ACVP/AAVLD Diagnostic Pathology Travel Award Session

Friday, October 30, 2020 | 10:00 a.m. – 12:00 p.m. CDT

Friday, October 30, 2020
10:05 a.m. – 10:15 a.m. CDT

THEILERIA ORIENTALIS IKEDA GENOTYPE IS IDENTIFIED IN CATTLE IN SOUTHWESTERN VIRGINIA

Vanessa Oakes[1], Michael Yabsley[2], Diana Schwartz[3], Tanya LeRoith[1], Carolynn Bissett[4], Charles Broaddus[4], Jack Schlater[5], S. Todd[1], Katie Boes[1], Meghan Brookhart[1], Kevin Lahmers[1]

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Theileria orientalis Ikeda genotype/genotype 2 is a pathogenic, non-transforming hemoprotozoan. This genotype is increasingly implicated as a causative agent of bovine infectious anemia outside of the United States and is of significant economic importance in other countries. It is most frequently transmitted by *Haemaphysalis longicornis*. In 2018, this tick was reported in the eastern United States, including in Virginia. Cattle within several herds in Virginia that were parasitized by *H. longicornis* developed clinical signs consistent with hemoprotozoal infection. Blood collected from members of infected herds revealed the presence of intraerythrocytic piroplasms consistent with *Theileria* spp. Diagnostic tests for *Anaplasma* and *Babesia* spp. were negative. **Objective:** Identify the etiology of the anemia as *Theileria orientalis* Ikeda genotype/genotype 2. **Methods:** Blood was collected from multiple, unrelated cattle herds throughout Virginia, with no travel of individual cattle between herds. Conventional PCR was conducted on DNA extracted from whole blood. **Results:** Sequences of partial major piroplasm surface protein (MPSP) and the small subunit ribosomal DNA (SSU) genes were similar to GenBank accessioned sequences of *T. orientalis* Ikeda genotype/genotype 2. Phylogenetic analysis of these sequences revealed clustering of clinical samples with Ikeda genotype/genotype 2. **Conclusion:** This study confirms the presence of *Theileria orientalis* Ikeda genotype/genotype 2 in the United States. Recently completed transmission studies confirm *H. longicornis* as a competent vector for this hemoprotozoan. The increasingly described range of this tick throughout the US suggests that this disease is not limited to Virginia and may represent an emerging disease within the United States.

Friday, October 30, 2020
10:15 a.m. – 10:25 a.m. CDT

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF MESOTHELIOMA IN SIX LARGE FELIDS

Sarah Coe[1], Michael Garner[2], Matti Kiupel[1]
[1]Michigan State University Veterinary Diagnostic Laboratory and Department of Pathobiology and Diagnostic Investigation, Lansing, MI, USA, [2]Northwest ZooPath, Monroe, WA, USA

Background: Mesothelioma has been frequently reported in large felids. These neoplasms present a diagnostic challenge due to their highly variable morphology that mimics carcinomas or sarcomas at different locations. This report describes the immunohistochemical labeling pattern of mesotheliomas in six large zoo felids. **Objectives:** The goal of this study was to morphologically and immunohistochemically characterize mesotheliomas in six large zoo felids to determine if a panel of antibodies could be used to more accurately support the diagnosis of these neoplasms. **Methods:** Mesotheliomas from six large felids, including four clouded leopards, one Bengal tiger, and one cheetah were immunohistochemically labeled for vimentin, E-cadherin, pancytokeratin, Wilm's Tumor 1 (WT1), MUC-1, and calretinin. **Results:** The mesotheliomas of the four clouded leopards and the tiger represented the epithelial subtype, and the mesothelioma from the Cheetah was biphasic. The predominant morphologic pattern in the epithelial mesotheliomas was tubulopapillary, and three neoplasms had abundant papillary fronds. The epithelial component of the biphasic mesothelioma was predominantly solid. All six mesotheliomas had strong immunohistochemical labeling for vimentin, E-cadherin, and pancytokeratin. All cases had cytoplasmic labeling for WT1, and two of these also had positive nuclear labeling. The three mesotheliomas with distinct papillary fronds were weakly positive for MUC-1. These and one other epithelial mesothelioma were also positive for calretinin. **Conclusions:** This study demonstrates that the morphologic and immunohistochemical phenotypes of mesothelioma identified in humans and domestic species can occur in large felids and that a panel of antibodies can be utilized to support the diagnosis of these neoplasms.

Friday, October 30, 2020
10:25 a.m. – 10:35 a.m. CDT

MEDULLARY BONE IN MALE BUDGERIGARS

Nathan Hoggard, Linden Craig
University of Tennessee, Knoxville, TN, USA

Medullary bone, or laying bone, is a calcium-rich, labile bone normally occurring in female birds with each egg-laying cycle. The stimulus for deposition of medullary bone is the cyclical increase in serum estrogens produced by preovulatory ovarian follicles. Increased bone density due to deposition of medullary bone, particularly in pneumatic bones, has been termed polyostotic hyperostosis, even if physiological. This case series investigates the deposition of medullary bone in non-pneumatic (femur) and pneumatic (humerus) bones in sexually mature male budgerigars submitted for autopsy between 2017 and 2020. Of the seven budgerigars that met the case criteria, 4/7 (57%) had

deposition of medullary bone in one or more bones examined. All four male budgerigars with medullary bone had a testicular neoplasm, which was morphologically consistent with a Sertoli cell tumor or seminoma. Medullary bone was not present with other diseases. Medullary bone deposition in pneumatic and non-pneumatic bones can occur in male budgerigars with testicular neoplasia. Radiographic increases in medullary bone density, particular in the humerus, could provide antemortem indication of testicular neoplasia in male budgerigars.

Friday, October 30, 2020
10:35 a.m. – 10:45 a.m. CDT

A RETROSPECTIVE STUDY OF CANINE INTRANASAL MAST CELL TUMORS

Eileen Larsen, Juan Gutiérrez, Allison Watson
Colorado State University, Fort Collins, CO, USA

Background: Mast cell tumors (MCTs) are an uncommon primary neoplasm of the nasal cavity in dogs for which there is a paucity of existing literature regarding their clinicopathologic and molecular features. **Objective:** Our objectives included: 1) to retrospectively study the clinical findings, histopathologic and immunohistochemical features, and c-kit status of primary intranasal MCTs in dogs, and 2) identify potential prognostic factors. **Methods:** Canine biopsy submissions to Colorado State University between 2010-2019 with intranasal neoplasms diagnosed as MCTs with no history of cutaneous or oral MCT were considered. Immunohistochemistry for CD117 and Ki67, and PCR for internal tandem duplications at exons 8 and 11 of the *c-kit* gene were performed. **Results:** Twenty-one (1.1%) out of 1,852 primary nasal neoplasms were MCTs. Metastases were reported in 11 cases, with the submandibular lymph nodes representing the most common site. One case had distant metastases to abdominal viscera. Survival time ranged from 20 days to 1.5 years with only 3 (20%) dogs alive 1 year after the onset of clinical signs. Cases with a <1 year survival tended to have mitotic counts of ≥8, metastasis to regional lymph nodes, and/or atypical CD117 immunostaining patterns. Only one case had a *c-kit* mutation at exon 11. **Conclusion:** Canine intranasal MCTs appear to be clinically aggressive. In this study, dogs with regional lymph node metastasis had a shorter survival time. A mitotic count of ≥8 may serve as a useful prognosticator for canine intranasal mast cell tumors.

Friday, October 30, 2020
10:45 a.m. – 10:55 a.m. CDT

POST-CARDIAC ARREST SYNDROME IN A DOG

Alexander Aceino, Kira Bourne, Ann-Mari Osgood, Raquel Rech
Texas A&M University, College Station, TX, USA

Post-cardiac arrest syndrome occurs following resumption of spontaneous circulation (ROSC) after prolonged, successful cardiopulmonary resuscitation (CPR). In human medical literature, it has been documented to result in numerous difficult-to-manage, pathophysiologic events. These changes are due to a combination of whole-body ischemia, reperfusion, myocardial dysfunction, and systemic inflammatory response syndrome resulting in microvascular fibrin thrombi, continued tissue hypoxia, hypovolemia, sepsis, and multiorgan failure. A 10-year-old, male neutered, terrier mix

presented for thoracoscopic surgical excision of a cranial mediastinal mass. As the trocar was advanced from a trans-xiphoid approach, the patient decompensated into ventricular fibrillations. Cardiac massage with intermittent intrathoracic defibrillations were continued for 22 minutes until ROSC was achieved. Over the next five days in the ICU, the patient initially improved then suddenly demonstrated a decline in mentation, intermittent ventricular tachycardia, intermittent ventricular premature complexes, and forelimb paresis due to a thrombus in the left axillary artery confirmed via ultrasonography. The patient acutely arrested. At necropsy, widespread thrombosis and infarction were observed within the heart, kidneys, intestine, spleen, pancreas, and brain. The cranial mediastinal mass and thrombosis of the left axillary artery were confirmed. Microscopic examination confirmed widespread fibrin thrombi with secondary infarcts resulting in hemorrhage and necrosis. The renal infarcts were deemed to be 2-3 days old while the brain infarcts ranged from a few minutes to 1-2 days old. The cranial mediastinal mass was consistent with a benign thymic cyst. The clinical history, gross and microscopic lesions, and timeline of events generated the diagnosis of post-cardiac arrest syndrome.

Friday, October 30, 2020
10:55 a.m. – 11:05 a.m. CDT

T-ZONE LYMPHOMA WITH CUTANEOUS PRESENTATION IN A GOLDEN RETRIEVER

Catharine Cowan, Janna Yoshimoto, Jeremy Dossey, Paula Schaffer, Anne Avery, Kelly Hughes
Colorado State University, Fort Collins, CO, USA

Background: An 8 year-old male castrated Golden Retriever enrolled in the Morris Animal Foundation Golden retriever lifetime study (MAF_GRLS) presented for pruritic skin disease in the axilla, pinna, prepuce and perineal areas which was refractory to treatment with diphenhydramine, antibiotics, steroids, and lokivetmab. Skin scrapes were negative for infectious organisms, and *Sarcoptes* antibody titers were negative. Three months later generalized lymphadenopathy developed. Lymph node biopsy, fine needle aspirate and a skin biopsy were collected for diagnosis. **Objective:** Characterize and subclassify lymphadenopathy and skin disease. Determine if disease could be detected in earlier samples obtained during the lifetime study. **Methods:** Histopathology, immunohistochemistry (IHC), flow cytometry and PCR for antigen receptor rearrangements (PARR) were used to characterize disease from the lymph node, skin biopsy, and peripheral blood. **Results:** Histopathology and IHC were consistent with T zone lymphoma (TZL), lacking expression of CD45 in both the lymph node and skin. Flow cytometry from the lymph node revealed a homogeneous expansion of T cells lacking CD45, diagnostic for TZL. PARR from the lymph node and skin identified the same sized clonal T cell receptor rearrangements. PARR from previous peripheral blood samples did not identify a clonal population. **Conclusions:** This case is an uncommon example of a cutaneous manifestation of TZL with concurrent lymph node involvement. Indolent cutaneous T-cell lymphoma/lymphocytosis has been reported in dogs, however there has not been a clear association with TZL. Lack of earlier peripheral blood involvement may aid in understanding cutaneous manifestation of the disease in the future.

Friday, October 30, 2020
11:05 a.m. – 11:15 a.m. CDT

NEURONAL CEROID LIPOFUSCINOSIS IN A CAT

Alan Mulder II, James Stanton
University of Georgia, Athens, GA, USA

A two year old, female spayed Siamese cat presented to the rdvm with a four month history of progressive, abnormal behavior, including urinating outside of the litter box, becoming anti-social, hyperexcitable, and exhibiting mild ataxia. Gross necropsy was unremarkable. Histopathology reveals the majority of neuronal and astrocytic cytoplasm expanded by amphophilic to eosinophilic globules which often peripheralized the nuclei. The cerebral white matter and lamina of the gray matter is severely spongiotic, and the associated neurons are hypereosinophilic and angular (neuronal necrosis). Electron microscopy was performed on cerebral tissue. Within the cytoplasmic matrix of neurons are one or multiple bundles of small, electron dense C-shaped to S-shaped lines. This pattern is indicative of the curvilinear profile (CLP), or Type 2 of neuronal ceroid lipofuscinosis (NCL). NCL is a lysosomal storage disease that has been described in several domestic species and humans, and clinical signs involve progressive cognitive decline, motor dysfunction, epileptic seizures, and eventually death or euthanasia. Although therapeutic advances have delayed onset of clinical signs, there is no current cure for this disease.

Friday, October 30, 2020
11:15 a.m. – 11:25 a.m. CDT

NEISSERIA SP. INDUCED EMBOLIC NECRO-SUPPURATIVE PNEUMONIA IN 3 DOMESTIC CATS

Christopher Bolt[1], Arno Wuenschmann[1], Sunil Mor[1], Vikash Singh[1], Hallie Richards[1], Kelly Gehlhaus[2]
[1]Department of Veterinary Population Medicine, Veterinary Diagnostic Laboratory, University of Minnesota, St. Paul, MN, USA, [2]VCA Bloomington Animal Hospital, Bloomington, MN, USA

Clinical Background: Three cats, age 2 to 11 years-old, presented to the University of Minnesota Veterinary Diagnostic Laboratory over a three-year period following euthanasia or death due to marked respiratory distress, depression, and dehydration. Antemortem thoracic radiographs revealed multiple, nodular, soft tissue opacities throughout the lung fields in all cases. Gross and Histologic Findings: On postmortem examination of all three cats, approximately 60-80% of the lung parenchyma were expanded by widely-disseminated, multifocal to coalescing, well-demarcated, beige to tan, raised, semi-firm nodules. Histologically, large numbers of variably degenerate neutrophils, large amounts of fibrin, moderate numbers of macrophages, and moderate amounts of cellular and karyorrhectic debris effaced and replaced the pulmonary parenchyma multifocally. Affected areas contained aggregates of Gram-negative extra- and intracellular coccoid bacteria. Bacterial Identification: MALDI, 16s rRNA sequencing and whole genome sequencing revealed that the bacteria isolated under aerobic conditions from the lung of all cats are novel Neisseria sp. Whole genome based and 16S rRNA based phylogenetic analysis revealed that the most similar species is

Neisseria animaloris. **Conclusions:** Infection with Neisseria sp. induces a fairly characteristic pneumonia in cats that radiographically and grossly resembles metastatic neoplasia. Pathogenesis and reason for the sporadic, rare nature of the disease is poorly understood, with hematogenous spread favored given the widely-disseminated pulmonary distribution. In younger cats, Neisseria spp. pneumonia should be considered among the top etiologic differential diagnoses in cases of lower respiratory disease with a disseminated, nodular lung pattern.

Friday, October 30, 2020
11:25 a.m. – 11:35 a.m. CDT

DERMAL TUMORS IN FOUR PUERTO RICAN CRESTED TOADS (PELTOPHYRNE LEMUR)

Sierra Imanse[1], Caitlin Burrell[1], Sarah Cannizzo[2], Tara Reilly[2], Kimberly Rainwater[2], Martha Delaney[1]
[1]Zoological Pathology Program, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Brookfield, IL, USA, [2]Fort Worth Zoo, Fort Worth, TX, USA

Puerto Rican crested toads (*Peltophryne lemur*) (PRCTs) are critically endangered due to habitat loss and competition with invasive species. Captive breeding serves as a barrier against extinction. Dermal tumors were found in four male, 6-9 year old PRCTs from a breeding colony between July and October 2019. In three cases, tumors were solitary, 0.4 to 1 cm diameter, well-demarcated, smooth, raised, soft, pink, and non-ulcerated; excisional biopsies were obtained. In the fourth case, the lesion presented as diffuse swelling and pallor of the left forelimb, and humane euthanasia was elected. Bacterial and fungal cultures of the masses had no growth. Histologically, all tumors were roughly nodular, nonencapsulated, expansile, mildly infiltrative, and densely cellular with elevation but no invasion of the overlying epidermis. Neoplastic cells were predominantly arranged in loose sheets with few streams and whorls all supported by fine fibrovascular stroma. Cells were round to occasionally spindloid and some contained cytoplasmic metachromatic granules. Nuclei were small and oval with coarse chromatin. Anisocytosis and anisokaryosis were mild and mitoses were not observed. The toad with the forelimb mass had regionally invasive and disseminated disease with neoplastic cells in subjacent muscle, coelomic connective tissue and viscera. No infectious organisms were identified with acid fast staining. Cytoplasmic metachromatic granules were suggestive of mast cell origin. Previous reports of tumors in PRCTs are limited to a single report of a cutaneous mast cell tumor. Continued monitoring of PRCT managed populations for neoplasia will be critical for effective management and conservation of this endangered species.

Friday, October 30, 2020
11:35 a.m. – 11:45 a.m. CDT

A NASAL NEUROECTODERMAL TUMOR IN A LLAMA

Nataly Mamaliger, Alicia Olivier, Wes Baumgartner, Gretchen Hardwick
Mississippi State University College of Veterinary Medicine, Starkville, MS, USA

This report describes a suspect esthesioneuroblastoma in a two-year-old female Lama that presented for a sinonasal mass. At post mortem examination there is marked

deformation and effacement of the left maxilla and orbit by a homogenous, friable, tan infiltrative mass. The mass deviates the nasal septum and extends through the cribriform plate to compress the left frontal cortex. Similar masses (indicative of metastasis) are present within the lungs, thyroid, liver and spleen and the cervical and subiliac lymph nodes. Histologically, the masses are composed of tightly packed lobules of neoplastic cells which frequently form rosettes and pseudorosettes. Neoplastic cells have a centrally located ovoid nucleus with dense chromatin and one to two indistinct nucleoli. The mitotic rate is high (8 mitotic figures per high power field) and mild cellular and nuclear pleomorphism. Neoplastic cells are strongly immunopositive for vimentin and variably immunopositive for chromogranin and S100. Neoplastic cells are immunonegative for GFAP and cytokeratin. Based on these findings, the nasal mass is identified as a neuroectodermal tumor most consistent with an esthesioneuroblastoma. Esthesioneuroblastoma are aggressive, malignant neoplasms derived from germinal olfactory neuroepithelial cells found lining the ethmoturbinate region of the nasal cavity. Esthesioneuroblastoma are rare in veterinary literature but have been described in several domestic species including dogs, horses and camelids, however this is the first report in a llama. Additionally, this particular tumor was highly aggressive with widespread multiorgan metastasis.

ASVCP - ISACP Clinical Pathology Focused Scientific Session

Friday, October 30, 2020 | 10:00 a.m. – 3:00 p.m. CDT

Friday, October 30, 2020
11:00 a.m. – 11:15 a.m. CDT

LIPOPROTEIN PROFILE OF TRANSUDATES IN DOGS AND CATS

Flavio Herberg de Alonso[1], Dallas Hollis[1], Erica Behling-Kelly[2], Dori Borjesson[1]
[1]University of California, Davis, CA, USA, [2]Cornell University, Ithaca, NY, USA

Background: Transudate effusion is a common clinical manifestation of disease in companion animals, but the underlying pathogenesis is not always clear. In people, transudative chylous effusions (TCE) are characterized by the presence of chylomicrons in the effusion secondary to disorders including liver cirrhosis and nephrotic syndrome, among others. TCE has not been described in animal patients. **Objective:** Establish effusion lipoprotein patterns in dogs and cats with pleural or abdominal transudates. Determine if TCE can manifest in these species. **Methods:** Body fluid and serum samples from 9 cats and 22 dogs with transudative effusion (total protein <3.0 g/dL and nucleated cell count <3,000 cells/µL) were collected from samples submitted to the UC Davis teaching hospital lab between April 2019 and May 2020. Triglyceride and cholesterol concentration were determined in fluid and serum. Lipoprotein electrophoresis was run on fluid samples. **Results:** Fluid lipoprotein electrophoresis indicated two distinct patterns, one rich in lighter (VLDL and LDL, group I) and another rich in denser (IDL and HDL, group II) lipoproteins. Cases in group I had fluid cholesterol ranging from <4 to 8 mg/dL (versus 25 to 233 in group II, n=27) and were associated with severe chronic kidney disease, acquired portosystemic shunts or protein-losing enteropathy. Cases in group II were primarily associated with heart disease or intracavitary masses/neoplasia. **Conclusions:** Preliminary data indicate that

a distinct lipoprotein and fluid cholesterol pattern may be present in body fluids from patients with effusion secondary to chronic liver, kidney and GI disease.

Friday, October 30, 2020
11:15 a.m. – 11:30 a.m. CDT

FLOW CYTOMETRIC ANALYSIS OF EQUINE BRONCHOALVEOLAR LAVAGE FLUID CELLS IN HORSES WITH AND WITHOUT SEVERE EQUINE ASTHMA

Heng Kang[1], Dorothee Bienzle[1], Gary Lee[1], Érica Piché[1], Laurent Viel[2], Janet Beeler-Marfisi[1]
[1]University of Guelph, Department of Pathobiology, Ontario Veterinary College, Guelph, ON, Canada, [2]University of Guelph, Department of Clinical Studies, Ontario Veterinary College, Guelph, ON, Canada

Background: Severe equine asthma (SEA) is a common lower airway inflammatory disorder that is severely debilitating in older horses. Alveolar macrophages (AMs) survey inhaled particulates from barn sources, which can cause them to switch from an anti-inflammatory to a proinflammatory phenotype. In some horses, this switch appears aberrantly prolonged and may contribute to the development of SEA. However, validated antibodies to query the cells involved in the pathogenesis of SEA are lacking. **Objectives:** To validate that specific commercial antibodies react with equine leukocytes. To discriminate leukocytes in bronchoalveolar lavage fluid (BALF) using a multi-color flow cytometric assay. **Methods:** Monoclonal antibodies against CD90, CD163 and CD206 were tested for reactivity with equine leukocytes by immunocytochemistry and flow cytometry. Antibodies against equine CD5 and B cells were used to distinguish lymphocytes by flow cytometry. Flow cytometry was performed on BALF cells from healthy and SEA-susceptible horses before and one day after exposure to moldy hay. **Results:** Antibodies against CD90 successfully labeled equine neutrophils. Antibodies against CD163 and CD206 were equine macrophage markers. The expression of CD163, a hemoglobin-haptoglobin receptor expressed on equine monocytes and macrophages, was increased on AMs after moldy hay challenge in both groups of horses but the increase was significant in SEA-susceptible horses only ($p = 0.02$). **Conclusions:** Flow cytometry is an effective tool for analyzing equine BALF leukocytes. The greater expression of CD163 on AMs, particularly in SEA-susceptible horses, suggests an association with lung inflammation. However, its role in the pathogenesis of SEA remains to be elucidated.

Friday, October 30, 2020
11:30 a.m. – 11:45 a.m. CDT

LIPOFUSCIN IN CANINE BLOOD NEUTROPHILS OR MONOCYTES IS ASSOCIATED WITH MODERATE TO SEVERE HEPATOCELLULAR INJURY

Kimberley Sebastian, Cynthia Lucidi, Michael Scott
Michigan State University, East Lansing, MI, USA

Background: Lipofuscin-like cytoplasmic inclusions of neutrophils and monocytes are uncommonly encountered in routinely stained canine blood smears; their incidence, origin, composition, and significance have not been reported. In people, similar “green granules of death” have been associated with moderate to severe hepatocellular injury

and mortality rates of 30-65%. **Objectives:** To describe clinicopathologic abnormalities, diagnoses, and outcomes of dogs with these inclusions, and to determine if they had features of lipofuscin. **Methods:** Cases were identified prospectively to assess unstained inclusions, and retrospectively to investigate clinicopathologic associations in additional dogs. Cases were excluded if a chemistry profile was unavailable or if neither a clinical nor post-mortem diagnosis was available. Inclusions in smears from prospective cases were assessed for autofluorescence (5/7) and with routine (7/7) and Prussian blue stains (5/7). For retrospective cases, the hospital database was searched (3-year period) for dogs with acute hepatic injury, and their archived blood smears were examined. **Results:** All 11 dogs with inclusions (7 prospective, 4 of 97 retrospective cases) had marked inflammation and moderate to marked increases in serum ALT activity. Inclusions were Prussian blue-negative and had broad-spectrum autofluorescence supportive of lipofuscin. Clinical diagnoses were acute hepatic failure (5), hepatic carcinoma (2), pancreatitis with hepatocellular injury (3), and sepsis (1); 73% of affected dogs died within 9 days of admission. **Conclusions:** Detection of iron-negative, blue-green granular cytoplasmic inclusions in blood neutrophils or monocytes of dogs supports the presence of lipofuscin inclusions and suggests hepatocellular injury, often severe. Reporting these inclusions is recommended to guide clinical management.

Friday, October 30, 2020
11:45 a.m. – 12:00 p.m. CDT

CYTOLOGICAL FEATURES OF PITUITARY ADENOMAS IN DOGS: 8 CASES

Michelle DeCourcey[1], Tina Owen[1], Annie Chen-Allen[1], Linda Martin[1], Margaret Miller[2], David Kim[1], K. Jane Wardrop[1], Cleverson Souza[1]
[1]Veterinary Teaching Hospital, Washington State University, Pullman, WA, USA, [2]Indiana Animal Disease Diagnostic Laboratory, Purdue University, West Lafayette, IN, USA

Background: Adenomas are the most common tumor of the pituitary and hypophysectomy is becoming a more available treatment option. Cytologic review of intra-operative samples of pituitary tumors will likely become more applicable. **Objective:** Description of the cytomorphologic features of pituitary adenomas in dogs that were removed via transsphenoidal hypophysectomy. **Methods:** Cytology of 8 cases of pituitary adenoma were reviewed between 2015 and 2020. The final diagnosis for these cases was based on histopathology and immunohistochemistry in selected cases. The case set includes 4 melanotroph and 4 corticotroph pituitary adenomas. **Results:** All samples from pituitary adenomas contained high numbers of bare nuclei. In general, the intact cells were round to polygonal and occurred individually and in small, tight clusters. These cells had round to oval, eccentric nuclei with finely stippled chromatin and 0-3 prominent nucleoli. They had moderate to abundant amounts of lightly basophilic to amphophilic, grainy cytoplasm with distinct borders and variable numbers of discrete vacuolation. In general, these cells displayed mild to moderate anisocytosis and anisokaryosis. Occasional binucleation, rare aberrant mitotic figures, and nuclear molding were also observed. The samples from the melanotroph pituitary adenomas tended to have more features of atypia, with occasional marked anisocytosis and anisokaryosis, and variably sized and shaped nucleoli. Four of the samples contained

evidence of previous hemorrhage indicated by the presence of erythrophagocytosis and hemosiderin-laden macrophages. **Conclusion:** Intra-operative cytology of pituitary adenomas can be useful prior to confirmation with histopathology.

Friday, October 30, 2020
12:30 p.m. – 12:45 p.m. CDT

ASSAY OF HAPTOGLOBIN, THE PLASMA ACUTE PHASE PROTEIN, BY HEMOGLOBIN BINDING: OPTIMIZATION OF A NOVEL CHROMOGEN

Blanka Ljubić[1], Vladimir Mrljak[1], Romana Turk[1], Nicola Brady[2], David Eckersall[2]
[1]University of Zagreb, ZAgreb, Croatia, [2]University of Glasgow, Glasgow, United Kingdom

Background: Haptoglobin (Hp) can be assayed by a biochemical assay based on its binding to hemoglobin (Hb) and preservation of the Hb peroxidase activity with the peroxidase-catalysed reaction of 4-aminoantipyrine and 8-anilino-1-naphthalone sulfonic acid as chromogen. The resulting product has low stability potentially leading to poor precision. New peroxidase substrates are available that may improve the Hp assay. **Objective:** Assess an alternative peroxidase substrate for the assay of Hp. **Method:** The substrate, N,N'-bis(2-hydroxy-3-sulfopropyl)-tolidine (SAT-3, Dojindo, Japan) in buffer was optimized for use on an Architect c4000 Analyser (Abbot, USA) so that 3 ml of sample was mixed with 157 ml of Hb reagent, 80 ml of SAT-3 solution including H_2O_2 was added and absorbance at 660 nm determined. Validation was by determination of precision, limit of quantification, interference and detection of clinical changes by monitoring plasma Hp in healthy dairy cows (n=10) and those with clinical mastitis (n = 10). **Results:** The SAT-3chromogen product was stable for several minutes post-reaction with intra-assay and inter-assay CVs below 6.5%. The limit of quantification assay was 0.05 g/L. Lipemia had no effect on Hp results to 0.16 g/L while hemolysis increased the apparent Hp concentration above 0.07 g/L of Hb. Haptoglobin in plasma from healthy cows (n=10) was <0.05 g/L in all samples, while the median (range) in plasma from cows with clinical mastitis (n=10) was significantly greater (P=0.0011) at 0.15 (<0.05-0.84) g/L. **Conclusion:** The use of SAT3 is effective as a peroxidase substrate for the assay of Hp in plasma.

Friday, October 30, 2020
12:45 p.m. – 1:00 p.m. CDT

HAPTOGLOBIN AS AN INDICATOR OF ON-GOING ACUTE INFLAMMATION IN PRE-SLAUGHTER COWS

Funmilola Thomas, Kabirat Okeowo, Eyitayo Ajibola, Olukayode Akintunde, Folusho Egunleti, Samson Rahman
Federal University of Agriculture Abeokuta, Abeokuta, Nigeria

Background: Haptoglobin (Hp) is a major acute phase protein (APP) of cattle and its measurement has been exploited in the diagnosis and prognosis of various diseases in bovines. **Objective:** The goal of this study was to apply the evaluation of Hp levels to determine the presence of ongoing inflammation in animals meant for human consumption. **Methods:** Seventy-three serum samples obtained from immediately pre-slaughtered cows in a major slaughter slab located in the South Western Nigerian city,

Abeokuta, were analyzed using a validated ELISA for Hp. Hematological parameters (PCV, WBC and differential) were also analyzed and a physical examination assessment of each animal's body condition was carried out. **Results:** Hp was detected in moderate levels in 56% of animals examined. The range of values for Hp concentration was 31.58 to 117.40 µg/ml with a median of 45.05 µg/ml. There was no significant correlation between Hp concentration and hematology values or body condition scores of the cattle (Spearman's Rho, P>.05). **Conclusions:** Overall, results suggest that increases in Hp may be more related to stress induced on the cattle from transportation and from conditions in the pre-slaughter holding pens, although the emergence of new acute inflammatory episodes cannot be totally ruled out. Assessment of other stress markers and a broader panel of acute inflammation indices would further shed light and add value to the benefits of Hp as an indicator of acute inflammation in pre-slaughter animals.

Friday, October 30, 2020
1:00 p.m. – 1:15 p.m. CDT

ACUTE PHASE PROTEINS IN MILK FROM COWS WITH CLINICAL MASTITIS CAUSED BY DIFFERENT PATHOGENS

Elizabeth Schmidt[1], Felipe Dalanezi[1], Sâmea Joaquim[1], Felipe Guimarães[1], Simony Guerra[1], Bruna Lopes[1], Ronaldo Cerri[2], Chris Chadwick[3], Helio Langoni[1]
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Background: Mastitis has multiple etiologies that lead to an inflammatory reaction. Milk acute-phase proteins (APPs) have been identified as biomarkers of mastitis in cows. **Objective:** The objective of this study was to determine the profile of APPs from milk samples from cows with clinical mastitis caused by different pathogens. **Methods:** SPARCL immunoassay was used to measure the concentrations of microplate haptoglobin (Hp), alpha-1-glycoprotein (AGP), and C-reactive protein (CRP) in mastitic milk samples. Assays were validated using bovine APP antigens and standard calibrators. Precision (intra-assay, inter-assay) was determined as the coefficient of variance (CV) in triplicate assays of four samples (n=12) with varying concentrations of the biomarker. **Results:** Intra- and inter-assay CVs were below 8% and 15%, respectively, for all APPs except AGP (inter-assay CV 30.2%). Mastitis caused by *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* or environmental *Streptococcus* had the highest concentrations of Hp; *E. coli* and *S. aureus* also had the highest concentration of AGP. Mastitis caused by *Mycoplasma spp*. had moderate concentrations of Hp and AGP. Milk with environmental *Streptococcus* and *K. pneumoniae* had moderate concentrations of AGP. CRP was significantly higher in milk from cows with *E. coli* or *K. pneumoniae,* as compared to *S. aureus*, environmental *Streptococcus*, and *Mycoplasma spp*. Mastitis caused by *Enterococcus spp*. and coagulase-negative *Staphylococcus* had the lowest concentrations of Hp, AGP, and CRP. **Conclusion:** SPARCL accurately quantified APP concentrations in milk from mastitic cows. CRP and AGP may be as reliable as Hp in diagnosing clinical mastitis caused by different pathogens.

Friday, October 30, 2020
1:15 p.m. – 1:30 p.m. CDT

BRONCHIAL BRUSH CYTOLOGY, ENDOBRONCHIAL BIOPSY, AND IMMUNOHISTOCHEMICAL MEASUREMENT OF SALSA AS DIAGNOSTIC TOOLS FOR SEVERE EQUINE ASTHMA

Gary Lee, Janet Beeler-Marfisi, Laurent Viel, Érica Piché, Heng Kang, William Sears, Dorothee Bienzle
Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

Background: Horses with severe equine asthma (SEA), previously also known as heaves and recurrent airway obstruction, have severe neutrophilic inflammation of the lower airways, which leads to airway remodeling. Cytologic evaluation of bronchoalveolar lavage (BAL) fluid is the most common confirmatory test for horses with clinical features of SEA. The value of alternative diagnostic modalities such as assessment of bronchial brushings and immunohistochemical detection of disease-associated biomarkers for the diagnosis of SEA, or for the assessment of airway remodeling, remains undetermined. **Objective:** Evaluate brush cytology, endobronchial biopsies, and immunohistochemical results for the salivary scavenger and agglutinin (SALSA) protein as diagnostic tools for SEA and airway remodeling. **Methods:** Samples of BAL, brush cytology and endobronchial biopsies were collected from six asthmatic and six non-asthmatic horses, before and after an inhaled asthmatic challenge. The intensity and location of SALSA in endobronchial biopsies was assessed with image analysis software. **Results:** Both BAL and brush cytology evaluation identified a significant increase in the proportion of neutrophils following challenge in asthmatic compared to non-asthmatic horses ($P<0.001$). Evidence of airway remodeling was not identified in either brush cytology or endobronchial samples. Staining intensity of SALSA in mucosal and submucosal epithelial cells, and surface mucus, did not differ significantly between asthmatic and non-asthmatic horses. **Conclusions:** These findings illustrate the potential of bronchial brush cytology as a diagnostic modality and suggest that SALSA is not suitable as biomarker in horses with SEA.

Friday, October 30, 2020
1:30 p.m. – 1:45 p.m. CDT

INAPPROPRIATE REGENERATIVE RESPONSE IN DOGS NATURALLY INFECTED WITH BABESIA ROSSI

Chandini Seejarim, Yolandi Rautenbach, Emma Hooijberg, Andrew Leisewitz, Johan Schoeman, Amelia Goddard
University of Pretoria, Onderstepoort, South Africa

Background: Despite hemolytic anemia being the main consequence of *Babesia rossi* infection in dogs, the bone marrow response is mild in the face of severe anemia. A similar finding has been described in *falciparum* malaria and has been ascribed to either a decreased production of erythroid precursors or an inability of erythroid precursors to respond to hormonal stimulus. **Objective:** To compare the admission absolute reticulocyte count (ARC) in dogs naturally infected with *B. rossi* with dogs suffering from immune-mediated hemolytic anemia (IMHA), unrelated to babesiosis, as well as healthy control dogs. **Methods:** A retrospective observational study looking at the records

generated on a hematology analyzer, the ADVIA 2120 (Siemens). The hematocrit (HCT) and ARC for 103 dogs with babesiosis was compared to 16 dogs with IMHA and 14 control dogs. **Results:** The HCT for the *Babesia* (0.16 L/L; *P*<0.001) and IMHA (0.15 L/L; *P*<0.001) groups were significantly lower than the control group (0.52 L/L). Compared to the control group (42.1 $x10^9$/L), the ARC was significantly higher in the *Babesia* (82.1 $x10^9$/L; *P*=0.006) and IMHA (256.7 $x10^9$/L; *P*=0.004) groups. The ARC was significantly lower in the *Babesia* group compared to the IMHA group (*P*=0.011), despite no difference for HCT between groups. **Conclusions:** The regenerative response in dogs naturally infected with *B. rossi* is inappropriate, despite the severity of anemia observed, compared to dogs with IMHA. These findings may indicate a direct effect by the organism on the bone marrow, as reported in *falciparum* malaria, or other factors which require further investigation.

Friday, October 30, 2020
1:45 p.m. – 2:00 p.m. CDT

UTILITY OF AMIKACIN TO DISSOCIATE PLATELET CLUMPS IN BLOOD SAMPLES FROM CATS AND DOGS

Demitria Vasilatis, Naomi Walker, Dori Borjesson
University of California-Davis, Davis, CA, USA

Background: Pseudothrombocytopenia is a well-known laboratory phenomenon in which platelets aggregate *in vitro* and lead to spuriously low platelet counts by an automated hematology analyzer. This phenomenon may lead to the erroneous diagnosis of thrombocytopenia, resulting in costly and unnecessary testing and treatment. **Objective:** The objective was to determine if adding amikacin to blood samples post-collection could disaggregate platelet (PLT) clumps. **Methods:** Prospective study. EDTA blood samples collected from 28 cats and 17 dogs were obtained from the hospital population at UC Davis Veterinary Medical Teaching Hospital. Samples had platelet clumps on blood smear review and were thrombocytopenic per analyzer count. Amikacin (5mg/ml) was added to samples post-collection and the CBC and blood smear review were repeated. CBC parameters were compared between amikacin and saline treated aliquots, and the original EDTA blood. **Results:** The addition of amikacin to cat blood with platelet clumps significantly increased the PLT count (average 134%, p= <0.0001), decreased the MPV (average 14%, p= <0.0001) and visibly reduced platelet clumps on smear review. The addition of amikacin did not significantly increase PLT count in dogs (p= >0.05) although MPV was marginally increased by 1% (p=0.02). No other CBC parameters were affected by the addition of amikacin. **Conclusions:** The addition of amikacin to blood samples that contain microscopically visible platelet clumps may be a cost-effective, convenient solution for pseudothrombocytopenia in cats. Future studies in a larger cohort of dogs with fewer comorbidities may be needed to determine the utility of amikacin.

Friday, October 30, 2020
2:00 p.m. – 2:15 p.m. CDT

EVALUATION OF AN ANTIBODY-BASED FLOW CYTOMETRY METHOD FOR TOTAL AND DIFFERENTIAL WHITE BLOOD CELL COUNTS IN CHICKENS

Priscila B S Serpa, Natalia Strandberg, Meagan Abraham, Darrin Karcher, Andrea Pires dos Santos
Purdue University, West Lafayette, IN, USA

Background: The presence of nucleated erythrocytes and thrombocytes in birds precludes the use of current automated hematological analyzers. **Objective:** Evaluation of an antibody-based flow cytometry (FC) method for total and differential leukocyte counts in chickens as an alternative for time-consuming manual methods. **Methods:** Blood samples from laying pullets (n = 40) were used for 200-cell differential counts and total counts using phloxine. For FC, with a “no-lysis-no-spin” approach, a mixture of CD45 and CD41/CD61 was used to determine the total count, and CD45, KUL01, Bu-01, and CD3 for the differential count. **Results:** For total count, the methods had proportional (slope 0.1013, 95% CI 0.054-0.160) and constant bias (intercept 6.92 k/ µL, 95% CI 5.95-7.94), large systematic error (-7.56 k/µL, SD = 10.25 k/µL), and poor correlation (r = 0.52). Poor correlation was also observed in granulocyte and monocyte counts (bias –1.4 and -0.6 k/µL, respectively). No correlation or agreement were detected for lymphocyte counts. Immunocytochemistry (ICC) using the antibodies at the same concentrations as in FC showed sensitivity/specificity of 96%/100% of CD45 to WBC, 75%/100% of BU-01/CD3 to lymphocytes, and 74%/99% of KUL01 to monocytes, indicating a high association between FC and ICC. Blood smear evaluation showed leukocyte and thrombocyte clumping in almost all of the samples. **Conclusion:** Although these results disqualify this antibody-based FC method for leukocyte counts in chickens, the imprecision and subjectivity of manual techniques should be taken into account whenever they are used as a gold standard for the development of new techniques.

Friday, October 30, 2020
2:15 p.m. – 2:30 p.m. CDT

COMPARISON OF IRON STAINING AND SCORING METHODS ON CANINE BONE MARROW ASPIRATES

Grace Pawsat, Deanna Schaefer, Michael Fry, Schneider Liesel
University of Tennessee, Knoxville, TN, USA

Background: Insufficient iron for erythropoiesis can occur in multiple conditions, including absolute iron deficiency, which is often caused by chronic external hemorrhage in dogs. Distinguishing this from other causes of iron-restricted erythropoiesis allows appropriate interventions to identify sources of hemorrhage and provide supplemental iron. Decreased marrow iron assessed by Prussian Blue staining is a method to diagnose absolute iron deficiency but scoring systems for marrow iron are not validated in dogs. **Objectives:** Our objectives are to evaluate 1) technical performance of two iron scoring systems used in human medicine and 2) effects on iron scores when Wright stained marrow slides are destained and restained with Prussian Blue. **Methods:** Two marrow aspirate slides were included from each of 12 ill dogs,

where marrow was collected during clinical evaluation. One slide was directly stained with Prussian Blue ("non-destained"). The other was first stained with Wright stain then destained. Three blinded observers scored iron in each of the 24 randomized slides using the Gale method (scale 0-6) and the sideroblast method (percentage score). Slides were then rerandomized and rescored. **Results:** For the Gale method, interobserver agreement was fair and intraobserver agreement was very good. There was less agreement using the sideroblast method, with a significant observer effect. Iron scores were slightly but significantly lower in destained slides compared to non-destained slides. **Conclusions:** Interobserver and intraobserver agreement was acceptable for the Gale method, but the sideroblast method should be used cautiously. A destaining procedure before Prussian blue staining may modestly decrease marrow iron scores.

Friday, October 30, 2020
2:30 p.m. – 2:45 p.m. CDT

BONE MARROW AND HEMATOLOGIC FINDINGS IN DOGS TREATED WITH PHENOBARBITAL

Tiffany Scott, H. Bailin, L. Jutkowitz, Michael Scott, Cynthia Lucidi
Michigan State University, East Lansing, MI, USA

Background: Hemic abnormalities have been reported in dogs treated with phenobarbital, but detailed descriptions of associated aspirate and core bone marrow abnormalities are lacking. **Objective:** Characterize hematologic findings and clinical outcomes of phenobarbital-treated dogs undergoing bone marrow evaluation. **Methods:** Dogs undergoing marrow evaluation for hematologic abnormalities that developed while receiving phenobarbital were identified (2008-2019). Dogs were excluded if marrow samples lacked diagnostic value, phenobarbital was withdrawn >1 day before marrow collection, a same-day CBC was lacking, or dogs had concurrent illness or therapy associated with development of cytopenias. Archived slides for each case were reviewed. **Results:** Twelve dogs met inclusion criteria: 8 pancytopenic, 2 anemic/thrombocytopenic (AT), 1 neutropenic/thrombocytopenic (NT), and 1 approaching neutropenia. Neutropenia was marked (<700/μL) in 8 dogs; all neutrophil concentrations were ≤4,300/μL. Of the 10 anemic dogs (Hct=12-42%, median=28.5%), 3 had mild reticulocytosis (of 7 tested). One had inappropriate rubricytosis with erythroid dysplasia. The 9 neutropenic dogs had evidence of ineffective neutropoiesis: neutrophilic hyperplasia with left-shift (9) and/or neutrophagocytosis (5). Seven of the 10 anemic dogs had evidence of ineffective erythropoiesis: erythroid hyperplasia (6) with left-shift (2) and/or rubriphagocytosis (5). No thrombocytopenic dog had megakaryocytic hypoplasia; 7 had megakaryocytic hyperplasia. One AT dog had marked myelofibrosis. The noncytopenic dog had equivocal myeloid hypoplasia with neutrophagocytosis. Median time to resolution of cytopenias was 14 days (n=10). **Conclusions:** Most phenobarbital-treated dogs suspected of marrow disease had cytopenias with ineffective neutropoiesis and/or erythropoiesis, whether phenobarbital-induced or not. Features to differentiate phenobarbital toxicity from other causes of ineffective hematopoiesis were not found.

Friday, October 30, 2020
2:45 p.m. – 3:00 p.m. CDT

TTHREE-YEAR SURVEILLANCE OF CANINE TOTAL RETICULOCYTE COUNT AND RETICULOCYTE HEMOGLOBIN ABNORMALITIES

Dennis DeNicola, Julia Chase, Peter Hendrickson, David Szlosek, Jeremy Hammond, James Russell, Graham Bilbrough, Helen Michael
IDEXX Laboratories, Inc, Westbrook, ME, USA

Background: The value of a complete reticulocyte profile has increased over the last 10 years, especially now that reticulocyte counts and reticulocyte hemoglobin (RETIC-HGB) is readily available at the reference laboratory and in-clinic with the IDEXX ProCyte Dx® Hematology Analyzer (PDx). Prevalence studies have indicated that clinically valuable reticulocyte information is provided in approximately 37% of all canine CBCs performed in-clinic on the PDx. **Objective:** To define the prevalence of reticulocyte profile abnormalities over a three-year period and potentially document consistent findings over this time period. **Methods:** One million canine CBCs were collected randomly each year from the global PDx fleet of analyzers in 2017, 2018 and 2019. The prevalence of reticulocytosis with and without anemia, decreased RETIC-HGB without anemia or reticulocytosis and the prevalence of an indices profile with increased MCV, decreased MCHC and increased RDW in anemia cases with reticulocytosis were determined for each year. **Results:** For 2017, 2018 and 2019, respectively, prevalence of anemia with reticulocytosis was 18.7%, 18.9% and 19.6%; prevalence of reticulocytosis without anemia was 8.4%, 8.9% and 9.7%; the prevalence for decreased RETIC-HGB in the absence of anemia or reticulocytosis was 15.4%, 13.1% and 13.6%; the prevalence of finding increased MCV, decreased MCHC and increased RDW in anemia cases with reticulocytosis was 4.0%, 3.5% and 3.1%. **Conclusions:** This study documents the high incidence of abnormal reticulocyte profiles in dogs and the need for reticulocyte counts for characterizing anemia. The addition of a complete reticulocyte profile to the canine CBC provides value to the veterinarian.

ACVP Veterinary Student Selected Platform Presentation Session

Friday, October 30, 2020 | 12:30 p.m. – 1:00 p.m. CDT

Friday, October 30, 2020
12:30 p.m. – 12:35 p.m. CDT

DEVELOPMENT OF A DEEP LEARNING METHOD FOR DISCRIMINATING ANTERIOR NASAL CAVITY DEGENERATIVE, REGENERATIVE, AND INFLAMMATORY LESIONS IN THE RAT

Samuel Neal[1], Jogile Kuklyte[2], Adam Power[2], Dan Rudmann[1]
[1]Charles River Laboratories, Ashland, OH, USA, [2]Deciphex, Dublin, Ireland

Nasal cavity assessment is essential for both the safety assessment of environmental and workplace exposure agents and pharmaceuticals. The nasal cavity in the rat is complex and in a standard 30-day rat toxicology study at least 240 sections are examined by the pathologist. Nasal cavity lesions are diverse, and efforts to ensure consistent diagnostics and scoring is time consuming. We hypothesized that a deep

learning artificial intelligence (AI) algorithm would provide a decision support tool for the pathologist that would increase diagnostic quality and efficiency. Whole slide images (WSI) of nasal cavity levels I and II from control and treated animals were scanned at 40x on a Leica AT2 scanner and uploaded to Deciphexs' Patholytix Preclinical Study Browser. Training annotations were performed for 14 different classes at 10x magnification based on INHAND criteria and used by Deciphex to conduct supervised training of a convolutional neural network (CNN). Model performance was quantified on both a pixel and object basis by using confusion matrices and F1 scores for AI generated masks. The developed algorithm performed well for 12/14 classes (F1 scores exceeding 0.70). A test set of unannotated nasal cavities was evaluated by 4 ACVP board-certified pathologists and the algorithm and consensus supported qualification of the algorithm performance on an object-basis. The AI algorithm was incorporated into an intuitive digital workflow for the toxicologic pathologist and has potential to provide both functionality and decision support that increases efficiency and consistency.

Friday, October 30, 2020
12:35 p.m. – 12:40 p.m. CDT

HOW DOES WEIGHT LOSS AFFECT INFLAMMATION IN THE OBESE MURINE MAMMARY GLAND?

Brittney Moore, Genevra Kuziel, Lisa Arendt
University of Wisconsin - Madison, Madison, WI, USA

Obesity is a metabolic condition characterized by chronic adipose tissue inflammation, which increases the risk of developing breast cancer in women. Additionally, obese breast tissue has increased adipose tissue fibrosis and immune cell recruitment and is associated with breast tumor progression and metastasis. Although it is recommended that obese women undergo a weight loss regimen to reduce their risk of breast cancer, the impacts of weight loss on the mammary gland are not well understood. We predict that weight loss will reduce mammary gland fibrosis and macrophage recruitment. Briefly, we used a mouse model to examine the effects of weight loss on the mammary gland. Picrosirius red staining, to label collagen, was coupled with immunohistochemistry to monitor changes in tissue fibrosis and immune cell recruitment. We generated an obese mouse model by feeding a high fat diet for 16 weeks, which resulted in significant weight gain in mice; we then induced weight loss by feeding a low-fat diet for 5 weeks. Mammary gland collagen deposition and macrophage recruitment were increased in obese mice relative to lean mice, suggesting that obesity contributes to increased mammary gland fibrosis and inflammation, but the impact of weight loss on these processes remains unclear. Elucidating the relationship between weight loss, tissue fibrosis, and inflammation in the obese mammary gland will lead to a better understanding of obesity's contributions to breast cancer progression and may guide future treatment recommendations.

Friday, October 30, 2020
12:40 p.m. – 12:45 p.m. CDT

INVESTIGATING CROSS-REACTIVE ANTIBODIES TO SARS-COV-2 IN BCG-VACCINATED MICE

Aubrey Specht[1], Sherry Kurtz[2], Karen Elkins[2], Gillian Beamer[1]
[1]Cummings SVM at Tufts University, North Grafton, MA, USA, [2]Food and Drug Administration, Silver Spring, MD, USA

The Bacillus Calmette-Guérin (BCG) vaccine, composed of live attenuated *Mycobacterium bovis,* remains the only vaccine available for human use against tuberculosis. However, the impact of this vaccine extends beyond tuberculosis: a growing body of evidence supports non-specific, cross-protective immunological benefits of BCG-vaccination against a broad spectrum of pathogens unrelated to *mycobacterium*, including viruses. One proposed hypothesis for such cross-protective benefits includes antibody cross-reactivity to a wide array of antigens. We hypothesized immunization with the BCG vaccine may deliver a humoral response against the spike protein on SARS-CoV-2. An in-house ELISA, coated in recombinant spike protein (rSpike) was optimized to investigate cross-reactive antibodies to SARS-CoV-2 by measuring the concentration of IgG reactive to the spike protein from the sera of BCG-vaccinated mice. The concentration of IgG reactive to the rSpike in BCG-vaccinated mice (n=7) was compared with the concentration of IgG from serum of an unvaccinated mouse (n=1). Preliminary results indicated 4 of 7 sera samples from BCG-vaccinated mice possess more IgG reactive to the rSpike than the serum of the unvaccinated mouse. Results suggest the BCG antigen induces cross-reactive antibodies capable of binding the spike protein on the SARS-CoV-2 pathogen. These results will help elucidate the mechanism by which BCG-vaccination offers cross-protective immunological benefits, and aid in the global search for defense against COVID-19 through two perspectives: 1) development of a vaccine optimizing the humoral immune response to the pathogen and 2) awareness of the impact cross-reactive antibodies may have in generating false positives in serological tests from BCG-vaccinated humans.

Friday, October 30, 2020
12:45 p.m. – 12:50 p.m. CDT

BALD EAGLE MORBIDITY AND MORTALITY IN THE SOUTHEASTERN UNITED STATES: A FIVE-YEAR RETROSPECTIVE

Aidan O'Reilly[1], Nicole Nemeth[1], Mark Ruder[1], Heather Fenton[1,2]
[1]University of Georgia, Athens, GA, USA, [2]Ross University, Basseterre, Saint Kitts and Nevis

Bald eagles (*Haliaeetus leucocephalus*) are at risk of anthropogenic causes of mortality, most commonly through various sources of trauma (e.g., collisions, electrocution, and gunshot). As both apex predators and facultative scavengers, bald eagles also frequently suffer from toxicoses, notably lead, anticoagulant rodenticides, and insecticides. However, published reports on causes of mortality in bald eagles in the southeastern U.S. are scarce. We retrospectively evaluated diagnostic findings of bald eagle cases submitted from 21 states (primarily southeastern) from January 2015 to

June 2020 and categorized primary and contributing causes of mortality. Diagnostic findings included gross and histopathology and case-specific ancillary test results when performed. Among 267 bald eagles examined, non-infectious causes of mortality (209; 78.3%) were more common than infectious (21; 7.9%), with 37 (13.8%) that died of unknown causes. The majority (127; 47.6%) of noninfectious causes were attributed to trauma, most commonly blunt force (e.g., vehicular collision; 21). Seventy-three (27.3%) eagles died of toxicoses, including lead (44), anticoagulant rodenticides (18), and avian vacuolar myelinopathy (i.e., cyanobacterium toxin; 5). Additionally, numerous toxicants were detected at low levels in 110 eagles. Infectious causes were most often viral (11; most commonly, West Nile virus). This study demonstrates challenges faced by bald eagles in the southeastern U.S. including potential, population-level impacts of human-associated activities. Although trauma was the most common source of mortality, frequent detection of a variety of toxins is concerning. Specifically, the potential health impacts of low-level and possibly chronic toxicant exposure in bald eagle populations warrants further investigation.

Friday, October 30, 2020
12:50 p.m. – 12:55 p.m. CDT

RETROSPECTIVE ANALYSIS OF LABORATORY DATA AS PROGNOSTIC FACTORS FOR SURVIVAL IN CANINE SPLENIC HEMANGIOSARCOMA

Erin Paul, Andrea Pires dos Santos
Purdue University, West Lafayette, IN, USA

Hemangiosarcoma (HS) makes up 5-7% of malignant tumors in dogs and has a poor prognosis due to metastatic disease. HS originates from pluripotent endothelial cells and relies on angiogenesis for growth. Initial slow growth allows time for invasion of surrounding tissues and hematogenous dissemination. Visceral HS of the spleen or right auricle is more common than non-visceral HS, which develops in the skin or muscle. Treatment of visceral HS with surgery results in an average survival time of 1-3 months, and with chemotherapy, up to 6 months. However, reliable factors to help predict survival are difficult to elicit. In this study, electronic medical records from cases of splenic HS in dogs presenting to a veterinary hospital from 2010-2020 were analyzed to determine if signalment, CBC, and serum chemistry data had a significant relationship to overall survival time and could be utilized as potential prognostic markers. Twenty-three cases of splenic HS met inclusion criteria and were divided into three groups: G1, <90 days survival; G2, 90-180 days survival, and G3, >180 days survival. Kruskal-Wallis was used for group comparisons. As expected, presence of the tumor in multiple organs (multicentric) and gross metastatic disease were significantly different between groups (p<0.5). In addition, serum phosphorus levels were decreased in G2 compared to G1 and G3. This retrospective analysis supports the presence of metastasis as a useful prognostic indicator in canine HS. Other potential prognostic markers of HS in dogs are being analyzed, including IHC and microRNA-based markers in archived samples from these cases.

ACVP Focused Scientific Session Platform Presentation Highlights

Friday, October 30, 2020 | 1:00 p.m. – 2:15 p.m. CDT

Friday, October 30, 2020
1:05 p.m. – 1:13 p.m. CDT

PUSTULAR DERMATITIS WITH FOLLICULITIS AND ARTERITIS IN A GOAT ASSOCIATED WITH OVINE HERPESVIRUS-2

Jason Struthers[1], Clemence Chako[1], Stephani Ruppert[1], Kenneth Jackson[2], Patricia Pesavento[2]
[1]Animal Health Institute, Midwestern University, Glendale, AZ, USA, [2]School of Veterinary Medicine, UCD, Davis, CA, USA

A one-year-old Nigerian dwarf goat had multifocal alopecic crusting dermatitis for four months. This goat was the only affected animal among a mixed herd of 7 sheep and 4 goats. The goat had thickened and crusting skin with erosions that predominated on the ventrum and did not resolve with topical iodine and miconazole. Bacteriology of affected skin yielded no significant growth. Mycology and a fungal histochemical stain ruled-out fungal dermatitis. Biopsies of alopecic, hyperkeratotic, and hyperplastic skin had multifocal neutrophilic, eosinophilic, lymphocytic, and histiocytic dermatitis and mural folliculitis with multinucleated giant cells, necrotic keratinocytes, and spongiosis. The overlying epidermis had multifocal erosion with pustules and individual necrosis. In one section, the deep dermis had eosinophilic lymphocytic arteritis. Multiplex real-time PCR of fresh affected skin was positive for ovine herpesvirus-2 (OvHV-2) (Ct 34.30), while negative for alcelaphine herpesvirus-1, caprine herpesvirus-2, ibex Malignant Catarrhal Fever (MCF) virus, and white-tailed deer MCFV. Using a nucleic acid probe to the ORF 25 and 50 of OvHV-2, in situ hybridization localized abundant OvHV-2 to leukocyte nuclei in areas of arteritis, folliculitis, and erosions. OvHV-2 is one viral agent of MCF that affects several ungulates, but infection of goats is rare or rarely reported. OvHV-2 associated dermatitis in goats is potentially underdiagnosed, since it is not typically part of the differential diagnosis of caprine dermatitis. The distribution of the OvHV-2 nucleic acid in this dermatitis-folliculitis is evidence of interspecies transmission of a persistent endogenous ovine herpesvirus to goats.

Friday, October 30, 2020
1:13 p.m. – 1:21 p.m. CDT

NONINVASIVE SAMPLING ALTERNATIVES TO CONVENTIONAL BIOPSY FOR THE MOLECULAR DIAGNOSIS OF CUTANEOUS LEISHMANIASIS IN DOGS

Josilene Nascimento Seixas[1], Jessica Felix Moreira[1], Raquel Romano Palmeira Gonçalves[1], Marcos de Almieda[2], Jana M. Ritter[2]
[1]Federal University of Lavras, Lavras, Brazil, [2]Centers for Disease Control and Prevention, Atlanta, GA, USA

Background: Diagnosis of canine cutaneous leishmaniasis is often not straightforward, due to prolonged clinical course, pleomorphic nature of skin lesions, and concurrence with other dermatoses. Diagnosis frequently requires skin punch biopsy, which may be unfeasible in high-density, low-resource settings such as shelters and surveillance programs. Development of sensitive, noninvasive diagnostics would allow for increased

testing and improved diagnosis, especially in these settings. **Objective:** Determine the utility of noninvasive samples compared to skin punch biopsies for molecular diagnosis of cutaneous leishmaniasis. **Methods:** Biopsies of 41 dogs from a shelter in Lavras-MG, Brazil were evaluated by histopathology and immunohistochemistry (n=167) and tested for *Leishmania* spp. by PCR with sequencing analysis (=41). Additionally, noninvasive specimens, including adhesive tape impressions (n=14), hair samples (n=14), and mucosal swabs (n=48), from dogs with positive results on skin biopsies were also tested by PCR. **Results:** Among 41 dogs with skin lesions, 14 animals had biopsy sample positive for *Leishmania infantum* by PCR. Of these 14 animals, 11 (79%) had at least one noninvasive specimen positive, 10 (71%) had a positive mucosal swab, 7 (50%) had a positive adhesive tape sample, and 3 (21%) had a positive hair sample. Additionally, *Leishmania* spp. amastigotes were seen in 9 of the 14 PCR confirmed cases (64%) by histology and immunohistochemistry. **Conclusions:** Non-invasive specimens, especially mucosal swabs and adhesive tape samples, provide an alternative to invasive skin biopsy for diagnosis of cutaneous leishmaniasis and may be particularly useful in high-volume, low-resource settings.

Friday, October 30, 2020
1:21 p.m. – 1:29 p.m. CDT

BORRELIA BURGDORFERI BB059 IS REQUIRED FOR INFECTION AND CONTRIBUTES TO THE DEVELOPMENT OF ARTHRITIS IN MICE

Sebastian Carrasco[1], Meiping Ye[2], Youyun Yang[2], Suresh Ganesan[1], Alejandra Aguilar[1], Frank Yang[2]
[1]Massachusetts Institute of Technology, Cambridge, MA, USA, [2]Indiana University, Indianapolis, IN, USA

Borrelia burgdorferi (Bb), the pathogen of Lyme disease, differentially expresses a selected repertoire of surface proteins in vivo, which contribute to spirochetal infection and/or disease. Bb has several genes in the chromosome, like bb059, that are annotated as hemolysin-like proteins. However, the function of these proteins is largely unknown. To examine the role of BB059 during infection we first generated a BB059 deletion mutant (BB059-) of Bb. The BB059- was not able to establish infection in C3H/HeN mice after 28 days p.i.. Spirochetes lacking BB059 were unable to trigger an increase in white blood cell counts and cardiac and joint inflammation in these mice. In contrast, the BB059- was able to infect C3H/HeN-SCID and colonized multiple tissues. SCID mice infected with the BB059- exhibited high neutrophil counts, which was comparable to the neutrophilia noted in SCID mice infected with wild-type spirochetes. Histopathology of the joints from SCID mice infected with the wild-type strain were characterized by severe multifocal to coalescing neutrophilic and histiocytic tenosynovitis and arthritis with periosteal bone remodeling and osteophyte formation. However, the BB059- did not induce tenosynovitis and arthritis in SCID mice. The hearts of SCID mice infected with wild-type or BB059- spirochetes exhibited moderate multifocal neutrophilic and histiocytic aortitis and myocarditis/epicarditis. qPCR analysis showed that the BB059- exhibited a defect in colonization in the tibiotarsal joints of SCID mice. Taken together our findings suggest that BB059 is essential for Bb infection in immunocompetent hosts and plays an important role in the development of Lyme arthritis.

Friday, October 30, 2020
1:29 p.m. – 1:37 p.m. CDT

PATHOLOGY ASSOCIATED WITH SARS-COV-2 INFECTION IN RHESUS MACAQUES (MACACA MULATTA), BABOONS (PAPIO SPP.), AND COMMON MARMOSETS (CALLITHRIX JACCHUS).

Olga Gonzalez[1,2], Journey Cole[1,2], Yenny Goez-Gazi[2], Jessica Callery[1,2], Dolores Escalona[1,2], Jesse Martinez[1,2], Colin Chuba[1,2], Stephanie Earley[1,2], Shannan Hall-Ursone[1,2], Kathy Brasky[1,2], John Dutton[1,2], Elizabeth Clemmons[1,2], Tyneshia Camp[1,2], Dhiraj Kumar Singh[1,2], Hilary Staples[1,2], Kendra Alfson[2], Amanda Mannino[1,2], Michal Gazi[2], Benjamin Klaffke[2], Laura Parodi[1,2], Carmen Bartley[1,2], Vida Hodara[1,2], Priscilla Escareno[2], Luis Giavedoni[1,2], Ricardo Carrion, Jr.[1,2], Deepak Kaushal[1,2], Edward Dick, Jr.[1,2]
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Background: We report histology findings of SARS-CoV-2 infection in experimentally infected young and aged rhesus macaques, baboons, and common marmosets. **Methods:** Animals were infected by combined routes (ocular, intratracheal and intranasal). Rhesus macaques (3 dpi; n=4 and 14 to 17 dpi; n=12), baboons (14 to 17 dpi; n=12), and marmosets (14 to 17 dpi; n=12) were necropsied and tissues were evaluated histologically. **Results:** The lungs were the most severely affected tissue in all three species and the most common findings were interstitial or intra-alveolar neutrophilic and mononuclear inflammation (eosinophils were also present in the rhesus macaques and baboons), bronchitis and bronchiolitis with BALT hyperplasia, alveolar syncytia, increased alveolar macrophages, and type II pneumocyte hyperplasia. Vasculitis, necrosis, fibrin, and bronchiolization were less common, with rare microthrombi, endothelial syncytia, and scattered megakaryocytes. Other lesions included tracheitis, rhinitis, and myocarditis. Rhesus macaque lung inflammation was more neutrophilic at day three, transitioning to predominantly mononuclear infiltrates by two weeks; bronchiolitis decreased and alveolar fibrosis increased over time. Lung lesions, tracheitis, rhinitis, and myocarditis persisted at two weeks in young and aged rhesus macaques, baboons, and marmosets. Ocular changes (choroiditis and episcleritis) were common at two weeks; only episcleritis was observed in marmosets. There was vasculitis in several tissues in rhesus macaques. Some young baboons had perivascular edema in the brain and some aged baboons had choroid plexitis and encephalitis.

Friday, October 30, 2020
1:37 p.m. – 1:45 p.m. CDT

BI-TRANSGENIC CTLA4+/- PDCD1-/- MOUSE RECAPITULATES IMMUNE CHECKPOINT INHIBITOR-ASSOCIATED MYOCARDITIS

Lauren Himmel[1], Elizabeth Whitley[2], Spencer Wei[2], Wouter Meijers[1], Nana-Ama Anang[2], Margaret Axelrod[1], Elles Screever[1], Elizabeth Brunner Wescott[1], Douglas Johnson[1], James Mancuso[2], Matthew Wlekinski[1], Bjorn Knollmann[1], Jayashree

Srinivasan[3], Yu Li[3], Oluwatomisin Atolagbe[2], Xiayu Rao[2], Yang Zhao[2], Jing Wang[2], Lauren Ehrlich[3], Joe-Elie Salem[1], Justin Balko[1], Javid Moslehi[1], James Allison[2]
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Background: Immunotherapy has been a breakthrough anticancer strategy for many tumor types in both humans and to a lesser extent companion animals. Immune checkpoint inhibitors (ICI) are antibodies that target programmed death-1/ligand-1 (PD-1/PD-L1) and/or cytotoxic T lymphocyte antigen-4 (CTLA-4) and release the brakes on T cell responses, exerting cytotoxic antitumor effect. One unintended outcome of such efficacious treatments in humans is the development of immune-related adverse events, including fulminant myocarditis. ICI-associated myocarditis is uncommon, with nearly 300 human cases identified to date, but the fatality rate is high, at 30-50%. The underlying mechanisms and predisposing factors are not yet understood. **Objective:** A preclinical animal model would be useful to study the disease course, identify potential biomarkers, test interventions, and monitor outcomes. **Methods:** A transgenic mouse model on a C57BL/6J background was generated to mimic drug-induced abrogation of CTLA-4 and PD-1, and mice underwent comprehensive postmortem investigation. **Results:** We found that *Ctla4*$^{+/-}$ *Pdcd1*$^{-/-}$ mice had a mortality rate of 50-60% across 2 institutions, increased heart weights, an increased histologic inflammatory infiltrate score, and immunohistochemical evidence of T-cell-rich myocarditis. Cardiac inflammation involved all 4 chambers and was predominantly distributed along the endo- and epicardium, with a low incidence of concomitant atrial thrombus or aortic dissection. Lymphocytic infiltration into the myocardial tissue and cardiomyocyte necrosis demonstrated patterns similar to those observed in human patients with ICI-induced myocarditis. **Conclusions:** Taken together, our data provide evidence of phenotypic validity of this novel model for further study of ICI-associated myocarditis.

Friday, October 30, 2020
1:45 p.m. – 1:53 p.m. CDT

DEVELOPING A DEEP LEARNING CONVOLUTIONAL NEURAL NETWORK METHOD TO COUNT OVARIAN FOLLICLES IN RATS

Lauren Prince[1], Lindsey Smith[1], Cynthia Swanson[2], Steven Denham[3], Daniel Rudmann[4]
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Background: The rat is an important animal model in assessing potential ovarian toxicity. One method involves the manual enumeration of different ovarian follicle types in serial sections of ovaries from rats exposed to a test agent. The counting process is laborious and prone to variability. We hypothesized that a deep learning method using a convolutional neural network (CNN) would improve speed and reproducibility for this assay. **Objective:** To design, train, and qualify a convolutional neural network that can identify, distinguish, and count three types of ovarian follicles in rats. **Methods:** Ovaries collected from female rats were sectioned, stained with hematoxylin and eosin, scanned at 20x, and uploaded into Aiforia Create. Ovaries were annotated for three types of

follicles, small (primary and primordial), growing, and antral, based on the diameter of the follicle and number of layers of granulosa cells. Training annotations representing follicles with an intact ovum (all three types) and a nucleus (growing and antral) were counted, then this ground truth was verified by two trained experts. **Results:** The developed CNN AI model had less than 1% error in counting specific follicle types. Using a testing set, there was good concordance between follicle counts done by a group of blinded evaluators, AI model trainers, and the computer algorithm. Time saved was approximately 50% when comparing the automated and manual methods. **Conclusions:** The CNN AI model supported an automated counting procedure for rat ovarian follicles that could be incorporated into a toxicology study workflow and increase study speed and reproducibility.

Friday, October 30, 2020
1:53 p.m. – 2:01 p.m. CDT

RETROSPECTIVE ANALYSIS OF PHAEOHYPHOMYCOSIS IN AQUARIUM-HOUSED FISH, INCLUDING TWO PREVIOUSLY UNREPORTED FUNGAL SPECIES

Abigail Armwood[1], Jennifer Dill-Okubo[2], Alvin Camus[1]
[1]University of Georgia, Athens, GA, USA, [2]Bronson Animal Disease Diagnostic Laboratories, Kissimmee, FL, USA

Background: Novel and known dematiaceous fungi are emerging pathogens of fish cultured for human consumption and aquarium display. **Objective:** This retrospective study characterized associations between fish species, tissues affected, and where possible, fungal species causing phaeohyphomycosis in aquarium fish. **Methods:** Piscine submissions (2250) received by the Aquatic Diagnostic Service, University of Georgia from 2006 to 2020 were searched for phaeohyphomycosis. When fresh tissue was available (11), culture and/or sequencing of the internal transcribed spacer and large ribosomal subunit gene D1/D2 regions were performed for fungal identification. **Results:** Forty-seven cases of phaeohyphomycosis were identified (47/2250, 2.1%), representing 34 cartilaginous and bony fish species. The majority (45/47, 95.7%) involved bony fish and were overwhelmingly marine (41/47, 87.2%), with only a few freshwater species (4/47, 8.5%). The cartilaginous fish cases were two zebra sharks (*Stegostoma fasciatum*) (2/47, 4.3%). Northern seahorses (*Hippocampus erectus*) were most frequently affected (7/46, 15.2%). Syngnathiformes (11/47, 23.4%) and Perciformes (19/47, 40.4%) were the most commonly involved fish Orders. *Exophiala, Ochroconis,* and *Devriesia* spp. were identified, with *Exophiala* as the most common genus (8/11, 72.7%). *Exophiala lecanii-cornii* and *Devriesia imbrexigena* are reported for the first time in fish. Microscopically, lesions were typified by necrosis, granulomatous inflammation, and angioinvasion with thin, brown, septate hyphae possessing no distinguishing microscopic features or observed tissue associations. **Conclusions:** Phaeohyphomycoses result in sporadic infections in a diverse group of aquarium-housed fish species, with variable pathologic presentations, tissue distributions, and severities. *E. lecanii-cornii* and *D. imbrexigena* were previously unreported in fish.

Friday, October 30, 2020
2:01 p.m. – 2:09 p.m. CDT

PATHOLOGY AND CAUSES OF DEATH IN FREE-RANGING NORTH AMERICAN BEAVERS (CASTOR CANADENSIS) IN CALIFORNIA: A SUITABLE SENTINEL SPECIES FOR FRESHWATER ECOSYSTEMS

OMAR GONZALES VIERA, LESLIE WOODS
California Animal Health and Food Safety (CAHFS), Davis Laboratory, UC Davis, Davis, CA, USA

Background: North American Beavers (*Castor canadensis*) are semiaquatic rodents recognized as keystone species increasing diversity of ecosystems and sentinel species for freshwater ecosystem health. Materials and **Methods:** 16 free-ranging beavers (10 females, 6 males; 2kits, 6 juveniles, 8 adults) submitted for postmortem examination between 2008-2020 at the California Animal Health and Food Safety (CAHFS), UC Davis received full diagnostic workups, which in addition to gross and microscopic examination included immunohistochemical, bacteriological, parasitological, molecular, serological and toxicological tests. **Results:** Of 16 beavers examined, encephalitis was the most prevalent (8/16) cause of mortality with *Baylisascaris* sp. demonstrated in two and *Listeria monocytogenes* cultured in one beaver. A non-specific encephalitis/meningoencephalitis was diagnosed in five animals with multifocal astrocytic scarring, eosinophils, and malacia forming tracks in the brain most suggestive of *Baylisascaris* sp. larval migration. *Francisella tularensis* type B (etiologic agent of Tularemia) caused septicemia with multisystemic necrosis and peritonitis in one animal. Mesocestoides larvae-associated chronic peritonitis was diagnosed in one adult male beaver and trauma with multiple complete pelvic fractures and multifocal contusions were observed in another adult male beaver. Other pathological findings included granulomatous hepatitis with *Calodium* (*Capillaria*) *hepaticum* (1), cerebral *Toxoplasma gondii* cysts (1), cholangiocellular (1) and papillary renal (1) adenomas, castor gland squamous cell carcinoma (1), oxalate crystal-associated nephrosis (1), Brodifacoum (anticoagulant) (1) and *Leptospira interrogans* serovar Pomona (1) exposure. **Conclusion:** In California, beavers are exposed to various pathogens with human and domestic/wild animal impact. Investigating the mortality and pathology of free-ranging beavers represents a barometer of freshwater ecosystem health.

ACVP Focused Scientific Poster Session Highlights

Friday, October 30, 2020 | 2:15 p.m. – 3:00 p.m. CDT

Friday, October 30, 2020
2:20 p.m. – 2:25 p.m. CDT

MASSIVE BRANCHIAL HENNEGUYOSIS: A DISTINCTIVE MYXOZOAN-INDUCED GILL DISEASE OF CATFISH CAUSED BY MASSIVE INTERLAMELLAR INFECTIONS OF HENNEGUYA EXILIS

Justin Stilwell[1], Matt Griffin[2,3], John Leary[1], Lester Khoo[2,3], Alvin Camus[1]
[1]University of Georgia, Athens, GA, USA, [2]Mississippi State University, Stonevile, MS, USA, [3]Thad Cochran National Warmwater Aquaculture Center, Stoneville, MS, USA

Proliferative gill disease (PGD), caused by the myxozoan parasite *Henneguya ictaluri*, is the most prevalent parasitic disease of United States catfish aquaculture. Recently, an unusual myxozoan-induced gill disease caused by massive burdens of *Henneguya exilis* has been diagnosed within channel (*Ictalurus punctatus*) x blue (*Ictalurus furcatus*) hybrid catfish monoculture. Targeted metagenomic sequencing and *in situ* hybridization (ISH) were used to examine myxozoan community composition between massive branchial *Henneguya* infections and clinical PGD cases to identify myxozoan species contributing to pathology. Thirty ethanol-fixed gills from seven hybrid catfish massive branchial *Henneguya* cases were subjected to targeted amplicon sequencing of the 18S rDNA gene (Illumina MiSeq) and compared to a metagenomic dataset generated from clinical PGD cases. Further, serial sections of 21 formalin-fixed gills (3 per case) were analyzed by RNAscope® duplex chromogen ISH assays targeting 8 different myxozoan species. Metagenomic and ISH data were in agreement, indicating myxozoan community composition significantly differs between PGD and branchial henneguyosis cases, with different myxozoan communities contributing to disease pathogenesis. Findings indicate PGD in farm-raised catfish can consist of mixed species infections, while branchial henneguyosis was attributed to nearly pure infections of *H. exilis*. Other *Henneguya* spp. were rare in branchial henneguyosis, although *H. ictaluri* was identified by ISH in infrequent PGD-like lesions, supporting previous work evincing hybrids are susceptible to acute stages of *H. ictaluri*. Building upon previous pathologic descriptions and molecular characterization, this work provides the case definition for a potentially emerging, myxozoan-induced gill disease of farm-raised catfish, tentatively termed massive branchial henneguyosis.

Friday, October 30, 2020
2:25 p.m. – 2:30 p.m. CDT

HAVE YOU SEEN THIS LESION: GLOMERULAR “BLOOD CYSTS” AND MESANGIOLYSIS IN A CAT

Jessica Wong, Chrissy Eckstrand
Washington State University, Pullman, WA, USA

An 8-year-old, female, spayed, domestic short-haired cat presented for coughing and dyspnea. Examination revealed chylous pleural effusion, and idiopathic chylothorax was diagnosed after ruling out cardiac and infectious diseases, neoplasia, and congenital abnormalities. Surgical intervention was elected, including thoracic duct ligation, subtotal pericardectomy, and pleural port placement. Post-surgical cardiac arrhythmias, hypotension, and anuria occurred for several days, which improved with medical therapy and adjustment of the central line. Despite anuria, creatinine and lactate levels remained normal. Ten days post-surgery, the cat represented for dyspnea and pleural effusion and was euthanized. Necropsy revealed chronic-active pleuritis; multisystemic thromboses affecting the cranial vena cava and lungs; and myocardial infarction with fibrosis. Interestingly in the kidneys, ~25% of glomeruli had segmental microaneurysms with mesangial matrix dissolution (mesangiolysis) and expansion by erythrocytes and occasionally pigment-laden or mononuclear cells. By TEM, most glomeruli had fibrin within capillary lumina and normal podocyte foot processes and fenestrated endothelium. Some causes of mesangiolysis reported in humans include toxicities from snake venom and chemotherapeutics; circulatory disturbances including ischemia,

hypertension, and congenital heart disease; microangiopathies such as hemolytic-uremic syndrome and malignant hypertension. In veterinary medicine, there are few cases of porcine glomerular hyalinosis and mesangiolysis, and one case of mesangiolysis in a cat with Tetralogy of Fallot. In this case, it is suspected that the chronic pleuritis resulted in cranial vena cava thrombosis which embolized and infarcted the heart, leading to congestive heart failure. Thus, the glomerulopathy is considered a sequela of hemodynamic changes rather than a primary pathologic lesion.

Friday, October 30, 2020
2:30 p.m. – 2:35 p.m. CDT

NEUROLOGICAL CHANGES WERE ASSOCIATED WITH BRAIN DAMAGE AND DOWNREGULATION OF BDNF LEVELS IN MICE INFECTED WITH BOVINE ALPHAHERPESVIRUS 5

Milene Rachid
Departamento De Patlogia Geral, Instituto De Ciėncias Biológicas, Universidade Federal De Minas Gerias, Belo Horizonte, Brazil

Background: The infection with Bovine alphaherpesvirus 5 (BoHV-5) has been associated with neurological disease and meningoencephalitis in cattle, characterized by neuronal loss, infiltration of immune cells and, eosinophilic intranuclear inclusion bodies in astrocytes and neurons. Astrocytes have pro-inflammatory and anti-inflammatory functions and are related with the production of neurotrophic factors, which play an important role in the survival of neurons in different central nervous diseases. **Objective:** Our objective was to investigate a potential involvement of astrocytes and the levels of neurotrophic factors (BDNF), glial cell line derived neurotrophic factor (GDNF), and neural growth factor (NGF) with brain damage and neurological sequelae after experimental BoHV-5 infection in mice. **Methods:** Mice were infected with 10^7 $TCID_{50}$ of BoHV-5 by intracranial route and evaluated until day 3 post infection. After euthanasia, brains were removed for histopathological and immunohistochemical analysis, Western blot test, ELISA and viral titration (Protocol 272/11). **Results:** Infected animals had ruffled fur, conjunctivitis, serous nasal secretion, swollen chamfer, apatia, ataxia, hunched posture and circling. The infection promoted meningoencephalitis, neuropil vacuolation, and reactive gliosis. Cleaved caspase-3 immunopositive glio-inflammatory cells were visualized around some blood vessels and increased immunoexpression of glial fibrillary acidic protein (GFAP) was presented throughout the parenchyma. Infected animals had lower concentration of BDNF, compared with mock group and viral load of $1x10^{3,5}$ $TCID_{50}$/ml. **Conclusions:** Our results demonstrated that clinical signs were related to brain inflammation, astrocytosis and tissue damage after infection in mice. We suggest that BDNF could be involved with anti-apoptotic effect and neuroprotection during meningoencephalitis by BoHV-5.

Friday, October 30, 2020
2:35 p.m. – 2:40 p.m. CDT

PATHOLOGICAL FINDINGS AND PATHOGENESIS IN EXPERIMENTAL STREPTOCOCCUS PNEUMONIAE INFECTION IN MICE

Ozan Ahlat[1], Rifki Haziroglu[1], Gulsen Hascelik[2]
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Background: *Streptococcus pneumoniae* causes sinusitis, conjunctivitis, otitis media, especially pneumonia and meningitis. **Objective:** To examine the organ localization of the agent in experimental *S. pneumoniae* infection and determine the most convenient experimental application method. **Methods:** 0,01 ml *S. pneumoniae* serotype 3 ($1x10^7$ CFU/ml) was inoculated to female, Swiss albino mice by using intranasal, intracerebral, intravenous and intraperitoneal methods. After 24 and 48 hours following the inoculation, lung, heart, brain, liver, spleen, kidney were evaluated for microbiological, histopathological, immunohistochemical examinations. **Results:** In microbiological examination, it was noticed that the agent attained to all organs earlier in intraperitoneal method than intracerebral method. Histopathologically, in liver, neutrophil, mononuclear cell infiltration, hydropic-vacuolar degeneration, necrosis and hemorrhage were more apparent in intraperitoneal groups. In intracerebral groups, besides meningitis, hemorrhage and inflammatory cell infiltration composed mostly of neutrophils, were observed in brain. Severe hemorrhage was seen in lungs after 24 hours following intravenous and intracerebral inoculations. Anti-*S. pneumoniae* immunopositivity was more distinct in intracerebral groups in brain, liver, heart; in intraperitoneal groups in kidney and spleen; in intravenous groups in liver and kidney. In terms of TNF, IL-1α, IL-6 and IL-10 positivity was apparent in intracerebral groups in brain; in intraperitoneal groups in spleen and liver. In intravenous groups, after 24 hours, immunopositivity in brain and liver was more severe in TNF compared to other cytokines. **Conclusions:** For studies of pneumococcal pneumonia, intranasal method appears not to be effective over Swiss albino mice; whereas, for studies of pneumococcal meningitis, intracerebral method seems to be more effective.

Friday, October 30, 2020
2:40 p.m. – 2:45 p.m. CDT

SYMMETRIC DIMETHYLARGININE IS A SENSITIVE BIOMARKER OF GLOMERULAR INJURY IN RATS

Rebecca Kohnken, Lauren Himmel, Michael Logan, Richard Peterson, Sabyasachi Biswas, Christina Dunn, Bruce LeRoy
Abbvie, North Chicago, IL, USA

Background: Glomerular filtration rate is the gold-standard method for assessment of renal function but is rarely performed in routine toxicity studies. Standard serum biomarkers of renal function are insensitive and become elevated only with significant loss of organ function. Symmetric dimethylarginine (SDMA) is a ubiquitous analyte that is freely filtered by the glomerulus and can be detected in serum. It has shown utility for the detection of renal injury in dogs and cats in clinical veterinary practice, but the

potential utility of SDMA to detect renal injury in preclinical species or toxicity studies has not been thoroughly investigated. **Objective:** We sought to compare the sensitivity of SDMA to standard renal biomarkers to detect acute glomerular toxicity. **Methods:** We utilized a well-characterized glomerular toxicant, puromycin aminonucleoside (PAN), to induce podocyte injury and subsequent proteinuria in young male Sprague-Dawley rats. At the end of 1 or 2 weeks, blood, urine, and kidney tissue were collected for analysis. **Results:** One week following a single 50 mg dose, urea nitrogen, creatinine, and albumin were within reference intervals while SDMA was increased. Glomerular changes in these animals included periodic acid-Schiff positive globules within podocytes, podocyte hypertrophy by light microscopy, and podocyte degeneration with fusion of foot processes by electron microscopy. **Conclusions:** Our data indicate that SDMA may be a useful biomarker for early detection of glomerular toxicities in rats.

Friday, October 30, 2020
2:45 p.m. – 2:50 p.m. CDT

CATHETER-ASSOCIATED LESIONS IN RABBITS: POTENTIAL FOR CONFOUNDING STUDY RESULTS

Erica Carroll, Pamela Blackshear
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To minimize the number of rabbits used in drug safety studies, protocols were designed to collect blood samples from the same animals for clinical pathology to evaluate toxicity and toxicokinetics (TK), to measure drug levels over time. TK requires multiple blood draws during a 24-hour period. Use of indwelling catheters is considered more humane and effective to obtain samples than multiple venipunctures. However, it has been reported, that catheterized rabbits have developed lesions resembling infarcts, but thrombi were not observed. In this study, rabbits designated for toxicity evaluation were also used for TK blood draws, using a catheter in the medial auricular (ear) artery. The test article was delivered by dermal patch for 5 minutes/day for 28 days with a 14-day Recovery period. For TK analysis, 5 blood samples were drawn twice, one month apart, during a two-hour period. Findings unassociated with the test article and attributed to the presence of the catheter included degeneration/necrosis of the mandibular salivary gland (13 rabbits, including two controls), degeneration of the Harderian (paraocular) gland (13 rabbits, including 4 controls) and neuron necrosis in the brain in 4 females, including one control. One treated female had a thrombus adjacent to an infarct in the brain and one treated female had a pulmonary arterial thrombus, which supported the hypothesis that infarcts developed subsequent to catheter-induced microthrombotic emboli. Recommendations for studies in rabbits include use of separate groups for toxicity and TK evaluation, enabling humane catheter use without creating potentially confounding lesions in toxicity studies.

Friday, October 30, 2020
2:50 p.m. – 2:55 p.m. CDT

DISORDERS OF THE NERVOUS SYSTEM IN NEW WORLD CAMELIDS (GENERA LAMA, VICUGNA)

Chloe Goodwin[1], Daniel Rissi[1,2]
[1]Department of Pathology, University of Georgia, Athens, GA, USA, [2]Athens Veterinary Diagnostic Laboratory, University of Georgia, Athens, GA, USA

Background: New World camelids (NWC; llamas and alpacas) are popular in the United States. Neurologic diseases are commonly diagnosed in NWC in the southeastern United States. **Objective:** To characterize the neurologic diseases of NWC at the AVDL. **Methods:** The autopsy records of NWC examined from 2008 to 2020 were reviewed for cases of neurologic disease in llamas and alpacas. **Results:** A total of 67 cases (50 alpacas and 17 llamas) with neurologic disease were found. Females (38/67) and males (29/67) with ages ranging between 4 days to 15 years (mean age = 6 years) were affected. Inflammatory diseases occurred in 33/67 cases; infectious organisms were identified in 14/33 cases, including *Parelaphostrongylus tenuis* (5/14), *Listeria monocytogenes* (2/14), *Fusobacterium necrophorum*, unidentified mixed bacteria, *Cladophilophora bantianum*, rabies virus, Eastern equine encephalitis virus, suspect *Blastomyces* spp., and protozoal cysts (1/14 each). Lesions characterized by necrosis, scattered axonal degeneration, lymphocytic infiltration, hemosiderin-laden macrophages, and glial scars were observed in 27/67 cases. While these lesions were attributed to *P. tenuis* infection, no intralesional nematodes were detected and the diagnosis could not be confirmed. Necrosis and/or axonal degeneration with no apparent cause was found in 7/67 cases. Axonal degeneration due to intervertebral disc disease was diagnosed in 2/67 cases, and hepatic encephalopathy due to severe liver dysfunction was found in 1/67 cases. **Conclusions:** As highlighted by our study, *P. tenuis* is an important cause of neurologic disease in NWC, but other infectious diseases, including rabies, should also be considered.

Friday, October 30, 2020
2:55 p.m. – 3:00 p.m. CDT

VACCINE BREAKS: A RETROSPECTIVE STUDY OF CANINE DISTEMPER IN VACCINATED DOGS.

Arturo Oliver-Guimera, Kevin Keel
Pathology, Microbiology & Immunology. School of Veterinary Medicine. University of California, Davis, Davis, CA, USA

Background. The general perception is that canine distemper vaccines are effective at protecting dogs and most cases of distemper in the US are attributed to inadequate or incomplete vaccination. However, newly reported genotypes have been associated with largely anecdotal reports of distemper in vaccinated animals, raising concerns that vaccines might be ineffective against them. We assessed the historical occurrence of distemper in vaccinated dogs to better understand the likelihood of vaccine failure. Objective & Methods. We analyzed the archives of the Veterinary Teaching Hospital of the University of California, Davis to investigate the risk factors associated with perceived vaccine failure in dogs affected by canine distemper from 1980-2020.

Results. A diagnosis of distemper was confirmed in 43 vaccinated dogs , with no associated risk factors. There was no gender bias, but many were young (46% < 6 months of age). The most common diagnostic methods used were histopathology and IFA (48,84% each one), followed by PCR (30.23%). Histopathology alone was used in only 5 cases (11.63%). Among those on which vaccinated date was provided, only in 31.82% the disease onset could have taken place before an immune response was formed after vaccination (less than 2 weeks). The most common presentation were neurological (65.12%) and respiratory (60.47%). Single system presentation of the disease was more common (58.14%) than multisystemic. Conclusions. Vaccine breaks are not as uncommon as we might think and the weak evidence of risk factors in this group suggests the need to study the effectiveness of current vaccination products.

ACVP, ASVCP and ISACP Virtual Poster Session

Clinical Pathology Poster Session

C-01: RETROSPECTIVE EVALUATION OF THE EFFECT OF CEREBROSPINAL FLUID TOTAL MICROPROTEIN LEVELS ON SURVIVAL IN DOGS AND CATS DIAGNOSED WITH ALBUMINOCYTOLOGICAL DISSOCIATION

Alexandra Draper[1], Laura Marshall[2], Timothy Williams[1], Joy Archer[1], Lisa Alves[1]
[1]Queen's Veterinary School Hospital, Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom, [2]Millenium Veterinary Practice, Braintree, United Kingdom

Background: Albuminocytological dissociation (ACD), increased cerebrospinal fluid total protein (CSF-TP) without a pleocytosis, is identified in dogs and cats with different neurological diseases. However, the association between survival and increased CSF-TP is unknown. **Objectives:** 1) Identify conditions commonly associated with ACD in dogs and cats, 2) investigate if higher CSF-TP concentrations, or other relevant factors, are associated with one-year survival. **Methods:** Retrospective study identifying dogs and cats with ACD (Cisternal: CSF-TP >0.30g/l, Lumbar: >0.45g/l with TNCC and RBC within RI) from 2008-2019; recording signalment, weight, TPR, inflammation, neuroanatomical localization, CSF-TP, sampling-site, final diagnosis, aetiological classification and one-year survival. Corrected CSF-TP was calculated as CSF-TP minus 0.3 (cisternal) or 0.45 (lumbar). Descriptive statistics were produced, CSF-TP differences between groups (e.g. neuroanatomical localizations) were evaluated using Mann-Whitney U test or one-way ANOVA (post-hoc testing), and Cox proportional hazards model used for survival data. Statistical significance was $p<0.05$. **Results:** Forty-five animals had ACD, associated with 20 conditions; neoplasia (9) and intervertebral disc disease (5) (IVDD) the most common. There was no significant difference between the CSF-TP (or corrected CSF-TP) of 1-year survivors and non-survivors, nor between different neuroanatomical localizations or aetiological classifications ($p>0.05$). Neoplasia, when controlling for age, was the only covariate associated with a worse survival (HR: 6.04 (95% CI: 1.45-25.1) $p=0.013$). CSF-TP wasn't associated with age ($p>0.05$). **Conclusions:** ACD in dogs and cats is associated with a wide range of conditions, most commonly neoplasia and IVDD. Higher CSF-TP levels do not correlate with a worse 1-year survival, however neoplastic lesions do.

C-02: PREVALENCE OF FELINE BLOOD TYPES FROM CENTRAL AND SOUTHERN CHILE

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Background: The AB system is the predominant blood typing system for cats. It comprises blood types A, B or AB. Ann additional antigen that causes hemolytic reactions is known as the *Mik* antigen. Blood typing of cats has been performed in various parts of the world and the prevalence can vary according the geographic region. **Objective:** The aim of this study was to determine the prevalence of blood types in cats of central and southern of Chile. **Methods:** Expression of blood types was determined in a total of 176 cats (171 domestic and 5 purebred cats) of both sexes and various ages. For each animal, 1 mL of blood obtained from the jugular or saphenous vein was collected into EDTA tubes and processed immediately in the Veterinary Blood Bank. Blood types expression was determined using an immunochromatographic cartridge (Quick Test A+B, Alvedia, Lyon, France) according to the manufacturer's instructions. Prevalence were expressed in percentage. **Results:** The prevalence of blood types in the studied population was: 90.3% (159/176) type A, 2.8% (5/176) type B and 6.8% (12/176) type AB. **Conclusion:** Our results indicate a high prevalence of type A cats in central and southern of Chile, consistent with what has been reported the worldwide, followed by a low frequency of type B and AB bloods groups, as reported before in cats from Europe countries. This is the first study to provide information regarding blood type prevalence in Chilean cats.

C-03: HEMATOLOGICAL AND SERUM BIOCHEMICAL VARIABLES IN LAMBS NATURALLY INFECTED BY GASTROINTESTINAL NEMATODES IN AN INTEGRATED CROP-LIVESTOCK SYSTEM

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Background: Brazilian sheep populations have grown in recent years and gastrointestinal (GI) nematode infections are the major sanitary problem in sheep production. **Objective:** The aim of this study was to evaluate natural infection by GI nematodes of lambs in an integrated crop-livestock system (ICLS), by means of coproparasitological analysis and a panel of hematological and serum biochemical analytes. **Methods:** Corriedale lambs were divided into three groups (G) of ten animals each, defined according to the eggs per gram of feces (EPG) and packed cell volume (PCV) obtained after the first sampling. Stool samples (tested for EPG and coproculture) and blood (complete blood count and serum biochemical analytes) were collected at four different time points (M) during the 70-day experimental period. *Haemonchus*, *Trichostrongylus*, and *Cooperia* genera were recovered in coprocultures. **Results:** There were differences ($P<0.05$) for EPG between the three groups at M1 and

M2. PCV was significantly decreased (P<0.05) for G1 in M1 and G2 in M2. There was no significant difference in total leukocyte count or MCHC between the different time points. Triglycerides, cholesterol, HDL-cholesterol, total calcium, and magnesium concentrations, and lipase activity were not significantly different between groups. **Conclusions:** Hematological and serum biochemical biomarkers were useful for monitoring GI nematode natural infection in lambs when associated with EPG in ICLS with supplementation, confirming no change in overall health status. These findings could provide a prophylactic alternative to controlling worms in the production of sheep in pastures, especially in an endemic area for these nematodes.

C-04: IN VITRO EFFECTS OF DALTEPARIN AND THROMBOMODULIN ON THROMBIN GENERATION IN PLASMA FROM HEALTHY DOGS AND DOGS WITH HYPERCOAGULABLE DISEASES

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Background: Currently available coagulation testing in veterinary medicine fails to reliably and consistently identify a hypercoagulable state. **Objective:** To compare the *in vitro* effects of thrombomodulin and dalteparin on thrombin generation in healthy dogs and dogs presented for diseases associated with a hypercoagulable state. **Methods:** 10 healthy dogs (control group) and 10 sick dogs considered hypercoagulable (diseased group) were selected based on history, physical examination, complete blood work and hemostatic testing. Platelet-poor plasma was obtained from citrated whole blood, aliquoted and frozen at -80˚Celsius for less than 12 months. Thrombin generation was measured on thawed plasma using the Calibrated Automated Thrombogram assay. Measurements were performed without an anticoagulant and with the addition of thrombomodulin and dalteparin at three dilutions (0.2; 0.4; 0.6 U/ml). Endogenous thrombin potential (ETP), lag time (lag), time to peak (TTPeak) and peak thrombin (Peak) were recorded and analysed using a mixed linear model ($p < 0.05$). **Results:** The addition of thrombomodulin and dalteparin significantly decreased ETP and peak thrombin generation in both groups. However, the effects of thrombomodulin and dalteparin were significantly less in the diseased group compared to the healthy group, except for peak at the highest dalteparin concentrations (0.4 U/ml and 0.6 U/ml). **Conclusion:** The addition of thrombomodulin highlights a protein C pathway deficiency in hypercoagulable dogs, by failing to reduce thrombin generation to the same extent as in healthy dogs. Similarly, the addition of dalteparin to the test highlights a decreased sensitivity of hypercoagulable dogs to an external anticoagulant when compared to healthy dogs.

C-05: RETICULOCYTE COUNTS AND HEMOGLOBIN LEVELS IN OBESE CATS

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Background: Aggregate reticulocyte counts (ARC) are used to evaluate the regenerative response in anemic cats. However, ARC may also be increased in non-

anemic cats with several pathologies, and in a low proportion of clinically healthy cats. Obesity is a growing problem in humans and cats, and it predisposes to several comorbidities. Hematologic alterations have been described in obese humans. **Objective:** To evaluate changes in ARC and red cell mass in obese cats. **Methods:** Seventy-five cats were recruited and divided in two groups according to their Body Condition Score (BCS), in a 9-point scale: “lean”, with BCS 4 or 5, and “obese”, with BCS 7 to 9. Health status was assessed by clinical history, physical examination and laboratory evaluation. A t-test was used to compare ARC, hemoglobin, hematocrit and red blood cell count (RBC) between the groups. A Spearman’s correlation test was used to investigate associations between these variables and the age. **Results:** Obese cats had a median ARC of 46,260 reticulocytes/µL, which was significantly higher than their lean counterparts, with a median of 18,220 reticulocytes/µL (p-value = 0.01). Hemoglobin was significantly higher in obese cats (means of 142.0 g/L versus 134.7 g/L; p-value = 0.04). Age was not correlated with ARC, hemoglobin, hematocrit or RBC in our cohort. **Conclusions:** This is, to our knowledge, the first study reporting an association between feline obesity and increased ARC. The median ARC in obese cats was high enough to potentially affect interpretation of results. Higher hemoglobin concentrations have been described in obese humans.

C-08: CHOLINE TOXICOSIS RESULTING IN A POSITIVE ETHYLENE GLYCOL TEST

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Background: FDA’s Center for Veterinary Medicine (CVM), Veterinary Laboratory Investigation and Response Network (Vet-LIRN) initiated an investigation into adverse event reports involving cats with gastrointestinal and neurologic signs that shared a common exposure to a single brand of canned cat food. One of sixteen reports involved a household of four cats that tested positive by an in-house commercial ethylene glycol (EG) test. The food manufacturer identified the cause as an isolated incident of excess choline addition; the product was voluntarily recalled. **Objective:** To investigate the positive EG test by testing brand product and to determine if adverse events were related. **Methods:** Vet-LIRN investigation involved medical record review, exposure interviews, and product testing for choline, choline chloride, EG, diethylene glycol, and propylene glycol. **Results:** Two male and two female cats (1.7 to 5 years old) presented with vomiting (n = 2) and wobbling (n =2). Serum chemistry findings included a normal BUN for all cats and hyperglycemia [258 (74-159 mg/dL)] and increased ALT [191 (12-130 U/L)] for one cat. The cats had no known EG exposure. Product testing showed choline and choline chloride content of 165-925 ppm on a dry matter basis (DMB) and 222,593 ppm DMB (American Association of Feed Control Officials upper limit- 2,400 ppm DMB), respectively. Glycols were below the detection limit. **Conclusions:** In-house commercial EG tests may cross-react with glycerol and glycols. EG was not detected in the food leading to a hypothesis that metabolism of choline into glycerin/glycols led to the positive EG results.

C-09: RAPID DIAGNOSIS OF EQUINE PIROPLASMOSIS BY FLOW CYTOMETRY-BASED METHOD: A PRELIMINARY STUSY

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Background and Objectives: Equine piroplasmosis (EP) is a tick-borne protozoal disease of equids, caused by *Theileria equi* and *Babesia caballi*. Infected horses present various signs including anemia, because the protozoa parasitizes red blood cells (RBCs). EP is not currently found in Japan or the United States but is endemic in tropical and subtropical regions. The growth in international horse trading is thought to increase the potential risk for EP outbreaks in non-endemic countries. Moreover, infected horses are temporarily permitted to compete in international equestrian events, such as the Olympics. The diagnosis of EP is currently performed by microscopic, molecular, and serological methods. Each of these techniques has its advantages and disadvantages, such as specificity, sensitivity, simplicity, and cost. In this study, we evaluated the utility of a flow cytometry–based method (FCM) for the rapid diagnosis of EP. **Methods:** *Babesia caballi* (USDA strain) and *T. equi* (USDA strain) were cultured *in vitro*. The numbers and ratios of infected RBCs (iRBCs) were counted by FCM and by microscopy methods. **Results:** FCM could detect iRBCs in approximately 1 minute. The minimum detection numbers were 176 iRBCs/μL for *B. caballi* and 184 iRBC/μL for *T. equi*. Results obtained by FCM correlated highly with those obtained by microscopy ($R^2 > 0.9$). **Conclusion:** FCM could recognize and count iRBCs, and it reported the infection ratio in approximately 1 minute. These findings indicate that FCM would be useful for EP diagnosis, especially for the monitoring of infected horses and for screening tests.

C-10: DIAGNOSIS OF RENAL LYMPHOMA BY WRIGHT-GIEMSA STAINED CYTOCENTRIFUGED URINE EVALUATION IN A CAT

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Background: Lymphoma represents up to 30% of neoplasms diagnosed in feline patients. Diagnosis of lymphoma in the urinary system by urine sediment examination has been described in a dog, but not previously in cats. **Objective:** Cytologic description of neoplastic lymphoid cells identified by Wright-Giemsa stained cytocentrifuged urine preparation in a cat. **Methods:** Concurrent serum, EDTA whole blood and urine samples were submitted from a 15-year-old spayed female domestic short hair cat exhibiting weight loss, polyuria and polydipsia. **Results:** Hematology findings included a mild normocytic, normochromic non-regenerative anemia and an inflammatory leukogram with a stress component. Chemistry abnormalities included a marked azotemia, moderate hyperphosphatemia and moderate hypermagnesemia. Concurrent urinalysis evaluation revealed inadequate urine concentration and moderate proteinuria. Wet-mount urine sediment examination revealed moderate numbers of leukocytes and erythrocytes. A uniform population of intermediate to large lymphocytes was observed on a fresh, Wright-Giemsa stained cytocentrifuged urine preparation.

These cells had small to moderate volumes of blue cytoplasm that occasionally contained clear vacuoles and fine azurophilic granules. Nuclei were ovoid to occasionally indented, with fine stippled chromatin and occasional distinct ovoid nucleoli. The patient was euthanized and abnormalities were restricted to the kidneys at necropsy. Bilateral renomegaly characterized by multifocal, pale-yellow, coalescing, poorly defined, homogenous nodules was identified. Microscopically, these nodules were composed of dense sheets of CD3 positive round cells, consistent with T cell renal lymphoma. **Conclusion:** This case represents an uncommon method of neoplastic lymphocyte identification and emphasizes the importance of Romanowsky-stained urine sediment examination in veterinary patients.

C-11: IMPACT OF TUMOR HETEROGENEITY ON THE CYTOLOGIC GRADING OF CANINE CUTANEOUS MAST CELL TUMORS

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Background: Histologic grade is an important prognostic tool in the assessment of canine cutaneous mast cell tumors (cCMCTs). Fine needle aspirate cytologic (FNAC) grade has been suggested as a sensitive and specific surrogate to histologic grade. However, its incorporation into routine clinical practice lacks universal acceptance due, in part, to concerns regarding its reproducibility and the uncertain impact of grade heterogeneity. **Objective:** To evaluate the influence of grade heterogeneity on the diagnostic accuracy, reproducibility, and clinical utility of grading cCMCTs using FNAC. **Methods:** Aspirates were collected from multiple locations within cCMCTs. Cytologic grade was assigned by three evaluators based on a previously developed grading system. Intratumoral grade heterogeneity was evaluated by assigning cytologic grade at multiple locations within each tumor. Overall tumor grade was based on the highest assigned grade. Fifteen cCMCTs were evaluated. Cytologic grade could be determined in 14 tumors. The grading system was 85.7% accurate for predicting histologic grade, overestimating tumor grade in 14% of cases. Agreement among pathologists was 98%. Within a single tumor, two evaluators assigned one site as high grade while the third investigator labeled the site low grade. Assessment of another site in the same tumor resulted in a consensus of low grade, indicating intratumoral grade heterogeneity. Tumor heterogeneity affected ultimate cytologic grade in 7% of cases, resulting in overestimation of final cytologic grade when compared to histologic grade. **Conclusions:** Cytologic grading of cCMCTs is a useful predictor of histologic grade. Intratumoral grade heterogeneity, while uncommon, may affect final cytologic grade.

C-12: QIMMEQ HEALTH: REFERENCE INTERVALS AND EFFECT OF SEX, SEASON AND MANAGEMENT ON THYROID HORMONES IN 144 GREENLANDIC SLED DOGS

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Background: Greenlandic sled dogs (GSDs) are a unique, genetically isolated population of dogs. GSDs have marked variation in activity depending on season; they are either inactive during summer or performing exercise of high intensity the remaining

part of the year. Metabolism, and thereby thyroid hormones, are affected by exercise and extreme environmental conditions. A breed-specific reference interval (RI) can be useful for diagnostics of potential thyroid-related pathologies. **Objective:** To determine RIs for thyroid stimulating hormone (TSH), thyroxine (T4) and free thyroxine (fT4) and evaluate the effect of sex, season and management in a population of GSDs. **Methods:** Physical exams and cephalic venous blood sampling were performed in summer and winter 2018-2019 in 265 adult GSDs managed privately or by the Danish navy. Serum biochemical analyses, including CRP, were performed. Using ASVCP guidelines, RIs were determined for TSH, T4 and fT4 (Immulite 2000, Siemens). Effects of sex, season and management were evaluated via Mann-Whitney test. **Results:** 144 GSDs were defined as healthy based on physical exam and biochemistry data. RIs were: TSH 0.04-0.55 ng/mL; T4 6.44-48.65 nmol/L; fT4 3.91-18.51 pmol/L. Female GSDs had significantly higher concentrations of T4 ($p=0.039$) and fT4 ($p=0.015$) compared to males. T4 concentrations were significantly higher ($p=0.003$) during summer compared to winter. TSH concentrations were lower in GSDs managed by the navy ($p<0.0001$) compared to privately managed dogs. **Conclusions:** RIs for TSH, T4 and fT4 were established in GSDs. The lower limits of the RIs for T4 and fT4 were lower than corresponding limits of existing RIs.

C-13: ACUTE PHASE PROTEINS ITIH4 AND HAPTOGLOBIN AS MARKERS FOR METRITIS IN DAIRY COWS

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Introduction: Metritis is a frequent cattle disease that compromises reproductive performance causing economical losses. During labor, the membranes of the female reproductive system are exposed to external milieu flattering bacterial colonization. Approximately 10-20% of cows are not capable of overcoming this infection spontaneously and develop the disease. Acute-phase proteins (APPs) are synthesized by the liver in response to proinflammatory cytokines during the onset of immune response and provide diagnostic and prognostic information of diseases. The objective of this study was to evaluate levels of the APPs ITIH4 and Haptoglobin (Hp) in cows with metritis of different severity during the peripartum period. **Materials and Methods:** A total of 139 Holstein dairy cows were monitored during the periparturient period and examined for the occurrence of metritis symptoms after parturition and their severity (mild, moderate and severe metritis). Blood samples were collected at 10 and 5 days pre-partum and at 1, 3, and 10 days post-partum. Species-specific immunoturbidimetric assays (Acuvet Biotech, Spain) were used for APP analysis. Data were analyzed using ANOVA in SPSS software. **Results:** Serum Hp and ITIH4 levels were low in prepartum cows. A significant increase of both proteins was observed after calving ($p<0.001$ and $p<0.05$, respectively). Cows with metritis showed increased Hp and ITIH4 levels versus healthy cows at three ($p<0.05$) and 10 days after calving ($p<0.001$). Magnitude of increase was correlated to disease severity. **Conclusions:** Hp and ITIH4 serum levels are useful biomarkers for diagnosis and monitoring bovine metritis during the onset of the disease.

C-14: PROTEIN BIOMARKERS FOR EARLY DIAGNOSIS OF CANINE HIP DYSPLASIA

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Background: Canine hip dysplasia (CHD) is one of the most commonly diagnosed orthopedic disorders in dogs. According to the Orthopedic Foundation for Animals (OFA) database, the prevalence of CHD can be as high as 70% in certain breeds. Dysplastic dogs are often diagnosed in the irreversible phase (i.e., presence of osteoarthritis), which limits effective preventative and joint preservation strategies. Proteins in bodily fluids have been used as biomarkers for early diagnosis of several other disorders, providing potential for disease prevention and improving efficacy of treatment. **Objective:** To identify protein biomarkers in serum and urine that differentiate dysplastic from normal dogs at an early age (i.e., prior to skeletal maturity). **Methods:** Whole blood and urine were collected from 14 client-owned dogs (8M, 6F) at 8 time points (3 to 24 months of age), and 7 biomarkers reflecting direct and indirect measures of joint health were measured. Radiographs were evaluated for joint pathology (hip dysplasia at 2 years: 3M, 1F) based on OFA grading criteria. Mann-Whitney U-test and single variable logistic regression analysis were performed with significance set at $p<0.05$. **Results/Conclusions:** At 5 months of age, male dogs showed marked differences in concentrations of targeted urine biomarkers between dysplastic and non-dysplastic hip cohorts such that a biomarker panel has significant predictive capabilities. Females exhibited a large variability in biomarker concentrations. Urine biomarkers have the potential to provide early, accurate diagnosis of CHD in male dogs. Ongoing analyses from a larger subset of puppies aim to validate this panel for clinical use.

C-15: A COMPARISON STUDY BETWEEN THE SIEMENS ADVIA 120 AND THE MANUAL METHOD FOR THE DIFFERENTIAL LEUKOCYTE COUNT IN SHEEP

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Background: While the ADVIA 120 is a widely-used hematology analyzer, it has not been previously validated for determining the differential leukocyte count in sheep. **Objectives:** To compare the differential leukocyte counts provided by the ADVIA 120 (A-diff) and the manual method (M-Diff) in sheep. **Methods:** EDTA-anticoagulated blood samples that were analyzed within 4h of collection were used in this study. Samples with inappropriately filled tubes, detectable clots and overtly erroneous ADVIA peroxidase cytograms were excluded. The A-Diff was compared to the M-Diff performed by two independent observers by counting 200 cells on Giemsa-stained blood smears. **Results:** Eighty-eight samples (44 rams, 44 ewes) were included. The extremely low basophil percentages did not allow for a meaningful method comparison for this leukocyte type. The correlation between the A-Diff and M-Diff was high for neutrophils

(r=0.873, P<0.001), lymphocytes (r=0.863, P<0.001), and eosinophils (r=0.750, P<0.001), and low for monocytes (r=0.212, P=0.048). The Passing-Bablok regression analyses revealed a constant error for eosinophils (1.17%; 95% confidence interval [CI]: 0.67%, 1.55%) and a proportional error for lymphocytes (0.84; 95% CI: 0.74, 0.95) and eosinophils (0.85; 95% CI: 0.74, 0.96). The Bland-Altman analyses revealed negative biases of 2.4% and 3.0% for neutrophils and lymphocytes, respectively, and positive biases of 3.2% and 0.8% for monocytes and eosinophils, respectively. **Conclusions:** The ADVIA 120 appears to perform generally well for determining the differential leukocyte count in sheep as compared to the manual method. However, any abnormal differential leukocyte count results should be confirmed by blood smear examination.

C-16: PRELIMINARY EVALUATION OF RENAL BIOMARKERS AS EARLY INDICATORS OF TREATMENT RESPONSE IN DOGS WITH LEISHMANIASIS UNDERGOING TREATMENT WITH MILTEFOSIN

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Background: Recent renal biomarkers like serum Cystatin C (sCysC), urinary Cystatin C/creatine ratio (uCysC/CR), urine N-acetyl-β-D-glucosaminidase/creatine ratio (uNAG/CR) and serum symmetric dimethylarginine (SDMA) may improve monitoring in dogs with chronic kidney disease secondary to Canine Leishmaniasis (CanL). **Objective:** To evaluate the effect of treatment with miltefosin on these renal biomarkers in dogs with CanL. **Methods:** Six dogs diagnosed with stage 3 and 4 CanL were analyzed at the beginning of treatment (day 1) and 28 days later. Cystatin C was measured in serum and urine by turbidimetric latex assay (Spinreact, Spain) and is expressed as uCysC/CR. NAG was determined by commercial kit (Diazyme®, Germany) and is expressed as uNAG/CR. SDMA was analyzed by commercial kit (IDEXX®, E.E.U.U.). A paired t-Test or a Wilcoxon Signed Rank Test (for those variables not meeting the paired t-Test assumptions) were used for comparisons between initial and post treatment values. A p value of <0.05 was considered statistically significant. **Results:** sCysC was 0.34±0.09mg/L(day 1) and 0.28±0.05mg/L(day 28); uCysC/CR was 4114.66±5964.30ug/g(day 1) and 1024.68±1165.58ug/g(day 28); uNAG/CR were 59.25±80.22UI/g(day 1) and 21.65±4.68UI/g(day 28) and SDMA was 19.33±8.77ug/dl(day1) and 15.00±7.07ug/dl(day28). A statistical difference (P<0.031) was observed between the pre- and post-treatment results for uCysC/CR but not for the other renal markers studied. **Conclusions:** uCysC/CR robustly decreases following treatment with miltefosin in dogs with advanced CanL. This indicates improvement of tubular function and makes this biomarker a good candidate to be explored in future studies as a tool for therapy guidance.

C-18: SAMPLE STABILITY AND HEPARIN INTERFERENCE USING A STAT PROFILE PRIME PLUS® VET CRITICAL CARE ANALYZER TO QUANTIFY IONIZED CALCIUM (ICA) AND IONIZED MAGNESIUM (IMG)

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Introduction: Validated methodologies are needed for the rapid measurement of iCa and iMg, which can be clinically useful in assessing medical disorders in horses. **Objective:** To evaluate stability of iCa, iMg, and other variables, and heparin interference in samples analyzed on the Stat Profile Prime Plus analyzer. **Methods:** Whole blood, plasma, and serum samples from ten horses were stored at 4°C and analyzed after 1,2,3,4,5,6,7,8,24,48 and 168 hours. ANOVA or mixed-effect model tests were performed. **Results:** All parameters had high stability up to 48h with low variability, except for K in whole blood ($p<0.001$). iCa and iMg decreased slightly during the first 8 hours. At 168h, slight differences were found in plasma Na and Cl, whereas tCa, tMg, iCa and iMg were stable. Na, Cl and iCa were higher in serum than in plasma or blood ($p<0.001$). Total Ca and iMg were similar in all sample types. Glucose and pH were higher in plasma and serum than in whole blood; lactate was lower in plasma. Glucose and pH decreased significantly and lactate increased significantly in whole blood at 168h; no variation was observed in plasma or serum. High stability was observed for pCO2, pO2, Hb, O2Hb, COHb, MetHb, SO2 and tBil. Interference by high heparin concentration was detected when comparing homemade syringes (~200-300U heparin/mL) and Vacutainers (20-30U/mL). **Conclusions:** Samples stored at 4°C can be used to determine electrolytes up to 8 hours after collection. Heparin interference should be taken into account if using homemade heparin syringes instead of Vacutainers.

C-19: FIRST MOLECULAR IDENTIFICATION OF BABESIA VOGELI IN DOGS FROM LIMA, PERU

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Background: Canine babesiosis is a vector-borne infectious disease caused by microorganisms from the genus *Babesia* spp. (*B. canis*, *B. vogeli*, *B. gibsoni* and *Babesia microti*-like) and transmitted by ticks. Last year, we received some cases with piroplasms in erythrocytes from dogs associated with anemia and thrombocytopenia. All the cases were related to Rhipicephalus sanguineus (brown dog tick) infestation without tick control. **Objectives:** Identify the species of *Babesia* using hematological and molecular techniques in dogs with a history of current tick infestation from Lima, Peru.

Methods: Peripheral and capillary blood samples were collected from 51 dogs with a history of current tick infestation. For hematological analysis, direct detection of hemoparasites (intraerythrocytic pyriform merozoites) was done for each animal using blood (peripheral and capillary) and buffy coat smears, the latter obtained after centrifugation of peripheral blood (12000 rpm/min x 5 minutes) and staining with Wright's stain. For molecular analysis, the 16S rRNA gene was amplified using Multiplex PCR to differentiate two species, *B. vogeli* (245 bp) and *B. gibsoni* (454 bp). Finally, all the negative samples were analyzed by specific 18S rDNA gene detection for *B. canis*, and all remained negative. **Results:** From fifty-one dogs tested, ten (10/51) were positive for *Babesia* spp. (19.6%) using hematological techniques. Using multiplex PCR, 13 dogs (13/51) were positive (25.4%) for *B. vogeli*. Three negative dogs by direct detection were positive for *B. vogeli* (7.3%) by PCR. **Conclusions:** This study confirms the presence of canine babesiosis caused by *Babesia vogeli* in Lima, Peru.

C-20: IMMUNE-MEDIATED ANEMIA AND ACUTE LIVER INJURY DUE TO LEPTOSPIROSIS IN A FERRET

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Background: Leptospirosis is a poorly described disease in ferrets. **Objective:** Description of the clinicopathological and pathological features of acute leptospirosis in a ferret. **Methods:** A 5-month-old spayed female ferret, was presented to a private hospital for generalized jaundice, depression and dehydration, samples for CBC and clinical biochemistry was submitted to a private laboratory. The ferret died shortly after. **Results:** CBC revealed regenerative anemia (0.26 L/L), with spherocytosis and discrete auto-agglutination indicative of immune-mediated hemolytic anemia, as well as neutrophilia and monocytosis due to inflammation. Serum biochemistry showed marked hyperbilirubinemia (910 mmol/L) with similar concentrations of conjugated and unconjugated bilirubin, hypocholesterolemia due to possible acute liver failure; increased ALT (1449 U/L) and AST (4900 U/L) indicative of severe hepatocellular damage, increased GGT (100 U/L) due to cholestasis; increased urea and hyperamylasemia suggestive of decrease glomerular filtration rate secondary to dehydration or acute renal failure. Creatinine could not be detected because of analytical interference from icterus. Microagglutination testing for leptospirosis was positive for Canicola (1:400) and Icterohaemorragiae (1:200) serovars. Leptospiremia was confirmed by iiPCR (*Leptospira lipL32* GeneReach, Taiwan). Postmortem examination showed severe multifocal vacuolar necrosis, severe periportal fibrosis and moderate bile duct hyperplasia. Acute tubular necrosis was seen in the kidney; and hemosiderosis was evident in the liver, spleen, kidneys and lymph nodes. Warthin-Starry staining did not reveal the presence of spirochetes in target organ tissues for *Leptospira*. **Conclusion:** Leptospirosis is not a common disease in ferrets, however, this report describes the acute presentation of the disease, in a susceptible individual.

C-21: CAPILLARY ZONE ELECTROPHORESIS OF AFRICAN GREY PARROT (PSITTACUS ERITHACUS) PLASMA

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Background: Capillary zone electrophoresis (CZE) is becoming increasingly used in human clinical pathology laboratories and, to a lesser degree, in veterinary laboratories. Previous studies have demonstrated increased resolution and lower CV over agarose gel electrophoresis (AGE) in mammalian species and limited exotic species. **Objective:** Validate a CZE method for the quantitation of protein fractions in plasma from the African grey parrot. **Results:** Using manufacturer protocols (Sebia Capillarys 2 Flex Piercing, Norcross, GA), protein fractionation was found to be significantly different from AGE with extended migration between prealbumin and albumin peaks and poor resolution of globulins. Subsequently, the protein buffer was replaced with that used in the Capillarys Urine kit; this buffer aids in sample migration by placing anodic and cathodic stop points. The resultant electrophoretogram resembled that observed in AGE inclusive of prealbumin, albumin, alpha, beta, and gamma globulins. CZE also allowed for the definition of two beta and two gamma globulin fractions. Inter- and intra-day CV analysis ranged from 0.9-5.4% with the exception of higher values for alpha globulins (7.9-17.9) due to the low concentration of these globulins in normal parrots. Significant differences were present in the all fraction values except the beta and gamma globulins versus AGE. Notably, the A/G ratio was higher by CZE versus AGE: 2.4 ± 0.3 and 2.0 ± 0.2, respectively. **Conclusions:** The impact of the increased resolution provided by CZE needs to be assessed by further studies. CZE and AGE are not equivalent methods, which necessitates the calculation of new reference intervals.

C-22: VALIDATION OF VETBIO-1 POINT OF CARE ANALYZER FOR THE DETERMINATION OF CRP LEVELS IN RABBITS

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Background: Acute phase proteins (APP) are valuable markers of inflammation used in health assessments and prognostication; C-reactive protein (CRP) is a major APP in the rabbit. **Objective:** The goal of this study was to validate the use of a point of care analyzer. **Methods:** Sixty samples were examined using the VetBio-1 rabbit CRP assay (Veterinary Biomarkers, West Chester, PA) and a previously described human immunoturbidimetric assay (Randox, Kearneysville, West Virginia). The VetBio-1 assay uses rabbit CRP specific antibodies and is calibrated to pure rabbit CRP. **Results:** The median (95% CI values) for the Randox method was 20.8 mg/L (12.3-33.9) and for the VetBio-1 was 3.2 mg/L (2.1-8.7). The Pearson's correlation coefficient was 0.93. A proportional error was detected using Passing-Bablok analysis. Bland-Altman analysis showed a bias (SD) of 32.6 (44.3). Inter-day and intra-day CV analysis ranged from 2.8% to 10.6%. The VetBio-1 assay was found to deviate from linearity with a proportional error when stepwise dilutions were examined. RI were calculated using n=30 samples from clinically normal rabbits using the robust method. The preliminary RI

for the VetBio-1 is 0.04-8.52mg/L. Notably, the VetBio-1 correctly identified all normal rabbits. In samples with a mild increase in CRP by immunoturbidimetry, the Vet Bio identified 50% of the samples. In samples with moderate to marked increases in CRP, there was complete agreement between the methods. **Conclusions:** This data indicates that the two methods are not equivalent. Additional studies should be undertaken to examine the clinical application of the VetBio-1 rabbit CRP assay.

C-23: EVALUATION OF PLATELET INDICES AND THEIR ASSOCIATION WITH GLYCEMIC STATUS IN HEALTHY AND DIABETIC DOGS: PRELIMINARY RESULTS

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Background: Altered platelet count (PLT) and function have been reported in diabetic human patients, implying their role in vascular events development. Platelet activation indices, such as mean platelet volume (MPV), have been found increased in diabetics and correlated with their glycemic status; however, platelet indices have not so far been investigated in diabetic dogs. **Objectives:** To investigate the PLT, MPV, and platelet mass (PM) in diabetic dogs compared to healthy controls, and their association with the major fraction of glycated hemoglobin (HbA1c). **Methods:** Anticoagulated (K_3EDTA) blood samples from 33 adult, clinically healthy and 14 diabetic dogs were included. Complete blood counts were performed with the ADVIA 120 within 2h of blood collection, while HbA1c (%) was determined using a previously validated capillary electrophoresis assay (Sebia Capillarys 2 flex-piercing). Platelet mass was calculated as MPV x PLT (fL/nL). **Results:** HbA1c was significantly higher ($P<0.001$) in diabetic (5.35% ± 1.02%) compared to healthy (1.69% ± 0.50%) dogs. Median MPV was not significantly different ($P=0.114$) between diabetic (13.6 fL, 10.1-22.6 fL) and healthy dogs (11.9 fL, 8.6-19.1 fL). Median PLT and PM were significantly higher in diabetic dogs (434.5x10^9/L, 176-987x10^9/L and 5,903 fL/nL, 2,552-12,239 fL/nL, respectively) compared to controls (297x10^9/L, 223-671x10^9/L; $P=0.039$ and 3,495fL/nL, 2,408-8,656 fL/nL; $P=0.001$, respectively). HbA1c was significantly positively correlated with PLT and PM ($r=0.298$, $P=0.042$; $r=0.340$, $P=0.019$, respectively), but no significant correlation was found with MPV ($r=0.199$, $P=0.180$). **Conclusions:** Canine diabetes mellitus is associated with increased PLT and PM, which are correlated with the glycemic status. Our findings might suggest a propensity of diabetic dogs to hypercoagulability similar to humans.

C-24: ALKALINE PHOSPHATASE ACTIVITY AND ISOFORMS IN CANINE AND FELINE EFFUSIONS – A PILOT STUDY

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Background: Bone, liver and corticosteroid-induced ALP isoforms are present in dog serum; liver isoform is present in cat serum. Neoplastic cells can produce atypical ALP

isoforms. **Objective:** We hypothesized that ALP activity and isoforms in canine and feline effusions might reflect the dominant pathogenesis of fluid accumulation. **Methods:** Dog (n=9) and cat (n=7) peritoneal/pleural effusions were analyzed for ALP isoforms using zymography. Results of routine effusion analysis were obtained from the laboratory (Vetlab, Belgrade) database. **Results:** Effusions were classified as modified transudate (MT) (n=4), exudate (n=3), neoplastic (n=4), feline infectious peritonitis (n=3), and chylous (n=1). Two ALP isoforms were detected: type-1 and type-2. In dogs, median ALP activity was higher in MTs (118 U/L, range 61-860 U/L) compared to exudates (22 U/L, 16-116 U/L), and neoplastic effusions (14 U/L, 10-22 U/L) (Kruskall-Wallis, p=0.067); 3 MTs had type-1 isoform and one had both ALP isoforms. Neoplastic effusions had variable patterns: no detectable isoforms (n=2), type-1 isoform (n=1), and both isoforms (n=1). In cats, an exudate with both isoforms had the highest ALP activity, the chylous effusion had type-2 isoform, and FIP effusions had undetectable ALP activity. Overall ALP activity was not correlated with effusion TP concentration or TNCC. **Conclusions:** Different patterns of ALP isoforms in effusions are observed in canine and feline effusions that might indicate underlying pathogenesis. Future studies are needed to determine specific ALP isoforms in effusions that result from known underlying diseases.

C-26: TUMOR-ASSOCIATED TISSUE EOSINOPHILIA IN AN AMELANOTIC MELANOMA IN A DOG

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Background: Tumor-associated tissue eosinophilia has been reported secondary to lymphoma, mast cell tumor, and carcinomas in dogs. Although it has been reported within a melanoma in a mouse model, tumor-associated tissue eosinophilia secondary to a melanoma has not been reported in veterinary literature. **Objective:** To describe tumor-associated tissue eosinophilia secondary to an amelanotic melanoma in a dog. **Methods:** A 12-year-old Standard Schnauzer presented to the WSU Oncology Service for evaluation of a mass on the tongue. The patient presented for coughing, gagging and decreased appetite that were attributed to the mass. Thoracic radiographs were unremarkable and there were no signs of allergies or parasitic disease. Fine needle aspirates of the mass were submitted for review. **Results:** The nucleated cells consisted primarily of round to plump spindeloid cells arranged individually and in tight clusters. These cells had oval, eccentric nuclei with finely stippled chromatin and 1-4 variably sized and shaped nucleoli. They had abundant amounts of basophilic, granular cytoplasm that frequently contained discrete vacuoles. These cells displayed moderate anisocytosis and anisokaryosis, and frequent aberrant mitotic figures were observed. Throughout the sample there was a moderately increased prevalence of eosinophils. There was also a mildly increased prevalence of neutrophils that contained intracellular mixed bacteria. Histopathology of the mass revealed a poorly differentiated malignant neoplasm. The neoplastic cells were positive for both PNL-2 and Melan-A, consistent with a melanocytic tumor, and the mass was diagnosed as a malignant melanoma.

Conclusion: Melanomas should be considered as a possible underlying cause of tumor-associated tissue eosinophilia.

C-27: ACUTE MYELOID LEUKEMIA IN A HORSE

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Background: Acute myeloid leukemia is rarely reported in horses. **Objective:** Description of the cytomorphologic features and immunophenotype of acute myeloid leukemia in a horse. **Methods:** A 4-year-old Quarter Horse presented to the referring veterinarian for lesions on the chest. A CBC was submitted to WSU Veterinary Teaching Hospital. The horse was euthanized shortly after, due to deteriorating quality of life. **Results:** A CBC revealed a leukocytosis (66.3 $x10^3$/microliter) with a mature neutrophilia (19.2 $x10^3$/microliter) and 68% large unclassified cells (45.1 $x10^3$/microliter). There was also a marked macrocytic, hypochromic anemia (PCV = 12%) and thrombocytopenia (23 $x10^3$/microliter). The large unclassified cells consisted of two cytomorphologically distinct populations. The first population had irregularly round, eccentric nuclei with stippled chromatin and 0-4 variably sized nucleoli and abundant basophilic grainy cytoplasm. The other population had amoeboid, reniform, or irregularly oval eccentric nuclei with coarse chromatin and 0-4 nucleoli. They had moderate amounts of lightly basophilic cytoplasm that frequently contained discrete vacuolation or fine magenta granules. Flow cytometry analysis revealed that the large unclassified cells were CD44+ and CD11b+, and negative for lymphoid markers. This is consistent with an acute myeloid leukemia, with preference for myelomonocytic (AML-M4) or monocytic (AML-M5) phenotype. Clinical features are also described. Necropsy with histopathology revealed a disseminated neoplasm in lymph nodes and viscera, composed of sheets of round cells. **Conclusion:** This case describes an acute myeloid leukemia in a young horse, which is rarely reported in the literature. As in previously reported cases, the prognosis for this horse was poor.

C-28: ALTERED PROTHROMBIN TIME (PT), ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT) AND PLATELET COUNT ASSOCIATED WITH DEATH IN HOSPITALIZED DOGS

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Background: The laboratory evaluation of blood coagulation aims to determine the causes and define the intensity of injuries in the hemostatic system. Identifying hemostatic alterations in hospitalized dogs can help make decisions and define the prognosis. **Objective:** The objective of this retrospective study was to evaluate whether the prolongation of APTT or PT and thrombocytopenia are associated with death of hospitalized dogs with several disorders. **Methods:** Medical records between 2014 and 2018 were retrospectively evaluated. A total of 218 hospitalized dogs were selected.

Out of the 218 dogs, 46 died during hospitalization. Selected dogs were classified in 8 groups according to prolonged PT, APTT, low platelet count and a control group with normal results. Fisher's exact test was used to verify association of death with presence of alterations in laboratory tests. The relative risk (RR) was calculated using the equation RR = CIexposed-/-CIunexposed, where CI represents cumulative incidence. **Results:** Dogs with a single alteration (prolonged APTT or PT or thrombocytopenia) did not differ from the control group. Groups of dogs with multiple alterations (thrombocytopenia and prolonged APTT, prolonged APTT and PT, and thrombocytopenia, prolonged APTT and PT) showed 3.06, 5.17 and 4.13 times more risk of death than the control group and a cumulative incidence of death of 29% (p value 0,02), 50% (p value 0,00) and 40% (p value 0,00) respectively. **Conclusion:** Dogs with multiple hemostatic parameter alterations had more chance of death and these alterations should be considered in hospitalized dogs.

C-29: PROGNOSTIC UTILITY OF CYTOMORPHOMETRY IN CENTROBLASTIC LYMPHOMA IN DOGS

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Background: Lymphoma is one of the most common neoplasms in dogs. Centroblastic lymphoma is found in dogs at different ages with predisposition to selected breeds and is the most frequent subtype of lymphoma in this species. The parameters that would permit an estimate of the prognosis in dogs with this cancer are still being sought. **Objective:** The purpose of the study was to evaluate cytomorphometric analysis as a subsidiary tool in estimating prognosis in centroblastic lymphoma in dogs. **Methods:** The material consisted of cytological smears of centroblastic lymphoma collected from dogs during routine cytological diagnostics. The smears were obtained from peripheral lymph nodes by fine-needle aspiration biopsy, stained with Giemsa and the B-immunophenotype was confirmed by immunohistochemistry with anti-CD3 and anti-CD79α antibodies. The dogs were grouped according to the total survival time: ≤6 months, 6-12 months and ≥12 months. Then 60 cytomorphometric measurements of nucleus and cytoplasm were performed. The data were analyzed statistically. **Results:** There were no significant correlations between cytomorphometric measurements and total survival time in dogs and there were no significant differences in cytomorphometric values between 3 groups of dogs with different total survival times. **Conclusions:** Cytomorphometry has no prognostic value in centroblastic lymphoma in dogs.

C-30: CHANGES IN ENERGY METABOLISM OF HEALTHY CATS WITH AGING

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Background: Energy metabolism declines with age in animals. Plasma metabolites and enzymes related to energy metabolism change with aging. **Objective:** The aim of this study was to evaluate changes in biomarkers for energy metabolism with aging in healthy cats. **Methods:** 67 clinically healthy cats were divided into five groups: Newborn

(0 to 1 year), Young (>1 to 5 years), Middle (>5 to 10 years), and Old (>10 to 17 years). Old cats were further divided into obese and thin groups. All cats were diagnosed as clinically healthy, and their body condition scores (BCSs) ranged from 3/9 to 9/9. Changes in concentrations of metabolites, inflammatory markers and hormones and enzyme activities related to energy metabolism were investigated in serum of each group. **Results:** Serum amyloid A (SAA) concentrations in the Old-thin group were significantly increased compared to those in the Young group. In Middle, Old-obese, and Old-thin groups, the malate dehydrogenase/lactate dehydrogenase (M/L) ratio, an energy metabolic indicator, tended to decrease compared to that in the Young group. In male Middle cats, The M/L ratio was significantly lower compared to that in female cats. In healthy cats, BCS and glucose, triglycerides, and SAA concentrations increased and ALB concentration and M/L ratio decreased with aging. **Conclusion:** These changes may indicate that energy metabolism in liver is attenuated with aging and may contribute to obesity with slight inflammation in healthy cats.

C-31: CHANGES IN PLASMA METABOLITE CONCENTRATIONS IN RIDING HORSES SUPPLEMENTED WITH AN ANTI-OXIDANT AND ANTI-INFLAMMATORY COMPOUND

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Background: Supplementation with anti-oxidant and anti-inflammatory compound is effective to increase lipid metabolism in livers of dogs and cats. The anti-oxidant and anti-inflammatory compound may be effective for improvement of hepatic energy metabolism in horses. **Objective:** A plant mixture containing *Rhus vernicius* and other herbs, Rv-PEM01-99, shows anti-oxidant and anti-inflammatory effects. The aim of this study was to evaluate the effects of supplementation with Rv-PEM01-99 in healthy riding horses and a riding horse with arthritis of the shoulders. **Methods:** 80 g/head/day of Rv-PEM01-99 was supplemented to riding horses for 4 weeks. Changes in plasma metabolite concentrations and enzyme activities were measured before and after the compound supplementation in the riding horses. **Results:** In 2 healthy horses receiving the compound, total protein, BUN, creatinine, total cholesterol, triglyceride, and glucose concentrations and AST, ALT, LDH, ALP and MDH activities were not changed significantly compared to those in healthy control horses without the compound. However, plasma NEFA and malondialdehyde concentrations tended to decrease after the compound supplementation for 4 weeks. And the compound supplementation relieved pain in the horse with arthritis of shoulders. Conclusions. The main component of Rv-PEM01-99 is a quercetin derivative, which shows strong anti-oxidant and anti-inflammatory effects. Supplementation with quercetin derivative also is effective to improve hepatic lipid metabolism in riding horses.

C-32: CYTOLOGIC FEATURES OF AN ACANTHOMATOUS AMELOBLASTOMA IN A DOG

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Background: An 8-year-old dog presented with a mass on the rostral mandibular gingiva. Mass was firm, smooth, well-circumscribed, and approximately 2x1x0.5 cm in size rostral to one canine tooth. **Objective:** Determine cytologic and histopathologic features of the gingival mass. **Methods:** Samples were obtained for cytological and histopathological examination. **Results:** Cytology revealed two distinct cell populations, consisting of uniform-appearing epithelial cell clusters and individual spindle cells. Epithelial cells had mild anisocytosis and anisokaryosis, round nuclei with finely stippled chromatin, no prominent nucleoli, high N:C ratio and low amount of pale basophilic cytoplasm. Slender spindle cells observed had oval nuclei with no prominent nucleoli and wispy cytoplasm. On histopathological examination, the lamina propria of the gingiva was dissected by irregular and anastomosing trabeculae and islands of neoplastic epithelial cells which were focally in connection with the hyperplastic overlying epithelium. In the periphery of islands and trabeculae, cells were arranged in palisades and nuclei had an antibasilar location. The epithelial cells had prominent intercellular bridges, low amount of cytoplasm and one round to oval nucleus. Anisocytosis and anisokaryosis were mild to moderate and 6 mitoses/10 HPF were present. Tumor cells reached the deep sample margin. Histopathological evaluation was consistent with an acanthomatous ameloblastoma. This locally aggressive neoplasm may extensively infiltrate and destroy the underlying bone. **Conclusion:** This is the first cytologic description of acanthomatous ameloblastoma correlating with histopathological findings. It is an important differential if both epithelial and spindle cells that do not fulfill cytologic criteria of malignancy are detected on cytology.

Diagnostic Pathology Poster Session

D-01: BILATERAL CORNEAL PIGMENTATION IN AN ARABIAN HORSE

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Background: A 12-year-old Arabian Gray stallion was referred to the SHS Veterinary Center for emaciation and protein loss syndrome. **Methods:** A physical examination was performed and ocular abnormalities were noted on direct examination included abnormal pigmentation of the cornea. **Results:** The Arabian horse was diagnosed with Equine Gastric Ulcerative Syndrome (EGUS), the horse died of colic three months after. On necropsy, both eyes were collected for histological study. Histopathologic features included migration of brown (melanin) pigment into the cornea. The pigment usually affected the surface of the cornea with involvement of both eyes. Neither inflammatory nor neoplastic cells were observed. Fontana-Masson stain was positive for melanin pigment and melanin bleaching using dilute hydrogen peroxide. Corneal epithelial cells do not normally contain melanin, however, the eyes of many animals, may possess a pigmented ring surrounding the corneal limbus. Unfortunately, a complete medical history could not be obtained, however, an inherited condition is possible in the Arabian

horse, which will be studied in the future in the progeny of this stallion. **Conclusion:** Gross and histologic appearance were consistent with a diagnosis of bilateral corneal pigmentation in an Arabian horse.

D-02: HYPOPLASIA OF DENTIN AND ENAMEL IN AN ARABIAN GELDING

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Background: Abnormalities of dental development occur quite commonly horses. They can present with a wide range of clinical signs, and detailed oral and radiographic examinations are often needed to diagnose a developmental dental abnormality. An 8-year-old Arabian gelding was referred to the SHS Veterinary Center for emaciation and protein loss syndrome. **Methods:** A physical examination was performed and the dental revision identified dentin and enamel hypoplasia. Transverse sections of teeth, 5 mm thick were cut from selected sites, were decalcified and prepared for routine histology with H &; E staining. **Results:** The clinical evaluation showed severe dentin and enamel hypoplasia in teeth 101, 102, 103, 401,402,403 and mild in teeth 201, 202, 203, 301, 302, 303. Histologically, abnormal enamel morphology was observed with branched pulp horns or abnormally shaped teeth. Hypoplastic dentin and enamel showed areas of decreased enamel thickness to total absence, failure in matrix formation with grooves and some areas of hypomineralization. Hypoplasia of dentin and enamel with defective enamel development can be the result of an inherited condition called amelogenesis imperfecta, or congenital enamel hypoplasia. Environmental factors and other problems in the development that can cause enamel hypoplasia include: trauma to the teeth, infection, calcium deficiency, deficiencies of vitamins A, C, or D and nutritional secondary hyperparathyroidism. **Conclusion:** Gross and histologic appearance, were consistent with a diagnosis of hypoplasia of dentin and enamel in an Arabian gelding.

D-03: MALAKOPLAKIA IN THE URINARY BLADDER OF TWO PUPPIES

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Background: Malakoplakia is a rare inflammatory disease described in humans and in several animal case reports including two pigs, two cats, a dog, and a macaque. In humans, malakoplakia most often affects the urinary bladder and is characterized by inflammation with von Hansemann type macrophages with or without Michaelis-Gutmann bodies. Malakoplakia is frequently associated with *E. coli* infection. **Methods:** Two puppies were diagnosed with malakoplakia based on history, gross and microscopic findings, immunohistochemistry, transmission electron microscopy (TEM), PCR, and sequencing of the bacterial 16s ribosomal RNA gene. **Results:** Cases included a 6-week-old Pug submitted for full necropsy with a history of straining to urinate and a biopsy from a 4-month-old English Bulldog with *E. coli* urinary tract infection. In both cases, the urinary bladder mucosa was markedly thickened by either nodular or frond-like projections. Microscopically, the submucosa was expanded by sheets of von Hansemann type macrophages characterized by abundant eosinophilic cytoplasm, intracytoplasmic PAS positive granules, intracytoplasmic rod-shaped

bacteria, and rare intracytoplasmic Prussian blue positive inclusions. Macrophages were positive for Iba-1 and CD18d. Michaelis-Gutmann bodies were not detected. TEM on the lesion from the Pug revealed bacteria in phagolysosomes of macrophages. PCR and sequencing of the bacterial 16s ribosomal RNA gene confirmed *E. coli* in tissue samples from the Pug. **Conclusions:** Microscopic findings and ancillary tests support a diagnosis of *E. coli* associated malakoplakia in the urinary bladder of two dogs.

D-04: IMMUNOHISTOCHEMICAL EXPRESSION OF ERG ONCOPROTEIN IN NORMAL CANINE TISSUES AND NONVASCULAR NEOPLASMS OR LESIONS

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Background: ERG oncoprotein is over-expressed in most canine vascular neoplasms. **Objective:** Immunohistochemical evaluation of ERG expression in canine nonvascular neoplasms, nonneoplastic lesions, and normal tissues. **Methods:** Sections from 646 formalin-fixed, paraffin-embedded canine samples of 202 normal tissues, 10 nonneoplastic hepatic lesions (nodular hyperplasia, peliosis, cirrhosis), and 434 benign or malignant neoplasms, including adenomas, carcinomas, mesothelioma, thymoma, mesenchymal, leukocytic (lymphocytic, histiocytic, plasmacytic, and mast cell), endocrine, testicular, ovarian, melanocytic, bone, hair follicle, and oral tumors, were tested with anti-ERG (monoclonal UMAB76, Origene, Rockville, MD) diluted 1/300 and incubated 60 minutes at room temperature. Heat-induced antigen retrieval (DIVA, Biocare Medical, Pacheco, CA) with a decloaker was used. **Results:** None of the normal tissues or nonneoplastic lesions was positive for ERG, other than the endothelium of all types of vessels (veins, arteries, lymphatics, hepatic sinusoids, and nodal sinuses). The only neoplasms with reactivity (nuclear labeling) for ERG antibody were 1/1 myelolipoma and 11/20 low- or high-grade, cutaneous or subcutaneous mast cell tumors. **Conclusions:** ERG immunohistochemical expression in normal, lesional, or neoplastic canine tissues appears to be highly specific for vascular differentiation with the exception of mast cell tumors in which neoplastic mast cells in about half the tumors had mild to strong reactivity.

D-05: CAPRINE ARTHRITIS-ENCEPHALITIS (CAE) IN A YOUNG GOAT

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A 7-month-old intact female mixed breed goat was euthanized and submitted for necropsy after a 2 week duration of progressive neurologic deficits. Postmortem examination revealed bilaterally dilated lateral ventricles lined by yellow, slightly granular material that replaced and effaced the ependymal and subependymal white matter, particularly affecting the right lateral ventricle. The brain was diffusely edematous. Microscopically, surrounding the lateral and third ventricles and extending into the internal capsule, thalamus, and cerebellum, were patchy to regionally extensive areas of subependymal white matter loss and cavitation and replacement with numerous Gitter cells, cholesterol clefts, mineral, and necrotic debris. Surrounding these areas, the neuropil was variably rarified and vacuolated with scattered necrotic neurons and increased numbers of plump glial cells. Vessels were frequently cuffed by

up to 10 concentric layers of lymphocytes and fewer plasma cells. Additionally, alveolar septa were lined by hyperplastic Type II pneumocytes, and thickened by perivascular and peribronchiolar infiltrates of macrophages and lymphocytes. Approximately 1% and 5% of macrophages within the brain and lung lesions, respectively, exhibited moderate to strong intracytoplasmic punctate immunoreactivity to caprine arthritis-encephalitis virus (CAEV). This classic case of concurrent lentiviral pneumonia and encephalitis in a goat kid is striking due to the severity of cerebral white matter loss. While forebrain cavitation is relatively common in adult sheep with small ruminant lentiviral infections, lesions in goats tend to be concentrated within the midbrain, cerebellum and spinal cord and uncommonly result in grossly evident cavitation.

D-06: POLYPHASIC MYODEGENERATION OF THE CRICOPHARYNGEUS MUSCLE IN A CASE OF CANINE CRICOPHARYNGEAL ASYNCHRONY

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Cricopharyngeal asynchrony is an uncommon disorder of dogs characterized by uncoordinated, weak contraction of the pharyngeal muscles during deglutition, and the histologic lesions of this condition are poorly characterized. Herein, we describe a case of cricopharyngeal asynchrony with histologic polyphasic myodegeneration affecting the cricopharyngeus muscles. A 3-year-old, female spayed Boxer presented to the Michigan State University Veterinary Teaching Hospital with a primary complaint of regurgitation and dysphagia. A fluoroscopic esophagram was performed, and a presumptive diagnosis of cricopharyngeal asynchrony was made. Following this visit, the patient was successfully managed with elevated feedings of soft food. One year later, the patient developed multiple liver masses, and was euthanized. On gross postmortem examination, the pharyngeal muscles had no abnormalities. Histologically, there was polyphasic degeneration and atrophy affecting approximately 25% of muscle fibers in the cricopharyngeus muscle. Degenerative changes included loss of cross striations and central placement of nuclei, and overt necrosis with karyolysis, cytoplasmic mineralization, and infiltration of myofibers with macrophages. There were also multifocal areas containing small, angular myocytes and endomysial and perimysial fibrosis. Cricopharyngeal asynchrony is a poorly understood condition from both an etiologic perspective, as well as morphologically; only a single prior case report (Langlois, et al., *Canadian Veterinary Journal*, 2014) has described associated histologic lesions, which were similar to the current case. The polyphasic nature of injury in both cases indicates repeated insults, as may occur in genetic disease or repeated toxin exposure. Further molecular testing is necessary to characterize the etiology of this condition.

D-07: CYTOLOGIC EVALUATION OF DIAGNOSTIC FLUIDS FROZEN WITH A CRYOPRESERVATION MEDIA

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Background: Cavitary fluid cytologic examination is a routine diagnostic tool capable of offering valuable clinical information. For best results, the fluid should be processed within hours of collection, but, if delayed, it is recommended the sample be kept cool and analyzed within 36 hours. Anecdotally, freezing of the sample is discouraged due to the likely loss of cellular integrity and thus devaluation of diagnostic worth. **Objective:** Determine the cytologic integrity of fluid samples following freezing for 24 hours at –20°C following mixture with a commercially-available mammalian cell cryopreservation media (C80EZ®, CryoCrate LLC). **Methods:** Fluid aliquots were mixed in equal parts with the following solutions; 10% glycerol solution, 10% DMSO solution, C80EZ with 10% glycerol, and C80EZ with 10% DMSO, with neat fluid acting as a control. Aliquots were frozen at -20°C for 24 hours, thawed, and cytologically compared to a freshly prepared, unfrozen sample. Differential cell counts and morphologic appearance of the cells were assessed for each aliquot of each fluid and compared to control fluid. **Results:** Compared to the non-frozen control fluid, C80EZ with 10% DMSO was subjectively and quantitatively determined to best maintain cytologic integrity followed by DMEM with 10% DMSO, C80EZ with 10% glycerol, and DMEM with 10% glycerol. Fluid frozen without a solution had the greatest loss of cellular integrity. **Conclusions:** For fluids that are unable to be evaluated the same day as collection, freezing with C80EZ/10% DMSO for up to 24 hours at -20°C can best help preserve cell morphology for accurate cytologic evaluation.

D-08: CUTANEOUS VIRAL PAPILLOMA WITH MALIGNANT TRANSFORMATION TO SQUAMOUS CELL CARCINOMA ASSOCIATED WITH CPV1 IN A LABRADOR RETRIEVER

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Background: Canine papillomaviruses are associated with benign endophytic papillomas, exophytic papillomas, and pigmented plaques, which can occur at mucosal or cutaneous sites. Canine viral papillomas that occur in the oral cavity of younger dogs are often associated with canine papillomavirus 1 (CPV1). Papillomas that occur at cutaneous sites are commonly associated with canine papillomavirus 2 (CPV2). Rarely, CPV2-associated lesions, particularly pigmented plaques, undergo malignant transformation to squamous cell carcinoma (SCC). Only one study to date has shown malignant transformation of a spontaneously occurring cutaneous papilloma associated with CPV1. **Methods:** A 2-year-old, female, spayed, Labrador retriever presented with a 1.8 x 1.7 x 1.7 cm, exophytic, pigmented, and alopecic mass on the left lateral metacarpus. Histopathology, PCR assay, and in situ hybridization testing of the mass were performed. **Results:** Histologically, a viral papilloma with transformation to SCC

and intravascular neoplastic emboli was diagnosed. DNA was extracted from two, 25-micron formalin fixed paraffin embedded scrolls of the biopsy. PCR was performed using two different sets of degenerate primers (My09/MY11 and CanPV/FAP64). Amplicons of an appropriate size (~400 base pairs) were generated using both primer sets. Sequencing of those amplicons and alignment to known canine papillomavirus types revealed sequences identical to CPV1. In situ hybridization for CPV1 E6/E7 revealed abundant viral nucleic acid within the papilloma and SCC. **Conclusion:** Canine papillomavirus 1 was identified in the neoplasm using PCR and ISH. To the author's knowledge, this is only the second case of a cutaneous viral papilloma with malignant transformation associated with CPV1.

D-09: HEMOCYTIC SARCOMA OF THE BODY WALL IN A CALIFORNIA KING CRAB

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Background: A California king crab (*Paralithodes californiensis*) with a history of being terminally anorectic and dropping four legs was found dead. A necropsy was performed and tissue samples were placed in 10% neutral buffered formalin for histologic examination. **Methods:** Tissues were processed routinely, and both histochemical (i.e. Brown and Brenn Gram, Fite-Faraco acid fast, Fontana-Masson, Giemsa, hematoxylin and eosin, Masson's trichrome, periodic acid-Schiff [PAS], phosphotungstic acid-hematoxylin, and von Kossa) and immunohistochemical (i.e. cytokeratin, vimentin, and lysozyme) stains were performed. **Results:** The body wall was ulcerated and subtended by a densely cellular, unencapsulated, invasive neoplasm composed of spindle cells arranged in intersecting streams and bundles embedded in a small to moderate amount of fibromatous stroma. Neoplastic cells were oval to elongate with fibrillar, pale eosinophilic cytoplasm that occasionally contained moderate numbers of small, spherical, brightly eosinophilic granules that were highlighted with PAS and Giemsa stains. Neoplastic cells had mild atypia and no evident mitoses. Immunohistochemical stains were noncontributory. Discussion: This neoplasm is consistent with hemocytic sarcoma of semi-granulocytic origin. Decapod crustaceans have three types of hemocytes: hyalinocytes, granulocytes, and semi-granulocytes. Neoplastic cells had PAS and Giemsa positive granules, which are present in both semi-granulocytes and granulocytes. Semi-granulocytes elongate and are associated with deposition of extracellular matrix during some immune responses. Neoplastic cells were elongate and associated with deposition of matrix. These findings suggest neoplastic cells were semi-granulocytic origin. Neoplasia is uncommonly reported in invertebrates. Mesenchymal neoplasia has not been previously reported in crabs.

D-10: TUMOR LYSIS SYNDROME IN A HORSE WITH LYMPHOMA

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An 11-year-old mare presented for a two-week history of inappetence and progressive azotemia. Bloodwork revealed markedly elevated urea nitrogen and creatinine, hyperphosphatemia, and hypocalcemia. The mare continued to decline despite treatment and euthanasia was elected. Postmortem examination identified numerous coalescing, firm, tan, nodules within the spleen, adrenal gland, lungs, and numerous lymph nodes. There were multiple wedge-shaped, red foci in the kidneys and few tan, gritty foci within the ventricular myocardium. Histologically, the nodules were composed of sheets of neoplastic lymphocytes effacing the normal parenchyma. Nuclei were often pyknotic. Approximately 60% of the neoplastic cells showed strong perimembranous immunoreactivity for CD79a. The renal interstitium was expanded by mononuclear inflammatory infiltrates and renal tubules were frequently lined by degenerate to necrotic epithelial cells. Multiple tissues, including the myocardium, lung, and intestines, contained variably sized foci of mineralization surrounded by fibrosis and mixed inflammatory cells. Based on clinical pathology and histopathologic findings in this horse, tumor lysis syndrome was suspected. Tumor lysis syndrome (TLS) is an uncommon condition in dogs and a well-recognized condition in humans which often occurs following chemotherapy, radiation, or corticosteroid treatment of lymphoproliferative neoplasia. TLS can also develop in the absence of treatment. Diagnosis of TLS requires the presence of two of the following: hyperkalemia, hyperuricemia, hyperphosphatemia, or hypocalcemia. TLS is clinically associated with arrythmias and acute kidney injury. Pathogenesis is related to rapid tumor cell turnover or extensive destruction, resulting in release of intracellular ions, nucleic acids, and metabolic byproducts into systemic circulation.

D-11: PROCESSING EQUINE TEETH FOR HISTOLOGY

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Background: Equine dentistry is a rapidly evolving field with an increasing number of recently recognized, often unique, equine dental diseases. The pathogeneses of these diseases, many of which are still being elucidated, are often tied to the complex and dynamic anatomy of the equine radicular hypsodont dentition. However, a simple and efficient process for preparing equine teeth is lacking for the veterinary pathologist to consult when processing and reviewing equine dental cases. **Objective:** To develop a simple and efficient process for preparing equine teeth for histology without the need of any specialized equipment. **Methods:** Equine teeth are fixed whole in 10 percent buffered formalin and then decalcified in a rapid acid decalcification solution (RDO Rapid Decalcifier). Incisors are allowed 7-10 days to decalcify sufficiently; normal cheek teeth may take up to 21 days to fully decalcify. Decalcified teeth are trimmed using a scalpel blade and all sections are processed using a routine soft tissue processing cycle in a tissue processor, microtomed at 5um thickness, and stained with hematoxylin and eosin. All sections are evaluated for quality assurance prior to reviewing for pathologic changes. **Results:** Sections are obtained from all major structures of the equine tooth, including the occlusal crown, gingival crown, reserve crown, and apical root. All major structures are discernable on light microscopy and the structure of each tooth and the surrounding periodontal tissues are preserved. **Conclusions:** Equine teeth can be

prepared for routine histology without the need of specialized equipment while maintaining adequate preservation of the microanatomy.

D-12: TROPHOBLAST EMBOLI IN THE LUNG OF A SNOWSHOE HARE

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Background: Trophoblast embolism is migration of placental trophoblasts into the maternal uterine blood vessels and dissemination to other organs such as lung, uterus, adrenal gland, spleen, and liver. Spontaneous trophoblast emboli occur in women and are reported in other species with hemochorial placentation like hamsters, chinchillas, gerbils, cotton rats, and porcupines. Although considered incidental in most species, increased incidence and numbers of trophoblast emboli are reported in women with gestational pathologies with arterial hypertension (pre-eclampsia and eclampsia). To our knowledge, trophoblast emboli have not been reported in lagomorphs. **Methods:** A wild, pregnant, adult, female, snowshoe hare (*Lepus americanus*) was found dead and submitted for necropsy. **Results:** Hemothorax was observed at necropsy. Histologically, the lung had multifocal hemorrhages in alveolar spaces and septa. Multinucleated cells, up to 60 um in diameter, filled random alveolar capillaries. These cells had microscopic morphology and immunoreactivity to CD31, CK AE1/AE3, and CK8/18 similar to trophoblastic cells within the placental labyrinth and lining and filling the lumen of maternal subplacental blood vessels, consistent with trophoblast emboli. **Conclusions:** This case shows presence of spontaneous trophoblast emboli in a snowshoe hare. Death was attributed to hypovolemic shock consequent to hemothorax, however a definitive cause of hemothorax was not found. There was no evidence of trauma. Anticoagulant rodenticides were not detected by liquid chromatography–mass spectrometry on liver. Rupture of small pleural or thoracic blood vessels secondary to trophoblast emboli was considered a possibility.

D-13: NEURONAL CEROID LIPOFUSCINOSIS IN A COQUEREL'S SIFAKA (PROPITHECUS COQUERELI)

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Background: Neuronal ceroid lipofuscinosis (NCL) is rarely reported in primates, with few reports in cynomolgus macaques. The pathogenesis of NCL is poorly understood. It is characterized by cytoplasmic accumulation of a lipofuscin-like pigment and cellular degeneration, particularly in the neurons of the cerebrum, cerebellum, and retina. Most cases of NCL are presumably inherited/genetic. **Methods:** A 6-year-old, female Coquerel's sifaka (*Propithecus coquereli*) had incoordination and seizures attributed to hydrocephalus, which was diagnosed by magnetic resonance imaging. Despite medical management, seizures progressed and the animal was humanely euthanized. Ventricular hydrocephalus was confirmed at necropsy. A full set of necropsy tissues were processed routinely and examined histologically with hematoxylin and eosin stain. Special stains (periodic acid-Schiff [PAS] with diastase, luxol fast blue [LFB], and acid-

fast), autofluorescent microscopy, and transmission electron microscopy were performed on cerebral sections. **Results:** Histologically, there was severe degeneration and necrosis of neurons and glial cells within the cerebrum and cerebellum. Affected cells contained abundant intracytoplasmic granular pigment that was LFB and PAS positive, and diastase resistant. The intracytoplasmic pigment was strongly autofluorescent. Ultrastuctural examination of the intracytoplasmic pigment consisted of membrane-bound granular osmophilic deposits and less often rectilinear profiles. These findings are diagnostic for NCL. **Conclusions:** NCL has not been previously reported in prosimians. NCL was considered the cause for clinical decline and histologic lesions in this animal. The cause for hydrocephalus was likely atrophy of cerebral tissue (hydrocephalus ex vacuo) due to neuronal degeneration and necrosis.

D-14: MEDULLARY BONE IN MALE BUDGERIGARS

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Medullary bone, or laying bone, is a calcium-rich, labile bone normally occurring in female birds with each egg-laying cycle. The stimulus for deposition of medullary bone is the cyclical increase in serum estrogens produced by preovulatory ovarian follicles. Increased bone density due to deposition of medullary bone, particularly in pneumatic bones, has been termed polyostotic hyperostosis, even if physiological. This case series investigates the deposition of medullary bone in non-pneumatic (femur) and pneumatic (humerus) bones in sexually mature male budgerigars submitted for autopsy between 2017 and 2020. Of the seven budgerigars that met the case criteria, 4/7 (57%) had deposition of medullary bone in one or more bones examined. All four male budgerigars with medullary bone had a testicular neoplasm, which was morphologically consistent with a Sertoli cell tumor or seminoma. Medullary bone was not present with other diseases. Medullary bone deposition in pneumatic and non-pneumatic bones can occur in male budgerigars with testicular neoplasia. Radiographic increases in medullary bone density, particular in the humerus, could provide antemortem indication of testicular neoplasia in male budgerigars.

D-15: INTRACRANIAL FIBROPAPILLOMA IN TWO GREEN SEA TURTLES (CHELONIA MYDAS)

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Background: Fibropapillomatosis (FP), caused by chelonid herpesvirus 5 (ChHV5), has been reported in sea turtles in warmer waters. Juvenile green turtles (*Chelonia mydas*) are primarily affected and ChHV5 is a major cause of mortality. ChHV5 is found in turtles with or without tumors. If present, tumors are observed externally and internally within the coelomic cavity. **Objective:** To describe histopathologic and molecular findings from two green sea turtles with external and internal fibropapillomatosis. **Methods:** Samples from necropsied free-ranging adult females (n

=2) from a rehabilitation facility were processed and stained with hematoxylin and eosin; immunohistochemistry for vimentin, S-100, and cytokeratin were performed on sections of the neoplasm. PCR for ChHV5 was performed on select formalin-fixed paraffin-embedded sections. **Results:** The green turtles exhibited neurologic signs and were euthanized. Histopathologic findings in the brain included an infiltrative neoplastic mass extending from the meninges composed of spindle cells forming streams, whorls, and islands. There was mild anisocytosis and anisokaryosis. Mitotic figures were not observed. Intracytoplasmic tumor cell immunoreactivity for cytokeratin and vimentin was rare and absent for S-100. One sea turtle was PCR positive for ChHV5. **Conclusions:** The neoplasms were consistent with sea turtle fibropapillomas. The brain is an unreported site and tumors likely arose from meningiocytes. The absence of detection of ChHV5 in one animal could reflect the stage of infection, degradation of nucleic acids over time, or the negative effects of fixation on DNA. Intracranial FP should be included as a differential for neurologic signs in sea turtles from FP endemic areas.

D-16: A RETROSPECTIVE STUDY OF CANINE INTRANASAL MAST CELL TUMORS

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Background: Mast cell tumors (MCTs) are an uncommon primary neoplasm of the nasal cavity in dogs for which there is a paucity of existing literature regarding their clinicopathologic and molecular features. **Objective:** Our objectives included: 1) to retrospectively study the clinical findings, histopathologic and immunohistochemical features, and c-kit status of primary intranasal MCTs in dogs, and 2) identify potential prognostic factors. **Methods:** Canine biopsy submissions to Colorado State University between 2010-2019 with intranasal neoplasms diagnosed as MCTs with no history of cutaneous or oral MCT were considered. Immunohistochemistry for CD117 and Ki67, and PCR for internal tandem duplications at exons 8 and 11 of the *c-kit* gene were performed. **Results:** Twenty-one (1.1%) out of 1,852 primary nasal neoplasms were MCTs. Metastases were reported in 11 cases, with the submandibular lymph nodes representing the most common site. One case had distant metastases to abdominal viscera. Survival time ranged from 20 days to 1.5 years with only 3 (20%) dogs alive 1 year after the onset of clinical signs. Cases with a <1 year survival tended to have mitotic counts of ≥8, metastasis to regional lymph nodes, and/or atypical CD117 immunostaining patterns. Only one case had a *c-kit* mutation at exon 11. **Conclusion:** Canine intranasal MCTs appear to be clinically aggressive. In this study, dogs with regional lymph node metastasis had a shorter survival time. A mitotic count of ≥8 may serve as a useful prognosticator for canine intranasal mast cell tumors.

D-17: A CASE OF INCLUSION BODY DISEASE OF BOIDS IN A BOA CONSTRICTOR FROM TRINIDAD AND TOBAGO

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Background: An adult, female, *Boa constrictor weighing* 4.8kg presented to the UWI-SVM for a post mortem evaluation. The history stated that the animal exhibited signs of lethargy, anorexia, weight loss, dysecdysis, stomatitis and a swollen head. Methodology: Medical treatment consisted of the parenteral administration of subcutaneous fluids, Gentamicin, vitamin B complex, Meloxicam and oral lavage. There was, however, no response to therapy, and the snake died. A necropsy was done and tissue samples measuring approximately $1cm^3$ were collected from multiple organs prior to fixation in 10% neutral buffered formalin. Tissue sections were routinely processed for histological analyses. **Results:** The most significant gross lesions included a necrotizing stomatitis, in which there were multiple ulcerated areas of red-brown friable tissue in the oral cavity overlaid by a foul smelling, pale yellow exudate, and a perioral mandibular swelling with oedema and similar appearance as the mouth. Microscopically, multiple organs (including liver, kidneys, and lungs) contained round to oval, brightly eosinophilic to magenta intracytoplasmic inclusion bodies that are characteristic of Inclusion Body Disease (IBD). **Conclusion:** This case would be the first histopathologic confirmation of the presence of IBD within boid snakes in Trinidad and Tobago. There is currently no effective treatment for IBD and it is always fatal in affected snakes. A clinical diagnosis may be possible with blood sampling or biopsy and subsequent microscopic assessment.

D-18: INTRAOCULAR ASTROCYTOMA IN AN ATLANTIC BOTTLENOSE DOLPHIN (TURSIOPS TRUNCATUS)

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Background: Neoplasia in marine mammals includes individual reports and population level (urogenitial carcinoma in sea lions). Ocular neoplasia is rarely reported in marine mammals. **Objective:** Describe histopathologic and immunohistochemical findings from an Atlantic bottlenose dolphin (*Tursiops truncatus*). **Methods:** A gross necropsy was conducted on the live-stranded dolphin that died spontaneously. Samples from the necropsied free-ranging immature male dolphin were processed and stained with hematoxylin and eosin; immunohistochemistry for vimentin, S-100, NSE, and GFAP were performed on the neoplasm. **Results:** The dolphin was emaciated, measured 170.0 cm and weighed 44.0 kg. There were shark bites including a large defect in the abdominal cavity with extruded intestine. The left eye was collapsed. Histopathologic findings from the eye included phthisis bulbi, corneal edema, ulceration, lenticular degeneration, and retinal disruption by neoplastic spindyloid to polygonal cells forming streams and occasional rosettes. Immunoreactivity for GFAP, vimentin, and S-100 was observed; NSE only stained neurons. Findings were consistent with an intraocular astrocytoma. **Conclusions:** Death was the result of interspecies aggression. The ocular neoplasm could have contributed to diminished visual acuity contributing to predation. Intraocular astrocytoma has not been reported in a dolphin; there is a report of cerebral astrocytoma.

D-19: HAVE YOU SEEN THIS LESION: GLOMERULAR "BLOOD CYSTS" AND MESANGIOLYSIS IN A CAT

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An 8-year-old, female, spayed, domestic short-haired cat presented for coughing and dyspnea. Examination revealed chylous pleural effusion, and idiopathic chylothorax was diagnosed after ruling out cardiac and infectious diseases, neoplasia, and congenital abnormalities. Surgical intervention was elected, including thoracic duct ligation, subtotal pericardectomy, and pleural port placement. Post-surgical cardiac arrhythmias, hypotension, and anuria occurred for several days, which improved with medical therapy and adjustment of the central line. Despite anuria, creatinine and lactate levels remained normal. Ten days post-surgery, the cat represented for dyspnea and pleural effusion and was euthanized. Necropsy revealed chronic-active pleuritis; multisystemic thromboses affecting the cranial vena cava and lungs; and myocardial infarction with fibrosis. Interestingly in the kidneys, ~25% of glomeruli had segmental microaneurysms with mesangial matrix dissolution (mesangiolysis) and expansion by erythrocytes and occasionally pigment-laden or mononuclear cells. By TEM, most glomeruli had fibrin within capillary lumina and normal podocyte foot processes and fenestrated endothelium. Some causes of mesangiolysis reported in humans include toxicities from snake venom and chemotherapeutics; circulatory disturbances including ischemia, hypertension, and congenital heart disease; microangiopathies such as hemolytic-uremic syndrome and malignant hypertension. In veterinary medicine, there are few cases of porcine glomerular hyalinosis and mesangiolysis, and one case of mesangiolysis in a cat with Tetralogy of Fallot. In this case, it is suspected that the chronic pleuritis resulted in cranial vena cava thrombosis which embolized and infarcted the heart, leading to congestive heart failure. Thus, the glomerulopathy is considered a sequela of hemodynamic changes rather than a primary pathologic lesion.

D-20: OLFACTORY GANGLIONEUROBLASTOMA IN A DOG

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Background: Neuroblastic tumors (neuroblastoma, ganglioneuroblastoma, ganglioneuroma) are a rare group of embryonal neoplasms that have been reported in man and veterinary species. These tumors can be categorized as peripheral, central, or olfactory. Unlike peripheral and central neuroblastic tumors (NTs), which are derived from neural crest cells that ultimately form the sympathetic nervous system, olfactory NTs (ONTs) arise from basal progenitors of the olfactory epithelium. ONTs are rare intranasal neoplasms in man and veterinary species, and only one canine olfactory ganglioneuroblastoma has been previously reported. Case presentation: A 7-year-old, male intact Alaskan Malamute presented for increased stertor and epistaxis. Computed tomography of the skull revealed a soft tissue mass within the left nasal passage that extended into the right nasal cavity and olfactory bulb. Multiple biopsies were obtained from the left nasal passage and submitted for histologic evaluation. **Results:** Histopathology revealed nodules of neoplastic primitive cells resembling neuroblasts, as

well as discrete clusters of neurons associated with Schwannian stroma. Immunohistochemically, neuroblastic cells and ganglia were strongly immunoreactive for microtubule associated protein-2 and modestly immunoreactive for TuJ-1 (Class III beta-tubulin); ganglia were strongly immunoreactive for synaptophysin, while neuroblastic cells exhibited mild immunoreactivity to synaptophysin and multicytokeratin. The immunohistochemistry, in combination with the neoplasm histomorphology, supports the diagnosis of an olfactory ganglioneuroblastoma with caudal extension into the skull base. **Conclusions:** This case report expands on the current literature on canine olfactory ganglioneuroblastomas by providing additional examples of positive immunohistochemical markers for, as well as diagnostic imaging documenting the invasiveness of, this tumor type.

D-21: TUMOR INFILTRATING LYMPHOCYTES IN 107 PET RABBIT MAMMARY CARCINOMAS – PERSPECTIVE CONSIDERATIONS

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Background: Tumor infiltrating lymphocytes (TILs) act as prognostic and predictive biomarkers in human breast cancer. International guidelines facilitate their standardized evaluation. Rabbits are considered a potential animal model for human breast cancer, and the rabbit immune system is closely related to that of humans. **Objective:** The aim of this study was to characterize TILs in pet rabbit mammary carcinomas according to guidelines and to examine whether results are associated with microscopic features indicative of a better prognosis. **Methods:** TIL evaluation was performed in hematoxylin-eosin stained sections of 107 rabbit mammary carcinomas. Statistical analyses examined the correlation of obtained results with histological and immunohistochemical tumor characteristics of presumed prognostic relevance. **Results:** Results showed a significant correlation between percentages of stromal TILs from the central tumor (CT) and infiltrative margin (IM). Within the CT, a higher maximal percentage of stromal TILs was significantly associated with a decreased mitotic count, a lower tumor grade, as well as a higher percentage of calponin immunopositive tumor cells. The latter was also significantly related to a lower mitotic count, as well as higher average numbers of stromal TILs from the CT and IM. **Conclusions:** Results show that higher percentages of stromal TILs are statistically associated with histological tumor features predictive of a more favorable prognosis and an increased expression of the tumor suppressor protein calponin. These findings lead to the hypothesis that TILs can act as favorable prognostic biomarker in rabbit mammary carcinomas. Further, they support the use of pet rabbits for comparative human research.

D-22: FELINE BRONCHORRHEA: HISTOPATHOLOGICAL, HISTOCHEMICAL, AND IMMUNOHISTOCHEMICAL INVESTIGATION OF A LOBECTOMIZED SPECIMEN TO DETERMINE THE CAUSE OF WATERY HYPERSECRETION

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Background: A 10-year-old castrated male Scottish Fold was presented with three-month anorexia and labored breathing. Radiography revealed ill-defined infiltrates in posterior lung fields. Bronchoscopy visualized watery hypersecretion. Bronchoalveolar lavage yielded macrophages and neutrophils with negative bacterial culture. PCR on an oropharyngeal swab detected no upper-respiratory pathogens. Feline bronchorrhea (FB) was clinically diagnosed based on airway hypersecretion with chronic dyspnea and radiographic diffuse patchy opacities. Lobectomy of both caudal lobes at 4-month intervals led to significant clinical improvement. **Objective:** Though cats having wet cough and IPF-like lesions have been reported, a histologic explanation for that type of cough is absent. Our objective is to increase understanding of the mechanism of wet cough of human idiopathic pulmonary fibrosis (IPF) patients by investigating a similar clinicopathological condition in cats. **Methods:** Histospecimens of the left caudal lobe stained with H&E, Masson trichrome, periodic-acid Schiff (PAS), Alcian blue pH 2.5, Alcian-blue PAS, colloidal iron, and Watanabe's silver methods were evaluated. Immunohistochemistry for cytokeratin AE1/AE3, TTF-1, α-SMA, Iba-1, and various MUCs was performed. **Results:** Histologic features of IPF were present. There was multifocal hyperplasia and bronchiole-like metaplasia (bronchiolisation) in the peripheral airway and alveolar epithelium. Fluid, lacking features of acid mucin, filled many alveoli with foamy macrophages. Occasional lack of TTF-1 expression and steady expression of MUC6 by the bronchiolised epithelial cells were remarkable. **Conclusions:** Our results showed that bronchiolisation, which has been seen in a minority of human IPF patients with unusual wet cough and poor prognosis, is also present in feline IPF-like patients.

D-23: POST-CARDIAC ARREST SYNDROME IN A DOG

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Post-cardiac arrest syndrome occurs following resumption of spontaneous circulation (ROSC) after prolonged, successful cardiopulmonary resuscitation (CPR). In human medical literature, it has been documented to result in numerous difficult-to-manage, pathophysiologic events. These changes are due to a combination of whole-body ischemia, reperfusion, myocardial dysfunction, and systemic inflammatory response syndrome resulting in microvascular fibrin thrombi, continued tissue hypoxia, hypovolemia, sepsis, and multiorgan failure. A 10-year-old, male neutered, terrier mix presented for thoracoscopic surgical excision of a cranial mediastinal mass. As the trocar was advanced from a trans-xiphoid approach, the patient decompensated into ventricular fibrillations. Cardiac massage with intermittent intrathoracic defibrillations were continued for 22 minutes until ROSC was achieved. Over the next five days in the ICU, the patient initially improved then suddenly demonstrated a decline in mentation,

intermittent ventricular tachycardia, intermittent ventricular premature complexes, and forelimb paresis due to a thrombus in the left axillary artery confirmed via ultrasonography. The patient acutely arrested. At necropsy, widespread thrombosis and infarction were observed within the heart, kidneys, intestine, spleen, pancreas, and brain. The cranial mediastinal mass and thrombosis of the left axillary artery were confirmed. Microscopic examination confirmed widespread fibrin thrombi with secondary infarcts resulting in hemorrhage and necrosis. The renal infarcts were deemed to be 2-3 days old while the brain infarcts ranged from a few minutes to 1-2 days old. The cranial mediastinal mass was consistent with a benign thymic cyst. The clinical history, gross and microscopic lesions, and timeline of events generated the diagnosis of post-cardiac arrest syndrome.

D-24: TALAROMYCES BONINENSIS MENINGOENCEPHALITIS IN A LABRADOR RETRIEVER

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A 6-year-old, male castrated Labrador Retriever presented for necropsy due to progressive neurologic dysfunction including cerebellar ataxia, tremors, lateral recumbency, and respiratory distress. The only gross necropsy findings were several chronic renal infarcts. Histopathology of the cerebrum, midbrain, cerebellum, brainstem, and cervical spinal cord revealed a severe, multifocal to coalescing, chronic granulomatous meningoencephalitis with intralesional fungal hyphae. The hyphae were approximately 5 micrometers in diameter with irregular septation, non-parallel walls, and acute angle branching with scattered bulbous swellings up to 10 micrometers in diameter, negatively stained on hematoxylin and eosin, and stained positively with Gomori methenamine silver stain. Panfungal PCR of the ITS and LSU regions yielded 100% matching identity with *Talaromyces boninensis* (basionym *Talaromyces helicus* var. *boninensis*). *Talaromyces spp.* are soil dwelling fungi characterized by the production of asci, sac-like reproductive structures containing ascospores. *Talaromyces helicus* has been reported twice previously in dogs, causing granulomatous lymphadenitis and fungal arthritis. The dog in this case had no history of immune compromise or immune modulating medication. This is the first report of disease caused by *Talaromyces boninensis* in any species.

D-25: NEURAL HYPERTROPHY AND HYPERPLASIA IN A LAMB WITH CHRONIC PANCREATITIS

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Background: Nerves can be severely reshaped in human pancreatic pathologies such as chronic pancreatitis or pancreatic cancer. In these two diseases, pancreatic nerves can become larger (neural hypertrophy) and/or increased in number (increased neural density/hyperplasia). This process, termed neural plasticity, is also associated with neuropathic pain. Although there are several animal models of chronic pancreatitis, pancreatic neuropathy is not well characterized in these models. Thus, the translational

value of these *in vivo* models cannot be entirely ascertained for the study of neural plasticity. **Objective:** This report describes spontaneous alterations characteristic of pancreatic neural plasticity in a lamb. **Results:** A clinically healthy fattening lamb was sent to a slaughterhouse in Quebec. Upon macroscopic examination of the carcass, a firm 10-cm white nodule was noted in the pancreas. Microscopically, severe and extensive interstitial fibrosis was present in the pancreas, and small groups of acinar cells were markedly atrophied. Pancreatic nerves presented significant changes, with large and tortuous nerve bundles infiltrating the examined tissue. Hyperplasia of mature ganglion cells was present, forming several aggregates in the parenchyma. **Conclusions:** Based on the histologic changes, a diagnosis of chronic, severe, sclerosing pancreatitis with neural/neuronal hypertrophy and hyperplasia was made. Even though chronic pancreatitis and pancreatic tumors are common in many animal species, to the authors' knowledge, spontaneous occurrence of associated pancreatic neural plasticity has not been reported in non-human species. Sheep might be a suitable animal model to study this pathology.

D-26: FELINE MESOTHELIOMA: A CASE REPORT AND COMPARISON OF CYTOLOGIC, IMMUNOCYTOCHEMICAL, HISTOPATHOLOGY AND IMMUNOHISTOCHEMICAL FINDINGS

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Mesotheliomas are an uncommon neoplasm arising from mesothelial cells in either the abdominal or thoracic cavities and are rarely diagnosed in cats. A 10-year-old spayed female domestic shorthair cat presented to the LSU oncology service for evaluation of a large amount of abdominal effusion. Abdominal ultrasound identified a large mesenteric mass with numerous ill-defined nodules. An abdominocentesis was performed with cytological and immunocytochemical findings consistent with a neoplastic effusion with large clusters of epithelioid cells that expressed pancytokeratin, vimentin and Wilms' tumor 1 antigens. Further diagnostics were declined, and meloxicam was prescribed until the cat died 23 days after initial presentation. At necropsy, the omentum was contracted into a firm mass adhered to multiple organs, accompanied by numerous small white nodules throughout the abdominal cavity. On histopathology and immunohistochemistry, neoplastic cells were found throughout the abdominal cavity; the majority were immunoreactive for cytokeratin, vimentin and Wilms' tumor 1 protein. The final diagnosis was an epithelioid mesothelioma. This case illustrates the utility of cytology, immunocytochemistry and its relation to histology and immunohistochemistry in the diagnosis of this disease.

D-27: ACUTE MYELOID LEUKEMIA IN A RHESUS MACAQUE

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A 24-year-old male rhesus macaque from a research breeding facility was clinically found dehydrated and in poor body condition. CBC revealed anemia (hematocrit

22.3%), severe thrombocytopenia (12 K/µL), 1% metamyelocytes and 1% myelocytes in the absence of lymphocytosis. The animal developed severe bruising on the left thorax and abdomen and was euthanized. Gross findings included extensive subcutaneous hemorrhage and an enlarged liver with rounded edges and prominent rib impressions. Histologically, bone marrow was densely cellular and composed of neoplastic blast cells with large nuclei, open chromatin, 1-3 nucleoli and a small amount of eosinophilic cytoplasm. Similar blastic neoplastic round cells were also present in sheets effacing normal architecture in multiple lymph nodes as well as the liver, kidney, testis, and choroid plexus. These neoplastic cells were immunonegative to CD3 and CD20, but showed diffuse positive cytoplasmic immunoreactivity to IBA-1, indicating monocyte/macrophage origin. Rhesus macaques are an important animal model of aging, and incidence of neoplasia has been shown to increase after age 20. However, round cell neoplasia of myeloid origin is rarely reported in rhesus macaques and nonhuman primates in general. This case represents an example of acute myeloid leukemia in an aged rhesus macaque.

D-28: MASSIVE BRANCHIAL HENNEGUYOSIS: A DISTINCTIVE MYXOZOAN-INDUCED GILL DISEASE OF CATFISH CAUSED BY MASSIVE INTERLAMELLAR INFECTIONS OF HENNEGUYA EXILIS

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Proliferative gill disease (PGD), caused by the myxozoan parasite *Henneguya ictaluri*, is the most prevalent parasitic disease of United States catfish aquaculture. Recently, an unusual myxozoan-induced gill disease caused by massive burdens of *Henneguya exilis* has been diagnosed within channel (*Ictalurus punctatus*) x blue (*Ictalurus furcatus*) hybrid catfish monoculture. Targeted metagenomic sequencing and *in situ* hybridization (ISH) were used to examine myxozoan community composition between massive branchial *Henneguya* infections and clinical PGD cases to identify myxozoan species contributing to pathology. Thirty ethanol-fixed gills from seven hybrid catfish massive branchial *Henneguya* cases were subjected to targeted amplicon sequencing of the 18S rDNA gene (Illumina MiSeq) and compared to a metagenomic dataset generated from clinical PGD cases. Further, serial sections of 21 formalin-fixed gills (3 per case) were analyzed by RNAscope® duplex chromogen ISH assays targeting 8 different myxozoan species. Metagenomic and ISH data were in agreement, indicating myxozoan community composition significantly differs between PGD and branchial henneguyosis cases, with different myxozoan communities contributing to disease pathogenesis. Findings indicate PGD in farm-raised catfish can consist of mixed species infections, while branchial henneguyosis was attributed to nearly pure infections of *H. exilis*. Other *Henneguya* spp. were rare in branchial henneguyosis, although *H. ictaluri* was identified by ISH in infrequent PGD-like lesions, supporting previous work evincing hybrids are susceptible to acute stages of *H. ictaluri*. Building upon previous pathologic descriptions and molecular characterization, this work provides the case definition for a potentially emerging, myxozoan-induced gill disease of farm-raised catfish, tentatively termed massive branchial henneguyosis.

D-30: T-ZONE LYMPHOMA WITH CUTANEOUS PRESENTATION IN A GOLDEN RETRIEVER

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Background: An 8 year-old male castrated Golden Retriever enrolled in the Morris Animal Foundation Golden retriever lifetime study (MAF_GRLS) presented for pruritic skin disease in the axilla, pinna, prepuce and perineal areas which was refractory to treatment with diphenhydramine, antibiotics, steroids, and lokivetmab. Skin scrapes were negative for infectious organisms, and *Sarcoptes* antibody titers were negative. Three months later generalized lymphadenopathy developed. Lymph node biopsy, fine needle aspirate and a skin biopsy were collected for diagnosis. **Objective:** Characterize and subclassify lymphadenopathy and skin disease. Determine if disease could be detected in earlier samples obtained during the lifetime study. **Methods:** Histopathology, immunohistochemistry (IHC), flow cytometry and PCR for antigen receptor rearrangements (PARR) were used to characterize disease from the lymph node, skin biopsy, and peripheral blood. **Results:** Histopathology and IHC were consistent with T-zone lymphoma (TZL), lacking expression of CD45 in both the lymph node and skin. Flow cytometry from the lymph node revealed a homogeneous expansion of T cells lacking CD45, diagnostic for TZL. PARR from the lymph node and skin identified the same sized clonal T cell receptor rearrangements. PARR from previous peripheral blood samples did not identify a clonal population. **Conclusions:** This case is an uncommon example of a cutaneous manifestation of TZL with concurrent lymph node involvement. Indolent cutaneous T-cell lymphoma/lymphocytosis has been reported in dogs, however there has not been a clear association with TZL. Lack of earlier peripheral blood involvement may aid in understanding cutaneous manifestation of the disease in the future.

D-31: PATHOLOGIC CHARACTERIZATION OF A LEUKOENCEPHALOMYELOPATHY IN TWO DOUBLE-RED-FACTOR SUN CONURES (ARATINGA SOLSTITIALIS)

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Genetic neurodegenerative disease has been rarely reported in birds. Two approximately 3-month-old, double-red-factor sun conures (*Aratinga solstitialis*) presented to the Texas A&M Veterinary Medical Teaching Hospital exotic service. The red factor of sun conures is a specific color mutation that results in the normal yellow color being almost entirely replaced by a dark red hue. Both birds presented for a several week history of neurologic deficits beginning at approximately 8 weeks of age that included weakness, paresis, twitching, torticollis, and seizures. Humane euthanasia was elected due to poor prognosis, and necropsies were performed. No gross findings

were noted within the nervous system. Microscopically, moderate axonal degeneration consisting of dilated myelin sheaths, swollen axons (spheroids), and digestion chambers was evident within the white matter of the spinal cord, cerebellum, and cerebrum. Gliosis and infiltration of foamy macrophages were also observed in affected areas. Neurologic deficits in double-red-factor sun conures have been recognized by conure breeders, but the pathology of the condition has never been characterized. This syndrome warrants further investigation.

D-32: CASE REPORT: ENDOCARDIAL FIBROSIS IN A DOMESTIC MIXED SHORTHAIR CAT

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Background: A 4-year old castrated male mixed short hair cat presented for autopsy following natural death. The cat was diagnosed with a heart murmur 3 years ago, and weight loss was reported three months prior to death. Per the owner, the cat laid on the floor, began labored breathing, and died within 3 hours. **Methods:** Tissues were examined grossly and histologically with hematoxylin and eosin stain. Special stains and immunohistochemistry were performed. **Results:** Autopsy findings included severe left ventricular concentric hypertrophy, mitral valve degeneration, and mild pleural effusion with pulmonary congestion. The heart weighed 32 grams (0.64% of body weight). The most significant histopathologic lesions were observed within the heart, including large raised endocardial plaques multifocally throughout the left ventricle, mild myofiber vacuolation with interstitial fibrosis of the papillary muscle, and expansion of the mitral valve spongiosa by myxomatous material. Trichrome method revealed endocardial thickening was predominantly densely packed and organized collagenous tissue and peripheral areas contained chondroid metaplasia. Elastin staining demonstrated that elastic fibers were not a significant component of the plaque. **Conclusions:** Heart weight and measurements collected during autopsy are suggestive of hypertrophic cardiomyopathy (HCM), and while there were no classical histologic findings of HCM, this remains the most likely differential diagnosis. Restrictive cardiomyopathy and fibroelastosis are unlikely based on histology and evaluation of special stains. The cause of atypical extensive collagenous plaques within the left ventricle is uncertain, but may be the result of chronic turbulent blood-flow secondary to HCM.

D-33: CANINE ADENOVIRUS TYPE-1 ENCEPHALITIS IN A PUPPY

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An 8-week-old, intact male, unvaccinated, Australian Heeler dog with seizures of acute onset was euthanized and submitted for postmortem examination. The vaccination history of the dog’s dam was unknown. The gross necropsy was unremarkable. The major histological finding involved lymphohistiocytic encephalitis, predominantly focused around blood vessels. The lesions were widespread throughout the gray and white matter of the cerebral cortices, cerebellum, thalamus, and brainstem. Rare glial cells within glial nodules and endothelial cells had solitary, large, central, basophilic, intranuclear inclusion bodies with chromatin margination. Similar inclusion bodies were

identified in various cells of the liver, renal glomeruli, and within lymphocytes, with only minimal inflammation or necrosis in these organs. Scattered endothelial cells within the brain and liver, occasional hepatocytes and Kupffer cells, as well as rare splenic lymphocytes exhibited strong intracytoplasmic to occasionally intranuclear immunoreactivity to canine adenovirus type-1 (CAV-1) antigen. The cerebral cortex had scattered endothelial cells with strong intracytoplasmic reactivity to generic adenoviral antigen. Neurological manifestation of CAV-1 infection in domestic dogs has been rarely reported. Previously reported cases predominantly had vascular changes within the brain, with minimal inflammation. In addition, the most striking lesions in the present case were in the brain, with mild changes in other affected tissues, including the liver. This report expands the number of documented cases of this rare manifestation of CAV-1 and reinforces this as a differential diagnosis for neurological disease in unvaccinated puppies.

D-34: NOT HORSING AROUND: ACTINOBACILLUS EQUULI INFECTION IN PIGS; A FIVE YEAR (2015 TO 2020) RETROSPECTIVE ANALYSIS OF CASES SUBMITTED TO THE IOWA STATE UNIVERSITY VETERINARY DIAGNOSTIC LABORATORY

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Background: *Actinobacillus suis* and *A. pleuropneumoniae* are the most common causes of actinobacillosis in swine cases submitted to the ISU VDL. Additionally, *A. equuli* has been reported in causing disseminated bacteremia or sepsis and valvular endocarditis in swine. The ecology of this bacterial organism is poorly understood within production systems and further characterization of the described pathologic lesions and manifestations were of interest in understanding this pathogen. **Objective:** Retrospectively analyze cases where *A. equuli* was isolated from fresh tissue lesions. **Methods:** *A. equuli* isolated from fresh tissue that included histopathology evaluation were selected for analysis (May 2015 to 2020). Diagnostic lesions, age, time of year, and associated pathogen cofactors were tabulated. **Results:** A total of 34 porcine tissues were selected with *A. equuli* detected as the sole bacterial pathogen in 15 (44%) cases. Pneumonia was the most common diagnosis, followed with sepsis, arthritis/synovitis, valvular endocarditis, pleuritis, and meningitis/encephalitis. In most cases (85%), *A. equuli* growth was considered moderate to high. Pigs 9 to 22-weeks-old were found to have lesions consistent with pneumonia and sepsis, while arthritis/synovitis was observed in 1 to 4-week-old piglets. Porcine reproductive and respiratory syndrome virus (PRRSV) and type A influenza were the most common viral cofactors detected, while *Streptococcus suis* was the most common bacterium co-isolated. **Conclusions:** Septicemia and pneumonia appear to be the most common diagnoses in finisher-age pigs, while arthritis/synovitis were processes observed in juvenile piglets with *A. equuli* infection. Overall, *A. equuli* may account for sporadic yet severe disease within swine production systems.

D-35: ORAL PAPILLOMATOSIS IN A ROTTWEILER DOG

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Background: Canine papillomatosis is an acute viral disease which commonly affects young dogs less than one-year-old. The causative agent is canine papillomavirus (CPV-1) which typically has an incubation period ranging from 4 to 8 weeks, after which the disease is often self-limiting. Case presentation: A six years old Rottweiler dog was presented to University Malaysia Kelantan Veterinary Clinic with a cauliflower-like mass at the tip of the mandible. Histopathological examination of the resected lump revealed the presence of epidermal hyperplasia with thickened stratum corneum. Orthokeratotic hyperkeratosis and ballooning degeneration of the keratinocytes were evident. The stratum granulosum layer of the epidermis contained basophilic keratohyalin granules. There was lymphoplasmacytic infiltration and fibrosis in the dermis. The lesion regressed after treated with Azithromycin and topical imiquimod cream. **Conclusion:** This case report described the histopathological features of canine oral papillomatosis in an adult dog with treatment resulting in regression of the papillomatous lesion.

D-36: NEURONAL CEROID LIPOFUSCINOSIS IN A CAT

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A two year old, female spayed Siamese cat presented to the rdvm with a four month history of progressive, abnormal behavior, including urinating outside of the litter box, becoming anti-social, hyperexcitable, and exhibiting mild ataxia. Gross necropsy was unremarkable. Histopathology reveals the majority of neuronal and astrocytic cytoplasm expanded by amphophilic to eosinophilic globules which often peripheralized the nuclei. The cerebral white matter and lamina of the gray matter is severely spongiotic, and the associated neurons are hypereosinophilic and angular (neuronal necrosis). Electron microscopy was performed on cerebral tissue. Within the cytoplasmic matrix of neurons are one or multiple bundles of small, electron dense C-shaped to S-shaped lines. This pattern is indicative of the curvilinear profile (CLP), or Type 2 of neuronal ceroid lipofuscinosis (NCL). NCL is a lysosomal storage disease that has been described in several domestic species and humans, and clinical signs involve progressive cognitive decline, motor dysfunction, epileptic seizures, and eventually death or euthanasia. Although therapeutic advances have delayed onset of clinical signs, there is no current cure for this disease.

D-37: NEISSERIA SP. INDUCED EMBOLIC NECRO-SUPPURATIVE PNEUMONIA IN 3 DOMESTIC CATS

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Clinical Background: Three cats, age 2 to 11 years-old, presented to the University of Minnesota Veterinary Diagnostic Laboratory over a three-year period following euthanasia or death due to marked respiratory distress, depression, and dehydration. Antemortem thoracic radiographs revealed multiple, nodular, soft tissue opacities throughout the lung fields in all cases. Gross and Histologic Findings: On postmortem examination of all three cats, approximately 60-80% of the lung parenchyma were expanded by widely-disseminated, multifocal to coalescing, well-demarcated, beige to tan, raised, semi-firm nodules. Histologically, large numbers of variably degenerate neutrophils, large amounts of fibrin, moderate numbers of macrophages, and moderate amounts of cellular and karyorrhectic debris effaced and replaced the pulmonary parenchyma multifocally. Affected areas contained aggregates of Gram-negative extra- and intracellular coccoid bacteria. Bacterial Identification: MALDI, 16s rRNA sequencing and whole genome sequencing revealed that the bacteria isolated under aerobic conditions from the lung of all cats are novel Neisseria sp. Whole genome based and 16S rRNA based phylogenetic analysis revealed that the most similar species is Neisseria animaloris. **Conclusions:** Infection with Neisseria sp. induces a fairly characteristic pneumonia in cats that radiographically and grossly resembles metastatic neoplasia. Pathogenesis and reason for the sporadic, rare nature of the disease is poorly understood, with hematogenous spread favored given the widely-disseminated pulmonary distribution. In younger cats, Neisseria spp. pneumonia should be considered among the top etiologic differential diagnoses in cases of lower respiratory disease with a disseminated, nodular lung pattern.

D-38: FELINE SEBACEOUS CARCINOMA: HISTOPATHOLOGY AND BIOLOGICAL BEHAVIOR IN 11 CASES

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Background: Sebaceous carcinomas (SbC), while common in dogs, are rare tumors in cats, and their biological behavior in cats is not clearly defined. **Objective:** To investigate clinical and histopathologic features of SbC in 11 cats. Methods. H&E-stained sections of solitary tumors from 11 cats diagnosed with SbC in the Penn Vet Diagnostic Laboratory database (2004-2018) were reviewed. Clinical data was obtained when available. **Results:** Affected cats were domestic shorthair (8/11) or purebred (Maine coon, Persian, Pixie-bob). Median age at diagnosis was 14 years (range 7-20 years). Eight of 11 SbC were located on the head or ventral neck. Histologically, SbCs comprised a variable mixed population of basaloid (reserve) cells and cells with sebaceous differentiation (finely dispersed cytoplasmic lipid vacuoles). Eight of the 11 SbC exhibited invasive growth, while three were well-circumscribed. One SbC had foci of squamous differentiation, and one exhibited lymphatic invasion. Median mitotic count was 17 mitoses/10 consecutive HPF (range 10-25). Clinical follow-up was obtained in 7 cats. Two cats were euthanized at 11 days and 2 months for poor quality of life. A third cat was euthanized due to a presumptive pulmonary metastasis. Regional lymph node metastasis was confirmed in one cat and suspected in another. The cat with confirmed lymph node metastasis was still alive 1.5 years after diagnosis. **Conclusions:** Eight of eleven feline SbC arose on the head or ventral neck. Metastasis occurred in a subset of

the affected cats. No clinical or histologic factor appeared to predict outcome in this small number of cats.

D-39: INVESTIGATION INTO SARCOCYSTIS INFECTION IN FREE-RANGING BLACK BEARS (URSUS AMERICANUS) AND GRIZZLY BEARS (URSUS ARCTOS HORRIBILIS) IN BRITISH COLUMBIA, CANADA

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Background: *Sarcocystis* are protozoan parasites that can cause a spectrum of lesions in various hosts. Hepatic sarcocystosis has been described in captive bears and a wild grizzly cub (*Ursus arctos horribilis*), but not yet in wild black bears (*Ursus americanus*). It is unclear whether this is a widespread concern for free-ranging bear populations. Increased yearling deaths in 2016 in British Columbia (BC), Canada prompted further diagnostic investigation into this parasite's role in bear mortality. **Objective:** This study aims to characterize the presence and lesions associated with *Sarcocystis* spp. in free-ranging bears in BC, Canada, submitted to the provincial diagnostic laboratory. **Methods:** From 2007 to 2019, 102 free-ranging black bears and grizzly bears were examined post mortem for sarcocystosis, using histopathology and molecular diagnostics to characterize infection. **Results:** Sarcocystosis was confirmed in 41 of 102 free-ranging black bears and grizzly bears. Lesions included multifocal necrotizing hepatitis, nonsuppurative encephalitis, myositis, or intramuscular sarcocysts without apparent lesions. *Sarcocystis canis* was identified by DNA sequencing in 19 cases, while the remaining were associated with other *Sarcocystis* spp. or identified only to genus level. Age was found to be a significant risk factor for *Sarcocystis* infection, with all cases of fatal sarcocystosis restricted to juvenile cubs <1 year old and yearlings more highly represented for *Sarcocystis* infection. Confirmed cases were distributed widely across BC, without notable spatial clusters apparent. **Conclusions:** This is the first report of fatal sarcocystosis in free-ranging black bears. Further research is needed to understand the epidemiology and significance of the disease.

D-40: CARDIAC PROTOTHECOSIS IN A BOXER DOG

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A 1-year-old, castrated male, black and white Boxer dog presented with signs of left-sided heart failure and was found to have increased troponin I, a dilated cardiomyopathy phenotype, a non-patent perimembranous ventricular septal defect (VSD), a right bundle branch block, ventricular arrhythmias, and periods of sinus arrest. The dog was also positive for Trypanosoma cruzi on an indirect fluorescent antibody test (1:80). At necropsy, cardiomegaly and a non-patent VSD were confirmed. The

myocardium had ill-defined areas of pallor, and occasional 1 mm diameter, raised, pale nodules were evident on the epicardium of the left and right ventricles. Histologically, the dog had severe, multifocal to coalescing, chronic, necrotizing, histiocytic, and lymphoplasmacytic pancarditis with intralesional algae consistent with Prototheca spp. Protothecosis is caused by an aerobic, unicellular, saprophytic achlorophyllic algae from the genus Prototheca, most commonly of the species wickerhammii or zopfii which are found in contaminated stagnant water. Prototheca has been isolated from cattle, fish, cats, humans, and dogs, with a higher prevalence in Boxer dogs in some studies. Protothecosis usually begins with colitis and then becomes systemic, resulting in a poor to grave prognosis. This case is unusual in that the infection was apparently confined to the heart.

D-41: LISTERIA MONOCYTOGENES INFECTION WITH SPIRURID INFESTATION IN A MONITOR LIZARD (VARANUS VARIUS)

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Clinical History: A 3-year-old male Monitor lizard had a 1.5 month history of severe weight loss and lethargy after being on an egg-only diet. Supportive care and assisted feeding were unsuccessful. After presenting with constipation, a tight coelom, and bilateral hindlimb paresis; euthanasia was elected. Gross examination: Grossly, the animal was emaciated. An adult nematode was found in the subcutis and adhered to the intercostal muscle. The coelom was distended by serosanguineous effusion. Multiple pale tan to yellow raised nodules were found in multiple organs including the heart, lungs, liver, spleen, kidneys, and the aboral colon. Histopathologic examination: Nodules identified grossly were determined to be pyogranulomas with intralesional bacteria. The intralesional bacteria was confirmed to be *Listeria monocytogenes* through immunohistochemistry, polymerase chain reaction, and culture. Numerous round to oval, 40-50 µm in diameter spirurid nematode eggs were noted in subcutis, skeletal muscle, heart, lung, liver, spleen, kidney and colon. The eggs did not evoke an inflammatory response. **Conclusion:** Two separate processes were identified in this animal. While parasitic infections are relatively common in lizards, Listeriosis has not been previously described in Monitor lizards. While egg deposition was high in some organs, especially the liver, infection by *Listeria monocytogenes* was the primary disease process in this animal.

D-42: A PUTATIVE EPIZOOTIC OF INTERSTITIAL PNEUMONIA IN A HERD OF YOUNG MINIATURE DONKEYS ASSOCIATED WITH ASININE HERPESVIRUSES 2 AND 5

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Background: Asinine herpesviruses 2 (AHV-2) and 5 (AHV-5) are gammaherpesviruses with equids being the natural hosts. While both viruses have been detected in healthy equids, AHV-5 has been associated with severe interstitial pneumonia in horses and in donkeys; with reports largely limited to individual cases. Case description: Two yearling miniature donkeys from a herd of five developed a mild cough and nasal discharge that progressed to respiratory distress. On thoracic ultrasound, extensive areas of pulmonary consolidation and abscessation were seen in both donkeys and an asymptomatic donkey from the same herd. All three were treated with antimicrobials, anti-inflammatories and supportive therapy. One donkey deteriorated and was humanely euthanized. The other two improved and were discharged. **Results:** Post-mortem examination of the euthanized donkey revealed severe bronchointerstitial pneumonia. qPCR analysis of nasal secretions from all three donkeys and lung tissue collected post-mortem from the deceased donkey were positive for AHV-2 and 5. The tracheal aspirates and bronchoalveolar lavage of the two surviving donkeys, and nasal secretions from two other asymptomatic donkeys from the same herd were also positive for AHV-5. All samples were negative for equine influenza A, equine herpesviruses 1, 2, 4 and 5, equine rhinitis virus A and B, asinine herpesvirus 3, and Streptococcus equi subsp. Equi. **Conclusion:** The qPCR results, clinical signs occurring concurrently in multiple individuals from the same herd, and necropsy findings strongly support a rare case of an epizootic of pneumonia in a herd of donkeys associated with AHV-2 and 5.

D-43: SUBEPIDERMAL BLISTERING/BULLOUS DISEASE IN AN ADULT QUARTER HORSE

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A 5-year-old Quarter Horse gelding was presented to the Texas A&M University Veterinary Teaching Hospital for evaluation of non-healing wounds and ulcerated lesions for more than 8 months, along the head, neck, and trunk. The mucous membranes were unaffected. Hereditar*y* equine regional dermal asthenia (HERDA) genetic test was negative. At necropsy, in the skin along the head, neck, and trunk, were multifocal, irregular crusts overlying ulcers and erosions. Histopathology showed multifocal areas of dermal-epidermal separation and cleft formation, with locally extensive areas of ulceration. The histologic findings were most consistent with a subepidermal blistering/bullous disease. Given this was an adult horse, the lesions were presumed to be due to bullous pemphigoid, which can be severe and rapidly progressive. Equine bullous pemphigoid is a rare autoimmune subepithelial, vesiculobullous dermatosis characterized by subepidermal vesicles and ulcers in the skin, oral mucosa, or both. Autoantibodies against the basal cell hemidesmosome antigen collagen XVII, also known as BPAg 2 BP180, bind to the antigen of the hemidesmosomes resulting in dermoepidermal separation and vesicle formation. A definitive diagnosis is based on direct immunofluorescence or immunohistochemical testing revealing IgG autoantibodies against the BPAg 2 (collagen XVII) antigen.

D-44: TORSION OF THE CAUDATE LOBE OF THE LIVER AND COINCIDENT NECROHEMORRHAGIC TYPHLOCOLITIS IN A CAPTIVE PATAGONIAN MARA, DOLICHOTIS PATAGONUM

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Background: Liver lobe torsion has previously been reported in rabbits but not, to our knowledge, in the Patagonian mara. **Objective:** To document the findings in a case of torsion of the caudate lobe of the liver, and coincident necrohemorrhagic typhlocolitis, in a captive adult male Patagonian mara, *Dolichotis patagonum,* that died suddenly. **Methods:** The mara underwent post mortem examination, with subsequent histopathology and microbiology. **Results:** At post mortem examination, the hepatic caudate lobe was torsed and was diffusely cream, with a “chalky” texture. The remaining hepatic parenchyma exhibited multifocal, irregularly sized red and gray foci less than 5 mm diameter. The cecum and colon exhibited red serosa and roughened, red, mucosa interpreted as necrohemorrhagic typhlocolitis. The adrenal glands bilaterally exhibited multifocal cortical hemorrhages. Histologically, the caudate liver lobe exhibited diffuse coagulative to lytic necrosis and multifocal fibrosis. The remaining hepatic parenchyma exhibited multifocal necrotizing and neutrophilic hepatitis. In the cecum and colon multifocal mucosal necrosis was accompanied by mixed inflammatory infiltrates that extended into the submucosa. Microbiological culture of cecal contents revealed a growth of *Salmonella* spp. Based on the post mortem findings and microbiology, *Salmonella* spp. was considered the likely etiology of the necrohemorrhagic typhlocolitis, multifocal necrotizing hepatitis, and adrenal hemorrhages. The hepatic caudate lobe torsion was chronic and was considered to be coincident. **Conclusions:** Caudate liver lobe torsion may occur in maras. Similar to the case in some rabbits, it appears that this mara survived for some time with the torsion. Salmonellosis was the likely cause of death.

D-45: A CASE OF PETER'S ANOMALY IN A DOMESTIC LONGHAIR CAT

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Background: Peter’s anomaly is a congenital and segmental defect in Descemet’s membrane and endothelium. This syndrome is proposed to be resultant from a defect of neural crest migration involving the corneal endothelium, corneal stroma, iris and/or iridocorneal angle. Case description: A 9-year-old, female spayed Domestic Long Hair cat was presented to the hospital for respiratory distress. Multiple firm, white masses were present in the tracheal wall, tracheobronchial lymph nodes, lung, kidneys and bone marrow, and a diffuse large B-cell lymphoma was confirmed histologically. In addition to these findings, the cat had a focal white, opacity in the axial corneal of the left eye with multiple radiating yellow uveal cords attached on parasagittal sectioning. **Results:** Microscopically, spanning the anterior chamber and communicating in between the posterior aspect of the axial cornea and anterior surface of the iris were thin uveal strands, composed of spindle cells, pigmented cells and thin-walled blood vessels. At the attachment and fused portion, the posterior cornea had an

approximately 5-mm long segment of disrupted Descemet's membrane and endothelial cells, and thinned disorganized corneal stroma. At the edge of each segment, the Descemet's membrane was scrolled, arborized or re-duplicated. These findings were consistent with Peter's anomaly. The left eye also had incidental multiple small iridal cysts, and there was no evidence of inflammation, cataract or glaucomatous changes. **Conclusion:** Herein we described an incidental unilateral Peter's anomaly in a cat confirmed by having strands of pigmented, vascular and mesenchymal tissue stretching from the iris to posterior cornea histologically.

D-46: FIRST REPORTED CASE OF RHINOSPORIDIUM SEEBERI INFECTION IN A DOG IN THE CARIBBEAN REGION

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Case Presentation A four year old, male, intact, mixed breed dog used for hunting was admitted to the Veterinary Hospital at the School of Veterinary Medicine, University of the West Indies, Trinidad and Tobago, for recurrence of a sessile fleshy nasal mass and sneezing. The mass had previously been removed surgically but regrew. Diagnostics Bloodwork was performed and was unremarkable. A fine needle aspirate of the mass revealed pyogranulomatous inflammation and organisms consistent with *Rhinosporidium seeberi*. This was confirmed with histopathologic examination, which showed severe chronic- active rhinitis with extracellular endospores and sporangia consistent with *Rhinosporidium* spp. Segments of this mass were also sent for molecular diagnostics. Discussion This is the first reported case of canine rhinosporidiosis in the Caribbean region in a hunting dog that likely roamed in a warm, humid environment suitable for survival of this organism. *Rhinosporidium seeberi* is an aquatic protozoan parasite of the order Mesomycetozoea that can cause nasal infection in humans, dogs, horses, and less commonly other animals such as cattle and cat. It is the only member of Mesomycetozoea that is pathogenic to mammals and birds. It is endemic in the tropics, as well as in southern India and Sri Lanka, and has been reported as well in the Americas, Europe, Africa, and Asia. Trinidad and Tobago are tropical islands located in the Caribbean Sea, close to South America. This geographical location provides the ideal environment for presence and survival of *Rhinosporidium seeberi*.

D-47: OXALATE NEPHROSIS IN A TONKIN BUG-EYED FROG (THELODERMA CORTICALE)

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Renal mineralization has been described in a few amphibian species. Renal calcium oxalate deposition has been noted in edible frog (Rana esculenta), Solomon Island leaf frog (Ceratobatrachus guentheri), and leopard frog (Rana pipiens) tadpoles fed diets high in oxalates (e.g. spinach) and has sometimes resulted in nephrosis with mechanical tubular damage and consequent granulomatous inflammation. Oxalate nephropathy has also been reported in free-ranging American bullfrog (Lithobates

catesbeianus) tadpoles in Japan. Reports of similar changes in mature amphibians are lacking. Tonkin bug-eyed frogs (Vietnamese mossy frog; Theloderma corticale) are native to northern Vietnam and China and listed on the IUCN Red List of Threatened Species. A captive-hatched adult male Tonkin bug-eyed frog was found dead with no premonitory signs of disease. No gross lesions were detected. Histologically, the kidneys were affected by a severe inflammatory process directed at tubules and the surrounding interstitium. Multifocally replacing/obscuring tubules and within the interstitium were aggregates of pale gray to colorless, refractile, birefringent, radiating angular crystalline material (calcium oxalate). Crystalline material was surrounded by large multinucleated giant cells, numerous macrophages, scant cellular debris, rare heterophils and minimal fibrosis. Death was attributed to severe tubulointerstitial nephritis with abundant intralesional crystalline material, morphologically consistent with calcium oxalate. No specific cause for oxalate nephrosis was evident in examined sections. Nutritional and/or toxic underlying etiologies were considered most likely though no source could be identified.

D-48: NATURAL CLINICAL INFECTION BY MYCOBACTERIUM INTRACELLULARE IN A DOMESTIC RABBIT (ORYCTOLAGUS CUNICULUS DOMESTICUS) IN OREGON, USA

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Background: Natural clinical mycobacteriosis in domestic rabbits (Oryctolagus cuniculus domesticus) is rare. Presentations include disseminated, respiratory and intestinal granulomatous disease. To date, apart from one case of chronic respiratory mycobacteriosis, all reported cases have been outside North America. However, fatal mycobacteriosis has been described in pygmy rabbits (Brachylagus idahoensis) in the Pacific Northwest of the United States. Case Description: A one-year-old domestic rabbit died after a brief history of gastrointestinal stasis and hypoalbuminemia. Necropsy revealed a moderately thickened, diffusely off-white sacculus rotundus and mild hepatomegaly with a few roughly linear, off-white foci. Histopathology confirmed severe granulomatous ileal enteritis with abundant intrahistiocytic acid fast positive bacilli and mural necrosis, and mild chronic hepatic coccidiosis. There were also scattered epithelioid macrophages and multinucleated giant cells containing rare acid fast positive bacilli in the liver, lung and spleen. Discussion: The histologic lesions are consistent with those reported in non-tuberculous Mycobacterium complex infections in rabbits. While the clinical abnormalities can be accounted for by the observed tissue changes, the gross and histologic changes attributable to Eimeria stiedae appeared much less severe than those due to Mycobacterium. This strongly suggests Mycobacterium had a more significant role in the associated overt clinical abnormalities. Studies in Europe indicate Mycobacterium infections in rabbits can represent a public health risk and reservoirs of infection for livestock. To the best of our knowledge, this is also the first report of naturally occurring fatal mycobacterial enteritis in domestic rabbits in North America. PCR identification of the species of Mycobacterium is underway.

D-49: DISSEMINATED ASPERGILLOSIS AND CONCURRENT LOCALIZED CUTANEOUS PHAEOHYPHOMYCOSIS IN A DOG (CANIS FAMILIARIS)

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Background: A 5-year-old, castrated male, Pit Bull mix canine presented for labored breathing acutely after nailbed infection. Patient was currently on prednisone and cyclosporine for immune-mediated hemolytic anemia. Thoracic ultrasound revealed moderate pleural and pericardial effusion. Owner elected humane euthanasia after no improvement with supportive care. **Objective:** To determine the cause of the pleural and pericardial effusions and its relationship to the nailbed infection. **Methods:** Antemortem pericardial fluid sample and nailbed fine-needle aspirate were submitted for evaluation. Postmortem examination was performed. Routine samples were fixed in 10% formalin, routinely processed, sectioned, and stained with hematoxylin and eosin and gomori methenamine silver stain. Liver, kidney, lung, pleural and pericardial fluid, and nailbed samples were submitted for mycology. Pericardial fluid sample was submitted for polymerase chain reaction. **Results:** Purulent discharge from the nailbed, serosanguinous effusions within the thorax and pericardial sac, nodular thickening of the mediastinum, pericardium, and pleura, and renal granulomas were detected grossly. There was pyogranulomatous inflammation and mixed fungi in the pericardial fluid, mediastinum, pericardium, and kidneys and similar inflammation and pigmented fungi in the nailbed. Fungal culture yielded *Aspergillus terreus* in the visceral organs and *Curvularia lunata* in the nailbed. PCR of the fungus identified *Aspergillus citrinoterreus* in the pericardial fluid. **Conclusions:** The patient suffered from concurrent disseminated aspergillosis from primary respiratory infection and localized cutaneous phaeohyphomycosis in the nailbed from *C. lunata*. Infection with these fungi tend to occur in immunocompromised individuals. The patient was currently on immunosuppressive drugs resulting in increased susceptibility to secondary fungal infections.

D-50: PRESUMPTIVE SARCOMA WITH DIFFUSE INVOLVEMENT OF THE SKELETAL MUSCLE IN A GROUP OF CAPTIVE PUERTO RICAN CRESTED TOADS (PELTOPHRYNE LEMUR)

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Seven (3 females, 3 males, 1 undetermined) captive Puerto Rican crested toads (PRCTs) ranging from 8 to 11.5 year were received for post-mortem examination. All had history of weakness/lethargy, and 5 were euthanized due to poor prognosis. Postmortem examination showed poor body condition (5/7), dysecdysis (3/7), and presence of nodules in the musculature or subcutis (3/7). Histology revealed an infiltrative population of round/polygonal to spindle cells affecting mainly the striated muscle in multiple areas (7/7), with variable involvement of visceral organs. Cells had indistinct cytoplasmic borders, moderate amounts of cytoplasm occasionally containing up to 5 µm eosinophilic granules (5/7), and 1-3 mitoses per ten high-powered fields.

Granulomatous inflammation was ruled out as no infectious agents (Ziehl-Neelsen, Gram, Periodic Acid-Schiff), were observed. Bacterial culture could not be performed due to lack of frozen tissues. Given the infiltrative nature and lack of other inflammatory cells (i.e., lymphocytes or plasma cells), lesions were considered neoplastic. Tumors showed no sarcoplasmic striation (phosphotungstic acid hematoxylin), and of those with granules detectable by haematoxylin and eosin, three showed metachromatic reaction of cytoplasmic granules with toluidine blue and Giemsa (mast cell differentiation). Transmission electron microscopy from the only submitted case confirmed presence of membrane-bound electron-dense cytoplasmic granules. No organisms were observed. Results suggest that these tumours may be myeloid sarcomas with multiple differentiation. Blood smears for evaluation of a concurrent leukemia were not available, and bone marrow sections were unremarkable. This is the first report of a possible myeloid neoplasm in a population of captive PRCTs.

D-51: OUTBREAK OF EXTRAINTESTINAL PATHOGENIC ESCHERICHIA COLI-INDUCED NECROTIZING PNEUMONIA IN DOGS

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Background: Extraintestinal pathogenic *Escherichia coli* (ExPEC) causes significant human and animal morbidity and mortality affecting extraintestinal organs. ExPEC is a cause of fatal pneumonia in various animal species including dogs, cats, a horse and a tiger. **Case Description:** Within a month, five dogs of various ages and breeds were presented for acute respiratory distress due to pneumonia. The clinical course of the five dogs was rapidly progressive in spite of aggressive medical treatment. Primary postmortem findings were similar among all of the animals. Grossly, the lungs were diffusely dark red. Microscopic examinations revealed severe acute necrotizing pneumonia with gram-negative short bacilli. Hemolytic *E. coli* was isolated from the lung tissue of three dogs. *Streptococcus canis* and *E. coli* were isolated from one dog. Gram, Giemsa, and Steiner stains also visualized the bacteria. There was no bacterial growth in one of the dogs, but similar lung lesions were observed microscopically. No canine distemper morbillivirus, canine herpesvirus, and canine influenza orthomyxovirus antigens or nucleic acids were detected. All the isolates, genotyped by PCR, carried genes characteristic of ExPEC including cytotoxic necrotizing factor 1 (cnf-1), and did not express genes for shiga toxins, or heat stable or heat labile toxins. **Summary:** The lesions and microbiological findings from an outbreak of *Escherichia coli* ExPEC necrotizing pneumonia in dogs are documented.

D-52: SUDDEN DEATH IN A DOG DUE TO PRESUMED POSITION-INDUCED HYPERTHERMIA: A FORENSIC INVESTIGATION

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A dog was found dead in a crate at a boarding facility, 12 hours after being taken outdoors to urinate. She was in a near-fetal position, with no water. The crate measurements were too small for her size, leaving her unable to assume normal body positions or comfortably reposition without being in full contact with the crate sides. She was forced to remain curled on her side with an unnaturally bent lower back, unusually extended hindlimbs, and chin tucked against her chest. At necropsy, the oral mucosa was deep red, the gingival mucosa was blue, blood vessels on the tongue were congested, and red spots were present in the oropharynx. There was diffuse lung congestion, and hemorrhage in the kidneys. Although these are nonspecific and the dog's behavior was not observed immediately before death, these findings can be seen in heat stroke. The urinary bladder was also empty. Post-mortem radiographic changes were consistent with noncardiogenic pulmonary edema. The post-mortem findings, circumstances, environment, and positioning of the dog led the forensic team to opine that death likely resulted from multiorgan dysfunction secondary to hyperthermia and dehydration, due to enclosure in an inappropriately small crate without water. The unnatural position she had to assume in the crate for 12 hours, combined with her dense coat and lack of water, provided optimum conditions to result in hyperthermia. The dog's inability to appropriately extend her neck prevented appropriate ventilation and thus thermoregulation. This resulted in respiratory difficulty, respiratory failure, loss of consciousness, and death.

D-53: IDENTIFICATION OF POWASSAN VIRUS INFECTION IN DOGS IN THE WESTERN GREAT LAKES REGION

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Background: Within the past decade, particularly within the Midwestern and Northeastern United States, there has been an increase in reported human cases associated with Powassan virus (POWV). This is a tick-borne flavivirus that, in severe cases, results in fatal meningoencephalitis in humans. POWV also infects a variety of mammals; however, natural infection has not been reported in domestic dogs. **Objectives:** To 1) identify the seroprevalence of POWV and 2) determine if POWV is a potential cause of neurologic disease in domestic dogs. **Methods:** We developed an ELISA to detect canine antibodies against POWV in 36 clinically neurologic, 230 non-neurologic systemically ill/immunocompromised (unhealthy), and 193 healthy dogs that presented to the University of Wisconsin-Madison Veterinary Hospital in 2017. Positive samples were tested using plaque reduction neutralization tests (PRNT). **Results:** POWV antibodies were identified in 1 neurologic, 2 healthy, and 5 unhealthy dogs. All ELISA-positive dogs had PRNT-70% antibody titers supportive of infection with POWV. The single ELISA and PRNT positive neurologic dog was a 4-year-old female spayed Akita, suspected clinically of tick-borne disease induced non-ambulatory tetraparesis with diffuse localization. Prior to this study, postmortem histologic examination revealed lymphoplasmacytic perivascular cuffing multifocally throughout the brain and spinal cord, consistent with meningoencephalomyelitis of unknown etiology. **Conclusion:** Powassan virus antibodies were identified rarely in healthy and systemically ill dogs as well as one neurologic dog diagnosed with meningoencephalomyelitis. Serologic

evidence of POWV suggests that domestic dogs can serve as a surveillance tool. Additionally, POWV is a possible cause of neurologic disease in this population.

D-54: A NASAL NEUROECTODERMAL TUMOR IN A LLAMA

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This report describes a suspect esthesioneuroblastoma in a two-year-old female Lama that presented for a sinonasal mass. At post mortem examination there is marked deformation and effacement of the left maxilla and orbit by a homogenous, friable, tan infiltrative mass. The mass deviates the nasal septum and extends through the cribriform plate to compress the left frontal cortex. Similar masses (indicative of metastasis) are present within the lungs, thyroid, liver and spleen and the cervical and subiliac lymph nodes. Histologically, the masses are composed of tightly packed lobules of neoplastic cells which frequently form rosettes and pseudorosettes. Neoplastic cells have a centrally located ovoid nucleus with dense chromatin and one to two indistinct nucleoli. The mitotic rate is high (8 mitotic figures per high power field) and mild cellular and nuclear pleomorphism. Neoplastic cells are strongly immunopositive for vimentin and variably immunopositive for chromogranin and S100. Neoplastic cells are immunonegative for GFAP and cytokeratin. Based on these findings, the nasal mass is identified as a neuroectodermal tumor most consistent with an esthesioneuroblastoma. Esthesioneuroblastoma are aggressive, malignant neoplasms derived from germinal olfactory neuroepithelial cells found lining the ethmoturbinate region of the nasal cavity. Esthesioneuroblastoma are rare in veterinary literature but have been described in several domestic species including dogs, horses and camelids, however this is the first report in a llama. Additionally, this particular tumor was highly aggressive with widespread multiorgan metastasis.

D-55: INTRALUMINAL AORTIC CHONDROSARCOMA WITH METASTASIS TO THE ILIAC BIFURCATION MIMICKING SADDLE THROMBOSIS IN A DOG

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There are five cases of canine intraluminal aortic chondroid neoplasms in the veterinary literature. We report an aortic chondrosarcoma in a dog and review the clinical and pathologic features of this unusual neoplasm, which often mimics cardiac or thromboembolic disease in its presentation. A 3-year-old Labrador Retriever presented with chronic progressive exercise intolerance and coughing. Recent signs included reluctance to move, polyuria/polydipsia, and facial twitching. On neurologic examination, there was discomfort on lumbosacral palpation and intermittent hindlimb weakness with exertion. Aspartate aminotransferase and creatine kinase were slightly elevated, but tick titers and acetylcholine receptor antibody levels were normal. Thoracic radiographs revealed a focal bulge of the left craniolateral cardiac silhouette. Following an episode of vomiting, the patient developed rapidly progressive dyspnea, elevated cardiac troponin, and tachyarrhythmias leading to cardiopulmonary arrest. Postmortem examination revealed a tan, firm, nodular to gritty intraluminal mass occluding the aortic

arch. Additional masses were located at the iliac bifurcation and in the right renal artery. Histologically, they consisted of lobules of spindle to stellate cells within lacunae in a chondroid matrix. The final diagnosis was an intraluminal aortic chondrosarcoma arising from the tunica intima of the aortic arch with metastasis to the distal abdominal aorta and right renal artery. Additional findings included polyphasic myocardial degeneration, renal infarction, and severe aspiration pneumonia with septicemia. Intraluminal aortic neoplasia should be considered in dogs with suspected thromboembolic disease and unexplained hind end weakness or cardiac insufficiency, with attention paid to imaging of the aortic arch and iliac bifurcation.

D-56: AN OUTBREAK OF FATAL HERPES SIMPLEX VIRUS IN FREE-RANGING BLACK-TUFTED MARMOSETS (CALLITHRIX PENICILLATA)

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Background: Herpes simplex virus (HSV) infection is a fatal zooanthroponosis mainly reported in captive non-human primates (NHPs). Reductions of natural habitats are resulting in increased numbers of black-tufted marmosets in urbanized areas and exposing these free-ranging populations to new pathogens and diseases. **Objective:** Characterize epidemiological and pathological findings in an acute fatal outbreak of HSV infection in free-ranging black-tufted marmosets in the Federal District, Brazil. **Methods:** Epidemiological, clinical, gross, histopathological, and immunohistochemical (HSV I and II) findings were evaluated. **Results:** Seven marmosets died within three days on a small farm in a peri-urban area of Brasilia, Brazil. Rural workers regularly fed marmosets in a forested area near the buildings, and there was close contact between animals and humans. Apathy, salivation, incoordination, ataxia, and vocalization were the main clinical findings. At necropsy, marmosets had enlarged lymph nodes and splenomegaly (7/7), and rarely ulcers on the tongue (1/7). Microscopy showed necrotizing meningoencephalitis (6/7), ulcerative necrotizing glossitis (1/7), and necrotizing hepatitis (1/7). Intranuclear basophilic inclusion bodies were detected in neurons, hepatocytes, and epithelial cells of the tongue, which showed strong immunostaining by an immunohistochemical assay that detects HSV. **Conclusions:** HSV infections are uncommonly reported in free-ranging marmosets. The close contact between marmosets and humans in anthropized areas has increased as a result of the destruction of natural habitats and may enable the spread of pathogens between species. Therefore, interactions between NHPs and human populations in urban and peri-urban areas must be minimized to prevent zoonotic diseases and promote conservation of NHP species.

D-57: INVESTIGATING EPIZOOTICS: ACUTE FATAL TOXOPLASMOSIS IN URBANIZED FREE-RANGING BLACK-TUFTED MARMOSETS (CALLITHRIX PENICILLATA)

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Background: Deaths on free-ranging non-human primates (NHPs) is a public health concern in endemic areas for Yellow Fever (YF). Investigations of outbreaks are determinant to the differential diagnosis between YF infections and other acute diseases such as toxoplasmosis. **Objective:** Characterize an outbreak of acute fatal toxoplasmosis in free-ranging urbanized marmosets, an uncommonly reported condition. **Methods:** Acute deaths in free-ranging marmosets were investigated in the Federal District, Brazil. Epidemiological, pathological, and immunohistochemical (*Toxoplasma gondii*) findings were determined. **Results:** Three adult black-tufted marmosets were found dead in a suburban area of Brasilia, Brazil, and were collected for pathological and immunohistochemical evaluation according to the National Epizootic Surveillance Program for the Control of Yellow Fever. Marmosets had been observed on trees and searching for food on the ground. Dogs and cats also frequented the same region. Grossly, hepatomegaly with an enhanced lobular pattern and splenomegaly were the main findings. The liver showed mild to moderate centrilobular to midzonal vacuolar degeneration, random necrosis within mild lymphohistiocytic inflammation, and scarce intralesional banana-shaped tachyzoites. Spleen had mild follicular necrosis, numerous free tachyzoites, and occasional protozoan cysts. Mild interstitial pneumonia was also detected. Numerous free tachyzoites and cysts showed strong immunostaining for *Toxoplasma gondii*. **Conclusions:** The differential diagnosis of YF in epizootics of acute liver necrosis in urbanized free-ranging marmosets is essential for the Public Health Surveillance Services to prevent human disease. High susceptibility of Callitrichids to toxoplasmosis promotes a challenge to the diagnosis, which develop acute fatal infections characterized by minor hepatic lesions and rare tachyzoites.

D-58: UNUSUAL IMMUNOHISTOCHEMICAL STAINING CHARACTERISTICS IN A CANINE RHABDOMYOSARCOMA

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Background: A necropsy was performed on an 11.5-year-old male neutered poodle cross euthanized for progression of a suspected gingival amelanotic melanoma, which had been unresponsive to radiation therapy. Necropsy identified neoplastic masses in the buccal mucosa, right atrium, lungs, adrenal glands, and omentum. **Objective:** Our objective was to immunohistologically characterize the neoplasm. **Methods:** Routine hematoxylin and eosin staining was performed on the original gingival biopsy and representative sections of gingiva, lungs, heart, adrenal glands, and omentum. Immunohistochemistry (IHC) for sarcomeric actin, smooth muscle actin (SMA), desmin,

myoglobin, MyoD1, and myogenin was performed on the original biopsy, and IHC for skeletal muscle actin, desmin, and MelanA was performed on postmortem samples. **Results:** Histologically, the neoplasm was a poorly-demarcated, unencapsulated, densely cellular, infiltrative mass composed a pleomorphic population of polygonal, round, and spindle cells. Anisocytosis and anisokaryosis were marked, with frequent binucleation. Neoplastic cells in the postmortem tissues exhibited strong cytoplasmic immunoreactivity for sarcomeric actin, but not for desmin or MelanA. Tissues in the initial biopsy showed strong cytoplasmic immunoreactivity for sarcomeric actin and desmin, and weak cytoplasmic immunoreactivity for myoglobin, but no immunoreactivity for MyoD1, myogenin, or SMA. **Conclusions:** The diagnosis of rhabdomyosarcoma is confirmed by positive immunoreactivity for sarcomeric actin, and myoglobin, and negative immunoreactivity for SMA and MelanA. Negative immunoreactivity for MyoD1 and myogenin is unusual; in a published review of immunohistochemical expression in canine rhabdomyosarcoma, 4/4 cases expressed myogenin, and 6/6 cases expressed MyoD1. Accurate diagnosis of rhabdomyosarcoma may require an immunohistochemical panel.

D-59: DISSEMINATED ACTINOMYCETES SPECIES INFECTION IN TWO PET CHINCHILLAS

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Two cases of disseminated infection due to actinomycetes are described in two chinchillas. A 13-year-old chinchilla with gastrointestinal obstruction was presented to the Foster Hospital for Small Animals Emergency Service at the Cummings School of Veterinary Medicine at Tufts University. She was lethargic, inactive, hypothermic, bradycardic, and had fluid-filled intestines. Because of her guarded prognosis, the owner elected euthanasia. At post-mortem examination, hundreds of nodules were scattered throughout the abdominal wall, gastrointestinal serosa, kidney, liver, diaphragm, and pelvic cavity, where they coalesced and circumferentially encompassed the bladder, uterine body, and rectum. On histopathology, nodules consisted of pyogranulomatous inflammation with gram-positive, acid-fast filamentous bacterial organisms with right-angled branching morphology that was most consistent with *Nocardia* species. Two months later, a 4-year-old chinchilla was presented to the Pathology Service for post-mortem examination after being found dead. At post-mortem examination, a focal ulcer was present on the left metatarsal pad. Multiple nodules were present throughout the myocardium and on one mitral valve leaflet. Cytologic evaluation of the nodules identified filamentous rods, most consistent with *Actinomyces* or *Nocardia* species. On histopathology, these nodules were pyogranulomas containing filamentous bacteria. In the lungs, liver, and kidneys, filamentous bacteria were present in vessels, with associated necrotizing pyogranulomatous vasculitis. Necrotizing encephalitis with filamentous bacteria was seen in the brain. Filamentous bacteria were also present in the foot ulcer, which was presumed to be the initial site of infection. A Gram stain performed on sections of pyogranulomas was inconclusive, and a Fite's acid-fast stain was negative.

D-60: MALIGNANT MESOTHELIOMA IN A LION

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Abstract: Case Report: An 18-year-old African lioness is presented to Mississippi State University for necropsy after euthanasia due to increased respiratory rate and effort, inappetence, and weight loss. Gross examination reveals voluminous amounts of cloudy, yellow fluid in the thoracic cavity and diffusely atelectatic lungs. The caudodorsal thoracic cavity contains a large mass filled with serosanguinous fluid and necrotic debris. The external surface of the mass and the visceral and parietal pleural surfaces of the thoracic cavity are multifocally covered by variably sized tan plaques and cauliflower shaped nodular masses. Mediastinal and perihilar lymph nodes are diffusely enlarged. Histologically, pleural nodules consist of a central collagenous stromal core supporting numerous arborizing and papilliferous fronds lined by rows of neoplastic cuboidal cells. Neoplastic cells display mild to moderate anisocytosis and anisokaryosis. Cells have a large nuclear to cytoplasmic ratio with moderate amounts of eosinophilic cytoplasm and a prominent oval nucleus with a small single nucleolus. The mitotic rate is 11 (2.37mm^2). Regional lymph nodes demonstrate evidence of metastasis. Gross and histologic lesions are diagnostic for malignant mesothelioma. The lining of the cystic mass is necrotic, precluding definitive diagnosis. Discussion: Mesothelioma is a rare pleural tumor originating from the mesothelium of the thoracic, peritoneal, and pericardial cavities. Spontaneous mesothelioma is rare but sporadic cases are reported in multiple animal species. Although a recognized cause of malignant mesothelioma in both humans and animals is prolonged or heavy exposure to asbestos, this lion had no known history of asbestos exposure.

D-61: EQUINE PROTOZOAL MENINGOENCEPHALITIS ASSOCIATED WITH NEOSPORA SPECIES IN A CAPTIVE BRED ZEBRA (EQUUS ZEBRA)

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Background: This report describes the clinical and pathologic changes of equine protozoal meningoencephalitis (EPM) in a 6 year old, female, zebra (Equus zebra) in Arizona. The zebra had a three year history of progressive neurologic signs that included hind limb ataxia, head tilt, and circling. **Objective:** To determine the cause of progressive neurologic signs in a captive-bred zebra (Equus zebra). **Methods:** On postmortem, sections of brain and brainstem were fixed in 10% formalin, routinely processed, and stained with hematoxylin and eosin. Immunohistochemistry and PCR were completed for *Sarcocystis neurona* and *Neospora* species. **Results:** On gross examination the spinal cord, brain, and vertebral column were unremarkable. On histopathology, the brain and brainstem had multifocal areas of lymphohistiocytic meningoencephalitis associated with numerous 25-30 micrometer in diameter protozoal cystic structures with a discernible outer wall containing numerous 2x4 micrometer oval to crescent-shaped tachyzoites. Immunohistochemistry indicated that the protozoal organisms stained positive for *Neospora* species and negative for *Sarcocystis neurona* antigen. PCR of the brain for *Neospora* species confirmed that the organism is most

likely *Neospora caninum*. **Conclusions:** Equine protozoal meningoencephalitis is a common infectious cause of neurologic signs in equines and is most frequently associated with *Sarcocystis neurona* and, less commonly, *Neospora* species. Neospora associated EPM is most commonly caused by *Neospora hughesi* and less commonly *Neospora caninum.* Furthermore, Neospora associated EPM is not well documented in wild and captive bred equids. To the best knowledge of the authors, this is the first report to describe Neospora associated EPM in a zebra.

D-62: PALISADING GRANULOMAS IN DOGS

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Background: Palisaded granulomatous dermatitis is a histologic pattern recognized in various cutaneous inflammatory lesions secondary to presumed collagen damage. Putative association with physical trauma and the predominance of this pattern has been clinically termed palisading granuloma. **Objective:** To characterize the histopathologic appearance and cellular infiltrate of palisading granulomas in dogs and document salient clinical features. **Methods:** Retrospectively, cases were identified via surgical biopsy database search from 1/1/2007 to 1/1/2019. Inclusion criteria were nodular lesions of the dermis or subcutis with a palisading histiocytic infiltrate targeting dermal collagen. Infectious causes were ruled out via standard histochemical stains (i.e. Gram, PAS/GMS, and Ziehl-Neelsen acid fast) and/or clinical data. Immunohistochemical (IBA1, CD204, E-cadherin) and histochemical (Masson's trichrome) stains were assessed with semiquantitative scoring to confirm and define histiocytic populations and dermal degeneration, respectively. Medical records were also assessed for signalment, clinical features, treatment outcome and comorbidities. **Results:** 36 cases were included. IBA1 and CD204 demonstrated moderate or strong positivity in all cases. E-cadherin was negative in 33 cases and weakly positive in three cases. Moderate or strong evidence of collagen degeneration was identified in 32 cases, mild evidence in three cases, and one case was negative. The majority of cases (31/36) were from large breed dogs (≥ 50 lbs). Focal nodules (31/36) were most common with a predominance for the head/face (19/36) and extremities (18/36). No evidence of recurrence was identified. **Conclusion:** Palisading granulomas are a distinct, non-neoplastic, histiocytic-predominant inflammatory entity in dogs associated with altered dermal collagen and favorable prognosis.

D-64: METASTATIC ORBITAL JUVENILE EMBRYONAL RHABDOMYOSARCOMA IN A LABRADOR RETRIEVER

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An 11-month old dog had a three-week history of rapidly progressive periorbital swelling. She was presented to the Foster Hospital at the Cummings School of Veterinary Medicine at Tufts University, where physical examination revealed right-sided orbital and maxillary swelling and asymmetry, with exophthalmos and globe deviation. The right submandibular lymph node was enlarged. Chest radiography

demonstrated soft tissue opacities in the lungs and dorsal to the cardiac silhouette. Cytologic examination showed findings consistent with an anaplastic neoplasm in the right periorbital region, with metastasis to the lymph node. Neoplastic cells exhibited moderate anisocytosis and anisokaryosis and had a spindle-like or stellate morphology. Because of the poor prognosis, the owners elected euthanasia. At postmortem examination, the right maxillary bone was expanded and effaced by a large, poorly demarcated mass that effaced cortical bone, extended into the maxillary sinus, infiltrated the zygomatic process and pterygoid muscle, and disfigured and compressed the right orbit, with exophthalmos and deviation of the globe. Similar masses effaced the submandibular lymph nodes, kidney, liver, and heart. Histopathologic findings were similar to those seen cytologically. The masses comprised bundles and streams of pleomorphic mesenchymal cells with myogenic differentiation. Neoplastic cells expressed positive immunolabeling for vimentin and desmin, and negative labeling for IBA -1. Overall, the findings were consistent with a diagnosis of orbital juvenile embryonic rhabdomyosarcoma with widespread metastases. Although rare, orbital rhabdomyosarcomas are highly malignant in young dogs. They frequently metastasize, and those reported in dogs younger than two years tend to be the most aggressive.

D-65: LYMPHOMA IN 2 CAPTIVE COPE'S GREY TREE FROGS (HYLA VERSICOLOR)

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Two frogs from a colony of captive-bred Cope's gray tree frogs (Hyla versicolor) originating from the same egg clutch in the North Carolina Aquarium System exhibited various degrees of ill-thrift over two years, warranting euthanasia of multiple frogs. These 3-4 year old frogs gradually developed acute, bilateral flaccid hindlimb paralysis, but retained mental acuity, appetite, and mobility in the forelimbs. After failing to improve with antibiotic, steroid,or non-steroidal anti-inflammatory therapy the two frogs were submitted for postmortem examination. In both cases, sheets of neoplastic round cells infiltrated and effaced the skin, liver, spleen, peritoneal adipose; other organs were variably affected in each frog. Neoplastic cells had distinct cell borders, scant eosinophilic cytoplasm, and round nuclei with coarsely clumped chromatin and an indistinct nucleolus. Anisocytosis and anisokaryosis are mild. Immunohistochemistry demonstrated moderate membranous immunoreactivity for CD3, and did not express CD20, vimentin, or pan-cytokeratin; internal controls reacted appropriately. Based on the histomorphologic findings associated with the positive immunohistochemistry labeling for CD3, the diagnosis of T-cell lymphoma was made. In general, neoplasia is infrequently diagnosed in amphibian species, with lymphoma reported in four clawed frogs (Xenopus spp), a cane toad (Bufo marinus), and an Alpine newt (Ichthyosaura alpestris). To our knowledge, these results describe the first cases of spontaneous T-cell lymphoma in anuran species and highlights the diagnostic utility of CD3 and CD20 antibodies.

D-66: EQUINE HERPESVIRUS-1 VIRAL MENINGOENCEPHALITIS IN TWO NANGER DAMA

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Two *Nanger dama* (Addra gazelle), a 5-year-old female and her 9-month-old male calf, presented to the Veterinary Diagnostic Laboratory at Michigan State University for necropsy following a history of pyrexia and varying neurological signs including acute death with muscle convulsions, right eye lid paresis, head tilt and circling. Grossly they had evidence of acute muscle degeneration in limb muscles (capture rhabdomyolysis). Histologically within the cerebrum and brainstem of the adult female were glial cells and macrophages surrounding hypereosinophilic, shrunken neurons. Affected neurons occasionally had marginized chromatin with an indistinct, smudgy, eosinophilic intranuclear viral inclusion bodies. Small numbers of lymphocytes and plasma cells were present in the meninges and expanding Virchow-Robins space. A herpesvirus was detected in the brain using generic herpesvirus primers. Genomic sequencing of the PCR product identified the virus as Equine herpesvirus-1. Histologically, the brain of the male calf had similar lesions. No viral inclusions were noted. Within the eye, the anterior chamber contained hemorrhage. A large numbers of neutrophils and macrophages were present in the retina effacing the ganglion cell layer, inner plexiform layer and inner nuclear layer. Neutrophils disrupted the wall of multiple retinal vessels. The cells of the inner nuclear layer and ganglion layer had eosinophilic intranuclear viral inclusion bodies. Immunohistochemistry for Equine Herpesvirus-1 was performed and labeled cells throughout the retina. This is the first report of Equine herpesvirus-1 associated neurologic disease in *Nanger dama*. These animals were housed with zebra, which are reported to be a reservoir of Equine herpesvirus-1.

D-67: MESOTHELIOMA IN A HORSE WITH BI-CAVITARY EFFUSION

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Background: Mesothelioma is rarely described in horses. **Objective:** Description of the cytomorphologic features of mesothelioma in a horse with histopathology and immunohistochemistry. **Methods:** A 16-year-old Thoroughbred presented to WSU with bi-cavitary effusion. Peritoneal and pleural effusion were submitted for review. **Results:** The peritoneal effusion had a nucleated cell count of 6,160 cells/microliter and a protein of 3.4 g/dL. Rare clusters of large, atypical polygonal to rounded cells situated in clusters were observed. These cells had round to oval, eccentric nuclei with finely stippled chromatin and 1-6 variably sized and shaped nuclei. They had moderate to abundant amounts of basophilic cytoplasm that frequently contained discrete vacuolation and occasionally had scalloped cytoplasmic borders. These cells displayed moderate anisocytosis and anisokaryosis, and occasional mitotic figures were

observed. The pleural effusion had a cell count of 6,270 cells/microliter and a protein of 3.2 g/dL. The predominant cells in the pleural effusion were similar to the large atypical cells described in the peritoneal effusion. Necropsy with histopathology revealed multifocal plaques on the serosal and peritoneal surfaces comprised of atypical mesothelial cells. The neoplastic cells were positive for vimentin and cytokeratin, consistent with mesothelioma. The lungs also contained thrombi that were suspicious for neoplastic cell emboli, which could explain the presence of high numbers of neoplastic cells in the pleural effusion. **Conclusion:** Although cytology may be helpful in identifying a neoplastic effusion, histopathology and immunohistochemistry are needed for a definitive diagnosis of mesothelioma.

Education Poster Session

ED-01: SURVEY OF INSTITUTIONAL TEACHING APPROACHES TO CLINICAL-YEAR CLINICAL PATHOLOGY INSTRUCTION AND COMPARISON WITH PRIOR SURVEY RESULTS.

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Background: Veterinary clinical pathology rotations in the final (clinical) year of veterinary school are taught separately from routine diagnostic service in many schools, but minimal published data is available on this topic or on approaches to teaching and assessment. **Objective:** Our goal was to characterize the structure, format, content, and approaches to teaching clinical pathology in the clinical year of veterinary school, with the aim of creating recommendations for future improvement in instruction. **Methods:** An online survey of veterinary institutions around the world was conducted in 2019. Questions focused on microscopy teaching format, personnel involved in instruction, and assessment methods in core clinical pathology rotations; and challenges and successes with clinical pathology instruction. Data analysis included comparison with results from a similar survey conducted in 1997. **Results:** 30 completed surveys were received from 10 countries. Rotation formats, content, and assessment varied greatly, but 27/28 (96.4%) rotations were taught separate from diagnostic services. Shifts in teaching strategies and rotation format since the 1997 survey included: (1) increased use of projection microscopy and decreased use of multi-headed microscopy; (2) more teaching by medical technologists and residents, and less teaching by faculty; and (3) increased numbers of students per rotation in 2019. **Conclusions:** Based on these findings, development of universal, specific learning outcomes and competencies and a standardized assessment strategy are recommended to improve consistency in content and enhance teaching efficiency. Evaluation of online teaching strategies and digital tools, and further comparison of traditional vs. digital microscopy instruction are also warranted.

ED-02: TELL THE CELL: AN ADAPTATION OF "GUESS WHO?" GAME FOR TEACHING CYTOLOGY TO CLINICAL PATHOLOGY RESIDENTS AND VETERINARY STUDENTS

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We developed an interactive game, "Tell the Cells", an adaptation of the famous board game "Guess Who?" as a tool to improve interactions among instructors, residents, and veterinary students, and to reinforce concepts, grow critical thinking, and evoke interest in Clinical Pathology. Originally, two players have a set of 24 face cards of people with different characteristics facing up in the boardgame. Each player draws one mystery card corresponding to one of the 24 face cards and alternates asking yes or no questions trying to figure out the opponents' mystery card first, flipping down the cards not compatible with the question asked. We used a free digital painting software to draw 24 cells from different origins (i.e., hematopoietic, epithelial, and mesenchymal) and adapted the game rules to increase complexity. In our version, questions may not pertain to morphologic characteristics, and therefore may be unknowingly incorrectly answered. Three outcomes are possible: player A guesses and answers correctly, receiving 3 points; guesses correctly but answers incorrectly, receiving 1 point; or has its cell guessed by player B, receiving zero points. Incorrect attempts to guess the opponent's cell will result in losing one point from the total score. The winner will be the one who sums 6 points first. The game prototype was brought to the 2019 ACVP/ASVCP Annual Meeting and tested during the Veterinary Student and Resident Forum. It was well-received, and we aim to apply it to a larger population of students to evaluate its impact on educational outcomes.

ED-03: IMPLEMENTING STRATEGIES TO EXPOSE COLORADO STATE UNIVERSITY UNDERGRADUATES, GRADUATE VETERINARY STUDENTS AND PATHOLOGY RESIDENT TRAINEES TO TOXICOLOGIC PATHOLOGY

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A large proportion of future positions for veterinary pathologists are projected to be within the toxicologic pathology industry. Using the ACVP members search tool, 514 of active ACVP members presently identify themselves as employed by industry in comparison to 456 in academia. Unfortunately, undergraduate and graduate veterinary school programs generally lack dedicated resources to support toxicologic pathology training due to both funding and staffing limitations. Only 1 training program in North America (Michigan State University) has a dedicated toxicologic pathology program. Organizations like the ACVP, STP, and IATP have initiated value added activities designed to support veterinary schools including the STP-ACVP coalition, externships, travel scholarships, speaker visits, and social media applications (Webinars). The expansion of digital pathology and distance learning has created an opportunity to expand the reach of these and similar efforts. A comprehensive program has been initiated with Colorado State University to model an approach to increasing student exposure to toxicologic pathology. Activities include lectures and seminars with pre-vet, veterinary, and graduate/resident groups and faculty; collaborative research and internship experiences; and distance training experiences using digital pathology technology. Preliminary outcome measures of this collaboration suggest a high return on investment for both the trainer and trainee. Details of the program will be provided as well as additional plans to expand and deepen activities that will support the training of future generations of toxicologic pathologists.

ED-04: HOW CLINICAL PATHOLOGIST ARE DOING ON QUARANTINE? IMPACT OF SARS-COV-2 PANDEMIC ON WORKING ACTIVITIES AND USE OF VIRTUAL CONTINUING EDUCATION TOOLS FOR CLINICAL PATHOLOGISTS OF BRAZIL.

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Background: Since pandemic arrived to Brazil, work activities of clinical pathologists have been impacted and virtual conferences/courses have been essential to continuum education during the social distance. **Objective:** To explore impact of SARS-CoV-2 pandemic on clinical pathologists' activities and use of virtual and distance education tools for continuous education. **Methods:** An online survey was carried out and a cross-sectional study was performed. Inclusion criteria comprehended graduated Brazilian veterinarians who were working on clinical pathology when first cases of Covid-19 were diagnosed. **Results:** 72 surveys were answered by veterinarians from different states of Brazil. 16,7% were from higher risk group and 40,3% lived with someone who is part of risk group. 50% had a reduction/partial suspension of working activities, 22,2% a total suspension and 26,4% kept without alteration. Referring to virtual education, 81,9% got knowledge about online conferences through social media, specifically Instagram (83,3%), WhatsApp (11,1%) and Twitter (4,2%). 72,2% attended 0 to 2 virtual clinical pathology conferences per week and 90,2% including related areas. About international conferences, only 22,2% attended virtual conferences without Portuguese subtitles and 20,8% attended lives despite time zone difference between countries. A total of 56,9% attended to pre-recorded conferences and 68,1% considered extremely important to compilate these conferences on a unique platform. **Conclusion:** This study highlights the importance of virtual education and language barrier on continued education for Brazilian clinical pathologists during the pandemic period.

Experimental Disease Poster Session

E-01: RHODOCOCCUS EQUI ENGAGES THE CYTOSOLIC DNA SENSING PATHWAY AND INDUCES TYPE I IFN EXPRESSION

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Background: *Rhodococcus equi* is a common cause of pneumonia in foals younger than 6 months. Macrophage expression of the type II interferon (IFN), IFN-gamma is required for R. equi clearance. Expression of type I IFN, important for antiviral immunity, can be induced by bacterial pathogens to transiently dampen the antibacterial IFN-gamma and establish a replicative niche. The balance between type I and type II IFN expression is a critical factor in the host response to bacterial pathogens, however it is unknown R. equi modulates the macrophage interferon response to induce type I IFN expression. **Objective:** Determine if *R. equi* infection induces type I IFN expression in macrophages and by which innate immune pathway. **Methods:** Type I IFN expression was measured in RAW246.7 macrophages infected with *R. equi*. To determine if the cytosolic DNA sensing pathway is required for type I IFN induction, macrophages deficient in TBK1, STING or cGAS were infected with *R. equi,* and IFN-beta measured

by qPCR. **Results and Conclusions:** *R. equi* infected RAW 246.7 and murine bone marrow derived macrophages showed induction of IFN-beta, interferon stimulated genes (ISGs), TNF-alpha, IL-1beta and IL-6. IFN-beta and ISG expression was not dependent on TRIF or MyD88. TBK1, STING and cGAS were required for induction of IFN-beta and ISGs. These results indicate that *R. equi* induced expression of type I IFN occurs by engaging the cytosolic DNA sensing pathway in macrophages.

E-02: INTERLEUKIN-1 ALPHA (IL-1A) AS A SURVIVAL BIOMARKER FOR PATIENTS WITH HUMAN PAPILLOMA VIRUS (HPV)-NEGATIVE HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCC)

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Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA, USA

Interleukin-1 alpha (IL-1α) is a cytokine involved in acute phase inflammation has been shown to be upregulated in many solid tumors including head and neck squamous cell carcinomas (HNSCCs). Previous studies have shown that IL-1α expression is generally associated with poor prognosis, but this has yet to be studied in HNSCC in the context of human papillomavirus (HPV) infection. HPV infection accounts for at least 12% of pharyngeal cancer, 3% of oral cancer, and 30–60% of oropharyngeal carcinoma cases. This study is aimed at investigating the prognostic value of nuclear and cytoplasmic immunohistochemical IL-1α and β expression in HPV positive and negative HNSCC. Tissue microarrays (TMAs) containing 78 HNSCCs were analyzed for IL-1α and IL-1β expression by immunohistochemistry. Expression scores were then correlated with survival outcomes. IL-1α expression was increased in HPV negative primary tumors but IL-1β expression was not statistically significant. Expression of these cytokines was also assessed in both primary and metastatic tumors. In primary tumors, IL-1α was elevated compared to metastatic sites. Interestingly, HPV negative patients with high IL-1α expression had a statistically significant decrease in overall survival compared to HPV negative patients with low IL-1α expression. These findings indicate that IL-1α may serve as a reasonable biomarker for survival in patients with HPF negative HNSCC.

E-03: RIPK1 KINASE INACTIVATION IN THE CONTEXT OF INTESTINAL SPECIFIC AND WHOLE BODY NEMO DEFICIENCY

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Background: Mutations in *NFκB essential modulator* (*NEMO*) underlie X-linked recessive epidermal dysplasia with immunodeficiency in people. Aside from epidermal defects and immune dysfunction, some patients develop colitis that is unresponsive to hematopoietic stem cell transplant. Mice with intestinal specific NEMO loss develop colitis and enteritis, which are protected by loss of RIPK1 kinase activity. The impact of RIPK1 inactivation in the context of whole body NEMO deficiency is unknown. **Objective:** The goal of this study was to determine how ubiquitous NEMO deficiency influences the development intestinal pathology and the response to RIPK1 kinase inhibition. **Methods:** $Nemo^{+/+}Ripk1^{+/+}$, $Nemo^{fl/fl}Ripk1^{+/+}$, and $Nemo^{fl/fl}Ripk1^{D138N/D138N}$ mice were crossed with $Rosa26.creER^{T2}$ and $villin.creER^{T2}$ mice to generate whole

body and intestinal specific *Nemo*$^{-/-}$ mice. Mice were treated with tamoxifen to induce Nemo deletion and were euthanized 3-4 days after the tamoxifen final dose. **Results:** Intestinal specific *Nemo*$^{-/-}$ mice developed ulcerative colitis and increased crypt cell death in the small intestine, which were both protected against by loss of RIPK1 kinase activity. While ubiquitous *Nemo* deletion resulted in histomorphologically similar colonic lesions, granulocytic enteritis was evident in the small intestine. RIPK1 kinase inactivation provided protection in the colon, but it did not impact the development of enteritis. **Conclusions:** The impact of NEMO deficiency is cell type specific, and the pathology associated with NEMO deficiency is influenced by the diverse effects in multiple cell types. While RIPK1 kinase inhibition may not benefit the entire clinical syndrome of *NEMO* mutant patients, it may be beneficial in treating colitis in post-transplant patients.

E-04: PATHOLOGICAL FINDINGS AND PATHOGENESIS IN EXPERIMENTAL STREPTOCOCCUS PNEUMONIAE INFECTION IN MICE

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Background: *Streptococcus pneumoniae* causes sinusitis, conjunctivitis, otitis media, especially pneumonia and meningitis. **Objective:** To examine the organ localization of the agent in experimental *S. pneumoniae* infection and determine the most convenient experimental application method. **Methods:** 0,01 ml *S. pneumoniae* serotype 3 ($1x10^7$ CFU/ml) was inoculated to female, Swiss albino mice by using intranasal, intracerebral, intravenous and intraperitoneal methods. After 24 and 48 hours following the inoculation, lung, heart, brain, liver, spleen, kidney were evaluated for microbiological, histopathological, immunohistochemical examinations. **Results:** In microbiological examination, it was noticed that the agent attained to all organs earlier in intraperitoneal method than intracerebral method. Histopathologically, in liver, neutrophil, mononuclear cell infiltration, hydropic-vacuolar degeneration, necrosis and hemorrhage were more apparent in intraperitoneal groups. In intracerebral groups, besides meningitis, hemorrhage and inflammatory cell infiltration composed mostly of neutrophils, were observed in brain. Severe hemorrhage was seen in lungs after 24 hours following intravenous and intracerebral inoculations. Anti-*S. pneumoniae* immunopositivity was more distinct in intracerebral groups in brain, liver, heart; in intraperitoneal groups in kidney and spleen; in intravenous groups in liver and kidney. In terms of TNF, IL-1α, IL-6 and IL-10 positivity was apparent in intracerebral groups in brain; in intraperitoneal groups in spleen and liver. In intravenous groups, after 24 hours, immunopositivity in brain and liver was more severe in TNF compared to other cytokines. **Conclusions:** For studies of pneumococcal pneumonia, intranasal method appears not to be effective over Swiss albino mice; whereas, for studies of pneumococcal meningitis, intracerebral method seems to be more effective.

E-05: EXPERIMENTAL INFECTION OF AQUATIC BIRD BORNAVIRUS-1 IN MUSCOVY DUCKS

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Background: Aquatic bird bornavirus-1 (ABBV-1), a novel orthobornavirus, was identified in 2009 in several wild waterfowl species, and has been associated with neurologic disease and non-suppurative inflammation of the nervous system. To date, no experimental infection has been attempted to document the pathogenesis of this virus in waterfowl. **Objective:** Evaluate the ability of ABBV-1 to infect, replicate, and cause disease in experimentally-infected Muscovy ducks (*Cairina moschata*), chosen as a representative waterfowl species. **Methods:** Day-old ducklings (n = 160) were divided into 4 groups (40/group), and inoculated with ABBV-1 by one of four routes: intracranial (IC, $6.6x10^4$ FFUs [focus-forming units]/bird), intramuscular (IM, $1.3x10^5$ FFUs/bird), oral (OP, $1.3x10^5$ FFUs/bird), and control (sham-infected). At 1, 4, 8, and 12 weeks post infection (wpi), 10 birds from each group were euthanized for tissue collection. **Results:** No clinical signs or gross lesions were observed. By 12 wpi, 100% of IC and IM inoculated ducks (7/group) tested positive for ABBV-1 by RT-qPCR in the brain, spinal cord, proventriculus, kidneys, and gonads. Lymphocytic perivascular inflammation was identified in the brain of all IC inoculated ducks (*n* = 3) at 4, 8, and 12 wpi, with the most severe lesions at 4 wpi. Immunohistochemistry confirmed presence of virus within tissues. **Conclusions:** ABBV-1 delivered intracranially and intramuscularly, but not orally, can infect Muscovy ducks, showing centripetal (*i.e.*, from the muscle to the central nervous tissue) and centrifugal spread (*i.e.*, from the brain to the peripheral tissues). Muscovy ducks represent a suitable model to study ABBV-1 pathogenesis.

E-06: RESPIRATORY SYNCYTIAL VIRUS (RSV) TROPISM IN BRONCHIOLAR EPITHELIUM

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Human Respiratory Syncytial Virus (RSV) is the most common cause of viral pneumonia of infants requiring hospitalization. RSV infects airway epithelium, but the cell types of the airway susceptible to infection are not fully understood. This study determines the tropism of RSV in bronchiolar epithelium by immunohistochemical detection of antigen in airways. Six newborn colostrum-deprived lambs were infected by Aerogen nebulizer with six milliliters of the clinical isolate hRSV Memphis 37 strain (3.5×10^7 FFU/lamb) and lung tissue was collected six days post inoculation. Viral antigen was present in the cytoplasm of nonciliated cells in bronchioles and ciliated cells of bronchi within the inner portions of the bronchiolar and bronchial lumens. These cells had cytoplasmic extensions in areas to the basement membrane zone of airways. Some cells with antigen had rounded cytoplasm, were partially or completely detached and other cells were completely detached and present in the airway lumen with variable numbers of neutrophils and cell debris. Syncytial cells also contained RSV antigen.

Epithelial cells of out layers lacked antigen as did cells undergoing hyperplasia. These studies demonstrate that RSV induces cell rounding, detachment and that dying cells contribute to debris within the bronchiolar lumen thus inhibiting airflow to and from the alveoli, the site of gaseous exchange. The reason for susceptibility of these cells to infection could be due to: a) state of development/maturation, b) cell subtype (Club or type II or other cell), c) innate or adaptive immune responses, or d) all of these.

E-07: FIBROBLAST GROWTH FACTOR-21 (FGF21) ANALOG, LY2405319, DECREASES BODY WEIGHT AND HEPATIC LIVER LIPID CONTENT IN OBESE AND OVERWEIGHT CATS

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Background: Obese cats are predisposed to metabolic dyscrasias, including hepatic lipidosis. Fibroblast Growth Factor-21 (FGF21) is an endocrine hormone that mediates the fat-liver axis by increasing the rate of hepatic fatty acid oxidation. LY2405319 is an engineered FGF21 analog that may have therapeutic benefit for obese cats. **Objective:** This study tests the hypothesis that an FGF21 analog increases weight loss and decreases biomarkers of lipidosis in obese cats. **Methods:** In this cross-sectional study, obese and overweight cats were fed *ad libitum* and administered either 10 mg/kg/day of LY2405319 (LY; n=4) or saline (control; n=3) for 14 days. Body weight, food, and water intake were quantified daily throughout and following the treatment period. Metabolic parameters, serum liver enzymology, hepatic triglyceride content (proton magnetic resonance spectroscopy), and hepatic viscosity (elastography) were evaluated before and following treatment. **Results:** Treatment with LY resulted in significant weight loss (~5.99%) compared to saline (~0.31%; $p<0.001$). LY-treated cats had a trend toward decreased liver lipid content (1.86% intrahepatic lipid decrease in LY-treated cats compared to a 2.89% increase in saline-treated cats; $p=0.055$) and significantly decreased serum alkaline phosphatase ($p=0.011$). No significant changes or trends were noted in liver viscosity, serum alanine aminotransferase activity, or serum metabolic parameters (glucose, triglycerides, and cholesterol). **Conclusions:** In obese and overweight cats, LY can safely induce weight loss and likely improves liver lipid content, but does not alter metabolic parameters. Thus, LY may have potential as a therapeutic for feline obesity and hepatic lipidosis.

E-08: NEUROLOGICAL CHANGES WERE ASSOCIATED WITH BRAIN DAMAGE AND DOWNREGULATION OF BDNF LEVELS IN MICE INFECTED WITH BOVINE ALPHAHERPESVIRUS 5

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Background: The infection with Bovine alphaherpesvirus 5 (BoHV-5) has been associated with neurological disease and meningoencephalitis in cattle, characterized by neuronal loss, infiltration of immune cells and, eosinophilic intranuclear inclusion bodies in astrocytes and neurons. Astrocytes have pro-inflammatory and anti-inflammatory functions and are related with the production of neurotrophic factors,

which play an important role in the survival of neurons in different central nervous diseases. **Objective:** Our objective was to investigate a potential involvement of astrocytes and the levels of neurotrophic factors (BDNF), glial cell line derived neurotrophic factor (GDNF), and neural growth factor (NGF) with brain damage and neurological sequelae after experimental BoHV-5 infection in mice. **Methods:** Mice were infected with 10^7 $TCID_{50}$ of BoHV-5 by intracranial route and evaluated until day 3 post infection. After euthanasia, brains were removed for histopathological and immunohistochemical analysis, Western blot test, ELISA and viral titration (Protocol 272/11). **Results:** Infected animals had ruffled fur, conjunctivitis, serous nasal secretion, swollen chamfer, apatia, ataxia, hunched posture and circling. The infection promoted meningoencephalitis, neuropil vacuolation, and reactive gliosis. Cleaved caspase-3 immunopositive glio-inflammatory cells were visualized around some blood vessels and increased immunoexpression of glial fibrillary acidic protein (GFAP) was presented throughout the parenchyma. Infected animals had lower concentration of BDNF, compared with mock group and viral load of $1x10^{3,5}$ $TCID_{50}$/ml. **Conclusions:** Our results demonstrated that clinical signs were related to brain inflammation, astrocytosis and tissue damage after infection in mice. We suggest that BDNF could be involved with anti-apoptotic effect and neuroprotection during meningoencephalitis by BoHV-5.

E-09: THE USE OF AN ENGINEERED DOXORUBICIN-LOADED FERRITIN NANOCAGE: A POSSIBLE TREATMENT FOR FELINE MAMMARY TUMORS

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Background: A ferritin nanocage (HFn) that selectively binds TFR-1 has been used in humans as an efficacious and safe intracellular delivery mean for doxorubicin. In veterinary oncology, there is no data on the use of nanocages to internalize drugs via binding to TFR-1, and its value in optimizing cancer treatment is unknown. **Objective:** We compared the efficacy of the treatment with doxorubicin (DOX)-loaded ferritin nanocage (HFn(DOX)) to the use of free DOX on a feline metastatic mammary cancer cell line (FMCm) expressing TFR-1. **Methods:** FMCm cells were treated with HFn(DOX), free DOX, and HFn alone at three different concentrations (0.01mM, 0.1mM, 1mM) and examined at three different timepoints (24h, 48h, and 72h post-treatment). The MTS cell proliferation assay was used to detect cell proliferation by measuring the cell metabolic activity. The experiment was carried out in quadruplicates and was repeated three times. **Results:** The results identified a trend, showing how FMCm cells treated with HFn(DOX) proliferated less than cells treated with free DOX. This difference was specifically observed at 0.01mM at 72h post-treatment ($p<0.05$), whilst at 0.1mM it was detected also 48h post-treatment ($p<0.05$). At the concentration of 1mM at all times set, no statistical difference was observed. To note that the use of HFn alone did not alter cell proliferation. **Conclusions:** The use of a doxorubicin-loaded ferritin nanocage with selective binding to TFR-1 induces TFR-1-expressing FMCm cells to internalize the treatment more rapidly, resulting in less cell proliferation.

E-10: EFFICACY OF CONCURRENT VACCINATION WITH MODIFIED-LIVE PRRSV-1 AND PRRSV-2 VACCINES AGAINST HETEROLOGOUS DUAL PRRSV-1 AND PRRSV-2 CHALLENGE IN LATE TERM PREGNANCY GILTS

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The objective of this study was to evaluate the effect of concurrent vaccination with a porcine reproductive and respiratory syndrome virus (PRRSV)-1 modified-live virus (MLV) vaccine and a PRRSV-2 MLV vaccine against a dual heterologous PRRSV-1 and PRRSV-2 challenge in late term pregnancy gilts. Gilts were concurrently administered PRRSV-1 and PRRSV-2 MLV vaccines at 21 days prior to breeding at separate anatomical sites and were inoculated intranasally with both PRRSV types at 93 days of gestation. Vaccinated gilts had a higher number of live-born and weaned pigs, and a decrease in stillbirths compared to the unvaccinated control group following a dual challenge. Concurrent vaccination resulted also in the reduction of both PRRSV-1 and PRRSV-2 viremia which correlated with an increase in the number of PRRSV-1 and PRRSV-2 specific interferon-γ secreting cells (IFN-γ-SC). We believe the T cell responses contributed to the reduction of both PRRSV-1 and PRRSV-2 viremia. The results presented here demonstrate that concurrent vaccination with PRRSV-1 and PRRSV-2 MLV vaccines improves reproductive performance, reduces viremia of PRRSV-1 and PRRSV-2, and induces protective T cell reactions against dual PRRSV-1 and PRRSV-2 challenge in late term pregnancy gilts without local and systemic adverse reactions related to concurrent vaccination.

E-11: INTEGRATED STRESS RESPONSE IN RAT CORNEAL ECTASIA

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Purpose: Keratoconus, a progressive, degenerative corneal disease, represents the second leading indication for corneal transplantation globally. We have previously demonstrated that components of the integrated stress response (ISR) are upregulated in human keratoconic donor tissue, and treatment of normal tissue with ISR agonists results in attenuated collagen production. We sought to establish an animal model to further elucidate the role of the ISR in the development of the keratoconic phenotype. **Methods:** Animal care and usage followed NIH guidelines and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Four-week-old female SD rats were randomly treated with topical SAL003 in hypotonic pegylated vehicle or vehicle every 48 hours for four doses, monitored daily for ocular inflammation, and euthanized at 1, 14, or 28 days after treatment was withdrawn. STT1, IOP, body weight, and blood glucose measurements were obtained at baseline and prior to euthanasia. Globes were subject to routine histopathology, immunocytochemistry for ATF4, and qPCR for *Col1a1* expression. ANOVAs were used to assess statistical significance ($\alpha = 0.05$). **Results:** There were no significant changes in ocular parameters or blood glucose. SAL003-treated eyes developed corneal ectasia, nuclear ATF4 expression within the axial cornea, and significantly decreased *Col1a1* expression (0.33+/- 0.11, $p = 0.0014$). **Conclusions:** *In vivo* treatment with an ISR agonist recapitulates key features of the activated ISR and keratoconic phenotype including ectatic corneal stroma, attenuated

collagen production, and increased nuclear ATF4 expression. Establishing a rodent model for keratoconus is essential for future evaluations of pathogenesis and therapeutic interventions.

E-12: QUANTIFICATION OF FECAL MUC5AC IN SWINE DYSENTERY

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Background: Swine dysentery (SD) induced by *Brachyspira hyodysenteriae* (Bhyo) is associated with mucohemorrhagic diarrhea and typhlocolitis in grower-finisher pigs. Marked *de novo* expression of mucin 5AC (MUC5AC) has been found in pigs with SD in previous studies. **Objectives:** Use an enzyme-linked immunosorbent assay (ELISA) to measure the quantity of MUC5AC in feces of pigs with SD as the disease progresses and to evaluate its potential as a diagnostic monitoring tool. **Materials and Methods:** A total of 36 gilts (12 controls and 24 inoculated with Bhyo) were included. ELISA of MUC5AC detection in feces was performed on samples obtained every other day from 4 days post inoculation and the level of detection was compared with the clinical fecal score. MUC5AC detection in fecal samples (ELISA) at the study endpoint was compared with detection in colonic contents (ELISA) and expression in colonic tissue samples (IHC). **Results:** There was a numerical trend for increased MUC5AC quantities in the feces as the disease progressed compared to the controls but with high variability. There was no correlation between colonic IHC results and fecal detection of MUC5AC by ELISA in pigs with SD. Significantly increased detection of MUC5AC was observed in fecal and colonic samples of pigs with SD at the endpoint compared to controls (both $P<0.0001$). Correlation of fecal MUC5AC quantities with clinical fecal scores was limited. **Conclusions:** Fecal ELISA for MUC5AC is not a reliable monitoring tool for disease progression in SD but may have value during overt clinical disease.

E-13: MORPHOLOGIC CHARACTERIZATION OF A PARANEOPLASTIC PERIPHERAL AXONOPATHY IN MICE WITH SUBCUTANEOUS PATIENT-DERIVED PANCREATIC DUCTAL ADENOCARCINOMA

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Background: The pathogenesis of paraneoplastic peripheral neuropathies remains poorly understood given their rarity and the lack of a well-established animal model. During maintenance of a patient-derived xenograft (PDX) model of pancreatic ductal adenocarcinoma (PDA) in NSG mice, a peripheral polyneuropathy was identified. While paraneoplastic peripheral neuropathies are often thought to be immunologically mediated, NSG mice are severely immunodeficient, suggesting a distinct pathogenesis. **Objective:** This study aims to characterize clinical signs and morphologic lesions in NSG mice with a paraneoplastic peripheral neuropathy to establish a model and better understand this disease. **Methods:** Twenty-six NSG mice aged 1 and 2.5 months were engrafted with a 32 mm^3 subcutaneous PDX PDA tumor in the right flank and examined for neurologic health. Upon observation of clinical neurologic signs, spinal cords and

sciatic nerves were collected and examined by light microscopy. Sciatic nerves were further examined by transmission electron microscopy (TEM). **Results:** Within 11-109 days after implantation of the PDX PDA, 19/26 mice (73%) developed unilateral or bilateral hindlimb paresis or paralysis with occasional forelimb involvement. Light microscopy and TEM revealed active degeneration of large myelinated axons with secondary myelin degeneration within the spinal ventral roots and sciatic nerve of affected mice, supporting a peripheral axonopathy with a motor neuron predilection. Clinical signs were observed over three passages with the PDX PDA. **Conclusions:** This study describes a unique and reproducible mouse model of paraneoplastic peripheral neuropathy that may be instrumental in understanding the pathogenesis of these conditions.

E-14: HISTOPATHOLOGICAL FINDINGS AND EXTRAVASCULAR PARASITES IN A MURINE MODEL OF AFRICAN TRYPANOSOMIASIS

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Trypanosoma brucei is the causative parasite of human and animal African trypanosomiasis, vector-borne neglected tropical diseases of One Health importance. Although *T. brucei* is a pathogen of the hemolymphatic and central nervous systems, parasites have been found outside of the vasculature in multiple other tissues. Although these tissue-resident parasites (TRPs) are poorly understood, research suggests that adipose tissue TRPs are involved in the chronic wasting syndrome of African trypanosomiasis. TRPs may also play a role in immune evasion and drug resistance. We investigated the histopathology of TRPs in a murine model of *T. brucei* infection. C57Bl/6J mice were infected with *T. brucei brucei* and euthanized on days 6, 10, and 14 post-infection. Samples of fat, kidney, heart, lung, and brain were collected for histopathology, and the presence and distribution of TRPs in each tissue were scored semi-quantitatively. The lung, heart, and gonadal fat were most commonly affected, with the highest scores. During early infection, TRPs were generally few in number and restricted to perivascular connective tissue, but were more abundant and widespread in later infection. Other findings included disruption of collagen networks, edema, and mononuclear inflammation. These findings are in agreement with reports of TRPs during natural infection. The presence of TRPs in multiple tissues and their association with other pathologies suggest that they may play an underappreciated role in disease. Notably, TRPs have an affinity for connective tissue, which may be important for parasite entry and persistence in tissues.

E-15: EXPERIMENTAL INFECTION OF AQUATIC BIRD BORNAVIRUS-1 IN CHICKENS

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Background: Aquatic bird bornavirus-1 (ABBV-1), a novel orthobornavirus, was identified in 2009 in several wild waterfowl species, and has been associated with

neurologic disease and non-suppurative inflammation of the nervous system. The virus has been since identified in multiple avian taxa. No experimental infection has been attempted to document the pathogenesis of ABBV-1 in commercial poultry. **Objective:** Evaluate the ability of ABBV-1 to infect, replicate, and cause disease in experimentally-infected White Leghorn chickens (*Gallus gallus domesticus*). **Methods:** Day-old chicks (*n* = 160) were divided into 4 groups (40/group), and inoculated with ABBV-1 by one of four routes: intracranial (IC, $6.6x10^4$ FFUs [focus-forming units]/bird), intramuscular (IM, $1.3x10^5$ FFUs/bird), oral (PO, $1.3x10^5$ FFUs/bird), and control (sham-infected). At 1, 4, 8, and 12 weeks post infection (wpi), 10 birds from each group were euthanized for tissue collection. **Results:** No clinical signs or gross lesions were observed. By 12 wpi, all brains (8/8) and most spinal cords (6/8) from IC inoculated chickens were positive for ABBV-1 by RT-qPCR. By the same time point, 3/9 spinal cords and 2/10 brains from IM inoculated chickens tested positive. Lymphocytic perivascular inflammation was identified in the brain of all IC inoculated chickens at 8 and 12 wpi, with increased severity at 12 wpi. Immunohistochemistry confirmed the presence of the virus within tissues. **Conclusions:** ABBV-1 delivered intracranially and intramuscularly, but not orally, can infect chickens. Chickens are susceptible to ABBV-1 infection and development of microscopic lesions, although clinical signs may require more time to appear.

E-16: DEVELOPMENT OF OCULAR PATHOLOGY IN A CANINE MODEL OF MUCOPOLYSACCHARIDOSIS IIIB

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Mucopolysaccharidosis (MPS) type IIIB is a rare lysosomal storage disease caused by a deficiency in the enzyme a-N-acetylglucosaminidase. This enzyme deficiency results in accumulation of the glycosaminoglycan (GAG) heparan sulfate in numerous tissues and organs, including the retina and optic nerve. Ocular manifestations of MPS IIIB include retinopathy, pigmentary retinal degeneration, optic atrophy, and optic disc swelling. Retinopathy is the most common finding in MPS IIIB patients. As modern therapies, such as enzyme replacement therapy, have the potential to improve and extend the lives of MPS IIIB patients, addressing ocular disease has taken on greater significance. The objective of the current study is to characterize ocular pathology in a canine model of MPS IIIB. This data will improve our understanding of the pathogenesis of this disease and identify outcome measures for future therapeutic studies. Entire globes from 2 to 26-month-old MPS IIIB affected dogs and age-matched controls were collected and evaluated microscopically for morphologic changes and accumulation of metabolic storage material. PAS-positive and Luxol fast blue-positive cytoplasmic inclusions were identified in vacuolated cells within various layers of the retina suggesting primary storage of GAG and secondary accumulation of gangliosides in MPS IIIB affected dogs. Lysosomal integral membrane protein 2 (LIMP-2) immunohistochemistry was performed and demonstrated expanded lysosomal compartments within the retinal cells of MPS IIIB affected animals compared to controls. Results of this study characterize ocular pathology in the canine model of MPS IIIB and provide foundational data for future therapeutic efficacy studies.

E-17: ACTIVATION OF THE TOLL-LIKE RECEPTOR 4 (TLR4) SIGNALING COMPLEX IN A CANINE MODEL OF MUCOPOLYSACCHARIDOSIS IIIB

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Mucopolysaccharidosis (MPS) IIIB is a primarily neuropathic lysosomal storage disease characterized by a deficiency in a lysosomal enzyme (N-acetyl-alpha-glucosaminidase) important for the stepwise degradation of the glycosaminoglycan heparan sulfate (HS). The pathogenesis of neuroinflammation and neurodegeneration in MPS IIIB is incompletely understood. We have previously demonstrated microgliosis, astrocytosis, and elevated proinflammatory cytokines (TNF-a and IL-1b) in select neuroanatomic regions of MPS IIIB affected dogs aged 2 to 26 months. Accumulated HS has been demonstrated to activate the toll-like receptor 4 (TLR4) signaling pathway in mouse models of MPS IIIB, resulting in production of proinflammatory cytokines, chemokines, and oxidative agents via microglia and monocytic origin cells. We hypothesize that a similar mechanism for the development of neuroinflammation is occurring in the canine MPS IIIB model with activation of microglia/glia and subsequent production of inflammatory effector molecules. Considering increased proinflammatory cytokines observed in the canine MPS IIIB model, activation of the TLR4 signaling pathway, and other innate immune system pathways, should be observed. We have evaluated the expression of select TLR4 signaling pathway components, including TLR4, MyD88, and NFk-B (phospho-p65), in the brains of MPS IIIB affected dogs by quantitative western blot analysis. We have correlated the protein expression of select TLR4 signaling components to microgliosis, astrocytosis, and increased levels of proinflammatory cytokines observed in MPS IIIB affected dogs. Demonstrating activation of the TLR4 signaling pathway in MPS IIIB could help identify new therapeutic targets and better define the role neuroinflammation plays in the pathogenesis of MPS IIIB.

E-18: EVALUATING MYCOBACTERIUM BOVIS IN TWO DISTINCT U.S. FERAL SWINE POPULATIONS

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Background: Feral swine on the Hawaiian island of Moloka'i have maintained a low prevalence of bovine tuberculosis (BTB) since 1997. In the continental U.S., feral swine inhabiting the southern states create a high-risk scenario for disease spillover and potential establishment of a novel BTB maintenance host. **Objective:** Identify diagnostic challenges of BTB in U.S. feral swine populations and evaluate BTB disease susceptibility and host status in North American suids from distinct genetic backgrounds using an experimental disease model. **Methods:** Disparities were analyzed between histopathology, PCR, and culture results from Moloka'i surveillance submissions obtained at the National Veterinary Services Laboratories in Ames, Iowa, from 1999-2008. Host status was inferred via comparative pathology, immunology, transmission, and genomics between European wild boar descendants from Texas and Polynesian swine from Hawaii experimentally infected with a low-dose, 50:50 mixture of two *M. bovis* spoligotypes originating from Moloka'i and Mexico. **Results:** Only 46% of

confirmed positive cases from Moloka'i surveillance samples were in agreement between all three diagnostic assays. Tuberculous lesions were observed in lymph nodes and lung of Texas origin swine experimentally infected with *M. bovis* and IFN-gamma was detected in PBMC restimulation assays in response to PPDb and ESAT6/CFP10. Culture and genotyping results are pending. **Conclusions:** Field-sampling/environmental constraints and certain histologic features likely impacted the success of BTB diagnosis in feral swine. Naturally infected BTB feral swine from Moloka'i and experimentally infected Texas feral swine produce similar lesions, implying a possible role in disease transmission.

Industrial and Toxicologic Pathology Poster Session

T-02: NOVEL POLYMER-CROSS LINKER ADHESIVES INDUCE FOREIGN BODY AND HYPERSENSITIVITY REACTIONS WHEN INJECTED INTO THE SUBCUTIS OF EIGHTY-EIGHT MICE

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Background: Tissue adhesives have become an important component of human and veterinary wound management; however, the availability of glues that retain their adhesive properties in liquid environments is limited. Novel synthetic adhesive polymers, mimicking those naturally secreted by marine mussels, have been developed to withstand these internal liquid environments, but in vivo administration and histologic evaluation of these compounds have yet to be assessed. **Objective:** The objective of this work is to characterize the acute and chronic, local and systemic histologic responses to subcutaneous injection of five different adhesive polymer-cross linker test article combinations in mice. **Methods:** Five separate test articles, characterized by one-of-three adhesive polymers combined with one-of-two cross-linkers, were individually injected into the subcutis of eighty-eight mice. A contralateral injection of saline served as the control. The local and systemic histologic effects of the adhesive polymer-cross linker test article were evaluated at 72 hours, four weeks, and twelve weeks. Two sections of skin with either test article or saline control were collected, along with sections of heart, lung, liver, kidney, and spleen, routinely processed, stained with hematoxylin and eosin, and evaluated histologically. **Results:** Adhesive polymer-cross linker test articles were associated with a significant foreign body reaction, including infiltration of neutrophils, epithelioid macrophages, and multinucleated giant cells, with granulation tissue and fibrosis. Eosinophil and mast cell infiltrations were also increased. **Conclusion:** Adhesive polymer-cross linker test articles incite subcutaneous foreign body and prolonged hypersensitivity reactions.

T-03: QUALIFICATION OF AUTOMATED CYNOMOLGUS MONKEY CSF ANALYSIS ON THE SYSMEX XN-1000V HEMATOLOGY ANALYZER

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Background: Multiple compounds are developed for direct delivery to the central nervous system by intrathecal drug administration. Cerebrospinal fluid (CSF) evaluation

is used to monitor neurotropic drugs during preclinical studies. CSF cell counts are usually assessed with a hemocytometer chamber and a differential count done on a cytocentrifuge smear. This method is fastidious while automated analysis is possible on the Sysmex XN-1000V hematology analyzer. **Objective:** To evaluate the performance of the Sysmex XN-1000V hematology analyzer for Cynomolgus Monkeys CSF analysis and compare with manual methods. **Methods:** Assay precision, linearity, carry-over, limit of quantitation (LOQ), stability and correlation with manual methods were evaluated for total nucleated cells count (TNC), red blood cell count (RBC) using cynomolgus monkeys CSF samples, whole blood and PBS, or manufactured CSF quality controls. The automated mononuclear/polymorphonuclear differential was assessed. **Results:** Intra-assay: CV <20% for TNC and RBC; with precision at the nearest hundred for RBC; linearity of dilution: down to 5% for TNC, 20% for RBC; carry over: ≤1.0%; assay stability: 4 hours at 4ºC. Lower LOQ: 1 cell/uL for TNC, 100 cells/uL for RBC. TNC and RBC evaluated with automated and microscopic methods were of similar clinical significance. Automated 2-cell differential was generally correct. **Conclusion:** The Sysmex XN-1000V hematology analyzer is a promising automated instrument that can perform CSF samples evaluation in a high throughput laboratory, providing TNC and RBC results comparable to hemocytometer method. Automated mononuclear/polymorphonuclear differentiation is a good indicator, but not sufficient, in comparison to manual microscopic differential, to provide clinical interpretation.

T-04: COXIELLA BURNETII WHOLE CELL VACCINE REACTOGENIC RESPONSE IN A MOUSE MODEL

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Coxiella burnetii is a Gram-negative, intracellular bacterium and the causative agent of Q fever. The only approved vaccine for humans, Q-Vax, is not licensed in the United States due to the high rate of local and systemic reactions in previously sensitized individuals. A greater understanding of the immunological components responsible for these reactions is needed to produce a safe and effective vaccine. We compared vaccine reactions in three mouse strains: a Th1-biased strain (C57Bl/6), a Th2-biased strain (BALBc), and a hairless, outbred strain (SKH1), to unsensitized controls. Mice were sensitized to *C. burnetii* by intratracheal inoculation of virulent *C. burnetii* strain Nine Mile I or subcutaneous injection of formalin-inactivated whole cell vaccine (WCV; Q-vax derivative) then vaccinated subcutaneously with WCV. Vaccine sites were monitored for 14 days then collected for histopathology and flow cytometry. Multiple vaccine doses were tested for elicitation. Lesions were evaluated with H&E and immunohistochemistry. Acute hypersensitivity reactions were elicited in infection- and vaccine-sensitized C57Bl/6 and SKH1, but not BALBc mice, compared to unsensitized controls. Flow cytometry of immune cells extracted from vaccine sites revealed increased numbers of IFNγ+ CD4 T cells indicating a Th1-mediated delayed-type hypersensitivity. Our mouse model of *C. burnetii* vaccine reactogenicity can be used to help develop novel *C. burnetii* vaccines that provide protection without causing adverse reactions.

T-05: ZINC TOXICOSIS IN TWO DOGS WITH GASTRIC FOREIGN BODIES (PENNIES)

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Background: Zinc toxicosis in dogs is commonly the result of ingestion of pennies minted in 1983 or later, which are composed of 97.5% zinc. Following ingestion, the acidic environment of the stomach releases free zinc, which forms zinc salts that are absorbed and induce intravascular hemolysis and necrosis in multiple organ systems. **Objective:** To characterize the gross and histologic findings in two dogs with zinc toxicosis. **Methods:** Two dogs (Dog 1, a 20-month-old spayed female Yorkshire terrier and Dog 2, an intact male Shih Tzu mix of unknown age) were presented for routine necropsy. **Results:** On gross examination, both dogs were severely icteric. The spleens were diffusely enlarged and meaty. Within the stomach of Dog 1, there were 4 pennies and there were 5 pennies in the stomach of Dog 2. Histologically, both dogs had similar lesions. There was multifocal centrilobular degeneration of hepatocytes and severe cholestasis in the liver. In the kidneys, there was multifocal tubular degeneration with hemoglobin casts. The pancreas was unremarkable in both animals. Mineral analysis of the liver revealed a zinc concentration of 1557.29 ug/g in Dog 1 and 953.3 ug/g in Dog 2 (Normal range: 75.00-225.00) **Conclusions:** The lesions in both dogs were characteristic of zinc toxicity. Following absorption from the duodenum, zinc salts rapidly distributed to multiple organs where they cause corrosive tissue effects. While pancreatic necrosis is typically the primary lesion in avian species, the pancreas was unremarkable histologically in both dogs and lesions were most prominent in the liver and kidney.

T-06: CATHETER-ASSOCIATED LESIONS IN RABBITS: POTENTIAL FOR CONFOUNDING STUDY RESULTS

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To minimize the number of rabbits used in drug safety studies, protocols were designed to collect blood samples from the same animals for clinical pathology to evaluate toxicity and toxicokinetics (TK), to measure drug levels over time. TK requires multiple blood draws during a 24-hour period. Use of indwelling catheters is considered more humane and effective to obtain samples than multiple venipunctures. However, it has been reported, that catheterized rabbits have developed lesions resembling infarcts, but thrombi were not observed. In this study, rabbits designated for toxicity evaluation were also used for TK blood draws, using a catheter in the medial auricular (ear) artery. The test article was delivered by dermal patch for 5 minutes/day for 28 days with a 14-day Recovery period. For TK analysis, 5 blood samples were drawn twice, one month apart, during a two-hour period. Findings unassociated with the test article and attributed to the presence of the catheter included degeneration/necrosis of the mandibular salivary gland (13 rabbits, including two controls), degeneration of the Harderian (paraocular) gland (13 rabbits, including 4 controls) and neuron necrosis in the brain in 4 females,

including one control. One treated female had a thrombus adjacent to an infarct in the brain and one treated female had a pulmonary arterial thrombus, which supported the hypothesis that infarcts developed subsequent to catheter-induced microthrombotic emboli. Recommendations for studies in rabbits include use of separate groups for toxicity and TK evaluation, enabling humane catheter use without creating potentially confounding lesions in toxicity studies.

T-07: SYMMETRIC DIMETHYLARGININE IS A SENSITIVE BIOMARKER OF GLOMERULAR INJURY IN RATS

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Background: Glomerular filtration rate is the gold-standard method for assessment of renal function but is rarely performed in routine toxicity studies. Standard serum biomarkers of renal function are insensitive and become elevated only with significant loss of organ function. Symmetric dimethylarginine (SDMA) is a ubiquitous analyte that is freely filtered by the glomerulus and can be detected in serum. It has shown utility for the detection of renal injury in dogs and cats in clinical veterinary practice, but the potential utility of SDMA to detect renal injury in preclinical species or toxicity studies has not been thoroughly investigated. **Objective:** We sought to compare the sensitivity of SDMA to standard renal biomarkers to detect acute glomerular toxicity. **Methods:** We utilized a well-characterized glomerular toxicant, puromycin aminonucleoside (PAN), to induce podocyte injury and subsequent proteinuria in young male Sprague-Dawley rats. At the end of 1 or 2 weeks, blood, urine, and kidney tissue were collected for analysis. **Results:** One week following a single 50 mg dose, urea nitrogen, creatinine, and albumin were within reference intervals while SDMA was increased. Glomerular changes in these animals included periodic acid-Schiff positive globules within podocytes, podocyte hypertrophy by light microscopy, and podocyte degeneration with fusion of foot processes by electron microscopy. **Conclusions:** Our data indicate that SDMA may be a useful biomarker for early detection of glomerular toxicities in rats.

T-08: ALPHA 2-MACROGLOBULIN MAY CONTRIBUTE TO INFLAMMATION ASSOCIATED APTT PROLONGATION IN CYNOMOLGUS MONKEYS

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Background: Cynomolgus monkeys exhibiting increases in one or more markers of inflammation commonly have small concurrent activated partial thromboplastin time (aPTT) prolongations. These small aPTT prolongations are generally not associated with other coagulation changes or gross evidence of hemorrhage and are commonly attributed to a secondary effect caused by inflammation. **Objective:** To review internal historical cynomolgus monkey data to identify correlations between biomarkers of inflammation and aPTT prolongations and to explore potential mechanisms through investigative in vitro studies. **Methods:** Historical cynomolgus monkey aPTT data were

compared to concurrent C-reactive protein (CRP), albumin, and fibrinogen concentrations to identify an association between inflammation and aPTT prolongation. Subsequently, aPTT was measured in normal cynomolgus plasma following the addition of various concentrations of CRP or a2-macroglobulin, two positive acute phase reactants in cynomolgus monkeys. **Results:** Increased CRP and fibrinogen and decreased albumin concentration were associated with small but significant aPTT prolongations ($p < 0.05$). Spiking normal cynomolgus plasma with increasing concentrations of α2 macroglobulin resulted in prolongation of aPTT values (>3.5 seconds at 1 mg/dL). Addition of increasing concentrations of CRP did not cause identifiable aPTT prolongations. **Conclusions:** In cynomolgus monkeys, inflammation can be associated with aPTT prolongation. Our results suggest that a2-macroglobulin, a broad-spectrum protease inhibitor likely contributes to this phenomenon in vivo, potentially through kallikrein inhibition. Small aPTT prolongations in cynomolgus monkeys may be a secondary effect related to inflammation. In the non-clinical safety setting, concurrent inflammation signals should be considered before directly attributing small aPTT prolongations to test item administration.

T-09: ACUTE DERMAL IRRITATION RESPONSE IN WHITE SINCLAIR, HANFORD, AND GOTTINGEN MINIPIGS

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Acute dermal irritation studies were conducted to characterize the response in lineages of miniature swine. A Pilot Study used Sinclair and Hanford minipigs (2 per breed, 3-4 months old, all females). A Larger study used Sinclair, Hanford, and Gottingens (6 per sex per breed, 3 per sex per breed at 3-4 months old or 9 months old). For both studies, animals were given 0.5 mL of test material to a 4 cm2 area for 4 hours. Animals were scored using a Modified Draize Scoring System and histopathology. Scoring was conducted daily up to a week. Materials tested included sodium hydroxide (NaOH), formaldehyde, benzalkonium chloride (BKC), and sodium dodecyl sulfate. **Results:** In the Pilot Study, only NaOH (4%) and BKC (15 and 30%) resulted in irritation, with Sinclairs displaying earlier irritation, and higher erythema and edema scores than Hanfords. At 45%, BKC induced irritation with no difference between lineages. In the Larger Study, irritation response in Sinclairs was greater than Hanfords. The irritation response for the Gottingens was similar to Sinclairs. At 9 months of age, the response of the Gottingens was much lower, while both the Sinclairs and Hanfords displayed a considerably greater response. **Conclusion:** When exposing 3-4-month old minipigs to known human irritants, Sinclairs and Gottingens displayed a greater response than Hanfords. However, 9-month old Sinclairs and Hanfords display more irritation than Gottingens. These tests suggest that Sinclairs may be a sensitive model for skin irritants over its lifetime and a superior animal model for human responses to chemicals.

T-10: CHARACTERIZATION AND COMPARISON OF ACUTE DEHYDROPYRROLIZIDINE ALKALOID TOXICOSIS IN C57BL MICE GAVAGED WITH RIDDELLIINE, SENECIONINE, SENECIPHYLLINE OR LASIOCARPINE

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Background: Dehydropyrrolizidine alkaloids (DHPAs) are a globally important group of plant-derived toxins, estimated to be found in 3% of flowering plants. Over 600 individual DHPAs have been discovered and many of these are hepatotoxic, pneumotoxic, nephrotoxic, genotoxic and under some circumstances, carcinogenic. Animal exposure occurs via contaminated hay or grain, or from grazing. Human exposure occurs from herbal supplements or teas, or contaminated grain, milk, honey, or eggs. Mice are less sensitive than other species to DHPA toxicosis, but are a useful animal model due to their size, availability, degree of genomic characterization and availability of genetically altered models. **Objective:** Characterize microscopic lesions and compare lesion severity following subacute exposure to select DHPAs in mice to obtain suitable dose(s) for genotoxicity and carcinogenicity studies. **Methods:** Six different doses of four purified DHPAs (riddelline, senecionine, seneciphylline and lasiocarpine) were gavaged to C57BL mice for ten days. The mice were necropsied on day eleven. **Results:** Hepatocellular hypertrophy in zone 2 and 3, and multifocal random, hepatocellular necrosis were observed. The severity of hepatocellular hypertrophy and necrosis increased with dose. Extrahepatic lesions were not observed. **Conclusions:** The order of hepatotoxicity, based on severity of necrosis as correlated with dose, from most hepatotoxic to least was senecionine, seneciphylline, riddelliine, and lastly lasiocarpine. The order of hepatotoxicity based on the lowest dose to produce microscopic lesions was seneciphylline (most toxic), followed by senecionine, riddelliine and lasiocarpine. This data indicates that despite similar chemical structures, there is variability in the toxicity of various DHPAs in mice.

T-11: A SPONTANEOUS CASE OF SUSPECTED PULMONARY EPITHELIOID TROPHOBLASTIC TUMOR IN A CYNOMOLGUS MACAQUE

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Background/Objective: Epithelioid trophoblastic tumors are rare tumors of the female reproductive tract with features resembling carcinoma. These tumors most frequently metastasize to the lung. The purpose of this study was to further characterize pulmonary masses noted in a cynomologus macaque at terminal necropsy using special stains and immunohistochemistry. To confirm the diagnosis, the pulmonary masses were evaluated with special stains and immunohistochemistry with markers against inhibin alpha, pan-cytokeratin, S100, and vimentin. **Methods:** A female cynomologus macaque was part of a treatment group for a safety assessment study and presented

for routine necropsy at study termination. Tissues were routinely processed following full necropsy examination. Immunohistochemistry was performed on sections of the tumor with primary antibodies directed against cytokeratins, inhibin alpha, S100, and vimentin. **Results:** Macroscopically, the lungs had multiple randomly distributed, pale tan, firm to hard masses, no other lesions were noted in this animal. On histological evaluation, the pulmonary masses were comprised of nests of polygonal cells separated by a moderate fibrovascular stroma embedded in lakes of fibrillar to PAS positive hyaline-like material with occasional mineralization. The neoplastic cells had strong cytoplasmic labeling with inhibin alpha and pancytokeratin. S100 and vimentin markers were negative. Other organs and tissues examined histologically were unremarkable and no primary tumor was noted. **Conclusion:** A presumptive diagnosis of epithelioid trophoblastic tumor was made based on preliminary histopathologic and immunohistochemistry evaluation. Rare spontaneous lesions provide valuable historical data and should be documented to help distinguish from potential test article-related effects.

T-12: REPRODUCTIVE FITNESS OF HONEY BEE QUEENS EXPOSED TO THIAMETHOXAM DURING DEVELOPMENT

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Background. Colony exposure to neonicotinoid insecticides can have negative effects on honey bee queen reproductive potential; however, it is unclear if these effects result from direct queen toxicity or from disrupted interaction between bees. Objective. The aim was to evaluate newly mated queens individually exposed to incremental doses of the neonicotinoid thiamethoxam (THI). M&M. We evaluated larval survival, sperm quality, and histomorphology of mandibular glands, the main pheromone producing organ of the honey bee queen. Individual queen larvae were exposed to 0ng, 5ng (highest calculated environmental dose) or 50ng (positive control) THI. After mating, the heads of surviving queens were formalin fixed, routinely processed, serially sectioned and stained with H&E. Gland morphology was evaluated using ImagePro software. Results. Total mandibular gland epithelial area was reduced by 26.2% in queens exposed to 50ng THI compared to negative controls (hypoplasia). Altered pheromone production in hypoplastic mandibular glands may disrupt colony cohesion and reduce queen longevity. High doses of THI also reduced larval survival and decreased sperm viability in mated queens. Conclusions. Histopathologic changes in the mandibular glands of queens in the positive control group warrant closer investigation of low dose THI effects on mandibular gland function which may precede detectable morphologic changes.

T-13: DRONE FERTILITY AND TESTICULAR DEVELOPMENT: IMPACT OF EXPOSURE TO A MITICIDE AND PESTICIDE

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Background: The acarian ectoparasite *Varroa destructor* has a major impact on honeybee colonies. Its control is critical to maintain their economic viability; however, several publications report a negative impact of acaricides on drone reproductive health. Neonicotinoids are systemic pesticides used to control harmful insects, but their persistence in the environment can have deleterious effects on non-target organisms. Some studies demonstrated severe effects on honeybee queens at field-realistic concentrations. **Objectives:** 1) Characterize the histomorphologic development of drone testes; 2) Assess the impact of a formic acid acaride and/or a sublethal concentration of imidacloprid (neonicotinoid) on sperm development and quality. **Methods:** Twenty honeybee colonies were distributed into four groups: 1-control group receiving a sugar solution, 2-treated with Mite Away Quick Strip™, 3-receiving 10 ppb of imidacloprid and 4-receiving both the varroacide and imidacloprid. Twenty-five drone larvae aged 6, 12 and 18 days old were collected in each colony and fixed in Bouin. Histopathology was performed on HE-stained slides. Sperm volume, viability and count was assessed on 50 drones per colony aged 15-20 days. **Results:** Testicular development is complete in emerging drones; testes then solely contain mature sperm. Upon evaluation of maturation steps in unexposed larvae and pupae, segmental testicular degeneration was observed in a few individuals. Exposure to formic acid and/or imidacloprid at the doses tested herein did not affect testicular development or sperm quality. **Conclusion:** Our results highlight the complexity of environmental toxicology studies and the importance of adequate characterization of normal development in bees to draw appropriate conclusions.

Natural Disease Poster Session

N-01: A CASE OF NATURALLY OCCURRING LYSOSOMAL STORAGE DISEASE IN A JACOB LAMB

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Lysosomal storage diseases (LSDs) are caused by autosomal recessively inherited deficiencies in lysosomal acid hydrolases. This leads to defects in the catabolism of their substrates and the accumulation of partially degraded insoluble metabolite within lysosomes. Sheep are a valuable animal model of this genetic disease and essential research tool. We report a 6-month-old lamb with a storage disease characterised by

the widespread presence of neuronal PAS-positive intracytoplasmic granules. A 6-month-old Jacob lamb from a small flock in south-east England with a history of neurological signs (ataxia, hind limbs paresis, tremors) and poor body condition score was submitted for autopsy. There was at least one other lamb of similar age with similar clinical signs, still alive at the time of autopsy. The farm had recently purchased a ram and these lambs were its first lambing season. The affected lamb was treated with penicillin-streptomycin and vaccinated for *Clostridium spp.* and *Pasteurella spp.* prior to euthanasia. A range of samples was collected, with histopathology and bacteriological and parasitic analyses performed. This case highlights a rare disease which should be considered when neurologic signs are detected in lambs. Characterisation of these cases contributes to research involving animal modelling and is vital, to better understand these diseases in veterinary and comparative pathology.

N-02: CHRONIC HEARTWORM PRESENTING AS SPONTANEOUS PNEUMOTHORAX

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A seven-year-old male castrated Great Dane had an acute onset of lethargy, increased respiratory rate and effort. The dog had been treated for dirofilariasis six years prior. Thoracic radiographs and computed tomography revealed pneumothorax and suspect bullae. Right caudal lung lobectomy was performed for histopathologic examination. Grossly, the lung lobe was diffusely collapsed and there were small foci of necrosis present at the apex of the lobe. Multiple vascular profiles were evident in the proximal bronchovascular bundles. Tissue sections were stained with hematoxylin-eosin. Verhoeff-Van Gieson was used to characterize lung vasculature. Histologically, there was partial to complete fibrous obliteration of multiple large pulmonary arterial lumens. Numerous small to medium caliber pulmonary arteries were similarly affected. Foci of subpleural ischemic necrosis (infarction) were found at the lung apex. Proximal bronchial arteries were markedly enlarged. There was multifocal severe interstitial fibrosis, marked alveolar capillary dilation, and increased numbers of capillary profiles. This case demonstrates an unusual clinical manifestation of dirofilariasis, with the resultant chronic occlusive pulmonary arteriopathy leading to infarction and pneumothorax six years after heartworm infection. Furthermore, the histopathology illustrates important concepts regarding the pulmonary vasculature. First, the lung is resistant to infarction, even in the face of occlusion of pulmonary arteries, because of the compensatory augmentation from the bronchial circulation. Second, because the bronchial circulation ends at the level of the alveolar capillaries, when its circulation is dramatically increased it may lead to the dilation of capillaries and increased numbers of capillary profiles similar to pulmonary capillary hemangiomatosis.

N-03: PHAEOHYPHOMYCOSIS CASES AT THE UNIVERSITY OF GEORGIA FROM 2010 TO 2020

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Background: Phaeohyphomycosis can be caused by numerous saprophytic, dematiaceous (pigmented) fungi. Infection is considered opportunistic. While the route of infection is poorly understood, respiratory, ocular, aural, and cutaneous wound routes have been suggested. Infection is rare in veterinary species and humans, and such patients are often immunosuppressed or immunocompromised. **Objective:** Analyze phaeohyphomycosis cases received by Athens Veterinary Diagnostic Lab and UGA Department of Pathology between 2010 and 2020. **Methods:** The laboratory information management system was reviewed from 2010 to 2020 for mammalian biopsy and necropsy cases with histologic diagnoses of pigmented fungi. **Results:** Fifty-two cases were included, with 46 biopsies and 6 necropsies. Biopsies were equine (46%), canine (33%), and feline (22%) origin, primarily of skin (89%), often originating from the head (24%) or limb (33%). Necropsies were feline (3), equine, canine, and camelid (1 each) origin, with encephalitis in 5 (83%). Three necropsies grossly described skin wounds, with 2 histologically confirmed as fungal dermatitis. Juvenile animals accounted for 80% of encephalitis cases. Fungal culture in biopsies (9%) and necropsies (83%) identified agents in 50% and 100% of attempts, respectively. **Conclusions:** While rare, accumulated phaeohyphomycosis cases in Georgia demonstrate a predominance of dermatitis in biopsies and encephalitis in necropsies. Relatively abrasion-vulnerable face and limb locations of skin lesions supports that pre-existing wounds likely predispose to infection. Encephalitis with concurrent skin wounds in 2 cases may support that systemic spread follows cutaneous infection. Encephalitis in predominantly young animals may indicate age or an immature immune status as predisposing factors.

N-04: PRIMARY ANGLE CLOSURE GLAUCOMA IN AMERICAN COCKER SPANIELS: PRELIMINARY GENETIC STUDY

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Background: Primary angle closure glaucoma (PACG) is a bilateral, painful disease without cure that often leads to vision loss. Despite PACG being a common cause of blindness in dogs, few genetic studies exist. The American Cocker Spaniel (ACS) is reported to have a high prevalence of PACG, and it is a popular breed in North America. Thus, the ACS is a compelling model for studying the genetics of canine PACG. **Objective:** To identify putative genetic markers associated with PACG in ACS. **Methods:** Thirty ACS diagnosed with PACG and 37 controls older than 10 years were included in this study. The phenotypes were confirmed with a complete ophthalmic examination that included gonioscopy, ultrasound biomicroscopy, and rebound tonometry, indirect ophthalmoscopy, and/or histology. When employing paraffin

embedded tissue, the clinical history and histology were carefully evaluated. The DNA was extracted from venous blood (30 cases, 25 controls) or paraffin-embedded tissue (12 controls) and was genotyped using Illumina canine HD 220K SNP array. **Results:** Thirty cases and 29 controls passed our quality filters and a genome-wide-association-study (GWAS) was performed. Out of the 12 DNA samples obtained from paraffin-embedded tissue, 4 passed our filters. No genome-wide significant single nucleotide polymorphism (SNP) was identified (P Bonferroni < 0.05). Genomic inflation (l) was 1.226. **Conclusions:** Similar to primary glaucoma in humans, PACG in ACSs is likely a complex trait and require a larger number of samples in order to identify PACG-associated loci. Further studies are warranted to understand the underlying genetic basis of PACG in ACSs.

N-05: DETECTION OF CANINE DISTEMPER VIRAL (CDV) RNA IN AN AFRICAN PYGMY HEDGEHOG (ATELERIX ALBIVENTRIS) WITH A CHRONIC DEMYELINATING MYELOENCEPHOPATHY

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A 3-year old African Pygmy Hedgehog was euthanized after a 4-month history of progressive gait abnormalities, head tilt, and weight loss. There had been no response to nutritional support and antimicrobial therapy. Lesions were not detected on necropsy evaluation performed the next day. Microscopically there was vacuolization of white matter tracts within the spinal cord and hind brain; especially within spinal lateral and ventral funiculi. Gitter cell infiltrates into myelin sheaths were observed. Lymphocytic infiltrates were not noted. Total RNA was extracted from hindbrain collected at the time of necropsy. The RNA was prepared for HiSeq Illumina sequencing using a standard procedure. We generated 227,789,112 paired-end reads. After Q/C steps and subtraction of Hedgehog sequence, remaining sequences were blasted against the NCBI viral data base. Viral hits were assembled for further analysis. We detect 7532 bp of CDV in 18 contiguous elements. The sequences were collectively the closest match with CDV A75/17, a neurovirulent strain that induces persistent CNS infection with demyelination, but grows poorly in culture. The clinical and pathologic findings are consistent with wobbly hedgehog syndrome (WHS), a common idiopathic disease in African Pygmy Hedgehogs. CDV needs further investigation as a possible cause of WHS.

N-06: RETROSPECTIVE ANALYSIS OF PHAEOHYPHOMYCOSIS IN AQUARIUM-HOUSED FISH, INCLUDING TWO PREVIOUSLY UNREPORTED FUNGAL SPECIES

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Background: Novel and known dematiaceous fungi are emerging pathogens of fish cultured for human consumption and aquarium display. **Objective:** This retrospective study characterized associations between fish species, tissues affected, and where

possible, fungal species causing phaeohyphomycosis in aquarium fish. **Methods:** Piscine submissions (2250) received by the Aquatic Diagnostic Service, University of Georgia from 2006 to 2020 were searched for phaeohyphomycosis. When fresh tissue was available (11), culture and/or sequencing of the internal transcribed spacer and large ribosomal subunit gene D1/D2 regions were performed for fungal identification. **Results:** Forty-seven cases of phaeohyphomycosis were identified (47/2250, 2.1%), representing 34 cartilaginous and bony fish species. The majority (45/47, 95.7%) involved bony fish and were overwhelmingly marine (41/47, 87.2%), with only a few freshwater species (4/47, 8.5%). The cartilaginous fish cases were two zebra sharks (*Stegostoma fasciatum*) (2/47, 4.3%). Northern seahorses (*Hippocampus erectus*) were most frequently affected (7/46, 15.2%). Syngnathiformes (11/47, 23.4%) and Perciformes (19/47, 40.4%) were the most commonly involved fish Orders. *Exophiala, Ochroconis,* and *Devriesia* spp. were identified, with *Exophiala* as the most common genus (8/11, 72.7%). *Exophiala lecanii-cornii* and *Devriesia imbrexigena* are reported for the first time in fish. Microscopically, lesions were typified by necrosis, granulomatous inflammation, and angioinvasion with thin, brown, septate hyphae possessing no distinguishing microscopic features or observed tissue associations. **Conclusions:** Phaeohyphomycoses result in sporadic infections in a diverse group of aquarium-housed fish species, with variable pathologic presentations, tissue distributions, and severities. *E. lecanii-cornii* and *D. imbrexigena* were previously unreported in fish.

N-07: INTERPATHOLOGIST DIAGNOSTIC AGREEMENT FOR CANINE GLIOMA

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Introduction/Objectives: Histopathologic assessment is necessary to determine the diagnosis and grade of canine glioma but can be affected by interobserver variability. Our objective was to assess interpathologist diagnostic agreement for canine glioma using recently published consensus diagnostic criteria. **Methods and Materials / Experimental Design:** 85 cases from the NCSU-CVM archives (2006-2018) previously diagnosed as glioma were independently reviewed by a panel of 5 pathologists (4 veterinary anatomic and 1 MD neuropathologist). H&E slides and immunohistochemistry for Olig2, GFAP, CNPase, and Ki67 were assessed. For cases diagnosed as glioma, subtype (oligodendroglioma, astrocytoma, or undefined glioma) and grade (high-grade or low-grade) were assigned. **Results:** Majority diagnosis of glioma subtype and grade was achieved in 73/85 cases. Overall percentage agreement for grade and subtype were 83.2% and 74.4%, respectively. Overall kappa scores for grade and subtype were 0.589 and 0.530, respectively. Category-wise kappa scores for astrocytoma and oligodendroglioma were 0.481 and 0.603, respectively. Kappa scores for high-grade and low-grade tumors were both equal to 0.589. **Conclusion:** Interpathologist agreement for grade and subtype of spontaneous canine intracranial glioma was

moderate. Agreements for astrocytoma and oligodendroglioma were moderate and substantial, respectively. There was no difference in agreement between high and low grade tumors. Impact Statement: Assessment of glioma by a single pathologist is likely sufficient in veterinary diagnostic pathology, though peer review or consulting with other pathologists is recommended in certain situations. Cases for which definitive classification remains challenging may benefit from further research on molecular features that differentiate glioma type and grade.

N-08: RNA-SCOPE IN SITU HYBRIDIZATION AS A NOVEL TECHNOLOGY FOR THE ASSESSMENT OF C-KIT M-RNA EXPRESSION IN CANINE MAST CELL TUMORS

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Background: Canine mast cell tumor (MCT) is a common neoplastic disease in dogs with a variable biologic behavior. Several studies have unsuccessfully focused on the investigation of definitive predictive factors for MCTs outcome. RNA has emerged as a means to discover novel biomarkers, as it is an ideal indicator of the dynamic genetic expression changes in a cell. RNAScope is a commercially available assay for RNA quantitative and less analytically subjective detection. **Objective:** We aimed to assess the feasibility of RNAScope in detecting and measuring the *c-KIT* mRNA in formalin fixed paraffin embedded (FFPE) canine MCTs. Also, we aimed to investigate significant correlation with other important prognostic markers in canine MCTs such as histologic grade and KIT protein localization. **Methods:** Correlations between *c-KIT* mRNA expression by RNAScope, histologic grade by Kiupel two-tier system and KIT immunohistochemical patterns were investigated using Spearman's Rho correlation in 60 FFPE canine MCTs. **Results:** We observed a good expression of *c-KIT* mRNA in neoplastic cells and a statistically significant correlation between histologic grade and *c-KIT* mRNA expression. No correlations were found between KIT immunohistochemical pattern and *c-KIT* mRNA expression or histologic grade. **Conclusions:** We have developed a fit-for-purpose, feasible RNAScope technology for the assessment of *c-KIT* mRNA in FFPE canine MCTs. Our results also provide a reference basis to better understand *c-KIT* mRNA expression in canine MCTs and strongly encourage further studies that may provide useful information about its potential and significant role as a prognostic and predictive biological marker for canine MCTs clinical outcome.

N-09: THE PANCREATIC ISLET MICROENVIRONMENT IN FELINE DIABETES MELLITUS

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Objectives: Diabetes Mellitus (DM) is a common condition that impacts quality of life of both owners and pets. The aim of this study was to identify DM-associated perturbations in the feline pancreatic islet microenvironment. Specifically, the utility of "clear, unobstructed brain imaging cocktails and computational analysis" (CUBIC) for three-dimensional (3D) analysis of the feline pancreas was investigated. **Methods:** Formalin fixed paraffin embedded tissues from cats with DM, or control cats without pancreatic pathology were retrospectively identified. Immunohistochemistry and immunofluorescence were used to assess changes in islets. For CUBIC, which was used to optically clear pancreas prior to confocal microscopy, pancreas from cats examined post-mortem was briefly fixed prior to storage in phosphate buffered saline. An image analysis pipeline was developed to analyse images acquired from two-dimensional immunofluorescence. **Results:** Diabetic cats have a significant reduction in synaptophysin-positive islet area. Intriguingly, DM-predisposed breeds exhibit a trend towards larger islet size compared to cats of other breeds. There is a significant decrease in intensity of insulin expression in diabetic cats and an inverse correlation between islet area and insulin expression intensity irrespective of diabetic status. Islet macrophage numbers do not vary between control and diabetic animals. CUBIC facilitates clear visualisation of pancreatic islets in 3D. **Conclusions:** Larger pancreatic islets may be associated with a pre-diabetic stage of insulin resistance and the presence of larger islets in non-diabetic cats may be associated with breed predisposition to DM. CUBIC offers an opportunity to investigate the microenvironment of the pancreatic islets in unprecedented detail in 3D.

N-10: PATHOLOGY AND CAUSES OF DEATH IN FREE-RANGING NORTH AMERICAN BEAVERS (CASTOR CANADENSIS) IN CALIFORNIA: A SUITABLE SENTINEL SPECIES FOR FRESHWATER ECOSYSTEMS

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Background: North American Beavers (*Castor canadensis*) are semiaquatic rodents recognized as keystone species increasing diversity of ecosystems and sentinel species for freshwater ecosystem health. **Materials and Methods:** 16 free-ranging beavers (10 females, 6 males; 2kits, 6 juveniles, 8 adults) submitted for postmortem examination between 2008-2020 at the California Animal Health and Food Safety (CAHFS), UC Davis received full diagnostic workups, which in addition to gross and microscopic examination included immunohistochemical, bacteriological, parasitological, molecular, serological and toxicological tests. **Results:** Of 16 beavers examined, encephalitis was the most prevalent (8/16) cause of mortality with *Baylisascaris* sp. demonstrated in two and *Listeria monocytogenes* cultured in one beaver. A non-specific encephalitis/meningoencephalitis was diagnosed in five animals with multifocal astrocytic scarring, eosinophils, and malacia forming tracks in the brain most suggestive of *Baylisascaris* sp. larval migration. *Francisella tularensis* type B (etiologic agent of Tularemia) caused septicemia with multisystemic necrosis and peritonitis in one animal. Mesocestoides larvae-associated chronic peritonitis was diagnosed in one adult male beaver and trauma with multiple complete pelvic fractures and multifocal contusions were observed in another adult male beaver. Other pathological findings included

granulomatous hepatitis with *Calodium* (*Capillaria*) *hepaticum* (1), cerebral *Toxoplasma gondii* cysts (1), cholangiocellular (1) and papillary renal (1) adenomas, castor gland squamous cell carcinoma (1), oxalate crystal-associated nephrosis (1), Brodifacoum (anticoagulant) (1) and *Leptospira interrogans* serovar Pomona (1) exposure. **Conclusion:** In California, beavers are exposed to various pathogens with human and domestic/wild animal impact. Investigating the mortality and pathology of free-ranging beavers represents a barometer of freshwater ecosystem health.

N-11: IMMUNOHISTOCHEMICAL PROFILES OF INFILTRATING LEUKOCYTES IN FELINE ORAL SQUAMOUS CELL CARCINOMA: A RETROSPECTIVE STUDY

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Feline oral squamous cell carcinoma (FOSCC) is a common and aggressive malignancy of cats for which very few successful treatments currently exist. A detailed understanding of the types of infiltrating leukocytes within these tumors may inform future treatment strategies. The objectives of this study are to describe the qualitative features of FOSCC, including subtype, and to characterize the tumor-associated leukocytes using immunohistochemistry, semi-quantitative scoring, and whole-slide image analysis, and to find correlations between these factors and patient outcome. Medical records were reviewed for clinical data. Histologic sections were evaluated categorized into subtypes, immunohistochemistry (IHC) for B cell, T cell, and plasma cell markers were performed, and qualitative, semi-quantitative, and whole-slide analyses of leukocytes were performed using an integer scale and digital analysis software. Oral squamous cell carcinoma was identified in 37 domestic cats from 2008-2018. Outcome data were available for 17 cases. The overall median survival time was 48 days. Tumors in the tongue were associated with significantly shorter survival time (16 days) compared to all other locations (152 days). All but one tumor was histologically consistent with conventional subtype. T cell infiltrates were present within tumor stroma and epithelium, while B cells and plasma cells were restricted to the stroma. No correlations were made between subtype, or numbers of infiltrating leukocytes, based on semi-quantitative scores, and outcome. Whole-slide analysis data showed correlation to the semi-quantitative scores. This validation of a method of measuring cellular infiltrates using artificial intelligence justifies the potential utility of this software in diagnostic pathology.

N-12: EMERGENCE OF BABESIA CONRADAE INFECTION IN COYOTE-HUNTING GREYHOUNDS IN OKLAHOMA

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Babesia species are intraerythrocytic apicomplexan parasites that infect a wide range of vertebrates and are transmitted by ticks or by direct blood-to-blood contact. *Babesia canis vogeli* and *Babesia gibsoni* infections have both been reported in

Oklahoma. To date, *B. conradae* infections have been limited to California and clinical signs include lethargy, anorexia, vomiting, and hemolytic anemia. In this study, we document 16 of 41 dogs from 4 separate kennels testing positive by 98-100% sequence homology to *B. conradae* by comparison of a partial 18S rRNA gene. All positive cases were coyote-hunting greyhounds and the outbreaks occurred from 2015 to 2020. Five dogs were clinically ill while the remaining 11 were subclinical. None of the dogs had history of travel to or from California. Treatment of positive dogs with atovaquone and azithromycin resulted in complete clinical recovery (negative follow-up PCR at 30 and 60 days post-treatment). Collectively, this study (i) documents the occurrence of *B. conradae* in Oklahoma, (ii) highlights this pathogen as a differential to be considered when clinical signs are present, and (iii) supports the use of atovaquone and azithromycin as effective treatment in these cases.

N-13: A RETROSPECTIVE ANALYSIS OF FATAL WEST NILE VIRUS ENCEPHALITIS IN PENNSYLVANIA HORSES FROM 2009-2019 AND CORRELATION WITH CLIMATE, HUMAN, AND AVIAN DISEASE

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Background: West Nile Virus (WNV) is a vector-borne flavivirus that can cause fatal infections in mammalian and avian hosts. Weather conditions (precipitation, temperature) can alter vector status and infection rates. Recently, our laboratory in the Mid-Atlantic region experienced an increase in fatal equine WNV encephalitis. **Objectives:** This study had three Objectives: identify, quantify, and characterize recent cases of fatal equine WNV encephalitis; determine any correlations between equine WNV cases and weather conditions; detect associations between equine, avian, and human WNV infections. **Methods:** WNV encephalitis cases were confirmed using PCR; lesions were characterized by using immunohistochemistry for WNV and inflammatory cell markers. Weather data was collected from National Oceanic and Atmospheric Administration. Human and avian information was obtained from the Center for Disease Control and PA Department of Health. **Results:** During the study period, 12 horses were diagnosed with fatal WNV encephalitis. Most cases occurred during 2018. Full histologic and immunohistochemical characterization of the lesions (location, severity and type of inflammation, presence and degree of necrosis, gliosis, demyelination) is underway. Human cases of WNV followed similar trends as horses, with the highest number of WNV infections during 2018. State-wide weather data shows increased levels of precipitation during 2018 compared to years when WNV cases were infrequent (2009). Data collection is in progress and statistical methods will be applied once complete. **Conclusions:** While many factors play a role in vector-borne diseases, our preliminary data indicates that precipitation in our region may have an impact on the development of fatal equine WNV infections.

N-14: PROPOSAL FOR CLINICAL AND HISTOPATHOLOGICAL GRADING SYSTEMS TO MORE ACCURATELY PREDICT OUTCOME IN CANINE APOCRINE GLAND ADENOCARCINOMA OF THE ANAL SAC

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Background: Canine apocrine gland adenocarcinoma of the anal sac (AGASAC) is a common malignant tumor with highly variable clinical progression. **Objective:** Primary indicators of poor outcome, namely tumor size, explain most outcome variability, but the risk is modified by sporadically present predictors. Our objective was to build grading systems, based upon multivariate models optimized for stability, flexible enough to capture the spectrum of disease, whilst usefully predicting outcome. **Methods:** Eighty-two AGASACA cases with clinical staging and survival data, were identified retrospectively. Fifty cases underwent histological review and Ki-67 evaluation. Published variables formed the global model and backwards elimination was applied in multivariate COX regression. Resultant variables were compiled into prognostic grading systems. **Results:** Tumor and lymph node size, and non-surgical management were independent risk factors of poor survival. Tumor size and presence of distant metastasis were independent risk factors for shortened progression-free interval (PFI). Grading systems to predict survival and PFI were created using those variables. The histopathological variables of necrosis, metastasis and/or vascular invasion, and a solid pattern were independent risk factors for poor survival. For PFI, histological evidence of metastasis and/or vascular invasion was the sole independent risk factor, but pattern and necrosis had input in the final model as non-significant variables. A histopathological grading scheme, applicable to both survival and PFI, was generated from the final model. **Conclusions:** The clinical and histopathological grading systems stratified AGASAC cases into groups with distinct hazard ratios and median survival time/median PFI and may be useful in guiding clinical decision making.

N-15: MULTI-SYSTEMIC SPIRORCHIDIASIS AND GASTROINTESTINAL PARASITISM IN LOUISIANA RED-EARED SLIDERS (TRACHEMYS SCRIPTA ELEGANS)

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Background: Recently, reports of mass mortality events have involved hundreds of red-eared sliders (*Trachemys scripta elegans*; hereafter, sliders) in Louisiana. Sliders are considered an invasive species in much of the U.S. but are native to much of the southeast, including Louisiana. **Objectives:** To characterize gross and microscopic

lesions and potential etiologies in sliders from three mortality events and assess for epidemiologic patterns. **Methods:** Postmortem diagnostic evaluation, including gross and histopathology and select ancillary tests, was performed on ten slider carcasses collected during die-offs in Louisiana from 2017-2020. **Results:** Mortality events occurred in February and March near agricultural lands (e.g., crawfish and rice production ponds, cattle pastures) in northwestern and southern Louisiana. Death in all sliders was attributed to multi-organ parasitism, most notably with spirorchids eggs associated with granulomatous inflammation and necrosis in stomach and intestine (9/10), liver (8/10), pancreas (5/10), kidneys (5/10), lungs (5/10), spleen (3/10), skeletal muscle (2/10), uterus (2/10), ovaries (1/10), heart (1/10), and skin (1/10). *Serpinema trispinosum* (Nematoda: Spirurida: Camallanidae) and *Neoechinorhynchus* sp. (Acanthocephala) were identified morphologically in gastrointestinal tissue of seven sliders. No viruses, including ranavirus, were detected in pooled tissues (0/10). **Conclusion:** Large die-offs of sliders were attributed to multi-systemic disease associated with severe spirorchid infections, posing a health risk to this species. Further, sliders pose a conservation threat to native turtle species in some regions of the U.S. via potential pathogen transmission in shared environments. Seasonal (late winter) or habitat (e.g., agricultural use) associations may represent a disease risk and warrant continued monitoring.

N-16: IMMUNE CELL DISTRIBUTION IN THE MAMMARY GLAND OF PRE- AND PERI-PUBERTAL LAMBS INDICATES A DYNAMIC MICROENVIRONMENT METHODS

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Methods. Immunohistochemistry and immunofluorescence were used to assess Ki67, Iba1, CD3, and CD20 expression in the mammary gland of pre- and peri-pubertal lambs. Expression patterns were analyzed by a combination of manual quantification and deep learning. “Clear, unobstructed brain imaging cocktails and computational analysis” (CUBIC) was utilized for three-dimensional analysis of immune cell location. **Results**. Mammary luminal epithelial proliferation is significantly higher in young lambs than in those approaching puberty. Getis Ord hotspot analysis demonstrates polarity of Ki67 luminal epithelial expression with expression focused at the leading edge of the advancing ducts. Intraepithelial macrophages frequently exhibit striking periodicity in their distribution with significantly reduced inter-macrophage distances in lambs approaching puberty. The developing ovine mammary gland is infiltrated by intraepithelial and stromal CD3-positive T lymphocytes that are significantly more numerous in older lambs. Getis Ord analyses demonstrate hotspots of Ki67 expression in large aggregates of T lymphocytes colocalized with groupings of Iba1 positive cells. Multifocally these aggregates have smaller numbers of CD20- positive lymphocytes at the centre and have distinct organization consistent with tertiary lymphoid structures. Conclusions. The lamb mammary microenvironment is dynamic with immune cell fluxes occurring towards puberty. The occurrence of tertiary lymphoid structures indicates

mammary pre-lactational subclinical pathogen challenge is potentially common in lambs in commercial systems.

N-17: A PROPOSED GRADING SCHEME FOR ENDOMETRIAL HYPERPLASIA (EH) IN LARGE CAPTIVE FELIDS

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Background: Endometrial hyperplasia (EH) is a pathologic condition of the uterus characterized by an increase in the endometrial gland-to-stroma ratio. In humans, severity of EH is associated with progression to endometrial cancer (EC). In domestic cats, endometrial hyperplasia is not a precursor to cancer, but is frequently associated with pyometra. In large captive felids, a similar association is common; however, the clinical and histologic findings are different when comparing lions (*Panthera leo*) to tigers (*Panthera tigris*). Clinically, lions more frequently have fulminant pyometra with more severe clinical disease, while tigers typically have low grade chronic changes without significant clinical disease. **Objective:** The objective of this retrospective study was to propose a reliable and reproducible histopathologic scoring system for endometrial hyperplasia that correlates with severity of clinical signs in the two species. **Methods:** Uterine sections from 9 lions and 17 tigers with EH and clinical pyometra were analyzed for: degree of endometrial hyperplasia, percentage of glands:endometrial stroma, glandular complexity, and adenomyosis. **Results:** Although only adenomyosis was significantly different (p=0.037) as a univariate analysis, a grading scheme composed of degree of endometrial hyperplasia, percentage of glands:stroma, glandular complexity and adenomyosis was significantly (p= 0.019) different between lions and tigers. Other histologic features including hemorrhage, fibrosis, degree and depth of inflammation, edema, and necrosis were evaluated but were not significantly different between lions and tigers. **Conclusion:** The grading scheme proposed here may have utility as a more objective evaluation of endometrial hyperplasia in both domestic and captive felids

N-18: CUTANEOUS MAST CELL TUMORS IN MINIATURE PIGS: A RETROSPECTIVE STUDY

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Background: Cutaneous mast cell tumors are poorly described in miniature pigs, and there is a need to better characterize these spontaneous neoplasms to guide diagnosis and treatment. **Objective and Methods:** This retrospective descriptive study characterizes the gross pathology, histopathology, histochemical staining, and anti-KIT immunoreactivity of cutaneous mast cell tumors in 11 miniature pigs (Sus scrofa domesticus) from the eastern United States, collected from 2003-2019. The animals were seven neutered males and four females (3 intact, 1 neutered), with an age range of 1-16 years. **Results and Conclusions:** Cutaneous tumors were papules, small nodules, or plaques (0.5 to 5 cm), single or multiple, frequently truncal, and often slow growing. In one pig, lymph nodes and internal organs were affected. Histologically, all

tumors involved the superficial dermis and some extended to the subcutis (4/11) and skeletal muscle (1/11). Most tumors were well-demarcated, unencapsulated, nodular or multinodular masses (8/11), and fewer were poorly demarcated plaques (3/11). Neoplastic cells were often well-differentiated with pale eosinophilic faintly granular cytoplasm. Anisocytosis and anisokaryosis were mild to moderate with occasional binucleation and rare multinucleation, and a low mitotic activity (<7 per 10 hpf; 10/11). Few or numerous eosinophils were present (10/11). Cytoplasmic granules stained most consistently with high pH (2.5-3) toluidine blue (9/10) when compared to low pH (0.5-1) toluidine blue (6/9) or Giemsa (7/10). Patterns of KIT immunoreactivity were strong perimembranous (4/8), focal perinuclear and stippled cytoplasmic (1/8), and diffuse cytoplasmic (3/8). Anti-KIT immunohistochemistry identified one case that was negative for histochemical stains.

N-19: GASTRIC MUCOSAL DEGENERATION AND NECROSIS IN PACIFIC LUMPSUCKERS (EUMICROTREMUS PACIFICUS)

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Pacific lumpsuckers (*Eumicrotremus pacificus*) are teleosts native to the coasts of Washington state, Alaska, and Japan. Lumpsuckers are popular aquarium species that lack scales and possess a ventral abdominal sucker utilized for substrate attachment. In 2019, twelve lumpsuckers were submitted for postmortem diagnostic evaluation. In eight cases, gastric mucosal degeneration and/or necrosis were noted ranging from mild (N=3) to moderate (N=2) or severe (N=3); two cases lacked gastric lesions, and in two cases, autolysis precluded meaningful evaluation. In severe cases, gastric glands were absent to short and blunted with marked loss of parietal cells. Most remaining parietal cells were hypereosinophilic, shrunken, smudged and fragmented with pyknosis or karyolysis (necrosis). Fewer cells were swollen with pale vacuolated cytoplasm (degeneration) or were attenuated with basophilic cytoplasm, an enlarged nucleus and vesicular chromatin (regeneration). In less severe cases, parietal cells were variably swollen with vacuolated cytoplasm either lacking or containing irregular clumped and smudged cytoplasmic granules. In five of the eight cases, stomach had variable numbers of 3-4 x 5-8 um lunate protozoa with a basophilic, polar nucleus and anterior recurrent posteriorly-directed flagellum (flagellate). Flagellates were typically distributed along the mucosal surface and within superficial glands. In severe cases, flagellates were present in deep glands. Flagellates were also detected in a single case lacking gastric lesions. All eight cases with gastric lesions had significant concurrent disease. Gastric mucosal degeneration/necrosis may a represent a nonspecific manifestation of debilitation, stress and/or hyporexia. Flagellate infection was likely opportunistic and may have exacerbated damage in some cases.

N-20: SPONTANEOUS OSTEOCARTILAGINOUS LESIONS IN CAPTIVE ENDANGERED PUERTO RICAN CRESTED TOADS (PELTOPRYNE LEMUR)

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Background: The Puerto Rican crested toad is a critically endangered species with estimates of less than 3,000 adult toads currently remaining in the wild. Under a unique Species Survival Plan, more than 800 captive toads are managed by over 32 separate US institutions. The North Carolina Zoo is a participating institution, receiving 45 toads in 2016. In late 2018, toads began developing musculoskeletal abnormalities which hindered their reproductive fitness prompting a thorough clinical and postmortem evaluation. **Objective:** To characterize gross and histopathologic osteocartilaginous lesions in this population of Puerto Rican crested toads. **Methods:** Seventeen Puerto Rican crested toads were subjected to complete postmortem examination. Histopathologic lesions were identified and scored at the following sites: vertebral column, coxofemoral, cubital, stifle, carpal, and tarsal joints. **Results:** Common presenting signs included joint luxation (n=10 [59%]), radiographically evident degenerative joint disease (n=6 [35%]), and decreased range of motion (n=4 [24%]). Histopathologically, toads exhibited osteochondrosis-type changes, most commonly within the vertebral column (n=13 [76%]) ranging from focal chondrocyte necrosis (n=5 [29%]) to articular cartilage flap formation (n=7 [41%]). Older toads frequently developed changes consistent with degenerative joint disease, most commonly within the hips (n=8 [47%]). Joint luxation occurred both in the presence and absence of cartilage changes. No evidence of severe renal disease or metabolic bone disease was captured. **Conclusion:** While metabolic bone disease is common in amphibian species, in this population of Puerto Rican crested toads, disturbances of endochondral ossification and degenerative cartilage changes predominated. Determination of an underlying etiology is under further investigation.

N-21: HISTOPATHOLOGY OF FREE-LIVING POPULATIONS OF THREE SPECIES OF FRESHWATER BIVALVES IN INDIANA

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Background: Freshwater mussels are one of the most endangered groups of animals in Indiana, with nearly half of the native species either extirpated or listed as “state endangered” or of “special concern.” Nationally, numerous freshwater mussel species are considered threatened. Freshwater mussel diseases are not well understood and few published accounts of freshwater mussel diseases with histopathology exist. Mass mortality events within mussel populations are increasingly recognized, often with undetermined etiology. **Objective:** Determine baseline histopathology in free-living populations of freshwater bivalves. **Methods:** One-hundred eighty individual bivalves representing three species—Plain Pocketbook (*Lampsilis cardium*), Fatmucket (*Lampsilis siliquoidea*), and Asian Clam (*Corbicula fluminea*)—were collected from three different locations within the Wildcat Creek watershed in central Indiana during June and July 2019. A cross-section through the visceral mass was obtained and immersed in 10% neutral-buffered formalin. Tissue was processed for routine histopathology and stained with HE. **Results:** A range of histopathologic changes were observed. Branchial acariasis occurred in 43/60 Fatmuckets and 22/60 Plain Pocketbooks. Infection with the

trematode *Bucephalus polymorphus* was recognized in 18/60 Fatmuckets, while infection of the gonadal duct with an unidentified trematode species was identified in 4/60 Fatmuckets and 18/60 Plain Pocketbooks. Additional infections with unidentified trematodes, bacteria, fungi, and ciliates were observed. No Asian Clams had evidence of infectious disease. Common degenerative changes included mineralization, neuronal lipofuscinosis, and gonadal atrophy/atresia. **Conclusion:** A range of histopathologic changes were observed, including infectious and degenerative lesions. Awareness of baseline lesions should inform future diagnostic investigations of mussel mortality events.

N-22: SINGLE-CELL RNA SEQUENCING OF CANINE KIDNEY ENABLES ESTIMATION OF CELL TYPE ABUNDANCE FROM BULK TISSUE TRANSCRIPTOME IN DIGITAL CYTOMETRY

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Background: Single-cell RNA sequencing (scRNA-seq) technology can profile thousands of individual cells from complex tissues, allowing characterization of cellular transcriptomes with higher resolution than the average bulk RNA-seq. Additionally, machine learning techniques can be used to deconvolute cell-type-specific gene expression from bulk RNA-seq data, which has not been published in dogs. **Objective:** We aim to identify cell-types in diseased canine kidney tissues and evaluate the performance of scRNA-seq data for the deconvolution of published bulk RNA-seq data. **Methods:** One gram of fresh kidney cortex was collected post-mortem from a dog with progressive chronic kidney disease (CKD). Cells were dissociated using the Multi Tissue Dissociation Kit and gentleMACS Octo Dissociator. The library was prepared using the Chromium Single-Cell 3′ Kit and sequenced on an Illumina NovaSeq. Data were analyzed through CellRanger, Seurat and clustermole R-packages. Lastly, CIBERSORTx was used to deconvolute published renal bulk RNA-seq data from 2 healthy controls and 6 dogs with CKD. **Results:** We recovered 9,592 cells after quality control and identified 15 cell-types, including major kidney cells (*proximal tubule cells, juxtaglomerular cells, collecting duct cells, and mesangial cells*) and immune cells (*macrophages and NK cells*). RNA-seq data deconvolution identified a decrease of a subtype of proximal tubule cells and an increase of distal tubule cells in dogs with advanced CKD. **Conclusion:** This study demonstrates the feasibility of scRNA-seq technology in dogs that can lead to the discovery of novel genes, pathways, and cell-types relevant to disease progression in CKD.

N-23: SPONTANEOUSLY OCCURRING CLONAL HEMATOPOIESIS IN THE CANINE: A NEW TRANSLATIONAL RESEARCH MODEL

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Background: Clonal hematopoiesis of indeterminate potential (CHIP) is a clinical entity of aging humans that possess cancer-associated mutations in white blood cells, without evidence of overt neoplasia. CHIP has been associated with an increased risk of hematologic cancers, cardiovascular disease, and all-cause mortality. We hypothesized that somatic mutations in specific genes associated with human CHIP would be detectable in the blood of aged dogs not known to have hematologic disorders. **Methods:** DNA from paired germline and whole blood samples from 94 canine patients age 11 and older was subjected to targeted next generation sequencing. Impact of the variants was predicted using Polymorphism Phenotyping version 2 software (PolyPhen-2, Harvard). **Results:** Somatic variants were detected in peripheral blood of three (3.2 %) dogs aged 12-15 years of age. All three dogs had a clinical history of solid cancer, a known risk factor in humans. All detected variants occurred in protein coding regions and were single nucleotide non-synonymous variants. Affected genes were *SF3B1, RUNX1*, and *KIT*. Following analysis by PolyPhen-2, the variants in *KIT* and *SF3B1* were predicted to be benign, while the variant in *RUNX1* was predicted to be damaging. **Conclusion:** Herein, we report the dog as the first spontaneously occurring animal model for CHIP. Spontaneous disease models better replicate the molecular complexity and heterogeneity seen in the human population in comparison to transgenic rodent models. Therefore, this discovery of a spontaneous CHIP animal model will provide an excellent research tool for both the human and veterinary research communities.

N-24: FISH HOST SPECIES SUSCEPTIBILITY INFLUENCES MYXOZOAN COMMUNITY COMPOSITION IN PROLIFERATIVE GILL DISEASE OF CATFISH AQUACULTURE

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Henneguya ictaluri, the cause of proliferative gill disease (PGD) in channel and hybrid catfish, is the most important parasite of commercial catfish aquaculture in the southeastern United States. Research indicates arrested sporogenesis occurs in channel (*Ictalurus punctatus*) × blue (*Ictalurus furcatus*) hybrid catfish, yet PGD persists in hybrid production systems. These reports suggest other myxozoans besides *H. ictaluri* may be associated with PGD. Further, it is hypothesized host susceptibility drives myxozoan diversity in catfish pond aquaculture systems and PGD outbreaks. This work investigated the influence of catfish host on myxozoan community composition within 1) naturally infected gill tissues and 2) pond water associated with

channel and hybrid catfish monoculture. For three years, DNA extracted from gills of diagnostic case submissions with PGD and water from experimental ponds dedicated to either channel or hybrid catfish monoculture were submitted for targeted amplicon metagenomic sequencing to compare myxozoan community composition and diversity between catfish species. Myxozoan community composition significantly differed between channel and hybrid systems in gill and pond water datasets. Detection of numerous described and unclassified species indicates PGD may involve mixed species infections. *H. ictaluri* was present in all channel and hybrid PGD cases but was not the most prevalent species in nearly half. Both datasets revealed hybrid catfish monoculture selectively suppresses *H. ictaluri* proliferation. This work suggests crop rotation strategies could mitigate disease by preventing *H. ictaluri* from reaching levels associated with catastrophic losses. Future work will investigate the potential contribution of other myxozoans to gill pathology in PGD outbreaks.

N-25: GLOBAL LEVELS OF DNA METHYLATION, HYDROXYMETHYLATION AND DNMT3A EXPRESSION AS POTENTIAL PROGNOSTIC BIOMARKERS IN CANINE MAST CELL TUMORS

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Background: Canine Mast cell tumors (MCTs) constitute up to 21% of all canine skin tumors. In Patnaik grade 2 and Kiupel low grade MCTs, biological aggressiveness is sometimes difficult to predict. DNA hypermethylation, hypomethylation and epigenetic enzyme dysregulation are involved in the progression of various cancers. Therefore, global DNA levels of 5-methylcytosine, 5-hydroxymethylcytosine and methylation enzyme expression could serve as prognostic biomarkers to predict MCT aggressiveness and better guide therapies. **Objective:** Quantify the global DNA methylation and hydroxymethylation levels as well as enzymes involved in DNA methylation and their relationship with canine MCT outcome. **Methods:** A tissue microarray with cores from 244 different tumor samples from 189 dogs, along with associated outcome data was immunolabelled for 5-methylcytosine, 5-hydroxymethylcytosine and DNMT3a. H-scores were generated for the cores using QuPath (*v0.1.2)*. **Results:** Patnaik grade 3 MCTs had significantly higher DNMT3a H-scores versus grade 1 and 2 MCTs. Kiupel Low grade and Patnaik grade 2 MCTs with high 5-methylcytosine H-scores also had poorer overall survival (OS). When not using grade to stratify cases, MCTs with high 5-methylcytosine H-scores had poorer disease-free interval (DFI) in all and in subcutaneous only cases. MCTs with high 5-methylcytosine H-scores had shorter OS in all, dermal, and subcutaneous MCT cases. High DNMT3a H-scores had poorer DFI and OS for all cases and shorter OS for dermal cases. For MCTs with no adjuvant therapy, high DNMT3a and 5-methylcytosine H-scores had shorter OS. **Conclusions:** 5-methylcytosine and DNMT3a have potential as prognostic biomarkers in canine MCTs, especially for intermediate histopathological grade.

N-26: RETROSPECTIVE ANALYSIS OF LESIONS IN PERDIDO KEY BEACH MICE (PEROMYSCUS POLIONOTUS TRISSYLLEPSIS)

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The Perdido Key beach mouse (*Peromyscus polionotus trissyllepsis*) is an endangered subspecies of the small old-field mouse native to Perdido Key, a barrier island off the Alabama and Florida coasts. The species is essential to the integrity of its coastal ecosystem but has been severely threatened due to habitat loss and fragmentation, an increased human presence on dunes, hurricane damage, and increased predation from feral and free-ranging cats, foxes, raccoons, and coyotes. There are only two previous reports of disease in the species: one describing a case of cutaneous B-cell lymphoma, and one describing the prevalence of chordomas within the captive population. From April 2013 to June 2020, formalin-fixed samples from 50 mice were submitted from Brevard Zoo to the Zoo and Exotic Animal Pathology Service at the Infectious Disease Laboratory at University of Georgia. Tissues were processed routinely and stained with hematoxylin and eosin. Selected cases were stained with additional histochemical and immunohistochemical stains, as needed. Observed disease processes included bacterial infectious disease, idiopathic amyloidosis, multiple neoplasms, and numerous degenerative lesions, such as glomerulosclerosis, renal interstitial fibrosis, fibrous osteodystrophy, and myocardial degeneration and fibrosis. Diagnosed neoplasms included pilomatricomas, chordoma, soft tissue sarcomas, adenocarcinoma, adrenal cortical tumors, and a follicular adenoma. This retrospective study demonstrates the varied lesions that afflict the Perdido Key beach mouse, particularly multi-organ amyloidosis and degenerative lesions. Understanding the common lesions that afflict this species will provide insight into causes of morbidity and mortality and better support the reestablishment of the species in the wild.

N-27: A 21-YEAR RETROSPECTIVE ANALYSIS OF CORMORANTS SUBMITTED TO ONTARIO/NUNAVUT NODE OF THE CANADIAN WILDLIFE HEALTH COOPERATIVE

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Background: Little is known about the main causes of morbidity and mortality affecting wild cormorant populations in Canada, including a description of possible risk factors associated with diseases. **Objectives:** 1) Summarize causes of cormorant mortalities diagnosed at the Ontario/Nunavut node of the Canadian Wildlife Health Cooperative (CWHC); 2) Identify the association between demographic or environmental factors and cormorant mortalities. **Methods:** Post-mortem reports from cormorants submitted to the Ontario/Nunavut node of the CWHC from 1998 to 2018 were retrieved, and the following were collected: demographic (e.g., age, sex) and environmental (e.g., date of death, location) data, final diagnoses (including cause of death), and ancillary laboratory

tests. Logistic regression was performed to assess risk factors associated with mortalities. **Results:** A total of 387 cases were retrieved (384 double-crested cormorants [*Phalacrocorax auritus*], 3 unspecified), for a total of 439 disease processes diagnosed (1.1 diagnoses/bird). Most common causes of death were avian avulavirus-1 (AAvV-1) infection (n=112, 25.5%), botulism (n = 77, 17.5%), emaciation (n = 46, 10.5%), parasitism (n = 26, 5.9%), and trauma (n = 22, 5.0%). Viral (25.7 %) and bacterial (18.9 %) infections were the most frequent disease processes overall. Strong positive associations were observed between juvenile cormorants and AAvV-1 outbreaks (OR = 2.92, 95% CI 1.39 – 6.14, P < 0.05). **Conclusion:** This study is the first large scale study regarding cormorant mortalities in Ontario, and it reveals causes of mortality similar to those observed in other North American surveys, including a high proportion of AAvV-1 infection in younger birds.

N-28: NEUROPATHOLOGY OF SYSTEMIC BACTERIAL INFECTION IN DOGS

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Background: Systemic bacterial infection (SBI) is a common cause of sepsis and death in dogs. The neuropathology of SBI in dogs is poorly characterized. **Objective:** To describe the neuropathology of SBI in 30 dogs. **Methods:** Autopsy reports from dogs submitted to the AVDL between 2010 and 2020 were retrospectively searched for cases of SBI. Cases with systemic lesions and concomitant central nervous system (CNS) involvement were included in this study. **Results:** Thirty cases were retrieved. The age of affected dogs varied from 3 days to 16 years. Females and males were affected in 18 and 12 cases, respectively, with no evident breed predisposition. The primary site of infection was determined in 17 cases, consisting mainly of pneumonia (7 cases), pyelonephritis (4 cases), and skin lesions (3 cases). Gross lesions in the brain were reported in 13 cases and consisted mainly of leptomeningeal hemorrhages (10 cases). The main histologic findings were suppurative or fibrinous meningoencephalitis with hemorrhage, vascular thrombosis, and necrosis; bacteria were seen in 2 cases. Extra-CNS lesions included suppurative mitral valve endocarditis (12 cases), pneumonia (12 cases), suppurative myocarditis (7 cases), splenic infarct (6 cases), and pyelonephritis (4 cases). Bacterial culture on fresh tissue samples yielded bacterial growth in 27 cases, including *Streptococcus canis* (6 cases), *Escherichia coli* (4 cases), *Klebsiella pneumoniae* (3 cases), and *Staphylococcus intermedius* (3 cases). **Conclusions:** This study highlights the main neuropathologic changes of dogs with SBI, as well as the most common extra-CNS lesions associated with the lesions in the brain.

N-29: FELINE LEUKOENCEPHALOMYELOPATHY IN BOBCATS (LYNX RUFUS) IN FLORIDA, UNITED STATES: PATHOLOGY AND POTENTIAL ETIOLOGIES

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Background: Since 2017, free-ranging bobcats (*Lynx rufus*) and Florida panthers (*Puma concolor coryi*) in Florida have been documented with neurologic disease characterized by ataxia and hind-limb paresis. **Objectives:** To characterize microscopic and ultrastructural lesions in bobcats affected by this recently documented neurologic disease and to investigate potential etiologies. Opportunistically collected carcasses from known-affected regions were also included. **Methods:** Gross and histopathology were performed on bobcat carcasses (n=17) and field-collected tissues (n=7) submitted from 2018-2020 (24 total bobcats). In addition, electron microscopy (EM) was performed on spinal cord from an affected bobcat and panther. Toxicologic testing on livers (n=5) included heavy metals, vitamin A, and gas and liquid chromatography-mass spectrometry analyses. **Results:** Five bobcats (21%) had diffuse white matter degeneration in lateral and ventral funiculi of spinal cord with dilated myelin sheaths, shrunken or absent axons, and digestion chambers. Occasionally, less severe lesions were in brainstem and cerebellar white matter, with microgliosis and perivascular, lymphocytic cuffing. Peripheral nerves were unaffected. EM revealed condensed and dying axons, vacuoles, with no evidence of hypo-, de- or remyelination. Three of five (60%) affected bobcats had copper deficiency; 2/5 (40%) had low vitamin A levels, and organochlorines were detected in 2/5 livers (40%). **Conclusion:** Lesions throughout the hindbrain and spinal cord white matter suggest a central nervous system axonopathy, with no clear associations or causes. Continued monitoring, including postmortem and toxicologic evaluation concurrent with environmental assessment, is imperative to better understanding the implications of this disease for wild felids in the southeastern United States.

N-30: MIRNA PROFILING OF CANINE APPENDICULAR OSTEOSARCOMA

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Background: Canine osteosarcoma is the most common tumor of bone, with most cases occurring in the appendicular skeleton. miRNAs are small, non-coding, regulatory RNAs that bind to messenger RNA and regulate translation. These molecules are potential diagnostic and prognostic biomarkers in human and canine neoplasms. miRNAs are found in both tissue and extracellular fluids. Previous work in our laboratory investigated the profile of miRNAs in plasma of osteosarcoma patients both pre- and post-amputation using RT-qPCR and demonstrated that many miRNAs are dysregulated at both timepoints compared to healthy dogs. Multiple miRNAs were also associated with clinical outcomes. **Objectives:** We sought to validate these findings with a confirmatory population of 27 pre-amputation samples. **Methods:** Thirty-five candidate miRNAs were measured in plasma using miRCURY LNA custom PCR arrays. X-tile analysis was used to obtain high- and low-expression groups before log-rank Mantel-Cox tests were performed to determine statistical significance. **Results:** As in the initial study, multiple miRNAs were associated with overall survival (14), disease-free interval (10), and 1-year survival (17). Increased expression of miR-214 was

significantly associated with poorer outcomes of all three parameters. **Conclusions:** We confirmed that miRNAs in plasma are dysregulated and associated with clinical outcomes in canine appendicular osteosarcoma. Increased expression of miR-214 was associated with poorer clinical outcomes, as demonstrated in our original population and other studies in the literature. In our previous work, miR-214 showed decreased expression post-amputation, suggesting that it is produced and released by the neoplastic cells, although this has yet to be demonstrated *in situ*.

N-31: HEPATIC LESIONS ASSOCIATED WITH IRON ACCUMULATION IN CAPTIVE KORI BUSTARDS (ARDEOTIS KORI)

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Background: There are anecdotal reports of iron storage disease in captive kori bustards (*Ardeotis kori*), but detailed descriptions of this disease are lacking. The goals of this retrospective, multi-institutional study are to (1) describe microscopic findings of iron accumulation in necropsy tissues of kori bustards and (2) use an adapted grading scale to score iron accumulation and associated hepatic lesions. **Methods:** Tissue sections from 20, adult, captive kori bustards were evaluated histologically with hematoxylin and eosin, Masson's trichrome, and Prussian blue stains, and scored for iron accumulation in the liver. **Results:** A diagnosis of hemochromatosis was made in cases with hepatic iron accumulation and concurrent hepatocellular necrosis and hepatic fibrosis. Hemosiderosis was diagnosed in animals with hepatic iron accumulation without necrosis and fibrosis. Twenty animals (age range 3-28 years old) were diagnosed with either hemochromatosis or hemosiderosis. Animals with hemochromatosis also had histologic evidence of iron accumulation in the kidneys, intestines, adrenal glands, and spleen. Hepatocellular carcinoma was diagnosed in two animals. **Conclusions:** Based on previous reports and the current study, iron storage disease may be a substantial condition in captive kori bustards. Future research is needed to understand risk factors for this disease, such as genetic predisposition and/or elevated dietary iron in captive versus wild diets.

N-32: DISORDERS OF THE NERVOUS SYSTEM IN NEW WORLD CAMELIDS (GENERA LAMA, VICUGNA)

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Background: New World camelids (NWC; llamas and alpacas) are popular in the United States. Neurologic diseases are commonly diagnosed in NWC in the southeastern United States. **Objective:** To characterize the neurologic diseases of NWC at the AVDL. **Methods:** The autopsy records of NWC examined from 2008 to 2020 were reviewed for cases of neurologic disease in llamas and alpacas. **Results:** A total of 67 cases (50 alpacas and 17 llamas) with neurologic disease were found. Females (38/67) and males (29/67) with ages ranging between 4 days to 15 years (mean age = 6 years) were affected. Inflammatory diseases occurred in 33/67 cases;

infectious organisms were identified in 14/33 cases, including *Parelaphostrongylus tenuis* (5/14), *Listeria monocytogenes* (2/14), *Fusobacterium necrophorum*, unidentified mixed bacteria, *Cladophilophora bantianum*, rabies virus, Eastern equine encephalitis virus, suspect *Blastomyces* spp., and protozoal cysts (1/14 each). Lesions characterized by necrosis, scattered axonal degeneration, lymphocytic infiltration, hemosiderin-laden macrophages, and glial scars were observed in 27/67 cases. While these lesions were attributed to *P. tenuis* infection, no intralesional nematodes were detected and the diagnosis could not be confirmed. Necrosis and/or axonal degeneration with no apparent cause was found in 7/67 cases. Axonal degeneration due to intervertebral disc disease was diagnosed in 2/67 cases, and hepatic encephalopathy due to severe liver dysfunction was found in 1/67 cases. **Conclusions:** As highlighted by our study, *P. tenuis* is an important cause of neurologic disease in NWC, but other infectious diseases, including rabies, should also be considered.

N-33: SPONTANEOUS NEOPLASIA IN FREE-RANGING WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) IN THE EASTERN UNITED STATES (1980-2020)

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Background: White-tailed deer (*Odocoileus virginianus*) are a popular game species and are widespread across much of North America. Despite high visibility during portions of the year and high densities in many suburban areas, spontaneous neoplasia is rarely reported in this species. **Objective:** We describe spontaneous neoplasia in free-ranging white-tailed deer across the southeastern U.S. **Methods:** We reviewed all white-tailed deer cases at the Southeastern Cooperative Wildlife Disease Study over a 40-year period to determine commonly and rarely diagnosed neoplasia and to assess demographic patterns. **Results:** Among all deer examined (n=4762), neoplasia was diagnosed in 1.66% (n=79) of deer in 17 southeastern and eastern states. The majority (65%) of affected deer were female and all were ≥1.5 years old. Twenty-four (30%) of neoplasms were cutaneous fibromas (43% female). Ten (13%) neoplasms were in the brain (90% female), including three oligodendrogliomas, two astrocytomas, two meningiomas, one hemangiosarcoma, one olfactory neuroblastoma, and one extramedullary plasmacytoma. Eleven tumors (13%) involved the skull (64% female), including five osteochondromas, two squamous cell carcinomas, two plasma cell tumors, one osteoma, one osteosarcoma. Less commonly diagnosed tumors included lymphosarcoma and urothelial carcinoma. **Conclusions:** Although neoplasia is not likely a substantial cause of morbidity and mortality in white-tailed deer, the frequency of brain and skull tumors is noteworthy given the concern over other potential causes of neurologic disease in free-ranging deer (e.g., chronic wasting disease, brain

abscesses). Fibromas often are incidental, unless they interfere with vision, respiration, or mobility based on size.

N-34: VACCINE BREAKS? A RETROSPECTIVE STUDY OF CANINE DISTEMPER IN VACCINATED DOGS.

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Background. The general perception is that canine distemper vaccines are effective at protecting dogs and most cases of distemper in the US are attributed to inadequate or incomplete vaccination. However, newly reported genotypes have been associated with largely anecdotal reports of distemper in vaccinated animals, raising concerns that vaccines might be ineffective against them. We assessed the historical occurrence of distemper in vaccinated dogs to better understand the likelihood of vaccine failure. Objective & Methods. We analyzed the archives of the Veterinary Teaching Hospital of the University of California, Davis to investigate the risk factors associated with perceived vaccine failure in dogs affected by canine distemper from 1980-2020. Results. A diagnosis of distemper was confirmed in 43 vaccinated dogs , with no associated risk factors. There was no gender bias, but many were young (46% < 6 months of age). The most common diagnostic methods used were histopathology and IFA (48,84% each one), followed by PCR (30.23%). Histopathology alone was used in only 5 cases (11.63%). Among those on which vaccinated date was provided, only in 31.82% the disease onset could have taken place before an immune response was formed after vaccination (less than 2 weeks). The most common presentation were neurological (65.12%) and respiratory (60.47%). Single system presentation of the disease was more common (58.14%) than multisystemic. Conclusions. Vaccine breaks are not as uncommon as we might think and the weak evidence of risk factors in this group suggests the need to study the effectiveness of current vaccination products.

N-35: ASSOCIATION OF FEATURES OF MALIGNANCY WITH EXPRESSION OF P16, 14-3-3 Σ, E-CADHERIN AND CD44 IN CANINE GASTRIC CARCINOMA

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Background: Canine gastric carcinoma is infrequent but accompanied by a generally poor prognosis. Tumor behavior and prognosis are closely aligned with features of malignancy including differentiation, anaplasia and invasion. Cell-cycle regulators and cell adhesion proteins, although widely implicated in oncogenesis, have not been investigated in depth in canine gastric carcinomas. **Objective and methods:** Twenty-two cases of canine gastric carcinoma were classified according to histological subtype, degree of differentiation and immunolabelled for cell-cycle regulators p16 and 14-3-3σ and cell adhesion molecules E-cadherin and CD44. The relationship between histological features of malignancy and expression were examined. **Results:** Tubular and papillary carcinomas were considered well-differentiated, signet-ring cell and mucinous as poorly differentiated and anaplastic as undifferentiated carcinomas.

Dysplastic and neoplastic cells expressed p16, with intensity greatest in poorly differentiated carcinomas. 14-3-3σ exhibited cytoplasmic and/or nuclear neo-expression with intensity greatest in undifferentiated tumors. E-cadherin was expressed with reduced intensity in the cytoplasm of neoplastic cells. CD44 was predominantly expressed on the cell membrane with intensity highest in the most well-differentiated carcinomas. Where present, intravascular neoplastic emboli generally labelled strongly for all markers. **Conclusions:** This is the first study to demonstrate expression of CD44 and 14-3-3σ in canine gastric carcinoma with both proposed as biomarkers predictive of metastasis in humans. Positive correlation between p16 and 14-3-3σ in intravascular emboli may be associated with their common, p53-dependent, transcriptional activation pathway and disruption of p53 function. In poorly differentiated cells, E-cadherin expression was cytoplasmically sequestered but overexpressed in tumor emboli, potentially promoting cell survival.

N-37: INFECTIOUS CHOLANGIOHEPATITIS IN A CAPTIVE MARMOSET COLONY

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Between November 2019 and July 2020, five adult common marmosets (*Callithrix jacchus*) presented for acute onset of severe lethargy and dull mentation. Additional clinical signs included weight loss, dehydration, hyporexia, and hypothermia. Blood chemistries taken at the time of presentation revealed markedly elevated hepatic and biliary enzymes, and complete blood counts revealed mild neutrophilia in 4 of 5 marmosets. All marmosets were unresponsive to rigorous supportive care and antibiotic therapy and either died or were euthanized within 48 hours of presentation. Gross and histopathologic examinations were performed for each marmoset and revealed severe, necrosuppurative cholangiohepatitis and proliferative cholecystitis with bacterial colonies and an absence of gallstones. Perimortem and postmortem cultures revealed single isolates of *E. coli* or *Pseudomonas aeruginosa*, or a combination of both. Other postmortem findings included bile duct hyperplasia, periportal hepatitis, bile peritonitis, ulcerative gastroenteritis and typhlitis, and interstitial nephritis. Source of infection and pathogenesis remain unclear; however, this level of change is highly suggestive of a subacute to chronic process despite the acute clinical presentation. To date, this type of severe, infectious cholangiohepatitis and proliferative cholecystitis has not been reported in captive marmosets. Genetic relatedness, the presence of co-infections, including viral, bacterial, and protozoal, and environmental factors are being investigated as potential contributing factors.

N-38: AN OUTBREAK OF FELINE INFECTIOUS PERITONITIS IN CAPTIVE SAND CATS (FELIS MARGARITA)

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Background: Feline infectious peritonitis (FIP) is a devastating disease of domestic cats worldwide. It is infrequently documented in non-domestic felids. The Columbus Zoo

and Aquarium received four juvenile Sand Cats from two litters in 2019. Shortly after their arrival, three of them rapidly declined despite therapeutic intervention and were either found deceased or humanely euthanized. Clinical and gross pathologic findings were suggestive of FIP. **Objective:** Diagnosis and characterization of suspected FIP in a series of three captive Sand Cats via histochemical, immunohistochemical, and electron microscopic evaluation. **Methods:** Slides generated from formalin-fixed paraffin-embedded tissues were stained using routine histochemical (H&E, PAS, Jones, and Trichrome) and immunohistochemical (feline coronavirus) protocols for microscopic evaluation. Additionally, transmission electron microscopy (TEM) was performed on select tissues. **Results:** There was variably severe pyogranulomatous vasculitis affecting multiple organs (lung, liver, kidney, intestine, lymph nodes, and/or meninges) in all cats. Special stains of affected kidney sections showed moderate to marked glomerular mesangial cell proliferation. TEM revealed podocyte and tubular epithelial degeneration and necrosis with no glomerular immune deposits. Immunohistochemistry of liver or kidney sections labeled feline coronavirus antigen in macrophages within inflammatory foci of all Sand Cats. **Conclusions:** The findings are consistent with the diagnosis of FIP. The pathogenesis of FIP remains poorly understood. Given that three of four Sand Cats were affected, and that all four cats were from only two litters, future work will focus on comparative RNA sequencing to elucidate genomic changes that may have contributed to the development of FIP.

N-39: THE PATHOLOGY AND INCIDENCE OF BUILDING-CAUSED BIRD MORTALITY ON MIDWESTERN UNIVERSITY'S GLENDALE CAMPUS

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Window collisions are a major cause of mortality among wild birds. To understand window-caused mortality and increase successful treatment outcomes, we determined the pathology from window collisions and the incidence of building-caused bird mortality on Midwestern University’s Glendale campus. We found that window-killed birds have characteristic gross pathology, most of which resulted in death. Eighty-six window-killed birds, from 17 species, were collected over 16 weeks yielding 74 reliable postmortem examinations to record sustained lesions and determine cause of death. We found that birds die from injuries of blunt force trauma sustained by and/or translated through their pectoral girdle/muscle area. Specifically, 77% had lower respiratory tract injury, 69% had hepatic/splenic injury, and 42% had cardiovascular pathology. Skeletal fractures were also common, including pectoral fractures (58%), pelvic fractures (11%), and long bone fractures (4%). In contrast, only 19% of birds had cranial injuries, suggesting that cranial injury is less common. Fifty-one (69%) of the examined birds had fatal pathologies from two or more of the aforementioned categories and, in 25 cases (34%), it was primary skeletal pathology that caused the fatal visceral injuries. Understanding the cause of death of window-killed birds will improve the treatment of injured birds admitted to wildlife rehabilitation centers, as not all window collisions are immediately fatal. The incidence of window collisions to identify troublesome areas among large buildings will also provide further insight into future remediation. Thus, subsequent work will investigate the role and design of large buildings and their effect on avian mortality.

N-40: DERMATOPHYTES INDUCE CALPROTECTIN EXPRESSION IN THE FELINE EPIDERMIS

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Background: Calprotectin is an antimicrobial heterodimer of S100A8 and S100A9 best known for its expression in myeloid cells and its role as a biomarker of inflammation. A recent genome-wide association study in our laboratory implicated a highly divergent *S100A9* allele (*S100A9*$_{alt}$) containing 13 amino acid substitutions in the susceptibility of Persian cats to severe, chronic dermatophytosis. **Objective:** We aimed to 1) characterize the expression of calprotectin in feline keratinocytes in both normal skin and in lesions of dermatophytosis, and 2) determine whether Persians with *S100A9*$_{alt}$ exhibit differences in calprotectin staining. **Methods:** Immunohistochemistry with Mac387 (calprotectin; S100A8/S100A9) antibody was performed on formalin-fixed paraffin-embedded skin from 5 healthy domestic shorthair (DSH) cats, 5 DSH cats with dermatophytosis, 5 Persian cats with dermatophytosis, and 5 cats with other skin diseases. The Persian group included cats with varying genotypes of an *S100A9* allele associated with dermatophytosis. Each sample was semi-quantitatively scored for number of keratinocytes stained and staining intensity, and groups were compared. **Results:** Keratinocytes of non-lesional cats exhibited little to no staining for calprotectin while keratinocytes of cats with dermatophytosis exhibited a marked increase in staining in sections with active lesions. A clear association was observed between dermatophyte infection and increased expression of calprotectin in feline keratinocytes. Persians with *S100A9*$_{alt}$ also exhibit Mac387 staining of keratinocytes. **Conclusions:** These findings suggest a novel role for the antimicrobial peptide calprotectin in feline host defense against dermatophytes at the skin surface. We are now testing whether amino acid substitutions in the divergent Persian S100A9 allele alter the pathogen specificity of calprotectin.

N-41: IMMUNOPHENOTYPING OF IRIDOCILIARY EPITHELIAL NEOPLASMS IN 21 DOGS

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Background: Neoplasms originating from iridociliary epithelium can be difficult to differentiate from other primary or metastatic intraocular neoplasms, especially if anaplastic. The paucity of studies evaluating expression of immunohistochemical markers in iridociliary neoplasms limits diagnostic use of immunophenotyping. **Objective:** The goal of this study was to evaluate the immunophenotype of iridociliary adenomas and adenocarcinomas using a panel of antibodies. **Methods:** A tissue microarray was created using five 0.6mm punches from each of 12 iridociliary adenomas, 9 adenocarcinomas, and 3 ciliary bodies from normal canine globes. Serial 5µm sections from the resulting microarray were immunohistochemically labeled for N-cadherin, P-cadherin, E-cadherin, vimentin, pancytokeratin [AE1/AE3], cytokeratin 7, cytokeratin 20, neuron specific enolase [NSE], S100, desmin, synaptophysin, glial

fibrillary acidic protein [GFAP], vimentin, and Melan-A, and treated with the Periodic acid-Schiff reaction. **Results:** 21/21 iridociliary neoplasms labeled with vimentin and had variably prominent PAS positive basement membranes. Within many neoplasms, there was heterogeneity of labeling for N- and E-cadherin, but 12/19 iridociliary neoplasms labeled predominately for E-cadherin and 8/20 for N-cadherin. 6/21 labeled for desmin,16/20 for NSE, 4/19 for S100, and 7/19 for P-cadherin. 1/9 adenocarcinomas labeled with pancytokeratin. None of the examined neoplasms labeled for other examined markers. **Conclusions:** Similar to previous reports, iridociliary neoplasms had expression of vimentin and often contained prominent basement membranes. Immunoreactivity for pancytokeratin and desmin was uncommon. Expression of E-cadherin may help differentiate iridociliary neoplasms from sarcomas. While GFAP and Melan-A are reportedly expressed by portions of normal iridociliary epithelium, none of our iridociliary tumors expressed these markers

Veterinary Student Posters

SP-01: HERE'S THE TEA ON T CELLS: EQUINE SUPPORTING LIMB LAMINITIS (SLL) IS ASSOCIATED WITH CD3+ T CELL INFILTRATION

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Supporting limb laminitis (SLL) is a debilitating, and often fatal complication of equine lameness. Altered weight-bearing disrupts the epidermal and dermal lamellae resulting in failure of the suspensory apparatus of the digit within the hoof capsule. The tissue damage triggers inflammation and lesions similar to human psoriasis. As reported, the interleukin (IL)-17 pathway, the major pro-inflammatory effector cytokine in psoriasis, is activated in SLL. Th17 cells, a subset of CD3+ T cells, produce IL-17 in psoriasis. Herein we show that lamellar CD3+ T cell infiltrates correlate with laminitis histopathological severity using immunohistochemistry (IHC) to identify and quantify T cells using an anti-equine CD3 primary antibody (a pan T cell marker) in archived lamellar tissue from SLL cases and controls. Positive cells were counted as a percentage of all cells in three separate locations along the primary epidermal lamella, axial, middle, and abaxial relative to the limb's axis, using digital image analysis (QuPath). Samples were assigned to histopathological subgroups (N=7 to 9) based on severity and stage of laminitis: Control, Developmental, Moderate Acute, Severe Acute (SA), or Severe Chronic (SC). ANOVA on Ranks and Dunn's test for multiple comparisons were used to demonstrate significantly higher proportions of CD3+ T cells in SA and SC cases vs controls. Scoring is in progress to investigate differences in CD3+ T cell tissue distribution between controls (mostly perivascular) and SA or SC cases (more diffuse). These data support the hypothesis that CD3+ T cells are positively correlated with SLL severity and IL-17 pathway activation.

SP-02: USING TECHNOLOGY TO BRIDGE THE SOCIAL DISTANCING GAPS IN A GLOBAL PANDEMIC

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In the pre-COVID-19 era, virtual veterinary teaching and mentoring were relatively uncommon, and often considered inferior to in-person alternatives. However, at ACVP 2019, we presented on how long-distance mentoring in pathology is not only feasible but enables as much interaction as in-person mentorship. Indeed, a few months later, the widespread emergence of COVID-19 throughout the United States after February 2020 required veterinary faculty to identify innovative ways to help students acquire skills and competencies from a distance and continue their professional development while campuses closed. Since then, virtual interaction has not only become more common, but has frequently been the only option for learning and mentorship. Use of virtual modalities such as video conferencing, digital slides, and live streaming for events has grown exponentially, leading to virtual teaching and mentorship being more plentiful than ever before. In this case study, we share how COVID-19 has shaped virtual interaction at our schools. We highlight how we have used virtual technologies, including new digital innovations, to enhance teaching and mentorship in pathology as a whole, and to bridge gaps left by social distancing. These include exchanging the multi-headed microscope for virtual classrooms with microscope live feeds, digital slide sharing, interaction with pathologists across the globe, increased accessibility to conferences, availability of a richer archive of lectures, and increased ability to connect virtually with different schools to explore residencies. COVID-19 innovations have highlighted how social distancing does not necessarily have to be disruptive, but instead, can enhance continued teaching, mentorship, and intellectual exploration.

SP-03: THE PATHOLOGY AND INCIDENCE OF BUILDING-CAUSED BIRD MORTALITY ON MIDWESTERN UNIVERSITY'S GLENDALE CAMPUS

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Window collisions are a major cause of mortality among wild birds. To understand window-caused mortality and increase successful treatment outcomes, we determined the pathology from window collisions and the incidence of building-caused bird mortality on Midwestern University’s Glendale campus. We found that window-killed birds have characteristic gross pathology, most of which resulted in death. Eighty-six window-killed birds, from 17 species, were collected over 16 weeks yielding 74 reliable postmortem examinations to record sustained lesions and determine cause of death. We found that birds die from injuries of blunt force trauma sustained by and/or translated through their pectoral girdle/muscle area. Specifically, 77% had lower respiratory tract injury, 68.9% had hepatic/splenic injury, and 41.9% had cardiovascular pathology. Skeletal fractures were also common, including pectoral fractures (58.1%), pelvic fractures (10.8%), and long bone fractures (4.1%). In contrast, only 18.9% of birds had cranial injuries, suggesting that cranial injury is less common. Fifty-one (68.9%) of the examined birds had fatal pathologies from two or more of the aforementioned categories and, in 25 cases (33.8%), it was primary skeletal pathology that caused the fatal visceral injuries. Understanding the cause of death of window-killed birds will improve treatment of injured birds admitted to wildlife rehabilitation centers, as not all window collisions are immediately fatal. The incidence of window collisions to identify troublesome areas among large buildings will also provide further insight into future remediation. Thus,

subsequent work will investigate the role and design of large buildings and their effect on avian mortality.

SP-04: NEURAL HYPERTROPHY AND HYPERPLASIA IN A CASE OF CHRONIC OVINE PANCREATITIS

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Background : Nerves can be severely reshaped in human pancreatic pathologies such as chronic pancreatitis or pancreatic cancer. In these two diseases, pancreatic nerves can become larger (neural hypertrophy) and/or increased in number (increased neural density/hyperplasia). This process, termed neural plasticity, is also associated with neuropathic pain. Although there are several animal models of chronic pancreatitis, pancreatic neuropathy is not well characterized in these models. Thus, the translational value of these *in vivo* models cannot be entirely ascertained for the study of neural plasticity. Objective : This report describes spontaneous alterations characteristic of pancreatic neural plasticity in a lamb. **Results:** Microscopically, lesions of chronic sclerosing pancreatitis were observed and associated with neural/neuronal hypertrophy and hyperplasia. Conclusions : Even though chronic pancreatitis and pancreatic tumors are common in many animal species, to the authors' knowledge, spontaneous occurrence of associated pancreatic neural plasticity has not been reported in non-human species. Sheep might be a suitable animal model to study this pathology.

SP-05: COMPARISON OF CYTOLOGIC AND HISTOLOGIC FINDINGS IN NOVEL SUBCUTANEOUS FIBROSARCOMA IN A CAPTIVE CHINCHILLA

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A previously healthy, three-year-old, intact male chinchilla (Chinchilla chinchilla) was presented with a single, approximately four-cm, firmly adherent nodule ventral to his left ear. A fine needle aspirate cytologic preparation revealed a population of individualized round cells with low numbers of heavily granulated mast cells. The round cells contained a moderate amount of basophilic cytoplasm and variable numbers of magenta to dark purple granules, variably located nuclei with finely stippled to lacy chromatin and one to three prominent nucleoli. Moderate anisocytosis and anisokaryosis were noted, as well as rare binucleate cells with rare nuclear molding. The cytologic interpretation was round cell neoplasia, with a poorly granulated mast cell neoplasm considered most likely. However, histologic examination of an incisional biopsy sample revealed a poorly-demarcated, highly-cellular, infiltrative, and non-encapsulated neoplasm composed of tightly packed spindloid cells. The neoplastic cells displayed characteristic interwoven and herringbone patterns within a moderately dense fibrovascular stroma. The cells lacked distinct margins and contained abundant eosinophilic fibrillar cytoplasm, oval to elongate nuclei with finely stippled chromatin, and a central magenta nucleolus. Moderate anisocytosis and anisokaryosis were observed. Immunohistochemical stains for smooth muscle actin, desmin, and glial

fibrillary acidic protein were negative, while approximately fifty percent of cells had faint reactivity to S100 IHC staining. These findings were consistent with a subcutaneous fibrosarcoma. This case highlights a compelling discordance between cytologic and histologic findings. To the authors' knowledge, this is the first report describing the cytologic and histopathologic diagnosis of a fibrosarcoma in a chinchilla.

SP-06: IDENTIFICATION OF NOVEL HTLV-1 ENV BINDING PARTNERS AND THEIR ROLE IN CELLULAR TRANSFORMATION TROPISM

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Human T-cell leukemia virus type 1 (HTLV-1) is a deltaretrovirus infecting approximately 20 million people worldwide, causing adult T-cell leukemia/lymphoma (ATL) and HTLV-associated myelopathy/tropic spastic paraparesis (HAM/TSP). While less than 10% of infected individuals develop disease, the virus exhibits a clinical latency period of several decades, making early viral detection an important component of disease prevention. While HTLV-2 exhibits similar genetic organization and expression patterns to HTLV-1, it has not been linked to disease. This may be attributed to the distinct transformation tropism of the viruses; HTLV-1 transforms CD4+ T lymphocytes, while HTLV-2 transforms CD8+ T lymphocytes. The genetic determinant of this transformation tropism and selective clonal expansion has been mapped to the viral envelope (Env-1/Env-2). We therefore hypothesize that differences in interaction between Env-1 and Env-2 and a cellular receptor/protein will induce unique downstream signaling events, leading to this distinct transformation tropism. In this study, we identified and characterized 19 Env-interacting proteins that may serve as both screening markers and therapeutic targets for HTLV-1. We concluded that Env-1 interacts with several unique cellular proteins in HTLV-1 transformed cells, warranting further investigation in future studies.

SP-07: SAMHD-1 MUTATIONS IN FELINE CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Lymphocytosis in cats can be a reactive or neoplastic process. When neoplastic, it is most often diagnosed as chronic lymphocytic leukemia (CLL) further characterized as CD4+ T-cell CLL. Sterile alpha motif and histidine/aspartic acid containing protein 1 (SAMHD-1) is a deoxynucleoside triphosphohydrolase (dNTPase) that regulates the availability of dNTPs for DNA replication. SAMHD-1 activity has implications for viral replication and response to chemotherapy, suggesting a role as a tumor suppressor. In humans with CLL, recurrent mutations in SAMHD-1 have been identified to contribute to disease progression. **Objectives:** This study examined SAMHD-1 for mutations in cats with lymphocytosis due to CLL or other causes. **Methods:** Samples from 42 cats with lymphocytosis were evaluated and classified as reactive or neoplastic by flow cytometry. Genomic DNA was extracted and a region of 2058 bp of SAMHD-1 comprising 4.25% of the gene was amplified. Products were

purified and sequenced. Sequences were analyzed for deletions, insertions, and single nucleotide polymorphisms (SNPs). Mutation frequency was compared between reactive and neoplastic cases using 2x2 contingency tables and Fisher's exact test, with $p < 0.05$ considered significant. **Results:** Multiple genomic regions contained mutations. The majority were in intronic DNA. In total, 33 different mutations were detected and 19 occurred repeatedly. Significant differences in mutation frequency and the cause of lymphocytosis were not identified ($p = 0.1071 – 1.000$). **Conclusions:** Mutations are present in SAMHD-1 in cats with CLL and cats with reactive lymphocytosis. The majority of mutations were located within introns which may affect gene splicing and gene expression.

SP-08: HISTOPATHOLOGY FOLLOWING CRYOABLATION OF SUBCUTANEOUS CANINE MASSES WITH A NOVEL CARBON DIOXIDE DEVICE

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Cryoablation is a technique which uses freezing temperatures followed by thawing to kill tumor cells. In veterinary medicine, cryoablation is typically only used for superficial lesions as human-grade devices capable of freezing large tissue volumes are prohibitively expensive. Kubanda Cryotherapy has developed a device that uses carbon dioxide, a low-cost, readily available gas, to perform a veterinary clinical trial as proof of principle in pets with subcutaneous masses for future translation to human breast cancer treatment. This device has been shown to form an iceball that reaches -40°C and causes necrosis in induced tumors in rats and normal pig livers. Six client-owned dogs with subcutaneous masses (> 2cm diameter) were treated with cryoablation in blocks of two dogs with escalating length of freeze-thaw cycles followed by surgical resection at 7 to 13 days. Histopathology was then performed on paraffin-embedded tissue. H&E stained slides taken primarily parallel to cryoprobe insertion were digitally scanned (Zeiss) and evaluated by manually tracing areas of necrosis, fibrosis, and granulation tissue (Proscia) by two blinded observers. Analysis revealed a central zone of necrosis surrounded by a transitional zone of pathologic change in each mass with average maximal necrotic zone width of 7.7 ± 1.3 mm and total pathologic change of 11.1 ± 1.2 mm with a trend towards increased necrosis with longer freeze times. This study of subcutaneous masses in pet dogs validated the safety of new, low-cost alternative to surgery and provides guidelines for appropriate selection of tumors for the future clinical trials.

SP-10: THE POWER OF PEER MENTORING IN PATHOLOGY AND PANDEMICS

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When starting new experiences, it's beneficial to have the guidance of someone who has been through it before; this is especially true for veterinary students interested in pursuing a specialty. That person can diminish the all-too familiar imposter syndrome and uncertainty many in the veterinary field face. However, students may find it difficult

reaching out to faculty for several reasons: apprehension, unfamiliarity, intimidation, or the fear of looking ‘stupid’ to someone they admire. The world of academia was further shaken up by COVID-19 and veterinary students faced novel predicaments, like not being able to meet faculty during office hours, distance education, social distancing, and state lockdowns. Peer mentors allow students to have guidance while avoiding many of these concerns. With similar schedules, the difference in availability between a peer mentor and a faculty member may be vast. Similarly, there’s less hesitation in contacting a peer and arranging to meet. While the new pandemic life puts a kink in some things, the accessibility of social media and video platforms allows for peer connections to be made, with an additional bonus of not feeling pressured to look professional for faculty. While the ability to wear pajamas is already enough of a selling point to many pandemic students, this report, based on personal experiences in student research, outlines additional benefits of peer mentorship. It also highlights factors to consider when pairing students, in order to enhance these pre-existing advantages. Such factors include academic stages, career goals, hobbies and interests, and personality types.

SP-11: GASTRIC NEUROENDOCRINE CARCINOMA IN A BEARDED DRAGON (POGONA VITTICEPS)

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Case Report A two-year-old, male, bearded dragon (*Pogona vitticeps*) was presented to the University of Florida’s Zoological Medicine service for evaluation of weight loss, anorexia, and marked hyperglycemia. Abnormal physical exam findings included pale mucous membranes and a palpable mass in the cranial coelom. Bloodwork revealed a mild anemia and confirmed the persistent, marked hyperglycemia. Ultrasonographic examination of the coelom showed a hypoechoic gastric mass and hepatic nodule. A fine needle aspirate of the gastric mass was performed for cytologic evaluation. The predominant cell population was fragile and appeared lysed, as free nuclei embedded in pale basophilic cytoplasmic material. The nuclei were mostly individualized but occasionally seen in partially intact, sometimes concentrically arranged aggregates, suggestive of acinar, rosette, or pseudorosette formation. A presumptive diagnosis of gastric neuroendocrine carcinoma was made based on the cytologic and clinical findings. The patient received palliative treatment and was discharged to be euthanized by the primary veterinarian due to poor prognosis. **Conclusions** Gastric neuroendocrine carcinoma is a neoplasm known to occur in bearded dragons. These tumors typically have immunohistochemical staining properties consistent with somatostatinomas. In both humans and bearded dragons, somatostatinomas are associated with hyperglycemia, anemia, and weight loss. Additionally, bearded dragons with gastric somatostatinomas often have metastatic lesions in the liver. Though immunohistochemistry was not performed in the present case, a presumptive diagnosis of somatostatinoma was achieved based on cytology, chemistry, imaging, and clinical findings. This case demonstrates the diagnostic utility of cytologic evaluation of intracoelomic lesions in bearded dragons with suspected gastric somatostatinomas.

SP-12: SENSITIVITY AND SPECIFICITY OF CYTOLOGY FOR THE DIAGNOSIS OF BACTERIAL SEPSIS IN BODY FLUIDS

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Bacterial sepsis is a relatively common, life-threatening condition with a high case-fatality rate. The current primary diagnostic tools for septic effusion in animal patients are bacterial culture and fluid cytology. While culture is the gold standard, it can take several days for results to be made available to clinicians, which can negatively impact a septic patient's chance at survival. The aim of this study is to evaluate the diagnostic accuracy of cytology in detecting bacterial sepsis in body fluids, which has not been thoroughly studied. We retrospectively reviewed 10 years of medical records at the Ohio State University's Veterinary Medical Center for mammalian patients with both cytology and bacterial culture of body cavity fluids (peritoneal and thoracic effusion), blood, joint fluid, or CSF. Preliminary results from 241 cases demonstrate an overall sensitivity of 42.3% and a specificity of 90.1% for the ability to detect bacterial sepsis by cytology. Interestingly, the trend for cytology of thoracic fluid (n=20) is somewhat opposite that of the overall diagnostic accuracy, with sensitivity and specificity of 80.0% and 50.0%, respectively. As more cases are included in the study, we expect to identify subtypes of cases or sample types that demonstrate better or worse diagnostic accuracy of cytology. Unsurprisingly, the overall data collected thus far indicate that cytology is rather insensitive, but specific for this purpose. The results from this study will allow better comparison between diagnostic accuracy of cytology and emerging diagnostic tests for the detection of bacterial sepsis in mammalian patients.

SP-13: IMMUNOHISTOCHEMICAL EVALUATION OF CORONAVIRUS AND ROTAVIRUS IN SHEEPS AND GOATS BOWELS FROM THE MEXICAN HIGHLANDS.

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Neonatal enteritis or neonatal diarrhea syndrome is one of the most common diseases causing high morbidity and mortality in goat kids and lambs. The presence of coronavirus and rotavirus in small ruminants in Mexico has not yet been described. Through immunohistochemistry, the presence of coronavirus and rotavirus was evaluated in intestine sections of goat kids and lambs from 0-120 days who were referred from Centro de Enseñanza, Investigación y Extension en Producción Animal en Altiplano from Facultad de Medicina Veterinaria y Zootecnia UNAM over a period of 10 years. Of 105 goat kids with enteritis and diarrhea; 19 (18%) were immunopositive to rotavirus, and 15 (14.2%) were immunopositive to coronavirus. Of 61 lambs that presented with diarrhea, 10 (16.3%) were immunopositive to rotavirus and 12 (19.6%) were immunopositive to coronavirus. Additionally, in goat kids, there was a combination of both viral agents in 7 (6.6%) cases, 4 (3.8%) with rotavirus and bacteria, 3 (2.8%) with coronavirus and *Coccidia* spp., and 1 (0.95%) with *Cryptosporidium* spp. and rotavirus. In 7 lambs (11.4%) there was a combination of rotavirus and coronavirus, 3 (4.9%) with rotavirus and bacteria, and 1 (1.63%) with concurrent coronavirus, rotavirus, *Coccidia* spp., and *Strongyloides* spp. Neonatal diarrhea syndrome in small ruminants

from the Mexican highlands between 0-120 days of age may be associated with viral agents, although coinfection with other enteropathogens was also identified.

SP-14: EFFECT OF CONCENTRATED PREPARATIONS VERSUS DIRECT SMEARS ON DIAGNOSIS OF SEPTIC FLUIDS BY VETERINARY STUDENTS

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Background: Veterinary student accuracy, confidence, and time to diagnosis of a fluid sample as septic or non-septic was evaluated using two slide preparation methods for fluid cytology: direct smears and concentrated preparations. **Objective:** We hypothesized that veterinary students would more accurately and quickly diagnose fluids as septic on concentrated preparations compared to direct smears. **Methods:** Thirty third- and fourth-year students, who had previously participated in an introductory sophomore clinical pathology course, completed a brief survey regarding cytology experience and reviewed 40 randomized slides (10 septic and 10 non-septic fluids with matched direct and concentrated smears). Slide evaluation time, student diagnosis, confidence, and slide photographs taken by participants of areas considered septic were recorded. **Results:** No difference in diagnostic accuracy between direct and concentrated smears was identified (AUC=57% for both preparations, p=0.77), although students agreed with the pathologist-determined diagnosis more often when viewing concentrated samples (63% ± 11% for concentrated and 56% ± 21% for direct, p=0.012). There was a moderate positive relationship between the accuracy of diagnosis (R^2=0.59) and senior status (p=0.002), comfort interpreting cytology slides (p<0.03), and if the student had completed the senior pathology rotation (p<0.02). Only 38% (121/319) of participant photographs correctly identified areas of sepsis. **Conclusions:** Under experimental conditions, concentrated cytology preparations do not increase the accuracy of a septic fluid diagnosis in veterinary students. However, since accuracy did increase with cytology experience, additional pre-clinical and clinical cytology training may benefit students prior to entering practice.

SP-15: MYOCARDIAL IBA1, MHC CLASS II, AND CD3 ARE DIFFUSELY INCREASED IN CANINE MYOCARDITIS CASES: A STEP TOWARDS ANTEMORTEM DIAGNOSTICS

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Canine myocarditis is rare but potentially serious, causing heart failure and death. Definitive diagnosis is made post-mortem based on the presence of myocardial necrosis, degeneration, and inflammatory infiltrates. However ante-mortem diagnosis is hampered by the numerous etiologies, non-specific course, and dearth of diagnostic criteria. The current human diagnostic gold standard is endomyocardial biopsy (EMB) pairing cardiohistology (Dallas criteria) with immunohistology to enhance detection of often multifocal disease. We sought to understand the expression of immune response

markers in the canine heart to establish similar immunohistologic criteria and lay the groundwork for antemortem definitive diagnosis of canine myocarditis. We hypothesized that myocardial MHCII, CD3, and Iba1, markers upregulated in human myocarditis, would be increased in canine myocarditis cases. Paraffin-embedded myocardial tissue from 20 histopathologically confirmed cases of adult and juvenile myocarditis and 18 controls (encompassing non-cardiac and non-inflammatory cardiac diseases) were obtained from the archives of the Cornell Animal Health Diagnostic Center. Heart tissue was immunoprocessed for MHCII, CD3, and Iba1, and the fraction of myocardium with optically detectable marker expression was determined using QuPath image analysis. All three markers were significantly increased compared to controls: Iba1 10.3x (Mann-Whitney (MW) $p<0.0001$), MHCII 4.6x, (MW $p=0.0068$), and CD3 3.8x (MW $p=0.0166$). These data show diffuse upregulation of immune response markers in the myocardium of dogs with myocarditis, with detection potentially independent of tissue sampling. This suggests that EMB and immunohistochemical detection of MHCII, CD3, and Iba1 may permit sensitive ante-mortem diagnosis of canine myocarditis.

SP-16: METASTATIC GASTRIC CARCINOMA IN AN ALPACA

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A ten-year-old, female alpaca presented to the University of Illinois Veterinary Teaching Hospital with a history of malaise, decreased appetite, weight loss, and straining to urinate and defecate. Abdominal ultrasound revealed a 15 cm mass in the cranial abdomen intimately associated with the GI tract with accompanying free peritoneal fluid. The fluid was submitted for cytology, revealing individualized and cohesive, round to polygonal cells with marked anisocytosis and anisokaryosis, along with few binucleated cells and bizarre mitotic figures, suggestive of carcinoma. Due to deteriorating condition and clinical diagnosis, owners elected humane euthanasia. Postmortem examination revealed a large, firm, tan, hemorrhagic mass associated with gastric compartments 1 and 2. Numerous, round, 0.5-2 cm diameter, firm, white nodules were observed throughout the liver. Histologic evaluation showed an unencapsulated, poorly circumscribed, and invasive neoplasm of the stomach. The cells were arranged in sheets and vague trabeculae, and were supported by fibrovascular to scirrhous stroma. The polygonal neoplastic cells showed marked anisocytosis with moderate amounts of cytoplasm and variably distinct cell borders. The nuclei contained coarsely clumped chromatin and prominent nucleoli, with some cells exhibiting multinucleation and karyomegaly. Frequent and often bizarre mitotic figures were present. Clusters of neoplastic cells were present within vascular and/or lymphatic vessels. Neoplastic cells effaced and expanded portions of the liver and tracheobronchial lymph node. Positive staining for cytokeratin confirmed the diagnosis of carcinoma. Gastric squamous cell carcinomas have been previously reported in South American camelids, although obvious evidence of squamous differentiation was not appreciated in this case.

SP-17: HEPATIC COCCIDIOSIS CAUSED BY EIMERIA STIEDAE IN TWO-MONTH-OLD RABBITS

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Tissues of three two-month-old rabbits were mailed to Mississippi State College of Veterinary Medicine Pathology Service in June of 2019 without a history. Their livers were severely altered by marked hyperplasia and bile duct expansion. The hyperplastic epithelium of the bile ducts contained numerous *Eimeria stiedae* (*E. stiedae*) organisms in different stages of development. Expansion of the bile ducts occurred due to accumulation of crenated unsporulated oocysts within them. There was marked fibrosis surrounding the affected bile ducts that extended to the adjacent hepatic lobules. The definitive diagnosis of this case was hepatic coccidiosis. Hepatic coccidiosis is important because it is a leading cause of morbidity and mortality among rabbits worldwide. The etiology of hepatic coccidiosis is a protozoan in the phylum Apicomplexa called *E. stiedae*. The transmission of this parasite is fecal-oral, starting with the ingestion of sporulated oocysts that undergo excystation prior to penetrating the mucosa of the small intestine. The parasites migrate through the blood and lymphatic system to the liver. The pathophysiology occurs due to this extra-intestinal migration to the liver, causing hepatomegaly and destruction of the hepatic architecture. The sporozoites then inhabit the bile duct epithelium, where they undergo merogony and gametogony. These colonizers produce unsporulated oocytes into the bile duct lumen that are shed in the feces and quickly sporulate. The main mechanism of controlling infection is strict sanitation because of adult subclinical carriers that provide a source of infection to young rabbits, who develop clinical symptoms and death.

SP-18: SINUSITIS AND CONJUNCTIVITIS IN A WILD TURKEY (MELAGRIS GALLOPAVO) ASSOCIATED WITH AVIBACTERIUM BACTERIA

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Background: In January of 2020, Colorado Parks and Wildlife (CPW) was contacted about sick and dying wild turkeys (*Meleagris gallopavo)* in Pueblo County, Colorado. Necropsy of one turkey revealed severe catarrhal and fibrinous sinusitis and conjunctivitis. Culture identified bacteria most consistent with *Avibacterium paragallinarum,* the cause of infectious coryza in domestic chickens. **Objective:** The objective of this study was to characterize the bacteria associated with disease and determine the extent of the disease within the flock. **Methods:** Eight additional wild turkeys from the flock (~ 25% of the flock) were culled for disease surveillance. Gross necropsy and histologic evaluation of the nasal and infraorbital sinuses was performed on all birds, along with aerobic culture and PCR for *Mycoplasma spp..* Mass spectrometry and whole genome sequencing was performed to further classify the bacterial isolates. **Results:** Infraorbital sinus swabs from four additional turkeys showed heavy growth of *Pasteurellaceae* bacteria, in conjunction with mild hyperplastic and lymphoplasmacytic sinusitis. Mass spectrometry of the bacterial isolates, and preliminary whole genome sequencing, most closely resembled *A. gallinarum.* **Conclusions:** Severe sinusitis and conjunctivitis were associated with *Avibacterium* in a wild turkey. The presence of this bacteria in other birds in the flock, in the absence of significant disease, suggests other factors may be involved in development of severe conjunctivitis and sinusitis. A whole genome sequence

comparison between isolates from the clinical and asymptomatic birds will determine if there are genetic differences in *Avibacterium sp*., which may exacerbate disease.

SP-19: CASE REPORT: ECTOPIC LINGUAL AMYLOID PRODUCING ONDONTOGENIC TUMOR IN A DOMESTIC SHORTHAIR

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An 11.5-year-old Domestic Short Hair spayed female cat presented for a left Total Ear Canal Ablation (TECA) and pinnectomy due to masses within the horizontal ear canal and on the pinna. Both tumors were previously diagnosed as ceruminous gland adenocarcinoma and mast cell tumor, respectively, and were histologically confirmed post excision. Upon intubation, a 1.1x0.6x0.5cm firm, white, oval nodular mass was noted on the right lateral tongue base. An excisional biopsy of this mass was performed. The tissue was routinely processed and stained with H&E. Histologically, islands and cords of odontogenic epithelium with stellate reticulum showing characteristic palisading and apical nuclei were observed. There was abundant fibrous stroma, with no visible dental hard tissue. Multifocally, eosinophilic, amorphic matrix exhibiting occasional mineralization was deposited within the epithelial portion. The matrix was consistent with amyloid in Congo red stain viewed under polarized light. Based on these characteristics, the mass was diagnosed as amyloid-producing odontogenic tumor (APOT). APOTs are uncommon in dogs and rare in cats. They are most frequently associated with the gingiva, mandibular and maxillary bones but have also been reported in the facial skin in cats. To our knowledge this is the first case of a lingual APOT. This case is also interesting due to the cat's predilection for facial tumors; there were three separate and histologically distinct facial masses. The FIV/FeLV status of this cat is unknown, however, this cat has had chronic ear infections, so an underlying immunosuppressive link between all three tumors cannot be ruled out.

SP-20: EIMERIA GILRUTHI: AN UNCOMMON CAUSE OF ABOMASAL COCCIDIOSIS IN THE UNITED STATES

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Eimeria gilruthi (formerly *Globidium gilruthi)* is an apicomplexan coccidia which was most recently documented in the United States of America in 2018 in a research setting in Tennessee. *Eimeria gilruthi* has not previously been described in Virginia to the authors' knowledge. *Eimeria gilruthi* is generally considered an incidental finding, but there are reports suggesting it is a pathogenic cause of abomasitis in small ruminants that may cause or contribute to morbidity. In this case an approximately 90 pound-ram lamb in good body condition was submitted for necropsy with history of diarrhea, anorexia, polydipsia, and excessive salt licking. Gross lesions included a gray abomasal mucosa with scattered petechiae and consolidation of the right cranial and middle lung lobes. Microscopically, the ram had multifocal necrotizing to granulomatous abomasitis, with numerous intralesional 150-400 um protozoal schizonts which had a thick eosinophilic wall and thousands of merozoites. Moreover, suppurative

bronchopneumonia (likely perimortem) and a systemic bacterial infection (with embolic pneumonia and multifocal necrotizing hepatitis) were identified, likely secondary to the coccidial abomasitis. Little is known about the life cycle or transmission of *E. gilruthi,* which has been documented in both sheep and goats. The dearth of literature on this parasite in the United States of America makes it a potentially neglected cause of abomasal coccidiosis and a differential diagnosis in small ruminant diarrhea.

SP-21: CONCURRENT CANINE DISTEMPER AND TYZZER'S DISEASE IN A GRAY FOX

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Two gray fox (*Urocyon cinereoargenteus*) littermates, estimated to be 1-2 months old, were found dead a few days apart and submitted for necropsy. Both foxes were in thin body condition, covered in fleas, and dehydrated. The lungs contained small areas of dark red to purple discoloration, and one fox had multifocal to coalescing, round, pale tan foci in the liver. In both kits, microscopic examination of the lungs revealed intranuclear and intracytoplasmic viral inclusion bodies consistent with those caused by canine distemper virus (CDV). Biliary duct epithelial cells in the second fox contained similar inclusion bodies. The liver of the second kit showed variably sized, discrete, pale areas of necrosis with admixed neutrophils randomly distributed throughout the parenchyma. Hepatocytes surrounding these foci occasionally contained faint, cytoplasmic, elongated bacteria. These bacteria were morphologically consistent with *Clostridium piliforme* and stained positively with a Steiner silver stain. Fresh and fixed sections of lung, liver, and kidney from both kits were submitted for CDV fluorescent antibody testing, and all examined sections were strongly positive. Concurrent canine distemper and Tyzzer's disease has been previously reported in the dog and raccoon. Immunosuppression caused by CDV presumably allows for a secondary *Clostridium piliforme* infection.

SP-22: XANTHOMATOSIS IN A YOUNG GOAT

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A 10-week-old Nigerian Dwarf doe presented to the Texas A&M Veterinary Medical Teaching Hospital for a laryngeal mass and possible pneumonia. A temporary tracheostomy was placed, and radiographs revealed possible laryngeal trauma or a retropharyngeal mass. Endoscopy revealed abnormal pharyngeal mucosa with multiple masses, and biopsy revealed a locally extensive, acute fibrinohemorrhagic laryngitis. A few days following the biopsy, the patient presented with dyspnea, and radiographs revealed a pneumothorax. Due to a poor prognosis, the owners elected humane euthanasia. Necropsy revealed a 1.5x1x1 cm, raised, lobulated, firm, white and red nodule on the epiglottis. Multiple small, tan to white, raised nodules were on the esophageal mucosa near the larynx, left arytenoid cartilage, thoracic diaphragm, and

thoracic wall pleura. A few similar nodules were in the kidneys, rumen, omentum, and within the falciform ligament. Histologically, the nodules were composed of macrophages, intra- and extracellular lipid accumulations, and small numbers of multinucleated giant cells, lymphocytes, and plasma cells. The lesions were supportive of a diagnosis of systemic xanthomatosis. Although xanthomatosis most often occurs in the skin, systemic xanthomatosis has been documented previously in humans and cats.

SP-23: CATHETER-ASSOCIATED AIR EMBOLISM OF THE BRAIN IN A HOSPITALIZED HORSE

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An 8-year-old Belgian Draft cross gelding was anesthetized for tenoscopy and laceration repair after sustaining a right hind digital flexor tendon sheath laceration. The horse remained hospitalized for several days, and on the fourth day following surgery the jugular catheter became disconnected from the extension set. The next day, the horse was blind bilaterally and circling to the left. Elevated cardiac troponin with mild tachycardia was also noted. The horse was treated supportively, but neurologic signs persisted and humane euthanasia was elected after three weeks without improvement. The most significant findings on gross necropsy were generalized cerebral edema, gross malacia, and hemorrhage of the left occipital cortex. Histopathology of the brain revealed regionally extensive cortical loss in both the left and right occipital cortex. Changes ranged from edema with necrosis and loss of outer cortical lamina to full thickness cortical necrosis that involved both grey and white matter. Foci of malacia consisted of lytic necrosis with loss of parenchyma and infiltration by gitter cells. Overall, necropsy revealed severe brain lesions consistent with an infarctive pathogenesis. The history and necropsy are compatible with air emboli in the brain. This is a rare complication of IV catheter use in horses.

SP-24: ISCHEMIC NECROSIS OF THE FEMORAL HEAD IN A PIGLET MODEL IS DETECTED BY QUANTITATIVE MRI ONE WEEK AFTER INJURY

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Legg-Calvé-Perthes Disease (LCPD) is a juvenile hip disorder affecting both children and dogs caused by interruption of blood flow to the developing femoral head, resulting in joint deformity. New imaging techniques are needed to assess the early-stage ischemic injury to better inform treatment decisions. Purpose: to evaluate the sensitivity of T1ρ and T2 mapping to detect ischemic injury to the femoral head in an induced LCPD piglet model. Hypothesis: T1ρ and T2 relaxation times will be increased in femoral heads undergoing ischemic injury compared to unaffected, contralateral hips. Eight 6-week-old piglets underwent surgery to induce unilateral global femoral head ischemia. One week post-operatively, the piglets were imaged *in vivo* at 3T MRI using 3D T1ρ and T2 mapping. Ischemia was confirmed using contrast-enhanced MRI. The T1ρ and T2 maps of the femoral heads were segmented into four regions of interest (ROIs): secondary ossification center (SOC); articular-epiphyseal cartilage complex

(AECC); primary physis; and metaphysis. Histological analysis using H&E staining found clear evidence of diffuse necrosis of bone marrow cells and chondronecrosis in the SOC and AECC in all operated femoral heads; osteocyte necrosis and decline was visible in the bone. Median T1ρ and T2 values were significantly increased in the operated vs. control femoral heads in the SOC (P=0.0032, P=0.0002, respectively) and AECC (P<0.0001, P<0.0001, respectively). There were no significant differences in the metaphysis and primary physis ROIs. These results support that T1ρ and T2 mapping are sensitive and potentially clinically-translatable techniques to detect early-stage ischemic injury to the femoral head.

SP-25: MYCOPLASMA ARGININI CO-INFECTION IN MYCOPLASMA BOVIS PNEUMONIA

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Background: *Mycoplasma bovis* has been implicated in uniquely presenting caseonecrotic lesions of the lung in cattle, although it is suspected that it does not act alone in the development of this lesion. The lesions of *M. bovis* pneumonia are commonly co-infected with *Mycoplasma arginini*. **Objectives:** This study determined the frequency of singular and co-infections of *M. bovis* and *M. arginini*, and the frequency of caseonecrotic lesions in singular versus co-infected cases. **Methods:** Bovine pneumonia cases with a positive culture for *M. bovis*, *M. arginini*, or both were gathered from the Animal Health Laboratory archive between 2007 and 2020. Of the included cases, those with histologic sections available were assessed for the presence of caseonecrotic lesions in lung tissue. **Results:** Of the 111 cases identified, 39 (35.1%) were infected with *M. bovis* alone, 9 (8.1%) were *M. arginini* alone, and 63 (56.8%) were co-infections of *M. bovis* and *M. arginini*. Of the 90 cases with histologic sections available, 0/9 (0%) of those infected with *M. arginini* alone had caseonecrotic lesions. In cases infected with *M. bovis* alone, 10/31 (32.3%) had caseonecrotic lesions. In the cases co-infected with *M. bovis* and *M. arginini*, 15/50 (30%) had caseonecrotic lesions. **Conclusions:** The majority of *M. bovis* pneumonia cases are co-infections with *M. arginini*, though there does not seem to be a difference in the presence of caseonecrotic lesions in histologic sections between *M. bovis* infected and co-infected cases. Caseonecrotic lesions were often absent from lesions of pneumonia infected with *M. bovis*.

SP-26: MYCOTIC PHARYNGITIS MIMICKING CHROMOBLASTOMYCOSIS IN A HORSE

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A 13-year-old Trakehner gelding presented to Texas A&M Veterinary Medical Teaching Hospital for evaluation of nasal masses. The horses had previously been diagnosed with nasopharyngeal cicatrix syndrome. On upper airway endoscopy, no worsening of the previously diagnosed cicatrix was noted, however, the mucosa of the pharyngeal wall rostral to the left guttural pouch opening had a nodular appearance. Several similar

nodular masses were also observed in the left nasal passage. These masses and a single pharyngeal mass were biopsied. Histologically, the masses were characterized by multifocal to coalescing pyogranulomas infiltrating and expanding the submucosa and extending into the mucosa, which was multifocally ulcerated. The pyogranulomas consisted of centers of neutrophils surrounded by macrophages and scattered multinucleated cells, with a rim of fewer plasma cells, Mott cells, and lymphocytes. Within the center of these pyogranulomas were multifocal clusters of 10-15µm, thick-walled, pigmented fungal organisms with chlamydospore-like conidia. The histopathologic findings were highly suggestive of chromoblastomycosis. Panfungal PCR targeting the ITS2 region followed by sequencing further characterized the fungi as belonging to the Teratosphaeriaceae family (Capnodiales). Members of the Teratosphaeriaceae family (Capnodiales) are rare opportunist human and plant pathogens that have been previously described in a single case report in a person with a cutaneous infection. Disease associated with this organism has apparently never been reported in the veterinary literature.

SP-27: DETECTION OF LYMPHOCYTES AND PLASMA CELLS IN FORMALIN-FIXED, PARAFFIN-EMBEDDED TISSUES OF MULTIPLE AVIAN SPECIES USING COMMERCIALLY AVAILABLE ANTIBODIES

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Background: Immunohistochemistry (IHC) is commonly used in veterinary diagnostic pathology. However, most commercially available antibodies are raised against mammalian antigens, limiting their applicability for avian species. **Objective**: To evaluate the cross-reactivity of commercially available antibodies raised against human Pax5, CD3, and MUM-1 (B-lymphocyte, T-lymphocyte, and plasma cell markers, respectively) in tissues of multiple avian species. **Methods**: Formalin-fixed, paraffin embedded lymphoid tissues (i.e., thymus, bursa, spleen, Harderian gland) from a variety of avian species were collected from archived postmortem cases submitted to the Ontario Veterinary College. Birds with obvious lesions in these organs or systemic immunosuppressive disease were excluded. The IHC protocol was conducted using commercially available antibodies [mouse monoclonal anti-Pax5 (clone 24) and anti-MUM-1 (clone MUM-1p); rabbit polyclonal anti-CD3] initially optimized on chicken tissues. Antibody specificity was assessed based on the known tissue distribution of the target cells (i.e., microanatomy). **Results**: Lymphoid tissues from 59 avian species in 13 families were included in the study, and the expected patterns of reactivity for the 3 antibodies were observed across most taxa. Anti-CD3 antibody was reactive in all genera. Anti-Pax5 antibody was reactive in all genera except 4 of 5 species of falcons (*Falco* spp.). Anti-MUM-1 antibody was reactive in all genera except canaries (*Serinus canaria*). Surprisingly, in domestic geese (*Anser domesticus*), anti-Pax5 reactivity was observed in T-lymphocytes in addition to B-lymphocytes. **Conclusions**: These results suggest that the Pax5, CD3, and MUM-1 are cross-reactive antibodies in many avian species, but discrepancies indicate that validation in control tissues is necessary prior to use.

SP-28: SYMMETRIC DIMETHYLARGININE (SDMA) CORRELATES WITH A PROPOSED HISTOLOGIC GRADING SYSTEM FOR CHRONIC KIDNEY DISEASE IN TIGERS (PANTHERA TIGRIS)

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Chronic kidney disease (CKD) is a highly prevalent cause of morbidity and mortality in felids, including not only domestic cats but a wide variety of non-domestic felid species such as tigers (*Panthera tigris*). In domestic cats, gross pathologic changes to the kidneys suggestive of chronic kidney damage are collaborated by palpation and imaging. These diagnostic techniques have not been shown to be as sensitive nor specific in the diagnosis of CKD in tigers. While the histologic changes related to CKD in tigers resemble those of domestic cats, the gross changes are not as reliable. Symmetric dimethylarginine (SDMA) is a renal biomarker produced as an end product of protein metabolism resulting from the methylation of L-arginine protein residues and is excreted almost primarily by the kidneys. SDMA has been shown to increase in domestic felids with CKD and decreasing glomerular filtration rate earlier than other renal biomarkers, such as creatinine and blood urea nitrogen. Correlating SDMA and other renal biomarkers with histopathologic changes to the kidneys would be of value in the diagnosis of tigers with CKD. We propose a semi-quantitative histologic scoring system incorporating fibrosis, tubular atrophy, and inflammation grading to approach evaluation of CKD in tigers. SDMA significantly correlated ($r = 0.667$) with histologic score using this method and may be better utilized in the future to evaluate severity of CKD in tigers and to provide an opportunity to diagnose renal insufficiency at an earlier stage than with traditional biochemical markers.

SP-29: BOVINE ADENOVIRUS 3 PNEUMONIA IN A WHITE-TAILED DEER FAWN

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A 7-month-old farmed white-tailed deer fawn (*Odocoileus virginianus)* died after several weeks of progressive deterioration associated with gastrointestinal parasitism and respiratory signs. A field necropsy was performed, and lung tissue was submitted to the Texas A&M Veterinary Medical Diagnostic Laboratory for histologic examination. Microscopically, the bronchi and bronchioles were dilated and filled with sloughed epithelial cells, necrotic cellular debris, and neutrophils. The bronchial and bronchiolar epithelium were hyperplastic with areas of necrosis, and many epithelial cells had large eosinophilic intranuclear inclusion bodies. Scattered throughout the alveoli were dense infiltrates of neutrophils with fewer macrophages, edema, and necrotic cellular debris. Alveolar septa were mildly hypercellular and expanded by edema. The findings were consistent with viral pneumonia with probable secondary bacterial involvement. Immunofluorescence using fluorescently labeled polyclonal antibodies to bovine adenovirus 3 and 5, respectively revealed a positive result for bovine adenovirus 3 (BAdV-3). BAdV-3 is an important pneumotropic pathogen in cattle and is often involved in bovine respiratory disease complex. Previous studies found that BAdV-3 can also

infect wild African buffaloes, alpacas, and goats, and a cervid adenovirus (CdAdV-1) is 76% genetically similar to BAdV-3 indicating that the virus is capable of cross-species transmission. Our findings suggest that BAdV-3 should be included in the differential diagnosis of respiratory disease in cervid species. Further research is warranted to evaluate the implications and distribution of this pathogen in deer species.

SP-30: A REVIEW OF AFRICAN PENGUIN (SPHENISCUS DEMERSUS) CHICK PATHOLOGY AT THE MARYLAND ZOO

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The Maryland Zoo currently houses the largest colony of endangered African penguins (*Spheniscus demersus)* in North America and is actively involved in efforts to understand the factors that affect chick survival in zoo-housed breeding colonies. The purpose of this study was to further classify and understand the pathologic processes contributing to African penguin chick mortality in a captive-bred colony. Pathology reports for African penguins under 3 years of age from 2006 to 2020 (N=27) were obtained from the Johns Hopkins comparative pathology database. Chicks were sorted into age groups and general causes of death were assigned based on the major gross and histopathologic findings. Of the 27 chicks, 59% died before reaching fledging age (3 months) and 26% died within seven days of hatching. Causes of death included failure to thrive (26%), trauma (15%), respiratory disease (15%), gastrointestinal disease (11%), congenital defects (11%), avian malaria (7%), hemorrhage/blood loss (7%), myocarditis (4%), and CNS disease (4%). The chicks whose cause of death was failure to thrive (N=7) underwent additional histologic examination to further investigate lesions that contributed to mortality. Common histopathologic findings in these chicks included bursal lymphoid depletion (6/7), cloacitis with abscess formation (4/7), and pulmonary hemorrhage, edema and congestion (7/7). The results of this study provide a thorough assessment of disease processes associated with penguin chick mortality at The Maryland Zoo and will inform future efforts aimed at enhancing chick survival in zoo-housed breeding colonies.

SP-31: FIVE NETWORKING TIPS FOR STUDENTS ON THE PATH TO VET PATH

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Networking is key to unlocking pathology experiences for veterinary students. Although internet research can provide a valuable introduction, the best insights into a pathology career come from shadowing and communicating with practitioners in the field. Events such as career fairs can provide formal introductions; however, students should not underestimate the power of informal contacts in their career development. Some veterinary students may, incorrectly, feel inclined to wait for pathology opportunities to arise as part of their course, whilst others are too intimidated to approach faculty or other accomplished professionals. Most pathologists, however, are keen to impart their

knowledge and share their enthusiasm. Here are five simple tips for successful networking: First, start early: experience leads to experience, every contact has connections to share, so a student's network can grow exponentially. Second, start on home ground: a student's own university offers immediate access to specialist guidance not available to students from other institutions. Furthermore, staff members have a vested interest in their students' careers. Third, never miss a chance: meetings, conferences, research placements and clinical experiences present numerous networking opportunities. Fourth, go virtual: social media holds a plethora of potential contacts with similar interests; this could expand a student's network worldwide. And finally, be open-minded: explore opportunities beyond personal interest; the catalyst for a future career may be entirely unexpected. By highlighting my personal experience as a veterinary student, this case study shares five ways in which networking can help students on the path to pursuing a career in veterinary pathology.

SP-32: DEVELOPMENT OF A DEEP LEARNING METHOD FOR DISCRIMINATING ANTERIOR NASAL CAVITY DEGENERATIVE, REGENERATIVE, AND INFLAMMATORY LESIONS IN THE RAT

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Nasal cavity assessment is essential for both the safety assessment of environmental and workplace exposure agents and pharmaceuticals. The nasal cavity in the rat is complex and in a standard 30-day rat toxicology study at least 240 sections are examined by the pathologist. Nasal cavity lesions are diverse, and efforts to ensure consistent diagnostics and scoring is time consuming. We hypothesized that a deep learning artificial intelligence (AI) algorithm would provide a decision support tool for the pathologist that would increase diagnostic quality and efficiency. Whole slide images (WSI) of nasal cavity levels I and II from control and treated animals were scanned at 40x on a Leica AT2 scanner and uploaded to Deciphexs' Patholytix Preclinical Study Browser. Training annotations were performed for 14 different classes at 10x magnification based on INHAND criteria and used by Deciphex to conduct supervised training of a convolutional neural network (CNN). Model performance was quantified on both a pixel and object basis by using confusion matrices and F1 scores for AI generated masks. The developed algorithm performed well for 12/14 classes (F1 scores exceeding 0.70). A test set of unannotated nasal cavities was evaluated by 4 ACVP board-certified pathologists and the algorithm and consensus supported qualification of the algorithm performance on an object-basis. The AI algorithm was incorporated into an intuitive digital workflow for the toxicologic pathologist and has potential to provide both functionality and decision support that increases efficiency and consistency.

SP-33: QUANTIFYING EPIDERMAL CHANGES OF NAÏVE AND SUBSEQUENT AMBLYOMMA AMERICANUM BITE LESIONS OF DOGS AND CATS

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Background: *Amblyomma americanum*, the lone star tick, readily bites and feeds on medium and large-sized mammals. **Objective:** To describe and quantify epidermal

changes caused by *A. americanum* attachment on dogs and cats. **Methods:** Punch biopsies (5 mm) were processed and stained with hematoxylin and eosin from 8 dogs and 8 cats during naïve and subsequent infestations. Epidermal thickness was measured every 0.25 mm from the center of the bite to the edge of the tissue cross section and compared using two-way ANOVA. Radii of ulcer and neutrophilic inflammation surrounding the bite were measured and compared with linear regression. Results were compared to the control (day 0 – no infestation) between day and sex for dogs and cats separately. **Results:** Two-way ANOVA results revealed multiple significant distance points for both dogs and cats over the course of the study. There were significant increases in epidermal thickness closest to the tick bite wound along with a general decrease in epidermal hyperplasia for distance points further away from the tick bite. Linear regression revealed significant difference between days for ulcer size in dogs in subsequent infestations. Stratum corneum neutrophilic inflammation increased around the tick bite wound over the course of the study. **Conclusions:** Tick attachment leads to significant hyperplastic, ulcerative, and inflammatory changes in the epidermis of dogs and cats thus supporting the use of tick preventatives for all dogs and cat.

SP-34: GROSS, HISTOPATHOLOGIC AND RADIOGRAPHIC LESIONS ASSOCIATED WITH LAMENESS IN SOWS EUTHANIZED FOR LAMENESS

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Background: Sow lameness decreases welfare and is costly to producers. Loose housing sows makes early detection of lameness difficult, but studies estimate prevalence is over 20%. **Objective**: To use necropsy, histopathology, radiology, and bacteriology to characterize lesions and better understand the etiopathogenesis of lameness in loose housed sows euthanized for lameness. **Methods**: Limbs from 26 lame sows were collected. Radiographs of distal limbs were taken. Joints and surrounding soft tissues were dissected. Gross lesions were photographed and documented. Tissues were processed and stained with H&E. Lesions were categorized as septic, osteochondrosis (OC), or degenerative osteoarthritis (OA). Joints were cultured for aerobic and anaerobic organisms. **Results**: Septic lesions (n=94) were more prevalent than OC (n=44) or OA (n=38). Lame limbs were significantly more likely to have a septic lesion than were non-lame limbs (OR=2.7 CI=1.1-7.0, $p<0.05$). Degenerative or OC lesions were not more likely in lame versus non-lame limbs ($p>0.05$). Septic lesions were more common in the distal (n=81) compared to proximal limb (n=15). Clinical lameness was attributed to septic processes in 23 animals. Cultures recovered mixed growth. *Trueperella pyogenes* was isolated in 12/21 (57%) of cultures. Radiography showed 58% sensitivity and 99% specificity for detecting septic lesions in the claw. **Conclusions**: The high occurrence of distal limb lesions, mixed bacterial growth, and frequent recovery of *Trueperella pyogenes* suggest a large portion of lesions may have a traumatic rather than hematogenous origin. Identified lesions differ from previous reports, indicating the need for further investigation of etiopathogenesis and earlier prevention methods.

SP-35: "LUMPY JAW", PNEUMONIA, AND SEPTIC MYOCARDITIS DUE TO ACTINOMYCES SPP. IN A SUGAR GLIDER (PETAURUS BRAVICEPS)

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A 2-year old female sugar glider (*Petaurus braviceps)* presented to Oklahoma Animal Disease and Diagnostic Laboratory (OADDL) for necropsy following acute respiratory distress and left-sided maxillary facial swelling. Necropsy revealed a facial abscess involving soft tissues and bone on the left maxillary region, hydrothorax, and suppurative pneumonia with pulmonary atelectasis. On histopathology, there were extensive areas of necrosuppurative cellulitis, panniculits, myositis, and osteomyelitis with myriad Gram-positive filamentous bacteria, occasionally surrounded with Splendore-Hoeppli material. Several regional blood vessels revealed necrotizing vasculitis with intraluminal filamentous bacteria. Multifocal necrosuppurative pneumonia with similar colonies of bacteria was seen on histopathology. The heart contained extensive necrosuppurative endocarditis and myocarditis with large numbers of similar bacteria in the right atrium. Culture revealed the causative organism to be *Actinomyces* spp., a gram-positive, rod-shaped anaerobe bacteria ubiquitous in the environment and commensal in many animal species. Best known as the causative agent of lumpy jaw in cattle, initial infection occurs through regional trauma in the oral cavity, allowing the bacteria to infect underlying tissue. Spread to the lungs is typically by aspiration of material containing the bacteria. Hematogenous spread is rare in animal species. Lesions consistent with lumpy jaw and secondary pneumonia associated with *Actinomyces* spp. have been documented in sugar gliders; however, septic myocarditis associated with actinomycosis has never been described in this species, making this case unique. Hematogenous dissemination of the bacteria from the mandibular lesion to the heart is the likely pathogenesis of the myocardial lesions in this sugar glider.

SP-36: PORCINE SAPELOVIRUS – CORRELATION OF HISTOPATHOLOGY AND VIRAL AGENT DETECTION VIA CHROMOGENIC IN SITU HYBRIDIZATION

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Background: Porcine Sapelovirus (PSV) is a ribonucleic acid virus found incidentally in feces of healthy swine, but also as a primary pathogen in neurologic tissue of pigs that present with ataxia, paresis, and other central nervous system signs. *In situ* hybridization (ISH) is a method of direct agent detection that's useful for pathogen identification. ISH utilizes a probe complementary to single-stranded nucleic acids to identify genetic sequences of organisms of interest in fresh or fixed tissues and can be performed using chromogenic enzymes or fluorescent markers. In ISH, cell and tissue-bound genetic material that match the complementary strand will hybridize, confirming the presence of that organism. **Objective**: Our objective was to validate a PSV chromogenic *in situ* hybridization (CISH) protocol as well as correlate histologic lesions and reverse-transcriptase polymerase chain reaction (rt-PCR) results for PSV with CISH results. **Methods**: Confirmed positive cases for PSV via rt-PCR were run with a programmed protocol on a Leica BOND RX (automated IHC/ISH stainer). A new probe sequence was generated for this protocol. **Results**: A protocol was developed,

optimized, and validated with strong staining and low background. While sections with histologic lesions were more likely to be positive, there was not a linear correlation between severity of inflammation and CISH positivity. Animals positive via rt-PCR on fresh tissue that had multiple formalin fixed tissue sections of spinal cord often had multiple sections negative by CISH, including sections with lesions. **Conclusions**: The protocol was successfully validated, giving context to diagnostic interpretation of the PSV CISH.

SP-37: A COMPARATIVE STUDY OF PAIN-RELATED BIOMARKER EXPRESSION IN SENSORY NEURONS IN THE DOG AND RABBIT

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Many of the molecular mechanisms that underlie chronic pain in mouse models have not been closely examined in other species. Further study of companion animal sensory neurons is needed to help address this gap in our knowledge. In mouse studies, several molecular markers have been used as reliable indicators of sensory neuron subtype, while characteristic changes in markers like Calcitonin-Gene-Related Peptide (CGRP) and Neuropeptide Y (NPY) have been explored as biomarkers of neuropathic pain. However, little is known about the expression of these markers in domestic animals even under normal conditions. To determine whether similar molecular markers are expressed in sensory neurons across different species, we analyzed post-mortem dorsal root ganglia (DRG) from healthy control dogs and rabbits. Techniques used to dissect lumbar DRGs in mice were adapted for larger animals, using the sciatic nerve as a landmark that circumvents variability in spinal anatomy. Protocols previously used for mouse cryosections, were successfully adapted to conduct multiplex immunofluorescence staining on formalin-fixed paraffin embedded tissues. Both the dog and rabbit have sensory neurons that vary greatly in diameter and smaller diameter neurons express CGRP or other pain-related markers, similarly to what is seen in mice. Unlike what is reported in mice, NPY may be expressed at low levels in large diameter DRG neurons in the dog and rabbit under normal conditions. Ongoing analysis will quantify relative abundance and co-expression of neuronal markers. These data lay the groundwork for future molecular studies that target sensory neurons in veterinary patients with pain-related disease conditions.

SP-38: IRON OVERLOAD: SEMIQUANTITATIVE HISTOLOGIC EVALUATION OF HEPATIC HEMOSIDEROSIS IN CHLOROCEBUS AETHIOPS SEBAEUS

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Wild African Green Monkeys (AGMs) are omnivorous arboreal hindgut fermenters, consuming a high polyphenol diet with an efficient iron absorbing gut. Hemosiderosis or iron overload (IO) is an iron accumulation syndrome that can advance to hemochromatosis, in which excess accumulation of iron results in organ dysfunction. As early as the 1960s, the common “incidental” finding of hepatic hemosiderosis has been reported in several primate species, including marmosets, tamarins, owl monkeys,

lemurs, rhesus macaques, and gorillas. IO in AGMs has not been previously reported. The goal of the present study was to investigate iron accumulation on 155 livers of AGM's, from the island of St. Kitts using histology (HE and Prussian Blue staining) as well as quantify the Fe using an adaptation of Brunt et al (2000) criteria used to evaluate IO human liver biopsies. Chi-square test of independence and Prevalence Test were used for statistical evaluation. From 155 animals, 49 (31.6%) were negative for iron (by Prussian blue staining), 27 (17.4%) had grade 1 IO, 26 (16.8%) had grade 2 IO, 22 (14.1%) had grade 3 IO, and 31 (20%) had grade 4 IO. Colony-born, geriatric (>15 years), and male monkeys had significantly high grades of liver iron compared to free-range, adult (4-15 years), and female monkeys, respectively. The histologic accumulation of iron in AGM liver samples observed in this study suggests that the recommended iron requirement may be excessive for certain life-stages in this species.

SP-39: MULTI-REGIONAL PHYLOGENETIC CHARACTERIZATION OF SKUNK AMDOPARVOVIRUS (SKAV)

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Amdoparvoviruses (APVs, family *Parvoviridae*) cause persistent infections in many small carnivores, with outcomes varying from subclinical to fatal disease. Skunk amdoparvovirus (SKAV) was first reported in 2017, and has been detected at high prevalence (65-87%) in striped skunks (*Mephitis mephitis*) across North America. Most infections are subclinical but fatal disease has been observed, and host spillover into mink has been reported. The APVs exhibit remarkable genetic diversity, but genetic factors mediating disease manifestations and host species jumps are unknown. The objective of this study was to clarify the genetic characteristics and range of diversity within SKAV by analyzing viral genomes from multiple geographic regions (California, New England, and British Columbia). We hypothesized that this would reveal geographic clustering of sequences, with regions of relative conservation and hypervariability across the ~4500nt SKAV genome. Cases were detected by conventional PCR, and full SKAV coding sequences were obtained by overlapping PCR amplifications and Sanger sequencing. Phylogenetic analysis revealed broad genetic diversity with clear geographic clustering, and demonstrated that diversity in SKAV is driven by relative variability in the non-structural (NS) gene, with relative conservation in the capsid-coding (VP) gene. Conservation of the capsid sequence is enigmatic, but has also been observed in related viruses and proposed as a mechanism to promote antibody-dependent enhancement (ADE) of infection and establishment of persistent infection. Structural gene conservation in SKAV could indicate that ADE is a potentially conserved mechanism of infection across the APVs.

SP-40: HOW CRITICAL IS IT TO PERFORM A MANUAL PLATELET CONCENTRATION ESTIMATE IN THE EXACT MONOLAYER OF A BLOOD SMEAR

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Background: Manual platelet concentration estimations on stained blood smears are commonly performed in the monolayer to verify automated analyser counts, however it is unknown how being just either side of the monolayer can affect counts. **Objectives**: The purpose of this study was to assess how critical it is to perform a manual platelet concentration estimate in the exact monolayer of a blood smear and the impact of this on clinical decisions. **Methods**: Manual platelet counts were performed within 3 separate zones (AM = after monolayer; CM = central monolayer; BM = before monolayer) on 36 separate blood smears which were also grouped by Haematocrit (HCT). These zones were compared with each other and the coefficient of variation (CV) was calculated for each count and used to compare how precision changes with HCT or zone used. Lins concordance correlation coefficient (CCC) was calculated for each zone to check for agreement with automated concentrations. **Results**: Mean manual platelet count differed significantly between the different zones ($F(1.356, 47.46) = 147.8$, $P<0.0001$). The CVs calculated from the different zones negatively correlated with platelet count ($P < 0.0001$, $r = -0.4698$). The mean platelet count was significantly lower in the AM than the CM, and were significantly higher in the BM. **Conclusions**: Slight changes in the zone used to conduct a manual platelet count to just outside of the monolayer can lead to significant variation with resulting platelet concentration. Care is therefore needed to ensure counts are conducted in the exact central monolayer.

SP-41: INCIDENTAL NEUTROPENIA IN CATS

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Introduction: General causes of neutropenia include overwhelming tissue inflammation, decreased granulopoiesis, or peripheral destruction. Neutropenia can be identified incidentally in some cats who appear otherwise healthy and can result in extensive diagnostics to identify an underlying cause. **MATERIALS & Methods:** Medical records of cats presenting to Ryan Veterinary Hospital at the University of Pennsylvania from January 2017 through 2019 with a neutrophil count below 2300/μL were reviewed. Cats with concurrent anemia or thrombocytopenia or with a presumed cause of neutropenia were excluded, i.e. concurrent disease and cats receiving chemotherapeutics or medications that could contribute to neutropenia. Blood smears were analyzed if only an instrument count was originally performed. **Results:** Neutropenia was detected in 100 cats of which thirty cats met inclusion criteria. The median age of included cats was 8.31 years, with 4 cats less than a year old. Three neutropenic cats were diagnosed as hyperthyroid prior to initiation of methimazole treatment. In two cats, the appearance of neutropenia aligned with pre-visit gabapentin administration. Three neutropenic cats had always received gabapentin prior to veterinary visits. Gabapentin administration records were inconsistent, so the information was not uniformly available. One cat underwent bone marrow aspiration; a lower myeloid to erythroid ratio was interpreted as decreased myelopoiesis in the face of neutropenia. No other trends were demonstrated by the data. **Conclusions:** Neutropenia in cats can be an incidental finding that may not warrant additional diagnostic investigation. This may result from normal biological variation, reference interval limitations, or effects of pre-visit medications.

SP-42: LARYNGO-TRACHEO-ESOPHAGEAL CLEFT IN A QUARTER HORSE FOAL

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A 12 hour-old male Quarter Horse foal presented to the Kansas State Veterinary Health Center for dysphagia and inspiratory stridor. Endoscopy revealed a fused trachea and esophagus. Euthanasia and necropsy were elected. On gross examination, the trachea and esophagus were fused from the intrapharyngeal ostium to 15cm caudal to the epiglottis, where a bifurcation was present. The dorsal and caudal arytenoid cartilage and dorsal and lateral cricoid cartilage displayed marked hypoplasia. The tracheal cartilage circumference was larger than would be expected. Dorsal laryngeal muscles were absent or hypoplastic, affecting the cricoarytenoideus dorsalis and arytenoideus transversus most severely. Histological examination revealed more profound hypoplasia of the tracheal cartilage than seen grossly. A gradual shift from respiratory epithelium to esophageal squamous epithelium is present, but, immediately caudal to the larynx, the respiratory epithelium takes on a thick, pseudostratified appearance, and the tracheal submucosa is infiltrated by skeletal muscle. Tracheal cartilage rings are atrophied, particularly at the periphery and near the margins of the esophageotracheal lumen. Diagnoses of a laryngeal esophageal cleft and aspiration pneumonia were made. Laryngo-esophageal clefts are a rare congenital abnormality seen in humans and veterinary species. Usually, they are associated with abnormal development in other organ systems; however, this foal had unremarkable findings in all other systems. Etiology is uncertain, but inheritance or sporadic malformation of the 4th branchial arch are suspected. This case displays an unusual presentation of an already rare congenital malformation and represents the importance of further research into the understanding and prevention of developmental anomalies.

SP-43: CHARACTERIZATION OF CANNABINOID RECEPTOR EXPRESSION IN CANINE TISSUES AND PBMCS VIA IMMUNOHISTOCHEMISTRY AND FLOW CYTOMETRY

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Interest in the endocannabinoid system (ECS) is ever-growing, particularly regarding immunosuppressive or analgesic therapeutics. The ECS includes cannabinoid receptors CB1 and CB2 and their endogenous ligands, the endocannabinoids. Cannabinoids, such as cannabidiol (CBD) and tetrahydrocannabinol (THC), are compounds from the marijuana plant (Cannabis spp.) that function as exogenous cannabinoid receptor ligands. These cannabinoids have risen to prominence as potential treatments, especially for autoimmune diseases. Most research has focused on human diseases but has recently expanded into veterinary medicine as demand increases among pet owners for the availability of cannabinoids for their pets. This project's aim was to

characterize cannabinoid receptor expression in canine peripheral blood mononuclear cells (PBMCs) and tissues, specifically spleen and brain. CB1 and CB2 expression in the spleen and brain of healthy dogs was assessed via immunohistochemistry using formalin-fixed, paraffin-embedded (FFPE) tissue. CB1 and CB2 expression in PBMCs isolated from whole blood samples from healthy dogs was assessed via flow cytometry. CB1 was detected in brain, spleen, and PBMCs, whereas CB2 was detected in spleen and PBMCs but not brain. In the brain, CB1 was most strongly expressed in neurons, astrocytes, and glial cells. In the spleen, both receptors were strongly expressed in lymphocytes and macrophages. PBMCs exhibited moderate expression of both receptors. Variation in receptor expression occurred between individual dogs. These findings provide important preliminary knowledge regarding the ECS in immune tissues of the canine patient and may have crucial implications for the significance of the ECS as a target for novel therapeutics, particularly cannabinoid-derived substances.

SP-44: HOW DOES WEIGHT LOSS AFFECT INFLAMMATION IN THE OBESE MURINE MAMMARY GLAND?

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Obesity is a metabolic condition characterized by chronic adipose tissue inflammation, which increases the risk of developing breast cancer in women. Additionally, obese breast tissue has increased adipose tissue fibrosis and immune cell recruitment and is associated with breast tumor progression and metastasis. Although it is recommended that obese women undergo a weight loss regimen to reduce their risk of breast cancer, the impacts of weight loss on the mammary gland are not well understood. We predict that weight loss will reduce mammary gland fibrosis and macrophage recruitment. Briefly, we used a mouse model to examine the effects of weight loss on the mammary gland. Picrosirius red staining, to label collagen, was coupled with immunohistochemistry to monitor changes in tissue fibrosis and immune cell recruitment. We generated an obese mouse model by feeding a high fat diet for 16 weeks, which resulted in significant weight gain in mice; we then induced weight loss by feeding a low-fat diet for 5 weeks. Mammary gland collagen deposition and macrophage recruitment were increased in obese mice relative to lean mice, suggesting that obesity contributes to increased mammary gland fibrosis and inflammation, but the impact of weight loss on these processes remains unclear. Elucidating the relationship between weight loss, tissue fibrosis, and inflammation in the obese mammary gland will lead to a better understanding of obesity’s contributions to breast cancer progression and may guide future treatment recommendations.

SP-45: CANTHARIDIN TOXICOSIS IN TWO HORSES FOLLOWING BLISTER BEETLE INGESTION

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Two horses presented to the Mississippi State University College of Veterinary Medicine Pathology Service in August 2020 with histories of sudden onset of colic-like symptoms. Post-mortem analysis revealed both horses had a dilated cecum and colon containing

profuse watery digesta. One horse had cyanotic mucous membranes, mottled dark red to purple lungs and white foam in the trachea. Both horse's esophagus were diffusely ulcerated, and the non-glandular portion of the stomach contained large areas of ulceration and multiple fluid filled intra-epithelial vesicles. Histopathology revealed extensive ulcerative erosions in the esophagus and non-glandular stomach, with infiltrations of rod and coccoid bacteria and neutrophils present in the necrotic epithelium and lamina propria. The liver and kidneys showed intermittent areas of necrosis. The alveoli were collapsed and contained neutrophils, suggesting pneumonitis. Large amounts of clear space surrounded the blood vessels, neurons, and glial cells of both brains, suggesting edema. The definitive diagnosis was cantharidin toxicosis secondary to blister beetle ingestion. Cantharidin is a toxin present in certain species of blister beetles that can cause gastrointestinal ulcerations when accidentally consumed in hay. Mucosal damage can result in septicemia, explaining the intermittent pneumonitis and hepatic necrosis seen on histology. The watery ingesta in the ceca and colon was caused by cantharidin's interference with protein phosphatase 2A receptors causing major gastrointestinal water loss and dehydration. This led to electrolyte imbalances which likely caused cardiac arrhythmias resulting in hypoxia in one horse. This case illustrates the importance of screening hay for blister beetles prior to baling.

SP-46: IDENTIFICATION OF A PAPILLOMAVIRUS IN A MALLARD DUCK WITH MULTIPLE MESENCHYMAL DERMAL TUMORS WITH CARTILAGINOUS DIFFERENTIATION

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Background: Papillomaviruses are known to be epitheliotropic and to induce epithelial tumors such as warts. They have been identified in various avian species. An adult female mallard duck (*Anas platyrhynchos*) was euthanized because of two sizable, beige, well-demarcated, firm masses; one in the subcutis and musculature of the thorax and one affecting the distal phalanx of the second digit of the right foot. **Objective:** To identify the specific cause of the two tumors observed. **Methods:** The masses were submitted, formalin-fixed, for histologic and ultrastructural examination, as well as next generation sequencing. **Results:** Both tumors had a lobular organization with cores of neoplastic cartilage separated and surrounded by densely cellular interlacing bundles of neoplastic spindle cells. Numerous neoplastic cells of both masses, particularly frequently the chondroblasts of the mass of the digit, had intranuclear basophilic inclusion bodies. The inclusion bodies observed on light microscopy consisted of viral replication and assembly complexes that averaged a size of 44nm in diameter. Next generation sequencing resulted in the whole genome assembly of a highly divergent papillomavirus that had some resemblance with *Fulmarus glacialis* papillomavirus 1 (FgPV1). **Conclusions:** FgPV1 was associated with a mesenchymal cell proliferation and cartilaginous differentiation in a single Northern fulmar. This mallard case of a papillomavirus associated with a cartilaginous spindle cell sarcoma corroborates the previously reported observation that some papillomaviruses infect mesenchymal cells and may induce mesenchymal neoplasms.

SP-47: MYXOID TUMOR OF THE PENIS IN A LABRADOR RETRIEVER

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A postmortem examination was performed on a 14-year old dog that had been a patient of the *Foster Hospital* at the Cummings School of Veterinary Medicine at Tufts University. The dog had a four-year history of dysuria, stranguria, and pollakiuria, and a 3-year history of complete urinary bladder atony. Previous neurological examination had revealed intact perineal and bulbospongiosus reflexes. At postmortem examination, a 6.0 cm x 6.0 cm x 7.0 cm mass was found immediately proximal to the os penis, 14 cm from the distal urethral orifice. The mass was poorly demarcated, ovoid, and gelatinous. It expanded and distorted the corpus cavernosum and corpus spongiosum, and compressed the urethra ventrally. The urethra proximal to the mass was markedly dilated. The urinary bladder was flaccid and markedly distended and its mucosal surface contained dozens of raised, red, foci measuring 1 mm to 3 mm diameter. Histopathologically, the mass comprised abundant background matrix that was clear to lightly basophilic, stained positive with Alcian blue stain, and was hypocellular with scattered bland spindle-shaped cells that lacked mitoses. Overall, the findings were consistent with a myxoid tumor. Given the bland appearance of the cells and the lack of other features typically associated with malignancy, such as invasion, necrosis, cellular pleomorphism, high mitotic activity, and the prolonged clinical course, a diagnosis of myxoma was favored over myxosarcoma. However, this differentiation can sometimes be difficult, and often unnecessary, because both tumors can have similar behavior. Reports of myxoid tumors of the penis are rare in animals.

SP-48: MANNHEIMIA HAEMOLYTICA RESPIRATORY DISEASE IN MATURE DAIRY COWS

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Background: *M. haemolytica* pneumonia outbreaks in mature dairy cows are anecdotally increasing in frequency. Despite welfare and economic challenges, the literature on pneumonia in mature dairy cows is sparse. **Objective:** Describe risk factors, herd characteristics, prevalence, seasonality, duration, and main pathogens involved in pneumonia in mature dairy cows, and to identify changes over time. **Methods:** 691 cases from 2007-2020 were retrieved from the laboratory database. Search criteria included bovine, lung, and suppurative inflammation, fibrinous inflammation, *Mannheimia, Histophilus*, or *Pasteurella*. Inclusion criteria were female dairy cows ≥ 2 years of age with pneumonia recorded as the proximate cause of death. Aspiration pneumonia or embolic pneumonia cases were excluded. **Results:** 112 cases were included in the study. Of 97 cases with bacterial culture data, *M. haemolytica* was isolated from 57 cases, *H. somni* from 3, *P. multocida* from 27, and other bacteria from 35. Of 52 cases tested for BRSV, 6 were positive. Of 14 cases tested for BVDV, 0 were positive. Of 54 cases tested for other viruses, 2 were positive for BHV-1. Common risk factors included introductions of new animals, cold temperatures, and the peripartum period. Year of submission was not correlated with number of *M. haemolytica* cases

(R^2=0.008). **Conclusions:** An increase in *M. haemolytica* cases was not observed. *M. haemolytica* was isolated in 62% of cases. This study identifies the major pathogens and associated risk factors for pneumonia in mature dairy cows, as a basis for understanding why outbreaks occur and for implementing appropriate therapy.

SP-49: RETROSPECTIVE ANALYSIS OF VETERINARY LEGAL NECROPSY CASES FROM JULY 2019 TO JULY 2020 IN CORPAVET, COLOMBIA

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Forensic veterinary pathology is an emerging area in Latin America, with recent legal framework against animal abuse, environmental crimes, or veterinary malpractice (Colombia Law 1774 of 2016). CORPAVET received approximately 2,300 cases per year in anatomic pathology (necropsies and biopsies) and in recent years, we have observed an increase in legal consults. We selected the legal necropsy cases in the CORPAVET casuistic in the period from July 2019 to July 2020, in order to know the main cause of deaths and promote knowledge and training to veterinarians in our country. Twenty-three percent (23%) of necropsies received in the laboratory during this period were legal cases (13/56). Between 2019 to 2020, the forensic cases increased from 2 to 11. Nine of them (69.4%, 9/13) were related to veterinary malpractice, including probable overdose of anesthesia (3 cases), postoperative hemorrhage (2 cases), and maluse of other medications (2 cases). Two cases were environmental crimes (15.3%, 2/13) for intoxication of wild animals during COVID-19 disinfection of public areas. This information is very useful to develop local training in order to avoid legal implications of veterinarians and stakeholders. During 2019, we had a workshop in Forensic Veterinary Pathology (October 30-31, 2019) with support from the Latin Comparative Pathology Group (LCPG) and Dr. Jason Brooks DACVP from PennState, and this training could be related to the requested forensic necropsies for 2020 in comparison to 2019.

SP-50: INVESTIGATING CROSS-REACTIVE ANTIBODIES TO SARS-COV-2 IN BCG-VACCINATED MICE

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The Bacillus Calmette-Guérin (BCG) vaccine, composed of live attenuated *Mycobacterium bovis,* remains the only vaccine available for human use against tuberculosis. However, the impact of this vaccine extends beyond tuberculosis: a growing body of evidence supports non-specific, cross-protective immunological benefits of BCG-vaccination against a broad spectrum of pathogens unrelated to *mycobacterium*, including viruses. One proposed hypothesis for such cross-protective benefits includes antibody cross-reactivity to a wide array of antigens. We hypothesized immunization with the BCG vaccine may deliver a humoral response against the spike protein on SARS-CoV-2. An in-house ELISA, coated in recombinant spike protein

(rSpike) was optimized to investigate cross-reactive antibodies to SARS-CoV-2 by measuring the concentration of IgG reactive to the spike protein from the sera of BCG-vaccinated mice. The concentration of IgG reactive to the rSpike in BCG-vaccinated mice (n=7) was compared with the concentration of IgG from serum of an unvaccinated mouse (n=1). Preliminary results indicated 4 of 7 sera samples from BCG-vaccinated mice possess more IgG reactive to the rSpike than the serum of the unvaccinated mouse. Results suggest the BCG antigen induces cross-reactive antibodies capable of binding the spike protein on the SARS-CoV-2 pathogen. These results will help elucidate the mechanism by which BCG-vaccination offers cross-protective immunological benefits, and aid in the global search for defense against COVID-19 through two perspectives: 1) development of a vaccine optimizing the humoral immune response to the pathogen and 2) awareness of the impact cross-reactive antibodies may have in generating false positives in serological tests from BCG-vaccinated humans.

SP-51: BALD EAGLE MORBIDITY AND MORTALITY IN THE SOUTHEASTERN UNITED STATES: A FIVE-YEAR RETROSPECTIVE

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Bald eagles (*Haliaeetus leucocephalus*) are at risk of anthropogenic causes of mortality, most commonly through various sources of trauma (e.g., collisions, electrocution, and gunshot). As both apex predators and facultative scavengers, bald eagles also frequently suffer from toxicoses, notably lead, anticoagulant rodenticides, and insecticides. However, published reports on causes of mortality in bald eagles in the southeastern U.S. are scarce. We retrospectively evaluated diagnostic findings of bald eagle cases submitted from 21 states (primarily southeastern) from January 2015 to June 2020 and categorized primary and contributing causes of mortality. Diagnostic findings included gross and histopathology and case-specific ancillary test results when performed. Among 267 bald eagles examined, non-infectious causes of mortality (209; 78.3%) were more common than infectious (21; 7.9%), with 37 (13.8%) that died of unknown causes. The majority (127; 47.6%) of noninfectious causes were attributed to trauma, most commonly blunt force (e.g., vehicular collision; 21). Seventy-three (27.3%) eagles died of toxicoses, including lead (44), anticoagulant rodenticides (18), and avian vacuolar myelinopathy (i.e., cyanobacterium toxin; 5). Additionally, numerous toxicants were detected at low levels in 110 eagles. Infectious causes were most often viral (11; most commonly, West Nile virus). This study demonstrates challenges faced by bald eagles in the southeastern U.S. including potential, population-level impacts of human-associated activities. Although trauma was the most common source of mortality, frequent detection of a variety of toxins is concerning. Specifically, the potential health impacts of low-level and possibly chronic toxicant exposure in bald eagle populations warrants further investigation.

SP-52: HEMATOLOGICAL AND PARASITOLOGICAL FINDINGS IN DOGS OWNED BY HOMELESS PEOPLE FROM BRAZIL

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“Best Friend” extension project aims to map zoonotic microorganisms circulating in Niterói municipality (Rio de Janeiro, Brazil) and assess the health status of street dogs owned by homeless people. This study presented hematological findings and the occurrence of blood parasites from fourteen dogs attended to in 2019. Mild hematological changes were observed, probably due to nutritional depletion or endoparasitism. One dog had a false positive result from *Leishmania* spp. in the screening test, but was not reactive by the enzyme-linked immunoassay method. None of the dogs had *E. canis* antibodies. *Dirofilaria immitis*, which causes a neglected tropical disease, was found in two dogs. Considering a One Health approach, it is important to encourage studies on the diagnosis, treatment, and prevention of blood parasites in street dogs, mainly those in close relationship with homeless people from Brazil and other developing countries.

SP-53: HISTOPATHOLOGIC FINDINGS IN NECROPSY CASES WITH COCCIDIOIDES SPP. INFECTION

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Background: Fungal infection with *Coccidioides* spp., also known as Valley Fever, is a prevalent problem in endemic regions such as Arizona and California. While *Coccidioides* may disseminate to any tissue, a recent comprehensive investigation and review of *Coccidioides* tissue tropism in veterinary medicine is lacking. **Objective:** The objective of this study was to characterize the histopathologic findings of Valley Fever cases submitted for necropsy at Midwestern University and compare the distribution of *Coccidioides* lesions between species. **Methods:** The Midwestern University Diagnostic Pathology Center case database was searched for necropsies that included a diagnosis of *Coccidioides*. This search resulted in 87 necropsies performed between July 2016 and June 2020. *Coccidioides*-associated lesion information was organized by tissue and species. **Results:** The majority of cases were canine (64%), while the remainder of cases were split between equine (13%), ruminant (10%), “other” species (8%), and feline (5%). For most species, the tissues with the highest tropism for *Coccidioides* included the lung, liver, lymph nodes, and spleen. Coccidioidomycosis was predominantly disseminated and was the cause of death in the majority of species (66-75%), except equines (9%). Dogs and “other” species showed an unexpectedly high predilection for central nervous system involvement (29%), while cardiovascular involvement (9%) was noted more frequently in horses. **Conclusions:** Although coccidioidomycosis may present in many forms, both classic and unique locations of *Coccidioides* tissue tropism were identified in several species. Results from this study will help add to the current knowledge base of *Coccidioides* pathology in veterinary medicine.

SP-54: COSTIMULATORY CHECKPOINT MOLECULE OX40 COMBINATION THERAPY AS A TREATMENT FOR CANINE SOLID TUMORS

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OX40 (CD134) is a transiently expressed costimulatory checkpoint molecule associated with T cell activation. Our laboratory generated monoclonal antibodies against recombinant canine OX40 with the intent of developing a clinical grade immunotherapeutic for dogs. Flow cytometry was used to assess OX40 protein expression. Resting T cells from dogs expressed little or no OX40 protein. Upon activation with Concanavalin A, OX40 expression was significantly upregulated on T cells. Functionally, *in vitro* treatment of naïve canine T cells with anti-OX40 antibody increased T cell survival, Granzyme B expression, and Interferon gamma production relative to an isotype matched control. To assess the therapeutic potential of anti-OX40 treatment we conducted a randomized case-matched prospective study in dogs with solid tumors. The study was designed to evaluate the effect of locally delivered OX40 immunotherapy and adjuvant, Toll-Like Receptor 9 (TLR9) agonist, in combination with stereotactic body radiation therapy (SBRT) on antitumor immunity when compared to SBRT alone. To date we have collected pre- and 2 weeks post- tumor biopsies from 5 patients (n=2, control), (n=3, combination therapy). Immunohistochemical (IHC) analysis of tumor biopsies showed a reduction in the number of T cell and Treg infiltrates between timepoints in the combination group while the control group exhibited an increase in overall T cell and Treg infiltrates. Our results suggest that the combination of OX40/TLR9 immunotherapy with SBRT decreases Treg populations in the tumor microenvironment. Tregs are responsible for immune suppression and their depletion in treated tumors could result in a stronger anti-tumor immune response.

SP-55: DETECTION AND CHARACTERIZATION OF PARAPROTEINEMIA IN CANINE B CELL CHRONIC LYMPHOCYTIC LEUKEMIA USING ROUTINE AND FREE LIGHT CHAIN IMMUNOFIXATION

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Background: Hyperglobulinemia is reported in ~25% of B cell chronic lymphocytic leukemia (B-CLL) cases, but few cases have been characterized by immunofixation. Increased monoclonal serum free light chains (fLC) is prognostic in human CLL, but incidence and prognostic utility of immunofixation-diagnosed monoclonal fLC in dogs is unknown. **Objective:** Characterize and determine the proportion of canine B-CLL cases with a clonal gammopathy using routine and fLC immunofixation. **Methods:** Protein electrophoresis was performed on plasma from 57 dogs with a B-CLL diagnosed by peripheral blood flow cytometry. Routine (targeting IgG, IgA, IgM, IgG4, and light chain) and fLC immunofixation was assessed in 48 and 54 patients, respectively. **Results:** There were 27 boxer dogs (which have poorer prognosis) and a variety of other breeds. Age ranged from 2 years to 16.7 years. Hyperproteinemia, >7.5g/dL, was present in 17/51 cases, range 4.8g/dL-11.3g/dL. A complete (monoclonal heavy and light chain) M-protein was diagnosed in 36/48 cases (75%, 95%CI 62-83%) with routine

immunofixation. Monoclonal fLC was detected in 15/54 cases (28%, 95%CI 15-39%) with fLC immunofixation. When both routine and fLC IF was available there were 22 IgM, 3 IgA, 1 IgG4, 1 IgG/IgM and 3 fLC only cases documented. 9 of the complete M-protein cases also had a fLC M-protein. Plasma fLC immunofixation suggested β-γ region interference, likely caused by fibrinogen. **Conclusion:** Most B-CLL cases had a monoclonal protein and were not hyperproteinemic. The majority were IgM and fLC were detected in a subset. Prognostic significance of heavy and fLC presence should be evaluated.

SP-56: CHARACTERIZING THE ASSOCIATION OF PROTEINURIA WITH DENTAL DISEASE IN DOGS AND CATS

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Background: Proteinuria is a common clinical finding in dogs and cats with periodontal disease and 27% of dogs display borderline to overt proteinuria. While proteinuria may be due to dental disease-associated inflammation, periodontal disease in humans and canines has been linked to chronic kidney disease, which may also lead to proteinuria. **Objective:** Our goals were to determine the prevalence of proteinuria in dogs and cats with periodontal disease, determine whether the magnitude of proteinuria is associated with dental disease severity, and evaluate urine protein fractions in a subset of periodontal disease patients. **Methods:** Records of patients presenting to Midwestern University for dental prophylaxis between 2015-2020 were reviewed and clinical history, dental disease grade, and pre-dental laboratory test results were documented. Urine protein fractions were prospectively evaluated via gel electrophoresis in a small subset of patients. **Results:** The majority of dogs and cats (87% and 73%, respectively) received a pre-dental urinalysis and 65% of dogs and 90% of cats were proteinuric. No association was noted between dental disease grade and proteinuria severity in dogs. While an association was noted in cats, the overall sample size was low. Urine protein electrophoresis was consistent with albumin excretion in proteinuric patients. **Conclusions:** Our study confirms a high prevalence of proteinuria in patients with dental disease. Periodontal disease grade does not appear associated with proteinuria severity in dogs, but may be positively associated in felines. Urine protein electrophoresis results indicate primarily albumin loss, which suggests intrinsic kidney disease, rather than an inflammatory proteinuria.

SP-57: IDENTIFICATION AND USE OF IHC TO CHARACTERIZE GIST IN A WHITE-SPOTTED BAMBOO SHARK WITH CONCURRENT GOITER.

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An adult female white-spotted bamboo shark with a three-year history of goiter was euthanized after developing buoyancy and locomotive problems. Histology slides were stained with hematoxylin and eosin (H&E) as well as immunohistochemistry for smooth muscle actin and c-kit. Gross examination revealed a 2cm round, white, firm mass in the wall of the proximal small intestine causing luminal obstruction. Histologically the poorly

demarcated mass was comprised of invasive haphazard streams of spindle cells consistent with a sarcoma. The intestinal mass had strong positive labeling for c-Kit and negative labeling for smooth muscle actin, identifying it as a gastrointestinal stromal tumor (GIST). Also present was an enlarged thyroid gland that histologically was diffusely expanded by variably sized follicular structures lined by a single layer of cuboidal epithelium (goiter). The goiter had features of a hyperplastic goiter transitioning into a colloid goiter, as well as capsular and focal vascular invasion consistent with a malignancy. This case provides an example of goiter and thyroid follicular carcinoma as well as the first reported GIST in a shark species. This is also the first reported use of smooth muscle actin and c-kit IHC to characterize a neoplasm in a shark species. Because of the relatively high prevalence of goiter among sharks in human care, better understanding of the pathogenesis is needed to provide optimal care. Further description and characterization of neoplasia in sharks will also better guide their care and may help conservation efforts by countering misinformed beliefs of the medicinal value of shark products.

SP-58: PULMONARY AND MESOTHELIAL NEOPLASIA IN A CAPTIVE LION (PANTERA LEO LEO)

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Neoplasia is common in the *Panthera* genus and is a leading cause of natural death or euthanasia decision. The reproductive, endocrine, integumentary and hemolymphatic systems account for most records of neoplasia, particularly in tigers (*P. tigris*), lions (*P. leo*) and their hybrids, as well as in leopards (*P. pardus*) and jaguars (*P. onca*). A 21-year-old, captive female West African lion (*P. leo leo*) with chronic respiratory disease, recurrent pyothorax, and terminal dyspnea and inappetence was found dead in her enclosure. The animal was necropsied and formalin-fixed tissues were submitted for histopathologic examination. The main gross findings were pleural effusion; multifocal nodules throughout the parietal and visceral pleurae, mediastinal fibroadipose tissue and pulmonary parenchyma; and intrahepatic biliary cysts. Based on the cytohistomorphologic features and the immunophenotypical profiles, the pleural nodules were compatible with mesotheliomas (TTF1-negative; variably positive for AE1/AE3 and CK5/6), while the intrapulmonary nodules were compatible with pulmonary carcinomas (TTF1-positive; AE1/AE3-positive; CK5/6-negative). Additional relevant histologic findings included acute to chronic inflammatory foci with variable necrosis in the heart, liver and kidney, which were most likely related to progressive neoplastic thromboembolic events coupled with hypoxia. While primary pulmonary carcinomas in captive *Panthera* is not uncommon, mesotheliomas appear to be a rarity, with a few cases reported. To the best of our knowledge, these findings represent a unique case of intrathoracic neoplasia in large felids, specifically involving concurrent mesothelioma and pulmonary carcinoma. Intrathoracic neoplasia should be included in the differential diagnosis of long-term, unresponsive respiratory disease in large felids.

SP-59: PRESUMPTIVE BASOPHILIC LEUKEMIA IN A MINIATURE PIG

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A 5.5-year-old, neutered male, miniature pig was presented to the Hospital for Large Animals at the Cummings School of Veterinary Medicine at Tufts University, with an 8-week history of weight loss, inappetence, and dullness, with episodes of vomiting and regurgitation. Abdominal ultrasound revealed marked hepatomegaly and abnormal renal architecture. Hematologic evaluation revealed severe leukocytosis (199.7 K/uL), characterized by marked neutrophilia (85.8 K/uL) and basophilia (83.8 K//uL), along with eosinophilia, monocytosis, and numerous variably-differentiated granulocytic cells, most consistent with basophilic lineage. These findings suggested a leukemic process and, due to poor prognosis and clinical deterioration, the pig was euthanized. At postmortem examination, the liver was severely enlarged, and its parenchyma effaced by myriad pinpoint to 2.0 cm, white-to-tan nodules. Similar nodules effaced the spleen, kidneys, mesentery, gastrointestinal tract, heart, lymph nodes, and lungs. Histologically, these lesions were composed of sheets of round cells supported by pre-existing stroma with scant to abundant eosinophilic cytoplasm frequently filled with eosinophilic granules, or infrequently, pale basophilic granules. The bone marrow was effaced by the same atypical population. Overall, the findings in this case are consistent with a diagnosis of leukemia and severe visceral infiltration. Although a basophilic origin/differentiation is favored, whether this process can be definitively differentiated from systemic mastocytosis or a severe cytokine-driven/inflammatory response is unclear without further diagnostic testing. Leukemias of basophilic or mast cell origin are rarely reported in large animals.

SP-60: IDENTIFICATION OF MYCOBACTERIUM SPP. IN CANINE CEREBROSPINAL FLUID

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A four-year-old, female spayed Australian Cattle Dog presented to the University of Missouri Veterinary Health Center for neck pain and lethargy. Three years prior, wooden splinters were removed from the left cervical and pharyngeal regions. Neurological examination showed mild dysmetria and general proprioceptive ataxia. Neuroanatomic localization was multifocal and meningoencephalomyelitis was suspected. MRI showed increased signal of T2 weighted images in the caudal brainstem and cranial cervical spinal cord, extending from the level of the medulla oblongata to C5. CSF collected at the cerebellomedullary cistern revealed 348 nucleated cells/microliter (mixture of mononuclear cells and neutrophils), 310 RBCs/microliter, and protein concentration of 423 mg/dL. Cytocentrifuged slides of CSF contained 41% lymphocytes, 31% large mononuclear cells, and 28% neutrophils. Occasional non-staining, acid-fast, rod-shaped bacteria consistent with *Mycobacterium* spp. were found in large mononuclear cells. Rifampin, clarithromycin, doxycycline, and anti-inflammatory prednisone was initiated. Clinical signs improved after 6 weeks of

therapy however organisms were still seen in CSF. Culture of both CSF samples failed to achieve growth of the organism. CSF analysis after 5 months of treatment was within normal limits. The patient was not known to be immunocompromised. This is the first report of apparently CNS-localized mycobacteriosis in a dog diagnosed via CSF analysis. Opportunistic *Mycobacterium* spp. are normally found in soil, water, or decaying material and are uncommon pathogens. The common route of infection in dogs is from inoculation via a penetrating injury. Based on prior history, foreign body inoculation of *Mycobacterium* spp. could have initiated the infection in this dog.

SP-61: MULTICENTRIC LYMPHOMA PRESENTING AS SCOLIOSIS IN A JUVENILE MALE BLUEGILL (LEPOMIS MACROCHIRUS)

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A 6-month-old male bluegill (*Lepomis macrochirus*) was submitted for necropsy with a history of progressive curvature of the spine and swimming oddly. Euthanasia was elected, and the fish was received whole in 10% formalin. Necropsy findings were confined to the clinically reported moderate scoliosis originating at the left pectoral fin and extending to the caudal fin. Histopathology revealed a disseminated neoplasm composed of monomorphic small lymphocytes consistent with multicentric lymphoma. The affected organ systems included the spinal column, nerve roots, and associated skeletal musculature. The severity of the spinal nerve root destruction is a probable contributor to multifocal denervation of the musculature, resulting in scoliosis. Lymphoma is the most prevalent hematopoietic tumor in fish, which in many cases manifests as epidermal plaques or nodules, with subsequent visceral dissemination. The present case is unique in the identification of disseminated lymphoma in the absence of cutaneous or visceral masses and which presented clinically as scoliosis. Environmental contaminants and viral carcinogens have been implicated in the pathogenesis of piscine lymphoma, and if involved, would have been present during developmental growth in this juvenile fish. Lymphoma has been extensively studied in northern pike and muskellunge; in these species, evidence suggests a retroviral etiology. In this case, next generation sequencing to mine for a potential viral etiology was not performed due to lack of available fresh tissues. Future investigations aimed at identifying potential tumorigenic viruses in bluegill neoplasia will need to be evaluated in light of known pre-existing integrated endogenous retroviral elements within their genomes.

SP-62: IONOPHORE TOXICITY IN QUAILS

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Seven different groups of quail from a Kansas gamebird farm were brought to Kansas State Veterinary Diagnostic Laboratory for necropsy between the dates of July 22, 2019 and September 25, 2019. The quail were exhibiting clinical signs such as muscle weakness, diarrhea, blood coming out of the nares, quivering, and being found in lateral recumbency. Some of the animals presented were brought in dead, and some were

euthanized upon arrival. Hundreds of birds may have perished, though a specific number is not known. The most consistent findings among the animals on gross examination were mildly decreased coelomic fat, bloody discharge in the nares, eyes, and oral cavities, and epicardial hemorrhage. Histological examination consistently showed varying severities of chronic myocardial degeneration with variable heterophilic and histiocytic myocarditis and myocyte degeneration and fibrosis of the skeletal muscle in all birds. Many birds also had bile duct hyperplasia, hepatocyte vacuolation, and degenerative changes in the liver. Several toxicology screens were submitted and the majority of them showed the presence of Salinomycin, an ionophore antibiotic, in varying concentrations. The findings on the necropsies and toxicology screens were consistently suggestive of ionophore toxicity, making that the primary diagnosis for most of the submitted animals. Ionophores are common feed additives in feedlot cattle and serve as a source of toxicosis for many veterinary species. Some of the common clinical signs of ionophore toxicity include anorexia, diarrhea, muscular weakness, and death. Salinomycin is also known to induce severe myocardial and skeletal muscle lesions

SP-63: WEST NILE VIRUS INFECTION-INDUCED DERMATITIS IN A JUVENILE FLAMINGO

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A ~1.5 month old American Flamingo was discovered deceased in an exhibit pool. The bird had an area of abrasion and blood on its ventral neck but was otherwise behaving normally and in good appetite with adequate adipose and muscling prior to its death. Gross necropsy indicated a thickened and hyperkeratotic region of skin along the ventral neck. Histopathology revealed a fibrinonecrotizing arteritis in the dermis of the affected skin with degeneration, necrosis, and hyperkeratosis of the overlying epidermis. Immunohistochemical staining for WNV showed positive intracytoplasmic staining within keratinocytes, macrophages, arterial smooth muscle cells, and occasional panniculus muscle cells. Additional findings included myocarditis, pancreatitis, scattered myositis, and pulmonary hemorrhage. West Nile Virus (WNV) is a globally distributed flavivirus, transmitted by mosquitos and capable of infecting a number of vertebrates. The virus is maintained primarily in avian reservoirs. Before the 1999 introduction of WNV to the United States, it primarily caused subclinical infections and self-limiting outbreaks in the Eastern Hemisphere. WNV has since been diagnosed in multiple captive flamingo collections in the Americas. In several reports, flamingos presented with disease and mortality before other species, indicating a possible role as a sentinel species for WNV in captive and free ranging wildlife populations. WNV infection commonly leads to inflammation, necrosis, and hemorrhage in the central nervous system and heart, but numerous other organs may show lesions. Although WNV is known to replicate in keratinocytes, to the authors' knowledge this is the first report of an animal presenting with WNV-induced dermatitis.

SP-64: RETROSPECTIVE ANALYSIS OF LABORATORY DATA AS PROGNOSTIC FACTORS FOR SURVIVAL IN CANINE SPLENIC HEMANGIOSARCOMA

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Hemangiosarcoma (HS) makes up 5-7% of malignant tumors in dogs and has a poor prognosis due to metastatic disease. HS originates from pluripotent endothelial cells and relies on angiogenesis for growth. Initial slow growth allows time for invasion of surrounding tissues and hematogenous dissemination. Visceral HS of the spleen or right auricle is more common than non-visceral HS, which develops in the skin or muscle. Treatment of visceral HS with surgery results in an average survival time of 1-3 months, and with chemotherapy, up to 6 months. However, reliable factors to help predict survival are difficult to elicit. In this study, electronic medical records from cases of splenic HS in dogs presenting to a veterinary hospital from 2010-2020 were analyzed to determine if signalment, CBC, and serum chemistry data had a significant relationship to overall survival time and could be utilized as potential prognostic markers. Twenty-three cases of splenic HS met inclusion criteria and were divided into three groups: G1, <90 days survival; G2, 90-180 days survival, and G3, >180 days survival. Kruskal-Wallis was used for group comparisons. As expected, presence of the tumor in multiple organs (multicentric) and gross metastatic disease were significantly different between groups (p<0.5). In addition, serum phosphorus levels were decreased in G2 compared to G1 and G3. This retrospective analysis supports the presence of metastasis as a useful prognostic indicator in canine HS. Other potential prognostic markers of HS in dogs are being analyzed, including IHC and microRNA-based markers in archived samples from these cases.

SP-65: GOING BATTY: CAUSE OF DEATH IN BATS IN BRITISH COLUMBIA, CANADA

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Background: White Nose Syndrome (WNS), wind turbines and habitat loss are key contributors to declines in wild bat populations in North America. Other causes of bat mortality, particularly in urban areas, are not well understood. **Objectives:** To determine common causes of death in bats in British Columbia. Recognizing causes of morbidity and mortality may identify mitigation measures to minimize mortality and increase resilience of bat populations. **Methods:** Between 2015 and 2019, 220 wild bat mortalities from 13 species, were analyzed at the Animal Health Centre. A full necropsy by a veterinary pathologist and rabies immunohistochemistry was performed on all bats and WNS testing was performed on bats found between November 1 and May 31. **Results:** Of the bats examined, 45% died from trauma, 22% from emaciation with no apparent significant concurrent disease, and 16% from infectious/inflammatory disease. Domestic cat predation accounted for 49% of traumatic deaths. Rabies was the most common infectious disease (9%), followed by pneumonia (4.5%). All bats were negative for WNS. Non-WNS fungal skin disease affected 17% of all bats and was considered clinically significant in 3%. This was particularly common in the spring. Fungi were histologically distinct from *P. destructans,* included at least 5 morphologically distinct hyphal types and were associated with inflammation in 65% of cases. **Conclusions:**

Trauma, particularly domestic cat predation, and emaciation are the most common causes of mortality in wild bats submitted for post-mortem in BC. Non-WNS fungal dermatitis is common in wild bats, particularly in the early spring.

Late-Breaking Posters

LB-01: CLINICAL AND PATHOLOGICAL FEATURES OF ACUTE BOVINE LIVER DISEASE (ABLD)

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Background: Acute Bovine Liver Disease (ABLD) is a poorly understood hepatotoxic disease affecting cattle in southern Australia. The cause of ABLD is unknown but is generally accepted to be a hepatotoxin with current research efforts focused on mycotoxin involvement. ABLD is characterized by its geographical restriction, Autumn to Winter occurrence and periportal hepatocellular necrosis on histopathological evaluation. **Objective:** The aim of this study was to characterize the clinical and pathological features of ABLD, with a view to highlighting diagnostic features and gain a greater understanding of the potential etiology and toxicological mechanisms. **Methods:** We describe the clinical and pathological findings of ABLD in 45 naturally affected cattle from 13 outbreaks occurring from 2010 to 2019 in Victoria, Australia. **Results:** Clinical signs commonly included a combination of mild photosensitization, hypogalactia and progressive neurological signs followed by death within 48 hours. Morbidity and mortality rates varied from 1.5 to 65.2% and 0.5 to 16.7% respectively. All affected animals had marked elevations in glutamate dehydrogenase, aspartate transaminase and gamma-glutamyl transferase activities. Macroscopic lesions included serosal petechiae and/or gastrointestinal hemorrhage and hepatomegaly with a pronounced reticular pattern. The principal histologic lesion was widespread, severe periportal hepatocellular coagulative necrosis with erythrocyte pooling, which often extended to panlobular necrosis. **Conclusions:** ABLD is characterized by clinicopathological and post-mortem changes of severe, acute hepatic injury and periportal to massive hepatocellular necrosis on histopathological evaluation. These findings suggest involvement of a potent intrinsic hepatotoxin that is either directly cytopathic or requires bioactivation by periportal-specific enzymes.

LB-02: TOLERANCE OF AMBIGUITY IN VETERINARY PATHOLOGISTS AND PATHOLOGY TRAINEES

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Ambiguity and uncertainty are ever present in veterinary medicine, including in the fields of veterinary clinical and anatomic pathology. However, tolerance of ambiguity (TOA) has been little investigated in veterinarians and, to our knowledge, not at all in veterinary pathologists. In this study, we used the 27-item Tolerance of Ambiguity of Veterinary Students (TAVS) scale to evaluate TOA in practicing pathologists and pathology trainees. We hypothesized that TAVS scores would be higher for practicing pathologists than for trainees, that scores would increase with years of experience in

the field for pathologists and year of study for trainees, and that scores would be similar for those in each discipline. 191 individuals participated (105 clinical pathologists, 23 clinical pathology trainees, 31 anatomic pathologists, 11 anatomic pathology trainees, 12 clinical and anatomic pathologists, 9 did not specify). TAVS scores were significantly higher for practicing pathologists than for trainees, and scores increased significantly with year of experience for pathologists but not with year of study for trainees. When comparing disciplines, TAVS scores for all clinical pathologists combined (including trainees) were significantly lower than the scores for all anatomic pathologists combined. Clinical pathology trainees had significantly lower scores compared to anatomic pathology trainees, with similar trends for practicing clinical pathologists compared to anatomic pathologists. Results suggest pathologists become more tolerant of ambiguity throughout their careers, although this may also be related to increasing TOA with age. Coping with ambiguity may be more difficult for trainees, especially clinical pathology trainees, than for experienced pathologists.

LB-03: SPONTANEOUS AUTOIMMUNE-MEDIATED ALOPECIA IN OUTBRED FEMALE SWISS WEBSTER MICE: A NOVEL MOUSE MODEL OF ALOPECIA AREATA

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Background: Alopecia areata (AA) is a complex, polygenic, immune-mediated disease of humans and other mammals and chickens and is one of the most common causes of non-scarring circumferential hair loss. While the underlying pathophysiology is not fully understood, the breakdown of immune privilege of the hair follicle is hypothesized to play a key role. A similar disease phenotype has been reported in a variety of mammals, with C3H/HeJ mice being the most commonly used animal model in current mechanistic and preclinical studies. **Objective**: Describe the gross, histopathologic and immunohistochemical findings in a group of female Swiss Webster mice clinically presenting with alopecia. **Methods**: The study was conducted on ten outbred female Swiss Webster (Tac:SW) mice, used as soil-bedded sentinels. All animals underwent a complete post-mortem examination, including macroscopic and histologic examination, as well as a routine pathogen screening. Skin samples of diseased and control animals underwent further immunohistochemical characterization. **Results**: This study describes a spontaneous and phenotypically identical disease process to human AA in female Swiss Webster mice. The results confirm the presence of an immune infiltrate centered around anagen hair follicles with intra- and perifollicular mononuclear cell infiltration. Immunohistochemistry identified the infiltrates as intrafollicular cytotoxic CD8+ and perifollicular CD4+ T-cells. **Conclusions:** This study highlights the potential of female Swiss Webster mice to serve as an alternative spontaneous disease model to the currently available animal models of AA. Furthermore, it raises awareness of the existence of a previously unknown spontaneous, skin phenotype in a commonly used outbred laboratory mouse stock.

LB-04: PRESUMPTIVE URINARY BLADDER SECRETORY B-CELL LYMPHOMA WITH AN IGG MONOCLONAL GAMMOPATHY AND BENCE-JONES PROTEINURIA IN A DOG

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Background: Urinary tract neoplasia can be diagnosed by urine evaluation in ~ 30% of cases, but diagnosis of myeloma related disease via urine evaluation is very rarely reported. History: A 12-year-old neutered male Labrador Retriever mix was presented for evaluation of a 1-week history of hyporexia and tenesmus with normal appearing stool. Possible stranguria was noted during exam and the caudal abdomen was firm on palpation. A focused ultrasound revealed significant thickening and a possible mass lesion of the dorsal bladder wall. Diagnostic findings: Urine sediment evaluation revealed low to moderate numbers of intermediate to large-sized lymphocytes with apparent nucleoli. PCR for antigen receptor rearrangement (PARR) on the urine sediment demonstrated a clonal immunoglobulin gene rearrangement, and flow cytometry identified an expansion of CD21+ and MHC Class II negative B-cells. Serum and urine protein electrophoresis and immunofixation revealed a serum and urine IgG monoclonal gammopathy with Bence-Jones proteinuria. MUM1 immunocytochemistry failed to label the neoplastic cells. Thus, a B-cell lymphoma was diagnosed and a plasma cell tumor was considered unlikely. COP chemotherapy protocol (prednisone, vincristine, and cyclophosphamide) was initiated but clinical signs persisted and euthanasia without necropsy was elected 21 days after initial submission of urine sediment our lab. Discussion: Extranodal lymphoma is rare, with few case reports describing urinary bladder-focused lymphoma. Multimodal diagnostic evaluation including cytology, PARR, flow cytometry, and electrophoretic evaluation allowed diagnosis of a rare condition.

LB-05: A SEVERE CASE OF INFLAMMATORY LINEAR VERRUCOUS EPIDERMAL NEVUS (ILVEN) IN A CHIHUAHUA MIX DOG

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Background: Inflammatory, linear, verrucous epidermal nevus (ILVEN) is a rare genodermatosis characterized by mostly unilateral, intensely pruritic and erythematous, wart-like epidermal nevi which follows the lines of Blaschko. ILVEN is caused by a somatic mutation that occurs after conception to the *NSDHL* gene. The disease is well characterized in humans but rarely reported in dogs. Here we characterize a rare case of severe ILVEN associated with bacterial infection in a two-year-old chihuahua mix dog. **Objective:** Our goal is to characterize a rare case of severe ILVEN in a two-year-old chihuahua mix. **Methods:** Due to disease progression, poor prognosis and unresponsiveness to treatment, the dog was humanely euthanized. Histopathology samples were collected from all major organs and representative sites of the skin (pinna, neck, abdomen, mammary gland and nipple, navel, pelvis, vulva, neck, and paw). Aerobic bacterial cultures were taken from both skin and mesenteric lymph nodes. **Results:** Severe epidermal proliferative wart-like papules were covering the vast majority of the right side of the face, neck, torso, abdomen, vulva and limbs.

Microscopically, the orthokeratotic hyperkeratotic epidermis was severely hyperplastic and projecting in thick papillomatous ridges. These were multifocally ulcerated and inflamed in association with large numbers of bacteria and generalized lymphadenomegaly. Cultures of skin and mesenteric lymph nodes lead to heavy growth of mixed bacteria. These were compatible with the clinical suspect of ILVEN.
Conclusions: ILVEN is a benign progressive proliferative cutaneous genodermatosis which can lead to recurrent secondary bacterial infection which can potentially lead to septicemia.

LB-06: VALIDATION OF A POINT-OF-CARE BENCHTOP ANALYZER FOR QUANTITATIVE MEASUREMENT OF C-REACTIVE PROTEIN IN CANINE SERUM AND PLASMA

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Canine C-reactive protein (cCRP) is an acute phase protein that increases dramatically with inflammation and has diagnostic utility in monitoring disease progression and response to treatment. Although current testing options are available, accessibility is limited. The objective was to validate the Vet Chroma™ canine specific point-of-care test (POCT) for the measurement of cCRP. The analyzer utilizes quantitative lateral flow immunofluorescence to measure the cartridge specific analyte. We hypothesized that the analyzer would measure cCRP within inherent biological variability. Serum and plasma from discarded canine patient samples, submitted initially for diagnostic testing, were used. Validation included intra- and inter-assay variation, linearity, spike recovery, and the effect of interfering substances and sample matrix. Spike recovery was performed using independently manufactured cCRP of a known concentration. Method comparison was also performed. Intra-assay variance ranged from 2.5-6.1%, and inter-assay variance ranged from 2.1-5.4%. The TE_{obs} ranged from 15.1-19.7%. The assay was linear over the manufacturer's analytical range with no evidence of bias. Recovery of cCRP in canine serum was acceptable at concentrations ≥31.25 mg/L. The presence of hemolysis, icterus and turbidity did not interfere with the assay. The cCRP concentrations of paired plasma and serum samples were close to equivalent. Comparison of the Vet Chroma™ cCRP assay to an established cCRP ELISA revealed significantly different results. We conclude that the Vet Chroma™ analyzer has acceptable test performance to measure serum or plasma cCRP concentration. The POCT format should advance the diagnostic use of cCRP by increasing accessibility and decreasing turn-around time and cost.

LB-07: MYCOBACTERIUM MICROTI INFECTION IN WILD BOAR (SUS SCROFA): EFFECT OF DIFFERENT HISTOLOGICAL FEATURES ON GRANULOMA MATURATION AND M. MICROTI MICROBIOLOGICAL ISOLATION

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Background: In Italy, retropharyngeal and submandibular lymph nodes (LNs) of hunted wild boars [WBs] (*Sus scrofa*) are assessed by veterinary public services to identify granulomas consistent with *Mycobacterium tuberculosis* complex (MTBC) infection. *M. microti* (MM) is an MTBC member whose infections are increasingly reported in humans and animals. MM identification is mostly based on direct molecular analysis, being in vitro growth extremely challenging. **Objective:** To study MM infection in WB by evaluating the effect of some histological features on granuloma maturational stage and MM microbiological isolation. **Methods:** 103 retrospective cases of WB LNs in which MM was identified by a specific PCR-RLFP assay, were considered. For each case, HE and Ziehl-Neelsen stained slides were retrieved together with MM microbiological results. In each section of LN, granulomas were counted, measured, and classified in four maturational stages. In each granuloma, multinucleated giant cells (MGCs) and acid-fast bacilli (AFB) were counted. Data obtained were statistically assessed. **Results:** 19/103 LNs were microbiologically positive for MM. The granuloma maturational stage was positively associated with granuloma size and AFB number, and negatively related to the number of MGCs and the number of granulomas in the LN. MM microbiological isolation was positively associated with the presence of AFB in the LN and negatively related to the presence of granulomas of the higher maturational stages in the LN. **Conclusions:** Histological features predict granuloma maturational stage and MM microbiological isolation, and may help to better understand host-pathogen interaction and epidemiological dynamics of MM infection in WB.

LB-08: IMMUNOPHENOTYPIC CHARACTERIZATION OF CANINE LYMPHOID NEOPLASMS BY CYTOLOGICAL EXAMINATION FOLLOWED BY IMMUNOFLUORESCENCE

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Background Cytologic evaluation of lesions is a largely employed diagnostic technique in small animal practice. Immunostaining of cytological preparation is crucial for differentiation of several lesions, including additional information cell histogenesis, immunophenotype, proliferation index and more. Canine lymphoid neoplasms are classified according to a myriad of criteria, among them the immunophenotype. **Objectives** To standardize immunofluorescence technique for canine lymphoid malignancies and evaluate its performance on cytological preparations obtained by fine needle aspiration. **Methods** Twenty slides of canine lymphoid malignancies (16 lymphomas and 4 bone marrow with lymphoid leukemia) aspirates were submitted for immunostaining, using acetone as fixative and fluorescein isothiocyanate as fluorophore. Antibodies against CD45, CD3, CD21 and CD79 were tested. To determine quality of samples the following criteria were examined: integrity, spacing between cells, cellular representativiness and erythrocytes presence (blood contamination). **Results** Immunophenotype with good immunoreactivity, yielding conclusive results was achieved in 13 of 20 samples (65%). Of the inconclusive ones, 71,42% had contamination with blood, and cell overlap in 42,85%, while conclusive samples with these problems were 15,38% and 23,07% respectively. Overall proportions of quality criteria were poorer in inconclusive samples. **Conclusions** Immunofluorescence is a quite simple and rapid technique. Immunostaining has an important role in veterinary

practice, once it can aid on differentiation neoplastic processes. Cytological examination is a useful diagnostic tool that can be routinely employed to investigate neoplasms in animal practice. High cell overlap and intense erythrocyte contamination could lead into difficult diagnosis or even turn impossible to interpret cytological immunophenotyping.

LB-09: FATAL PARAQUAT TOXICITY IN DOGS

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Background: Paraquat is a broad-spectrum herbicide unrestricted and commercially available in many Caribbean islands. Toxicity occurs in animals and humans with a poor prognosis and fatalities often result from progressive respiratory failure. **Objective**: To describe clinical and pathological findings of confirmed and suspected fatal paraquat toxicity in dogs of Saint Kitts. **Methods**: Dogs presenting to Ross University School of Veterinary Medicine with acute respiratory distress and histological features of diffuse alveolar injury were classified as paraquat toxicity suspects. Their medical records including history, laboratory results, radiographic, pathology, and toxicology data were retrospectively reviewed. **Result**: Eight suspect cases were identified between 2015-2018, representing 2% of canine autopsies over this period. Three cases were confirmed by liquid chromatography-mass spectrometry and initially presented for non-specific gastrointestinal signs (anorexia, abdominal pain, and vomiting) but progressed from tachypnea to respiratory distress within two days of presentation. All confirmed cases were azotemic on presentation and developed radiographic abnormalities compatible with interstitial lung disease. Histopathology showed lesions typical of paraquat toxicity including acute renal tubular necrosis, pulmonary edema and hemorrhage, interstitial pneumonia with hyaline membranes, type I pneumocyte necrosis, and type II pneumocyte hyperplasia. One suspected case presented with focal, ulcerative glossitis **Conclusion**: Accidental or malicious paraquat toxicity is not uncommon in dogs of St. Kitts and is therefore a serious animal and public health hazard that should be more closely regulated. It remains an important differential diagnosis for veterinary or human patients presented with unexplained anorexia, vomiting, azotemia, and progressive respiratory disease.

LB-10: DETECTION OF E26 TRANSFORMATION-SPECIFIC SEQUENCE VARIANT 1 (ETV1) MRNA EXPRESSION IN CECAL CANINE GASTROINTESTINAL STROMAL TUMORS BY IN-SITU HYBRIDIZATION

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Background: Gastrointestinal stromal tumor (GIST) is a common neoplasm of dogs, horses, and humans. On the basis of CD117 and DOG1 expression, GISTs are thought to originate from interstitial cells of Cajal (ICC) or their stem cell-like precursors in enteric neural plexuses. Detection of ETV1 mRNA expression by in-situ hybridization (ISH) is a useful marker for diagnosis of GIST in humans, particularly when DOG1 and CD117 expression are lost. **Objective:** Evaluate ETV1 mRNA expression in canine

GISTs and comparison with normal intestinal tissues and selected non-GIST intestinal neoplasms. **Methods:** ETV1 mRNA ISH of formalin-fixed and paraffin-embedded archived tissues obtained from 8 dogs with cecal GISTs, including 1 dog with a concurrent duodenal intestinal myogenic sarcoma, and dogs with intestinal leiomyosarcoma, leiomyoma, adenocarcinoma, and mast cell tumor. Samples of normal small and large intestine served as positive controls. **Results:** Strong cytoplasmic ETV1 signal was detected in myenteric and submucosa plexuses of normal intestine and normal intestine adjacent to each neoplasm. Similar ETV1 signal was detected in all GISTs as well as in a concurrent duodenal intestinal myogenic sarcoma and a presumptive leiomyosarcoma, but none of the intestinal leiomyoma, adenocarcinoma, and mast cell tumor. **Conclusion:** Canine cecal GIST and myogenic sarcomas express ETV1 mRNA at high level, while benign myogenic and non-myogenic malignant epithelial and non-epithelial intestinal neoplasms do not express ETV1. Taken together these preliminary studies suggest ETV1 expression is a specific marker of myogenic intestinal sarcomas.

LB-11: PATHOLOGICAL AND EPIDEMIOLOGICAL STUDY OF 46 CASES OF BLADDER TRANSITIONAL CELL CARCINOMA IN DOGS

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Transitional cell carcinoma (TCC) is the most common bladder tumor in dogs and humans, sharing pathophysiological and epidemiological aspects. **Objective:** We aimed to describe pathological and epidemiological aspects from 46 canine bladder tumors. **Methods:** This study included samples from UNESP and VetPat laboratory in Brazil and samples from the UniMI in Italy. The clinical records were assessed for information such as breed, sex and age. HE slides were classified according to WHO, 2004 and for the evaluation of presence of muscular infiltrating was performed, by two evaluators. **Results:** Of the total of 46 animals with TCC, 30 were females and 16 were males. the age ranged from 11 to 15 years, Poodle dogs were more frequent in the Brazilian set of cases, and mixed breed dogs in the Italian group. 76.1% of the tumors were papillary and infiltrating; followed by the non-papillary and infiltrating type (19.57%). Muscular invasion was accessed in 32 samples, and 50% of them were positive for this feature. **Conclusion:** There are similarities among canine and human bladder TCC regarding histological type and muscular invasion, which makes the dog a good natural model for the study of this disease.

LB-12: SARS-COV2 IN DOMESTIC CATS INDUCES LUNG PATHOLOGY DESPITE CLEARANCE OF VIRUS AND MINIMAL CLINICAL SIGNS

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Many patients that recover from COVID-19 report persistent respiratory signs; however, lung pathology following mild to moderate infections is poorly understood. It is extremely challenging to evaluate lung histopathology in human patients with non-fatal SARS-CoV-2 infections. **Objective:** A time course of lung pathology in domestic cats was examined to identify the effects of mild, non-fatal SARS-CoV-2 infections. **Methods:** Eleven domestic cats were inoculated with SARS-CoV-2, monitored for clinical signs, and euthanized at 3, 6, 10, or 28 days post-infection (DPI). Respiratory tract samples were collected for viral titers and histopathology. **Results:** No cats showed clinical signs. Viable virus was isolated from nasal turbinates, trachea, and in some cases lung, at 3 to 6 DPI, but not at later time points. Histology demonstrated moderate patchy interstitial pneumonia with damage to bronchiolar epithelium and partial to complete occlusion of bronchiolar lumens due to squamous metaplasia. Interstitial and perivascular inflammation was predominantly lymphocytic including both B and T cells and decreased significantly over time. Chronic lesions that persisted at 28 DPI included mild interstitial fibrovascular proliferation, bronchiolar remodeling with peribronchiolar fibrosis and luminal plugs of metaplastic epithelium. Alveolar septal thickening was due to vascular proliferation that lacked mature collagen and was immunopositive for endothelial markers, suggestive of angiogenesis. Unexpectedly, tissues from a single cat from the 28 DPI group demonstrated severe regional pneumonia with endothelialitis/vasculitis and hyaline membranes. **Conclusions:** SARS-CoV-2 infection in cats causes long-lasting lung pathology and may serve as an informative model to study the sequalae of non-fatal COVID-19 in humans.

LB-13: NEW IMMUNOASSAYS FOR THE DETECTION OF THE MOST COMMON FELINE SERUM AMYLOID A (SAA) VARIANTS

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SAA is a biomarker of inflammation in different species including cats. Several SAA variants were described in cats with substitutions at positions I29/K29, Q45/R45, A51/V51, N75/S75 (Van Rossum *et al*, 2003). The goal of this study was to develop immunoassays that recognize these SAA variants. SAA with the reference sequence (GeneBank AF136718) and four variants with single substitutions were expressed in *E. coli*. Monoclonal antibodies that recognized these SAA variants were selected. Two prototype sandwich immunoassays were developed that recognized SAA variants with the same efficiency. Immunoassay 1 required incubation with plasma samples at +37ºC. For immunoassay 2 incubation could be carried out at room temperature or at +37ºC. Plasma samples from healthy cats (n=19) and cats with inflammation induced by surgery (n=21) were analyzed by both immunoassays. SAA with the reference sequence was used as a calibrator. Dilution curves of feline plasma and recombinant SAA were parallel. SAA concentrations determined by the two immunoassays demonstrated high correlation ($r=0.99$, $p<0.05$). In healthy cats, median SAA concentration was 0.347 µg/ml (range 0.103-4.425 µg/ml) and 0.404 µg/ml (range 0.125-4.509 µg/ml) for immunoassays 1 and 2, correspondingly. In cats after surgery, median SAA concentration was 189 µg/ml (range 23-330 µg/ml) and 191 µg/ml (range

22-355 µg/ml) for immunoassays 1 and 2, correspondingly. In conclusion, we developed two new prototype immunoassays for accurate measurement of the most common SAA variants in feline plasma samples. Immunoassay 2 would be suitable for development of a point-of-care assay as it can be carried out at room temperature.

LB-14: UNTARGETED METABOLOMICS INVESTIGATION FOR FELINE HYPERTROPHIC CARDIOMYOPATHY-ASSOCIATED METABOLIC ALTERATIONS

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Hypertrophic cardiomyopathy (HCM) is the most common feline heart disease, associated with significant disease heterogeneity ranging from subclinical to premature death. HCM disease diagnosis and risk identification are hindered by a lack of prognostic tests. The molecular mechanism and triggers that affect HCM phenotype are unresolved, although metabolic and/or dietary factors are hypothesized to modulate pathologic hypertrophy. Untargeted metabolomics involves global detection and relative quantitation of metabolites associated with physiologic or biological states. As metabolomics has the potential to identify novel biomarkers for risk identification and assessment, we sought to characterize feline HCM-associated plasma metabolic alterations. Untargeted metabolomics was performed on an Orbitrap QE-HF system coupled with hydrophilic interaction (HILIC) and C18 reverse-phase chromatography to compare plasma samples from 21 HCM cats and 11 controls. Data were acquired in both positive and negative ion modes. Compound Discoverer 3.1 software was used to search multiple databases to annotate compounds based on m/z results and tandem mass spectrometry match to the spectral library. We found 719 and 909 annotated metabolites in feline plasma by HILIC negative and C18 positive ion modes. Principle component analysis for the HILIC data filtered by p-value <0.05 showed differentiation between groups; however, many of the significant changes reflected the detection of cardiac therapeutics (negative ion mode). While feline HCM is likely associated with myocardial metabolic alterations, these data suggest that tissue changes may not be readily detected by plasma metabolomics. Further investigation is needed to determine the relationship of systemic metabolic alterations with HCM pathophysiology and progression.

LB-15: IN VITRO VALIDATION OF PROTEIN GERANYLGERANYLATION AS A PHARMACOLOGICAL TARGET FOR THE TREATMENT OF CANINE MAMMARY GLAND TUMORS.

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Canine mammary tumours (CMTs) are the most common neoplasms in intact bitches, and no effective chemotherapeutic options are available for highly invasive and metastatic tumors. Recent studies show the potential involvement of dysregulated Hippo signaling in CMT development and progression. Protein geranylgeranylation (GGylation) is an important post-translational modification for many signaling molecules, and its blockade with statins has been shown to inhibit YAP/TAZ-mediated

transcriptional activity via activation of the Hippo pathway. In this study, we sought to determine if protein GGylation represents a valid pharmacological target for the treatment of CMTs. Two CMT cell lines (CMT9 and CMT47) were evaluated for their sensitivity to Atorvastatin and Fluvastatin. Results demonstrated statins to be cytotoxic to both cell lines, with ED50 values ranging from 0.95 µM to 23.5 µM. In addition, both statins lowered Hippo pathway effector proteins YAP and TAZ and Atorvastatin reduced the mRNA levels of key transcriptional target genes known to be involved in breast cancer progression and chemoresistance (*CYR61*, *CTGF* and *RHAMM*). Moreover, both statins effectively inhibited cell migration in CMT47 and anchorage independent growth in CMT9 and CMT47, but did not modulate matrix invasion. Finally, our FACs results showed that both statins increase apoptosis and promote cell cycle arrests. Taken together, our results show that statins activate the Hippo pathway in CMTs and modulate several aspects of CMT's molecular and cellular behaviours. These findings suggest that the targeting of Hippo pathway with statins could be a novel approach for the treatment of inoperable canine mammary gland cancers.

LB-16: DO CANINE PANCREATIC NEUROENDOCRINE NEOPLASMS RESEMBLE HUMAN PANCREATIC NEUROENDOCRINE TUMORS? A COMPARATIVE MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL INVESTIGATION INCLUDING A PANCREATIC MIXED NEUROENDOCRINE-NON-NEUROENDOCRINE NEOPLASM IN A DOG

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Background: Canine pancreatic neuroendocrine neoplasms (PanNEN) are proposed as model for human PanNEN, but the Ki67-index-based type (G1: <2%, G2: < 20%, G3: >20%) which they resemble is unclear. *ATRX* and *p53* gene mutations and somatostatin receptor 2A (SSTR2A) expression are confirmed in human PanNEN. Human pancreatic mixed neuroendocrine-non-neuroendocrine neoplasms (MiNEN) are extremely rare and only 1 canine case is reported. **Objective:** This study compares canine and human PanNEN according to grading, prognosis and selected molecular markers (*ATRX*, *p53*, SSTR2A) currently used in human tumors. **Methods:** Formalin-fixed canine PanNEN (15 primary, 2 metastases, 1 MiNEN) were routinely processed for histology and immunohistochemically labelled for synaptophysin, insulin, glucagon, Ki67, ATRX, p53 and SSTR2A. **Results:** All tumors were classified as pancreatic islet cell carcinomas based on local and vascular invasive growth. Cytoplasmic expression of synaptophysin and insulin confirmed the functional diagnosis insulinoma in all neoplasms. All tumors had a Ki67-index of <2.5% and membranous SSTR2A expression, nuclear ATRX expression (normal), but absence of p53 expression (normal). The non-neuroendocrine component of MiNEN showed cytoplasmic cytokeratin expression. **Conclusion:** Canine PanNEN share well-differentiated histomorphology, SSTR2A expression and absent p53 immunolabelling with human PanNETs G1, but differ in *ATRX* expression, functionality and have a worse prognosis. Human Ki67-based grading was not applicable to canine PanNEN. Membranous SSTR2A expression makes canine PanNEN and MiNEN potentially amenable to treatment with somatostatin analogs.

ATRX and *p53* gene mutation presumably do not play a pathogenetic role in canine PanNEN and MiNEN. Molecular analysis is necessary to confirm this.

LB-17: HARDERIAN GLAND ADENOCARCINOMA IN VANCOUVER ISLAND MARMOTS (MARMOTA VANCOUVERENSIS)

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Retro-orbital tumors were diagnosed in 4 of 28 Vancouver Island Marmots (VIMs, *Marmota vancouverensis*) necropsied at the Toronto Zoo from 1999 to 2020. Affected VIMs were three females and one male aged 8-10 year-old. Clinical signs were present in 3 out of 4 cases and included unilateral mucopurulent discharge, conjunctival swelling, exophthalmos, and buphthalmos. All tumors were unilateral and histologically infiltrative, invading the retrobulbar space (4/4) and the adjacent globe (1/4). Tumors were composed of polygonal epithelial cells arranged in packets, acini or occasionally tubules. The cytoplasm of numerous neoplastic cells was filled by variably sized, clear vacuolations. Mitotic figures ranged from 0 to 7 in 10 high-power (400x) fields. Metastases were found in three cases: lungs (3/3), brain (1/3) and a regional lymph node (1/3). In all tumors, neoplastic cells showed strong immunoreactivity to cytokeratin and none to vimentin or smooth muscle actin. All tumors were classified as carcinomas. The retro-orbital location and presence of vacuolated cells suggested a Harderian gland origin. In support of this diagnosis, similar proliferative changes (hyperplasia and dysplasia, or adenoma) were also present in the contralateral Harderian glands of two cases. Although Harderian gland adenocarcinomas have been reported in multiple rodent species, this is the first report in VIMs. In this series, Harderian adenocarcinomas were the main clinical concern leading to euthanasia of 3 marmots. Therefore, the retrobulbar space should be examined during necropsy of VIMs, and Harderian gland adenocarcinoma should be included in the list of differential diagnoses for this endangered species.

LB-18: PANCREATIC HISTOPATHOLOGICAL DIAGNOSIS IN CANINE AND FELINE POPULATIONS

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Background: Invasive techniques like histopathological analysis are often required for the diagnosis of clinically unspecific pancreatic conditions. **Objective:** To compare the rate of different pancreatic diagnoses of the feline and canine populations. **Methods:** 577 feline and 575 canine formalin-fixed pancreatic samples submitted between 2014-2019 were processed for histopathology and categorized as: 1: normal; 2: nodular hyperplasia; 3: mild pancreatitis (3a: lymphoplasmacytic; 3b: mixed/suppurative); 4: marked pancreatitis (4a: lymphoplasmacytic; 4b: mixed/suppurative); 5: neoplasia; 6: other. Cats were between 0.3-23 years-old, overrepresented by European shorthair (n= 395). Dogs were between 0.1-17 years-old, commonly represented by Labrador retrievers (n=39), Jack Russel Terriers (n= 32), German Shepherds (n= 28), and

Yorkshire Terriers (n= 27). **Results:** Of the cats, 37.4% had pancreatitis (3a: 79/216; 3b: 41/216; 4a: 11/216; 4b: 85/216) and 19.2% neoplasia (primarily epithelial: 86/111; endocrine: 3/111; secondary: 22/216). Of the dogs, 43.3% had a histologically normal pancreas in the sections examined, while 34.2 % had pancreatitis (3a: 34/197; 3b: 30/197; 4a: 13/197; 4b: 120/197) and 10.6% neoplasia (primary epithelial: 37/61; endocrine: 6/61; secondary: 18/61). The pancreas of German shepherds was often histologically irrelevant unremarkable (15/28) while pancreatitis was often seen in Labradors (17/39), Jack Russel Terriers (14/32) and Yorkshire Terriers (11/27). Tumors were diagnosed in 5/18 golden retrievers. **Conclusion:** Rates of pancreatic conditions should be taken into account when elaborating a differential list in these species. Pancreatic tumors are more common in cats, when compared to dogs and requires histopathological evaluation. The clinical relevance of mild pancreatitis must be interpreted within clinical context.