and inflammation/infection, neoplasia, and other pathology for cats. These results underline the rarity of the leukemoid reaction in dogs and cats and elucidate the most common causes. Further studies to explore treatment success rates, prognosis, and mortality rates of patients with the leukemoid reaction would be beneficial.

43: CONDOM ENTRAPMENT CAUSING DEFORMITY IN WILD RED-EARED SLIDERS

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Plastic waste and their environmental impacts have been a rising concern in recent years. Many types of plastic waste enter the waterways despite filtering systems in modern water treatment processes. Condoms are regularly flushed down the toilet and can escape the filtering systems in water treatment plants when overflows occur. Their impact on the environment and wildlife is not well understood. During a cold snap in Tennessee 200 red-eared sliders were found dead in an overflow pond at a water treatment plant, suspicious of having died from the anoxic conditions of the frozen pond. Six of the turtles were sent to the Southern Cooperative Wildlife Disease Study for evaluation, and two of them had marked malformations of the carapace and plastron resulting from constriction due to condom ring entanglement. It is unclear what percentage of flushed condoms escape modern water treatment plants. However, there is evidence from around the world that they regularly end up in waterways in various ways, and sewage treatment standards vary around the world. Findings on these turtles suggest that condom materials can be quite hardy in the environment and can pose a threat to wildlife.

44: POORLY DIFFERENTIATED UROGENITAL TUMOR AND CONCURRENT RENAL DYSPLASIA IN AN INDIAN RUNNER DUCK

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A 4-year-old intact male Indian runner duck presented to the OSU Boren Veterinary Medical Teaching Hospital for lethargy and inappetence. Ultrasound examination revealed a mass in the right coelom and free fluid within the coelomic cavity. The patient decompensated and CPR was performed but unsuccessfully. Necropsy revealed coelomic effusion and an 18x10x7 cm pale tan to off-white firm to soft, ovoid mass that markedly expanded the caudal coelomic cavity and obscured the urogenital tract. On cut section the mass was multifocally necrotic. Histologically, the mass contained both renal and testicular tissue. These tissues were markedly disrupted by a poorly demarcated, unencapsulated, densely cellular, infiltrative neoplasm composed of round to polygonal cells arranged in variably sized, circumscribed nests supported by a moderate fibrovascular stroma. Neoplastic cells had distinct cell borders, moderate amounts of pale basophilic cytoplasm, and round to oval nuclei. Anisocytosis and anisokaryosis were moderate with 15 mitotic figures in 10 40X fields. In other, less affected areas were small primitive tubules, loose, amphophilic mesenchyme, and foci of cartilaginous metaplasia, suggestive of concurrent renal dysplasia. Immunohistochemistry is needed to further characterize the neoplastic cell population. Spontaneous neoplasms are uncommonly reported in ducks, including Indian runner ducks.

45: HISTOLOGIC CHARACTERIZATION OF PURKINJE CELL DYSPLASIA IN A 1 DAY OLD NIGERIAN DWARF GOAT (CAPRA AEGAGRUS HIRCUS)

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HISTORY

A 1-day old female Nigerian dwarf goat (*Capra aegagrus hircus*) presented for lateral recumbency and inability to suckle. The kid was limp, unresponsive, and hypothermic with an erratic and rapid heart rate. Lungs auscultated harsh and wet. Despite supportive care and cardiopulmonary resuscitation, the kid passed naturally.

PATHOLOGIC FINDINGS

Postmortem examination found a small, irregularly shaped, smooth, round defect between the ventricles immediately under the atrioventricular valves. Histologic examination of the myocardium found multifocal nodular and linear aggregates of prominent Purkinje fibers and increased intercellular space within the left ventricular free wall. Other gross findings include partial atelectasis and renal medullary congestion. Purkinje cell dysplasia, in combination with the ventricular septal defect and ventricular non-compaction, is the presumed cause of death in this kid.

DISCUSSION

Purkinje cell dysplasia is a rare genetic disorder, characterized by an excess of purkinje fibers with histiocytoid appearances throughout the ventricular myocardium, resulting in cardiac arrhythmias and death. In humans, Purkinje cell dysplasia results in death within 2 years and disproportionately affects females. To date, only two reports of cardiac Purkinje cell dysplasia with ventricular non-compaction in veterinary species have been published: one in a 2-month old female Savannah kitten and one in a 4-month old female Holstein-Friesian calf. This case demonstrates the need to consider Purkinje cell dysplasia in female animals with sudden, unexpected deaths, particularly those with concurrent cardiac and/or congenital defects.

46: PROGRESSION FROM OMPHALITIS TO MENINGITIS AND NEURITIS IN A SHORTHORN HEIFER CALF

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BACKGROUND: A six-week-old shorthorn heifer calf was presented with stranguria and concern for a possible umbilical hernia. Antemortem ultrasound revealed an intact body wall with a hyperechoic lumen of the urachus and enlarged umbilical arteries. An omphaloarterectomy was successfully performed and noted moderate to severe abscessation of the right umbilical artery tracking to the right internal iliac artery. Despite recovering uneventfully, the calf was found recumbent four days later and unable to stand on her hindlimbs. After no improvement over four days of treatment, humane euthanasia was elected.

RESULTS: Postmortem examination found marked suppurative arteritis of the umbilical, uterine, and internal iliac arteries with extensive necrotizing and suppurative osteomyelitis at the level of L5 to the sacrum. Histologically, the localized spinal meninges were expanded by abundant neutrophils and mixed bacteria as well as an abscessed spinal nerve. There was no evidence of systemic embolic disease on either gross examination or histopathology.

CONCLUSION: The clinical signs were attributed to copious suppurative inflammation compressing the lumbosacral spinal cord due to direct spread of bacteria from the abscessed umbilical vasculature. Future considerations for the progression of urachal abscesses in calves should include the potential for neurologic involvement independent of systemic embolic disease. Urachal abscesses are not uncommon in calves, but do not typically cause extensive damage from localized spread. This case demonstrates the need for prompt surgical intervention after identifying the condition.

47: RETROSPECTIVE CLINICAL, GROSS, AND HISTOLOGIC CHARACTERIZATION OF FELINE EXOCRINE PANCREATIC NEOPLASMS

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Exocrine pancreatic adenocarcinoma (EPAC) is an uncommon, aggressive neoplasm in cats, but comprehensive data on clinical, gross, and histologic features are lacking. The aim of this study was 1) to evaluate a feline EPAC cohort for shared trends in clinical, gross, and histologic features and 2) define reproducible feline EPAC histologic sub-typing criteria. Retrospective evaluation of medical records, gross reports, and histopathology slides for 67 candidate cases was performed. Lesions were reclassified as EPAC (n=29), benign pancreatic neoplasms/pre-neoplastic lesions (n=11), ampullary carcinoma (n=7), biliary carcinoma (n=7), carcinoma of unknown origin (n=3), pancreatitis/pancreatodochitis (n=3), nodular hyperplasia (n=3), islet cell tumor (n=2), lymphoma (n=1), and hepatocellular carcinoma (n=1). EPAC histologic morphology was highly variable: at least six subtypes were identified, including acinar (n=17), cystic/papillary (n=7), anaplastic (n=2), ductal (n=1), adenosquamous (n=1), and intermediate (n=1). Common clinical signs were non-specific, including anorexia (n=9/29) and lethargy (n=6/29). The average age of EPAC cats was 12.6 YO, with an equal sex distribution and overrepresentation of DSH (n=21/29). Metastasis was common (n=26/29) with the liver most frequently involved (n=17/29); cystic/papillary subtype metastasis was less common (n=5/7). Few cats had abdominal effusion (n=9/29) and paraneoplastic alopecia (n=3/29). Concurrent pancreatic lesions included pancreatitis (n=18/29), nodular hyperplasia (n=16/29), and islet amyloidosis (n=11/29). The EPAC acinar subtype predominance correlates with previous literature. Feline EPAC histologic phenotype varied widely, making sub-typing challenging. Previously uncharacterized benign neoplastic/pre-neoplastic lesions were identified that resemble human pre-neoplastic lesions. Further immunohistologic characterization of feline EPAC subtypes and investigation into benign neoplasm/pre-neoplastic lesion pathogenesis is warranted.

48: PATHOLOGY OF FATAL PROTOPARVOVIRUS INFECTIONS AND THEIR COMORBIDITIES IN FREE-RANGING CARNIVORES IN CALIFORNIA

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Protoparvoviruses are DNA viruses distributed worldwide, are mainly species-specific, and are named according to their host/ reservoir. However, some have changed their host range causing fatal infections in wildlife species. This study documents cases of fatal parvoviral infections in free-ranging carnivores from California and assesses their main comorbidities. 16 raccoons (*Procyon lotor*), 2 gray foxes (*Urocyon cinereoargenteus*), and 2 mountain lions (*Puma concolor*) submitted to the California Animal Health and Food Safety Laboratory between 2010 and 2024 are included in this study. Necropsy reports were evaluated to collect gross and histopathological findings, demographic data, and categorize the parvoviral presentation and diseases or conditions that contributed to the fatal outcome. Only cases with suggestive parvovirus lesions and confirmed infection via immunohistochemistry, PCR/sequencing, and/or electron microscopy were included. The enteric presentation was the most prevalent (18/20, 90%); 13/18 cases had severe diffuse necrotizing enteritis with crypt necrosis, and 5/18 also had severe necrotizing enterocolitis. Only the mountain lions exhibited the neurological presentation, and both had moderate to severe multifocal nonsuppurative encephalomyelitis with gliosis. Ten cases had one or more comorbidities including enteric salmonellosis (6/10, 60%), enteric and septicemic *Escherichia coli* (2/10 each), *Klebsiella pneumoniae* septicemia, enteric *Cryptosporidium* spp., oral candidiasis, Pseudorabies, and polytraumatism (1 case each). This case series demonstrates that free-ranging carnivores from different families in California are vulnerable to fatal protoparvovirus infection, and the most common

comorbidities were secondary bacterial infections followed by other infectious diseases that might play an important role in the demise of these animals.

49: IDENTIFYING A MYCOPLASMA SP. AS THE CAUSAL AGENT OF SYNOVITIS IN BIG BROWN BATS (*Eptesicus fuscus*)

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Background:

Despite their important role in ecosystem health, little is known about infectious diseases in bats, aside from lyssaviruses and white nose syndrome. Big brown bats (*Eptesicus fuscus*) are increasingly observed with swollen joints in wildlife clinics and do not respond well to treatment, hindering successful release. The cause is not definitively known, but both an unnamed *Mycoplasma* species as well as a poxvirus, have been implicated.

Objective & Methods:

To determine the cause of synovitis in big brown bats, 25 big brown bats including 11 with enlarged joints and 14 “controls” with grossly normal joints were grossly and histologically examined and tested for both *Mycoplasma* spp. and *eptesipoxvirus* by PCR.

Results:

Mycoplasma was detected in 5/11 (45.5%) of bats with grossly enlarged and/or histologically inflamed joints. None of the bats with normal joints were PCR-positive for *Mycoplasma* ($p=0.0087$). Sequencing of the intergenic spacer region of *Mycoplasma* revealed that all five were identical to one another, shared poor similarity to other *Mycoplasma* species in Genbank (78% similarity to *M. iguana*) but were 100% identical to a *Mycoplasma* previously detected in big brown bats with polyarthritis. Microscopically the lesion consisted of neutrophilic synovitis, periosteomyelitis, fasciitis, and dermatitis similar to *Mycoplasma* infections in other species. Poxvirus testing is ongoing.

Conclusions:

This study is the first to concomitantly test for both suspected causative agents of this poorly understood syndrome in both clinical and unaffected big brown bats. Our data suggests that *Mycoplasma* is likely to be associated with swollen joints in this species.

50: INCIDENTAL OR NOT? A CASE OF BOVINE ENDOMETRITIS AND A PITUITARY CYST

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Presenting dead for necropsy, after a brief history of a 105-degree fever and ketosis, was a 3-year-old Holstein cow 39 days in milk of her second lactation. The major findings identified on necropsy were multifocal to coalescing necrotizing endometritis, focally extensive hepatic abscesses, multifocal embolic pneumonia, and a focal cyst-like structure in the pituitary gland. The normal parenchyma of the liver and the lung were replaced with focally extensive, and well-demarcated areas of liquefactive necrosis. The necrotizing endometritis was classified as a subacute to chronic lesion. Overall, the most significant findings, in this case, were the marked hepatic abscesses and embolic pneumonia with *Trueperella pyogenes* isolates cultured. The primary pathogenesis in this case was either a

hepatic abscess rupture with subsequent embolic pneumonia; or bacterial infection originating in the uterus leading to sepsis that seeded within the liver and lung parenchyma. Interestingly, the expansile cyst-like pituitary structure may have a detrimental effect on the hypothalamic-pituitary-gonadal axis impacting normal uterine involution, predisposing the uterus to a bacterial infection. Oxytocin, a hormone released from the pituitary gland, assists with uterine involution. Furthermore, in ruminants, the pulsatile release of oxytocin by the neurohypophysis stimulates the production of uterine PGF_{2α}, another key hormone responsible for proper uterine involution. Is it possible this cyst exerted enough pressure on the pituitary gland to induce a state of panhypopituitarism? This proposed pathogenesis could lead to insufficiencies in oxytocin and PGF_{2α}, resulting in improper uterine involution, predisposing a severe bacterial infection to harbor within the traumatized post-partum uterus.

51: COMPARISON OF CANINE PERIPHERAL BLOOD MONONUCLEAR CELL (PBMC) ISOLATION TECHNIQUES FROM WHOLE BLOOD: CPT™ VERSUS SEPMATE™ TUBES

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Background: Veterinary and translational veterinary research widely use peripheral blood mononuclear cells (PBMCs). These can be isolated by density centrifugation with commercial media like Ficoll and Histopaque-1077, allowing leukocyte separation into density-dependent layers for isolation. Although validated in humans, these media and methods have not been substantially studied in dogs. Therefore, the content purity of each layer may be impacted by any species-specific cell size, weight, or content changes.

Objective: Characterize the yield, viability, and composition of cells isolated from canine whole blood via Cell Preparation Tubes™ (CPT™) with FICOLL™ Hypaque™ and SepMate™ tubes with Histopaque-1077.

Methods: Whole blood from ten dogs was collected for parallel PBMC isolation with CPT™ and SepMate™ tubes containing Histopaque-1077, per manufacturer guidelines, and including additional platelet removal steps. Yield, viability, CBC, manual differential, and immunophenotypes were evaluated.

Results: SepMate™ tubes provided higher live cell yields (Sepmate™ 2.48E+06 *versus* CPT™ 9.75E+05 cells/mL), similar viabilities (Sepmate™ 96.7% *versus* CPT™ 96.1%), and subjectively better cellular preservation. Conversely, CPT™ isolations exhibited greater platelet contamination (Sepmate™ 3.7E+03 *versus* CPT™ 2.5E+04 cells/mL), lesser neutrophil contamination (Sepmate™ 25% *versus* CPT™ 5%), and significantly more CD 4+ T-cells (Sepmate™ 25.23% *versus* CPT™ 38.22). Both methods produced unexplained CD 4+ low T-lymphocyte and CD 11b+ only myeloid subpopulations, as well as overrepresented eosinophils (Sepmate™ 11.8%, CPT™ 6.4%) when compared to whole blood (5.8%).

Conclusions: SepMate™ tube isolation using Histopaque-1077 inconsistently and only partially depleted the samples of neutrophils when compared to CPT™ PBMC isolates in canine whole blood.

52: INTESTINAL TORSION AND PERFORATION IN A CAPTIVE SEA TURTLE DUE TO INGESTION OF PLASTIC TOY CAPSULE

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Background and Objective: Accidental or intentional ingestion of foreign objects is common in reptiles. Particularly in wild sea turtles, ingestion of plastic debris due to ocean pollution has become a major problem. In this study, detailed pathological findings of an aquarium sea turtle with lethal intestinal lesions caused by ingestion of an artificial plastic capsule are presented.

Material and Methods: An estimated 50-year-old, female turtle, a mixed breed of loggerhead and hawksbill turtles, presented with anemia and anorexia. Medical treatment did not provide any improvement. The turtle died approximately three weeks later. An autopsy and subsequent histopathological examination were performed at Nihon University.

Results: Large amounts of dark green, cloudy fluid containing muddy debris were observed in the coelomic cavity. The small intestine was twisted approximately 360 degrees at the axis of the mesentery and was perforated. The intestine proximal to the torsion was diffusely dilated (width up to 10 cm), with an approximately 6-cm-diameter, deformed plastic toy capsule within the lumen. The intestinal mucosa was covered by a pseudomembrane. Histologically, the pseudomembrane was composed of degenerative heterophils, fibrin, and necrotic tissue. Intestinal walls were thickened and transmurally infiltrated by large numbers of heterophils with marked edema.

Conclusions: Accidental or intentional ingestion of foreign objects impacts health conditions of zoo and aquarium reptiles. Not only raising awareness of ocean pollution, but also careful husbandry will improve animal welfare. This study provides new insights into pathological findings in these animals.

53: CLINICOPATHOLOGIC EVALUATION OF RADATION-INDUCED SKIN TOXICITY IN A PORCINE MODEL

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Background: Radiotherapy (RT) is a common cancer treatment, and many patients experience radiation-induced skin reactions (RISRs), having a significant negative impact on quality of life. Therefore, new strategies to reduce RISRs are critical. Studies in mice have shown FLASH-RT can deliver high doses eliminating cancer cells, while sparing normal tissues. Pigs being a highly relevant model, we evaluated clinicopathological skin changes after FLASH-RT and conventional radiation (CONV-RT). We hypothesized FLASH-RT would result in decreased skin toxicity and improved healing.

Materials & Methods: Four pigs received either CONV-RT or FLASH-RT on both the right and left flank using an IntraOp Mobetron electron linear accelerator. Skin punch biopsies were collected, and pigs were monitored for RISRs. Tissues were processed and microarrays created. Multiplex immunofluorescence (mIF) staining was performed to characterize immune cells and fibrosis. Slides were scanned and biomarkers quantified using HALO v.3.6. Data was analyzed via 2way ANOVA using GraphPad Prism v.10.

Results: FLASH-RT showed reduced acute clinical dermatitis characterized by reduced erythema, ulceration, and dry desquamation. Macrophages predominated in acute RISRs at all doses with a significant dose-dependent increase in M2 macrophages. Reduced chronic inflammation and fibrosis were observed clinically in FLASH-RT in comparison to CONV-RT.

Conclusion: FLASH-RT resulted in reduced acute and chronic skin toxicity clinically and improved wound healing. Macrophage infiltration is a critical component of the immune response in acute RISRs and dose dependent M2 polarization may be an early determinant of chronic inflammation, fibrosis, and delayed healing after RT.

54: CHARACTERIZING NEUROMA FORMATION AFTER TAIL DOCKING IN SHEEP

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BACKGROUND

Tail docking is a routine procedure performed in sheep that is known to cause both short- and long-term pain. Studies have found evidence of neuromas, characterized by abnormal proliferations of irregularly distributed nerve bundles, swirling patterns among axons, and fibrosis, at the site of docking in sheep tails. However, neuroma formation after a rubber ring is applied in the first days of life, a common method of tail docking, has not been described.

OBJECTIVE

This study seeks to describe the histological characteristics of neuromas formed in sheep tails docked using a rubber ring at 1-2 days of age, as compared to age-matched controls.

METHODS

Eighteen sheep (8 docked at 1-2 days of age, 10 undocked controls) were euthanized at 7.8 ± 0.2 months of age (mean $\pm$ SD) and their tails collected and decalcified for histology. FFPE sections were stained with H&E and trichrome, and immunohistochemistry was performed using stains for s100 and neurofilament heavy chain (NFH). Each tissue sample was evaluated for abnormalities characteristic of neuromas, receiving a severity score ranging from 0 (absent) to 4 (severe).

RESULTS

Neuroma-like proliferations were found in all docked tails, ranging from mild to severe. These proliferations were particularly prominent in the periosteal region surrounding the vertebrae. Neuromas stained positively for both s100 and NFH, and trichrome staining showed that neural proliferation was accompanied by Schwann cell proliferation and fibrosis.

CONCLUSION

This study provides evidence that tail docking with a rubber ring in sheep causes neuromas, a known source of chronic pain.

55: A COMBINED MOLECULAR AND GENOMIC APPROACH FOR THE IDENTIFICATION OF THE ETIOLOGICAL AGENT OF "DOPEY FOX SYNDROME"

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Background: "Dopey Fox Syndrome" (DFS) is an emerging neurological disease of unknown origin affecting UK wild red foxes (*Vulpes vulpes*). The disease is associated with non-suppurative meningoencephalitis, resulting in vision changes/blindness and neurological signs. The etiological agent has not been definitively established and, therefore, this study aimed to utilize a combined molecular and genomic approach to systematically investigate a number of candidate pathogens for involvement. These comprised parasites (*Toxoplasma gondii*, *Neospora caninum*, and *Angiostrongylus vasorum*) and viruses (Fox Circovirus, Canine Herpesvirus Type-1, Canine Adenovirus 1 and 2, Canine Parvovirus, Canine Distemper Virus, and Tickborne Encephalitis).

Methods: A total of 18 fox brain tissues were analyzed, representing 9 DFS cases and 9 controls. For the untargeted detection of pathogen DNA, samples were subjected to deep sequencing using MinION, Nanopore® technology. Additionally, a comprehensive panel of nested, hemi-nested, conventional and real-time PCRs was used to screen for each pathogen. Conventional PCR products were further characterized by Sanger sequencing.

Results/Conclusions: Nanopore technology was found to be inefficient in pathogen detection due to the abundance of host DNA. PCR-based assays detected *T. gondii* (77.7%, 14/18), FCV (22.2%, 4/18) and CAV-1 (33.3%, 6/18) in the study population. While there was no bias in the presence of infections between DFS cases and controls, the results of this study illustrate that these pathogens are circulating in foxes at an appreciable level. This study increases baseline knowledge of potential DFS agents and further work is warranted investigating the potential for their subtle and complex interactions in precipitating disease.

56: HISTOLOGIC EVALUATION OF TISSUE ARTIFACTS ASSOCIATED WITH EUTHANASIA METHODS IN BROOK TROUT

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Teleost fish are used in large numbers in aquaculture, research, as companion animals, and are vital to ecosystems. Histopathology aids accurate and specific diagnoses. Euthanasia procedures can create histologic changes with the potential to hide or create the appearance of lesions. Our aim was to characterize tissue changes due to various euthanasia methods commonly used in teleost fish. We hypothesized that euthanasia artifact would be found in tissues closest to the site of administration. 40 adult brook trout (*Salvelinus fontinalis*) were selected for use in the study. Each fish was randomly assigned to one of 5 treatment groups: euthanasia with intravenous, intracardiac, or intracoelomic euthasol, or intravenous or intracoelomic potassium chloride. Samples were taken from organs typically examined in a standard necropsy and stored in 10% neutral-buffered formalin. Tissues were then processed routinely and stained with hematoxylin and eosin (H&E). Raters were blinded to treatment conditions. Histopathologic examination identified possible artifactual changes in gill, liver, spleen, and heart. Tissues were coded based on degree and distribution of change. Preliminary results indicate that both intracelomic treatments were associated with increased artifactual changes in liver and spleen. In four cases, significant disruptions were also observed in the anterior kidney, with 75% of these marked artifacts belonging in the category of IV euthasol administration. These results provide veterinary pathologists baseline information about potential confounding lesions, and suggest how clinicians can consider artifactual lesions when selecting a euthanasia method.

57: MULTISYSTEMIC EOSINOPHILIC EPITHELIOTROPIC DISEASE (MEED) IN A QUARTER HORSE: A CLINICAL AND HISTOLOGICAL OVERVIEW

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A 3-year-old Quarter Horse mare presented with a history of progressive ulcerative lesions present on the neck bilaterally. Initial treatment was successful, with skin biopsy revealing eosinophilic and histiocytic inflammation with collagenolysis. Pruritic, ulcerative lesions returned a year later in the inguinal area with progression to lateral trunk, accompanied by chronic diarrhea, weight loss, and identification of nodular lesions in the liver and lungs via ultrasonography. Additional skin biopsy showed severe eosinophilic infiltration throughout a thickened dermis and panniculus with perivascular cuffing and evidence of collagenolysis and flame figures. Due to poor prognosis, humane euthanasia was elected. Gross findings included widespread multifocal tan nodules of varying sizes throughout the lungs and liver and multifocal ulcers with hemorrhage in the colon. Histologic findings from lung tissue samples showed multifocal eosinophilic granulomas with infiltration into the bronchiolar epithelium. Liver histology revealed a concentration of eosinophils within the bile duct epithelium, eosinophilic hepatitis, and multifocal eosinophilic granulomas. Compared to the liver and lung sections, the colon reflected a mild amount of eosinophil proliferation within the mucosa. The pancreas was unaffected with no eosinophils noted in the pancreatic duct epithelium. Histopathological diagnosis is consistent with multisystemic eosinophilic epitheliotropic disease (MEED). MEED is an extremely rare, chronic, eosinophilic condition that affects young horses with a poor prognosis and no effective treatment options. Primary locations for eosinophilic infiltration include the liver, colon, pancreas, and lung. Although the cause of MEED is not well-known, leading research suggests an autoimmune reaction with a possible parasitic or allergic etiology.

58: CUTANEOUS RHODOCOCCLUS EQUI INFECTION IN AN ADULT STANDARD BRED MARE

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A 9-year-old Standardbred mare presented with a two-month history of weight loss and progressive cellulitis affecting the left distal hindlimb. On presentation, there was segmental swelling affecting the pastern region, with multifocal nodular, tan foci up to 1.5cm in diameter with draining tracts, which focally coalesced to form an approximately 5cm in diameter, raised, friable ulcerated focus with pale tan discharge on the caudolateral aspect. Two punch biopsies of the ulcerated lesion were obtained and submitted to The Ohio State University Veterinary Dermatopathology Service. On histopathology, the dermis and subcutaneous tissues were effaced by nodular to coalescing inflammation composed of epithelioid macrophages admixed with abundant neutrophils, with low numbers of multinucleated giant cells. Many macrophages and multinucleated giant cells contained cytoplasmic vacuoles with numerous intracellular coccobacilli bacteria. *Rhodococcus equi* (heavy growth) was isolated by aerobic culture of aseptically collected skin tissue, consistent with the intracellular bacteria seen on histopathology. The mare was hospitalized, and the affected region was surgically debrided, at which time they identified involvement of the tendon sheath. Euthanasia was elected due to the comorbidities and guarded prognosis for return to normal. This case represents an unusual and rare presentation of *Rhodococcus equi* infection.

59: SUPRADIAPHRAGMATIC ACCESSORY LIVER IN A CHIMPANZEE

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Background: Nodules of ectopic liver are found in humans, primates and domestic animals with low incidence. They are primarily classified by size and relationship to the liver. Proposed etiologies include trauma, surgical complications or abnormal embryonic development.

Case presentation: A 47-year-old female chimpanzee (*Pan troglodytes*) was submitted for necropsy following chronic fibrosing cardiomyopathy. Numerous accessory liver nodules ranging from 0.5 cm to 5 cm were localized to the intrathoracic diaphragmatic surface with some loose adherence to both caudal lung lobes. These grossly normal nodules maintained negative pressure within the thorax despite their connection to the primary liver by transdiaphragmatic pedicles. Multifocal annular fibrosis of the diaphragm was noted at the base of these nodules. Histologically, these non-neoplastic, intrathoracic hepatic nodules demonstrated severe sinusoidal congestion with evidence of hydropic degeneration and hepatocellular regeneration. The hepatic sinusoids and portal areas had accumulated hemosiderin-laden macrophages and mononuclear infiltrate, respectively. These lesions had not been previously identified by routine imaging.

Discussion: This is the first report of ectopic liver in a non-human ape to our knowledge. Similar to reports in other species, these nodules were morphologically normal and likely incidental. However, the unique presence of dozens of accessory liver nodules contrasts the typical presentation of singular or dual herniations. Possible causes include age-related structural weakness of the diaphragm or acute abdominal trauma. Although rare and often asymptomatic, ectopic liver should be considered as a differential for caudal thoracic masses.

60: FORENSIC NECROPSY OF DOG WHOSE ABDOMINAL "MASS" WAS A MEGACOLON

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A 6-year-old intact female Pitbull-mix dog was owner-surrendered to a local shelter with a history of anorexia for multiple weeks and progressive weight loss. On clinical examination, the animal had a low body score, and imaging revealed a large mass in the abdomen. The dog was euthanized and

submitted for forensic necropsy with the specific request to investigate the cause of the weight loss and specify if neglect was a major contributory factor.

On gross examination, the colon was severely distended with a maximum circumference of 18cm and filled with 3.3kg of packed, dark brown, malodorous dry feces. Similar content dilated the cecum and the aborad ileum. The right kidney was compressed and deformed by the caecum with parenchymal atrophy and had additional chronic infarcts. Histologically, the cecal and colonic mucosa were markedly thin and compressed by the luminal content, which consisted of partially mineralized digesta colonized by mixed bacteria and plant material. The myenteric plexus was also thin with subjectively decreased numbers of nerve fibers and neurons. No other mass was identified.

The cause of death was the megacolon and the manner of death was classified as natural. This case illustrates how baseline veterinary care is of central importance, as it could have prevented/treated the additional endo- and ectoparasitism (in heart, intestines and skin) and provided management of the megacolon despite it being a therapeutic challenge and being a progressive condition.

61: MESENTERIC VOLVULUS IN TWO TORTOISES

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Mesenteric volvulus occurs when the bowel twists along the mesenteric axis around the root of the mesentery, causing cranial mesenteric artery occlusion and strangulation, dilation, and necrosis of the duodenum through the transverse colon. Mesenteric volvulus is well-documented in domestic animals but has, to our knowledge, only been reported in one chelonian. We present two cases of mesenteric volvulus in tortoises. Case 1 was a 28-year-old male Leopard tortoise (*Stigmochelys pardalis*) presented for lethargy and anorexia. Serial computed tomography identified progressive gastrointestinal dilation and thickening with vascular distension and air emboli; mesenteric volvulus was suspected, and euthanasia was elected. Postmortem examination identified a 360-degree rotation of the intestinal tract at the mesenteric root with segmental congestion to necrosis of the duodenum through the mid-colon. Midway through the affected bowel was a chronic stricture with severe distension and additional segmental volvulus of aborad intestine. Case 2 was a 3-year-old male Sulcata tortoise (*Centrochelys sulcata*) that died after 8 days of anorexia, lethargy, and vomiting/regurgitation. Antemortem radiographs identified marked dilation of the gastrointestinal tract. Postmortem examination found a 720-degree rotation of the intestinal tract around the mesenteric root, with well-demarcated segmental necrosis and fibrinous adhesions affecting the distal duodenum through proximal colon. Histologic evaluation in both cases confirmed segmental intestinal necrosis, hemorrhage, and edema. Histologic evaluation of Case 1's stricture identified fibrosis, ulceration, and heterophilic crusting with mixed bacterial colonization. Mesenteric volvulus should be considered in chelonian species with acute and progressive gastrointestinal signs, and antemortem diagnostic imaging may aid in diagnosis.

62: BORRELIA BURGDORFERI AS A CAUSE OF FATAL NECROTIZING AND LYMPHOPLASMACYTIC MYOCARDITIS IN A 6-YEAR-OLD FEMALE LABRADOR RETRIEVER DOG

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A 6-year-old female (spayed) Labrador Retriever was submitted for necropsy after a fatal cardiopulmonary event. Most significant post-mortem findings were confined to the thoracic cavity.

The lungs were enlarged, moist, diffusely red, and pliable, with subtle rib impressions more prominent on the caudal lobes. Sanguineous frothy fluid was present within the trachea and bronchi. The right ventricle was enlarged and presented multifocal, slightly shallow areas of reddish-brown discoloration, up to 1.5 cm in the largest dimension, on its epicardial surface. These foci, surrounded by poorly defined areas of pallor, were also found within the endocardium and extended deep into the underlying myocardium. Microscopically a severe necrotizing lymphoplasmacytic myocarditis with interstitial fibrosis was seen. *Borrelia burgdorferi* antigens were detected by immunohistochemistry (Rabbit anti *Borrelia burgdorferi* antibody) within the areas of myocardial inflammation. Lyme disease is endemic in the northeastern United States, and although seroprevalence in dogs from endemic areas is high, the disease is often subclinical. Clinical disease, when present, is manifested primarily by arthritis and nephritis. Lyme disease induced myocarditis in dogs, reported primarily in Europe, seems to be a rare occurrence. Lymphoplasmacytic myocarditis in this species is often interpreted as non-specific and likely the result of virus infections, mainly by canine parvovirus-2, canine distemper virus, or more rarely, West Nile virus. The finding of *Borrelia burgdorferi* antigens in the heart lesions of this dog suggests that Lyme disease associated myocarditis may be underdiagnosed and should be considered as a possible cause of heart disease in dogs from endemic areas.

63: DIAGNOSIS, VIRUS ISOLATION AND MOLECULAR CHARACTERIZATION OF CIRCULATING FIPV STRAINS FROM 2 SHELTER CATS OF LONG ISLAND

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Feline infectious peritonitis virus (FIPV) remains as one of the leading causes of morbidity and mortality in young cats from shelters and catteries worldwide. Since little is known about the molecular characteristics of currently circulating FIPV strains in Long Island, New York, as part of a major study, samples from two shelter cats (A15 and A37) with gross and microscopic lesions consistent with those of FIP were processed for virus isolation, molecular characterization and full-length genome decoding. The younger shelter cat, a 1-year-old male (A15) was found dead without previous signs of illness. Postmortem examination revealed gross and microscopic lesions characterized by vasculitis, necrosis, hemorrhage and pyogranulomatous inflammation confined to the colon and associated lymph nodes. The second cat, a 7-year-old spayed female (A37) had an identical clinical history and similar but widespread lesions, including peritoneal effusion, cecal, colonic, renal and hepatic involvement. The gross and microscopic diagnosis of FIP in these cats was confirmed by immunohistochemistry (IHC) demonstration of coronavirus antigen using mouse anti-FIPV3-70 monoclonal antibody. Virus isolation from saved frozen tissue was performed through several subsequent blind passages in MDCK and Vero cell lines. Confirmation of the FIPV isolation was done through qRT-PCR, IFA, western blot using N protein antibodies, and NGS of the full-length genome sequencing. Diagnostic surveillance, molecular characterization and sequencing analysis of circulating FIPV strains within animal shelters may help to identify unique evolving clinical and pathological manifestations of the disease and the development of more targeted prophylactic and therapeutic approaches to control it.

64: ARTIFICIAL INTELLIGENCE-BASED IMAGE ANALYSIS OF NODAL CANINE CUTANEOUS MAST CELL TUMORS

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Canine cutaneous mast cell tumors (cMCTs) are among the most common cancers in dogs. Lymph node metastasis is recognized as a negative prognostic factor and lymph node extirpation and histologic evaluation for metastasis is recommended for staging. The current cMCT lymph node classification system is based on a retrospective study that had a limited cohort size and relied on

subjective criteria. More objective, outcome-based assessment is needed to identify parameters that correlate with prognosis. The primary aim of this research is to generate an AI-based digital image analysis algorithm to qualitatively and quantitatively assess nodal mast cells in canine mast cell disease. A secondary aim is to establish baseline values of mast cell burden in dogs without MCTs. Using cohorts of 15 dogs with histologically diagnosed cMCTs with evidence of nodal metastasis and 10 negative controls, lymph nodes were labeled for cKIT and Ki67 and digitally scanned. Several iterations of annotations were performed, and digital slides were analyzed via Aiforia®, a machine learning platform, to assess the following parameters: lymph node mast cell burden (total cKIT+), proliferation quotient (Ki67+/cKIT+), and mast cell spatial distribution. Our AI model successfully identified cKIT+ mast cells, Ki67+/cKIT+ mast cells, and mapped mast cell density within lymph nodes. In future work, we will integrate this approach into a large-scale prospective study to compare nodal mast cell parameters with clinical outcomes. We believe that these parameters will correlate with long-term prognosis, establishing a more objective and accurate predictor of biological behavior.

65: DIFFUSE MYELOID LEUKEMIA IN AN 8-MONTH-OLD DOMESTIC CHICKEN

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Myeloid leukemia in domestic chickens is frequently associated with avian leukosis virus subgroup J. Other viral subgroups include A, B, C, D and recently, E, where only subgroup E is non oncogenic. Myeloid leukemia is a clonal malignancy seen in chickens 16 weeks or older. The disease is often subclinical, resulting in decreased egg production. Nodular lymphoid tumors can be seen in up to 4% of infected birds with cloacal bursa involvement considered pathognomonic. Prevention for myeloid leukemia and avian leukosis viruses involves eradication of genetically susceptible animals from breeding stock. On gross examination, the keel bone was prominent with decreased pectoral musculing. The right iris had a small foci of light yellow to white discoloration. The liver was markedly enlarged, pale with pale tan foci diffusely. The spleen was also markedly enlarged and mottled with pale tan foci similar to the liver. There were bilaterally enlarged, prominent nodules in the mid-cervical subcutaneous tissue. On histologic examination, all organ systems were found to have neoplastic round cell infiltration consistent with myeloid leukemia. Neoplastic cells were distinct with scant to moderate eosinophilic cytoplasm or cytoplasm that was filled with bright eosinophilic granules. Next-generation sequencing identified that only avian leukosis virus was present in the sample and further genomic analysis revealed that the virus identified in the case was closely related to two avian leukosis virus strains: IBDV2009 and TymS90.

66: MELANOMA WITH WIDESPREAD TISSUE METASTASES IN A 26-YEAR-OLD MARE WITH RESPIRATORY DISTRESS AND CONGESTIVE HEART FAILURE – TARGETED SEQUENCING CHARACTERIZATION OF GENETIC VARIATION

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A 26-year-old grey mare with multiple melanocytic tumors in the tail and perineum, progressive dyspnea and congestive heart failure was euthanized and necropsied. Significant findings were multicentric cutaneous melanocytic tumors with widespread metastases. Involvement of the pericardium, pleura, mediastinal and sternal lymph nodes and heart led to lymphatic obstruction and severe hydrothorax, compressive atelectasis, impaired respiratory function and congestive heart failure. The grey coat color in horses is characterized by progressive loss of pigmentation in the hair shaft and increased risk of melanoma with advancing age. Although the greying (grey) phenotype in horses is associated with an autosomal dominant 4.6kb duplication in the intron 6 of the syntaxin-17 (STX17) gene, further inspection through targeted sequencing demonstrated additional copy number variation at this locus, correlated with faster greying and higher

incidence of melanomas. Higher copy numbers in tumor DNA may result in increased malignant behavior and multiple metastases. Because of the widespread nature of the disease in this horse we have submitted samples of normal tissue and hair, as well as tumors for targeted sequencing following established protocols at Texas A&M University, School of Veterinary Medicine and Biomedical Sciences, to better characterize the genetic variation in this horse. Efforts to expand the current knowledge on the role of the genetic copy number variation in grey horses will assist equine veterinarians in early decision making for patients who may be predisposed to aggressive forms of melanoma.

67: ANORECTAL HEMANGIOSARCOMA IN A REX RABBIT (ORYCTOLAGUS CUNICULUS)

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An 8-year-old female spayed rabbit presented to University of Wisconsin Veterinary Clinic Special Species Department for a bleeding rectal mass that was first noted two weeks prior. A 2 cm soft, moveable, anorectal mass was identified on palpation of the caudal portion of the rectum with no erythema or ulceration. The caudal perianal skin was excoriated and scabbed from previous bleeding, and the circumferential anal skin was moderately edematous. Fine needle aspiration revealed a moderate to highly cellular population of homogeneous, irregular to rarely spindloid cells with moderate amounts of amorphous extracellular matrix, suggestive of hemangiosarcoma. The rabbit was euthanized at home 11 days after presentation. On necropsy, a 10 x 12 x 8 mm, mottled red to tan, nodular, soft mass elevated the dorsal mucosal surface of the anus. Histopathologic evaluation of the anorectal mass revealed a moderately to densely cellular mass of neoplastic mesenchymal cells that frequently formed variably sized, blood-filled, vascular channels morphologically consistent with hemangiosarcoma. There were many blood-filled channels near the ulcerative regions of skin accounting for the clinical bleeding. There was no gross or histological evidence of metastasis. Hemangiosarcomas are uncommon in rabbits, and to the authors' knowledge, this is the first reported case associated with the anorectal junction.

68: PERIURETHRAL RHABDOMYOSARCOMA IN AN ADULT NIGERIAN DWARF GOAT (CAPRA AEGAGRUS HIRCUS)

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A 3-year old Nigerian dwarf buck presented for straining to urinate and suspected urolithiasis following an urethral process amputation. Due to poor prognosis, the owner elected for humane euthanasia and the buck was presented for postmortem evaluation at Colorado State University. On gross examination, both kidneys were soft with markedly dilated renal pelvises filled with urine. Surrounding and encapsulating the proximal urethra and filling the pelvic canal was a white to tan, firm infiltrative mass that partially extended into the trigone of the urinary bladder. The sublumbal lymph nodes were enlarged and expanded by a similar mass. Histopathologically, the mass was composed of round neoplastic cells arranged in solid sheets and packets effacing the urethral wall, prostatic glands and extending into the bladder wall. Neoplastic cells had round to oval nuclei, 1-2 prominent nucleoli, scant pale basophilic cytoplasm and variably distinct cell borders. The sublumbal lymph nodes were up to 95% effaced with the same neoplastic population. Immunohistochemistry showed strong positive reactivity of neoplastic cells to vimentin and desmin and a diagnosis of alveolar rhabdomyosarcoma with sublumbal metastasis was made. Rhabdomyosarcoma is a malignant sarcoma that originates from skeletal muscle and is typically found in the head and neck region of juveniles. This mass either arose from the periurethral or prostatic skeletal muscle, which is an uncommon location in both humans and animals. Three cases of rhabdomyosarcoma in goats

have been reported in the literature, however, to our knowledge this is the first report of rhabdomyosarcoma in this location.

69: ROLE OF ENDOTHELIAL TLR9 IN THE PATHOGENESIS OF RADIATION-INDUCED LUNG FIBROSIS

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Background: Radiation-induced lung fibrosis (RILF) is a debilitating consequence of thoracic radiotherapy. While the pathogenesis of RILF involves a complex interplay of various cell types and signaling pathways, the role of endothelial Toll-like receptor 9 (TLR9) remains poorly understood.

Objectives: To investigate the specific contribution of endothelial TLR9 to the development and progression of RILF, offering insights into novel therapeutic strategies targeting this pathway.

Methods: Human Umbilical Vein Endothelial Cells (HUVECs) were irradiated and assessed using Western Blot and RNA-seq. Lungs from TLR9 knock-out and wild-type mice were collected and fixed with formalin after thoracic irradiation. H&E, Masson's trichrome staining, and immunohistochemical staining of F4/80, Ki67, and TGF β were performed.

Results: *In-vitro* results showed that ionizing radiation induces TLR9 expression. Bulk RNA sequencing analysis revealed that Cathepsin K (Ctsk) is upregulated in TLR9 knockdown cells. TLR9 deficiency reduced radiation-induced Ctsk levels. Histopathology revealed alveolar inflammation and fibrosis in the lungs of early-phase irradiated wild-type and TLR9 knock-out mice, with no significant differences between groups. Immunohistochemistry of F4/80, Ki67, and TGF β showed no statistically significant difference between TLR9 knock-out and wild-type mice.

Conclusion: Ctsk was a critical mediator of lung endothelial cell inflammation with correlation to TLR9 expression, though this was not apparent in the acute *in-vivo* study. As RILF is not instantaneous, it is important to understand both the acute and chronic stages of its development; information gained during this short-term study will serve as a point of comparison for future research involving lung harvest at 3- and 6-months post-irradiation.

70: AN INVESTIGATION OF ALKALINE PHOSPHATASE IN SITU HYBRIDIZATION FOR THE DIAGNOSIS OF OSTEOSARCOMA IN DOGS

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A microscopic diagnosis of osteosarcoma (OSA) requires the identification of tumor associated osteoid amongst neoplastic mesenchymal cells. This is a diagnostic challenge with small samples and tumors with minimal osteoid production. We hypothesized that alkaline phosphatase (ALP) in situ hybridization (ISH) detecting ALP RNA within poorly osteoid producing OSAs would aid in diagnosis. This experiment aimed to test the utility of ALP ISH for identifying OSA in dogs and distinguish it from other canine mesenchymal tumors (e.g. chondrosarcoma, fibrosarcoma, hemangiosarcoma, and histiocytic sarcoma). Cases were selected from the Washington Animal Disease Diagnostic Laboratory archives (CoreOne, VADDs), and categorized by tumor type by histopathology. OSAs were further categorized by productivity (non-productive vs. osteoid producing—minimal, moderate, marked). Formalin-fixed paraffin embedded samples and the RNAscope® assay were prepared and performed following manufacturer's protocol. OSA sections of moderate and marked osteoid

productivity displayed abundant ALP expression in neoplastic osteoblasts, while minimally productive OSAs were scant to abundant, though always had a positive detection. Nonproductive OSAs displayed variability in ALP expression, prompting the need to perform additional immunohistochemical staining (CD204, CD31). Scant traces of ALP expression were detected in lacunar cells of chondroblastic OSAs. ALP expression was appreciable in chondrosarcoma samples, while no ALP expression was seen in fibrosarcomas, hemangiosarcomas, or histiocytic sarcomas. Decalcified tissue samples and regions of necrosis and autolysis lacked ALP expression. From this retrospective study, ISH demonstrates great promise as a diagnostic tool for diagnosing OSAs in challenging cases. However, further investigation is needed to distinguish chondroblastic OSAs from chondrosarcomas.

71: SNAKE-ASSOCIATED SARCOCYSTIS PANTHEROPHISI N. SP. INFECTION IN THREE FOALS WITH SEVERE COMBINED IMMUNODEFICIENCY

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Three foals, bred for severe combined immunodeficiency (SCID) for immunology research at Washington State University spontaneously developed clinical illness and were euthanized between 2-3 months of age. At necropsy, all three had severe chronic multifocal to coalescing necrotizing hepatitis and fibrosis with intracytoplasmic protozoa in hepatocytes. One foal had multisystemic spread of the protozoa, with the most severe infection in the liver and lungs, and the other two also had adenoviral associated broncho-interstitial pneumonia. PCR and sequencing of the liver using universal *Sarcocystis* sp. primers for a segment of the 18S ribosomal RNA gene produced a 100% match for *Sarcocystis pantherophisi* n.sp., currently only isolated from the Eastern rat snake, which is believed to be the definitive host. Related species with snakes as definitive hosts typically use rodents as intermediate hosts. While speculative, exposure was presumably through bedding or feed, and the protozoal parasite was likely able to cause disease in these three foals due to their SCID status, leaving them unable to mount an effective adaptive immune response.

72: ELECTROPHORETIC URINE PROTEIN BANDING PATTERNS IN HEALTHY CALIFORNIA SEA LIONS (ZALOPHUS CALIFORNIANUS)

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Background: Kidney disease is common in California Sea Lions (*Zalophus californianus*; CSLs) and is linked to strandings. In dogs with proteinuric kidney diseases, urine protein banding patterns (UPBPs - number and molecular weight (MW) of urine proteins) generated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) can help characterize protein origin (glomerular, tubular) and severity of kidney damage. Expected UPBPs in healthy CSLs and the clinical utility of urine SDS-PAGE in sick CSLs are unknown.

Objective: Establish expected UPBPs in apparently healthy CSLs and identify significant sex and age group differences.

Methods: Urine SDS-PAGE was performed on urine supernatant from 76 apparently healthy CSLs [26 adults (19 males, 7 females); 35 juveniles (18 males, 17 females); 15 yearlings (6 males, 9 females)]. Bands in 4 pre-defined MW categories were quantified [low (LMW: 0-36.5 kDa), intermediate (IMW: 36.5-66.3 kDa), high (HMW: 66.3-200 kDa), very high (VHMW: >200)] and descriptive statistics for each category were determined for the entire population together and each

sex and age group separately. Significant differences between demographic groups were determined with Wilcoxon rank sum and Kruskal-Wallis tests.

Results: The population median (and range) of bands in each MW category were: LMW, 6 (0-8); IMW, 3 (1-5); HMW, 2 (0-5); VHMW, 0 (0-2). There were no significant differences between sexes. Yearlings and juveniles had significantly fewer HMW bands (2 (0-5)) than adults (3 (0-4)).

Conclusions: Expected UPBPs for apparently healthy CSLs established in this study will aid characterization of location and severity of kidney damage in diseased CSLs.