



Clinical Pathology

Sunday, October 29 | 2:30 PM – 2:45 PM

Session Chair: Melinda Camus

Sunday, October 29

2:30 PM – 2:45 PM

DETERMINING OPTIMAL STORAGE TIME AND TEMPERATURE FOR THE DETECTION OF RED BLOOD CELL AND PLATELET SURFACE-ASSOCIATED IMMUNOGLOBULIN BY FLOW CYTOMETRY IN HEALTHY HORSES

Miranda Frohlich¹, Nora Springer²

¹Colorado State University, Fort Collins, CO, USA, ²University of Tennessee, Knoxville, TN, USA

Background: Differentiating immune-mediated causes from other causes of anemia and thrombocytopenia is challenging. Flow cytometry can detect surface-associated immunoglobulin (slg) on red blood cells (RBC) and platelets (PLT) in dogs and horses. Sample storage parameters for ideal assay performance has not been evaluated in horses.

Objective: Identify optimal storage time and temperature of equine whole blood for the detection of RBC-slgl and PLT-slgl by flow cytometry.

Methods: Whole blood was collected from 10 healthy horses. RBC-slgl samples were stored at 4°C and PLT-slgl samples were stored at 4°C and room temperature. Direct immunofluorescence flow cytometry was performed to quantify RBC-slgl and PLT-slgl at 0, 4, 24, 48, and 72 hours post-collection. Five replicates per sample were run at each timepoint. Coefficient of variation and cutoff values converting measured quantitative data to a dichotomous result (negative or positive) were determined for 0 hour and compared to RBC-slgl and PLT-slgl at all other timepoints.

Results: RBC-slgl percent positive events were stable up to 72 hours storage. PLT-slgl percent positive events increased above baseline cutoff values at all timepoints and the effect was more pronounced in blood stored at room temperature.

Conclusions: Cutoff values for RBC-slgl remained similar across timepoints. PLT-slgl testing should preferably be performed within 4 hours of collection. In instances where this is not feasible, samples should be stored at 4°C and analyzed no later than 24 hours after collection.

Ideally, storage time-specific reference intervals or decision limits should be established by the laboratory running the test.

Sunday, October 29

2:45 PM – 3:00 PM

CLINICALLY VALUABLE COMMENTS WERE COMMON ON VETERINARIAN-ORDERED BLOOD SMEAR REVIEW FOLLOWING IN-CLINIC COMPLETE BLOOD COUNT

Helen Michael, Corie Drake, Kim Yore

IDEXX Laboratories, Inc, Westbrook, ME, USA

Peripheral blood smear review is commonly performed to confirm results from automated hematology analyzers or to identify cancerous cells, morphologic changes, infectious agents, and other changes that are not definitively classified by automated analyzers. Studies in human patients have shown variable value of blood smear review depending on the indication for smear review. The value hasn't been extensively evaluated in veterinary species. In this study, we evaluated blood smear reviews submitted to IDEXX reference laboratories during 2022 by a veterinarian and associated with a CBC performed on an in-clinic hematology analyzer. Initial CBC results, instrument flags, and blood smear review comments were analyzed. Comments added clinical value if they provided additional or confirmatory information to the automated CBC results that could impact clinical management. Reports for 4176 smear reviews were analyzed. 51.0% of reviews were associated with an instrument flag, most commonly for abnormal platelet distribution, left shift, abnormal WBC distribution, nucleated RBCs, and platelet clumps. The corresponding CBC showed cytopenia in 59.4% and cytolysis in 39.3%. Clinically valuable comments were present in 80.0% of reviews associated with instrument flags, 76.7% with cytopenia, and 85.4% with cytolysis. 17.1% of smear reviews had no instrument flags or abnormal cell counts; 48.6% of these smears had clinically valuable comments. The most common comments were associated with platelet clumping, and toxic change. Veterinarian-ordered blood smear review following an in-clinic CBC had a high yield of clinically valuable results, particularly when the corresponding CBC had instrument flags or aberrant cell counts.

Sunday, October 29

3:30 PM – 3:45 PM

ADVANCEMENTS IN AN EQUINE ADRENOCORTICOTROPIN HORMONE MEASUREMENT BY A NEWLY DEVELOPED RAPID, STALL-SIDE PORTABLE ASSAY

Lisa Neufang, Joseph Ramos, Luca Giori

University of Tennessee, Knoxville, TN, USA

Background: Pituitary pars intermedia dysfunction (PPID) is a neurodegenerative disease of senior horses. Loss of dopaminergic inhibition on the melanotropes of the pars intermedia leads to increased concentrations of proopiomelanocortin (POMC)-derived peptides. Diagnosis is challenging due to pre-analytical variables. Recent studies in human medicine, indicate that the Immulite 2000 detects and measures not only ACTH, but also other POMC-derived peptides.

Objectives: Develop an assay that could be performed under field conditions with similar sensitivity and specificity to the current gold standard Immulite 2000.

Methods: Using capture ELISA, two ACTH-specific monoclonal antibodies, CBL57 and EPR20361-248, were selected based on the recognition of separate epitopes, strong and rapid color change, minimal background interference, and no cross-reactivity. CBL57 serves as the solid surface for capturing and enriching ACTH from plasma. Biotin-conjugated EPR20361-248 served as the detection antibody. The concentrations of probes, dilutions and volumes of plasma, and incubation durations were optimized.

Results: The immunoassay detected unglycosylated human recombinant ACTH. However, the assay did not detect ACTH using plasma from positive PPID equine samples, as determined by the Immulite 2000.

Conclusions: The assay detects unglycosylated human recombinant ACTH, but not equine ACTH. Further studies are ongoing to identify 1) possible plasma matrix interferents, 2) determine whether the Immulite 2000 detects POMC-derived peptides in equine plasma, similar to people, and 3) whether the assay antibodies are unable to detect glycosylated forms of ACTH. This information would aid in optimization of the assay to be used in the for rapid and accurate identification of PPID-affected animals.

Sunday, October 29

3:45 PM – 4:00 PM

MICRORNA-BASED DIAGNOSTIC AND PROGNOSTIC BIOMARKERS OF CANINE VISCERAL HEMANGIOSARCOMA

Andrea Pires dos Santos, Kerstin Muner, Nelly Elshafie, Mary Nowak, Laura Machado Ribas, Luis Neves dos Santos
Purdue University, West Lafayette, IN, USA

Background: Hemangiosarcoma (HSA) is a devastating disease with a poor prognosis due to its aggressive nature and lack of specific clinical signs until metastasis develops. HSA can be diagnosed by histopathology. However, grading systems have poor predictive significance owing to the neoplasms' diverse histological properties, making it imperative to develop new prognostic tools. MicroRNAs are stable and abundant small non-coding RNAs that regulate gene expression and are altered in cancer, making them attractive biomarkers. **Objective:** We searched for microRNA-based biomarkers for early detection and prognosis of splenic HSA in dogs. **Methods:** Small RNA sequencing was performed in 18 cases divided into three groups based on survival time: Group 1 (G1; n=6) survived < 90 days, Group 2 (G2; n=7) 90 to 180 days, and Group 3 (G3; n=5) > 180 days, plus six spleen controls. Total RNA was extracted from FFPE splenic tissues, converted into sRNA libraries, and sequenced (Illumina). Analysis was conducted using CLC Genomics and reads mapped to miRBase. EdgeR was used for differential expression (DE) analyses. **Results:** 453 microRNAs were expressed across samples, and 90 were DE between controls and HSA groups independently of survival times (FDR<0.01). Moreover, three potential prognostic markers were revealed (FDR<0.01) across survival times, 26 if considering FDR<0.05. **Conclusions:** These microRNAs may be used as diagnostic and prognostic markers of canine HSA and will be validated and further explored. Predicting the potential outcomes of HSA can assist clinicians in determining appropriate treatment and setting realistic expectations regarding animal welfare and quality of life.

Sunday, October 29

4:00 PM – 4:15 PM

REFERENCE VALUES FOR SERUM SDMA CONCENTRATIONS IN CLINICALLY HEALTHY MALE RHESUS MACAQUES

JodiAnne Wood¹, Kendall Clark¹, Corey Drake¹, Chelsea Landon², Frances Sun², Mariya Morris³, Lee-Ronn Paluch³, Alyson Guy³, Colleen Thurman⁴, Rebecca Tierce⁵, Valerie Wong¹

¹IDEXX BioAnalytics, Westbrook, ME, USA, ²Duke University Medical Center, Durham, NC, USA, ³New York University & NYU-Regeneron Postdoctoral Training Program in Laboratory Animal Medicine, New York, NY, USA, ⁴Boston University, Boston, MA, USA, ⁵Columbia University, New York, NY, USA

Background: NG, NG'-Dimethyl-L-arginine, dihydrochloride (SDMA) is a serum biomarker for kidney disease. Serum SDMA concentration is useful for early detection of kidney disease in dogs, cats, horses, and some rat disease models. However, the utility of serum SDMA for detecting kidney disease in rhesus macaques is unknown. Furthermore, reference values for serum SDMA concentration in clinically healthy rhesus macaques are not available.

Objective: Our laboratory has previously validated a proprietary, high-throughput immunoassay for measuring SDMA concentrations in serum samples from rhesus macaques. This study aimed to determine the reference interval for serum SDMA concentrations in clinically healthy rhesus macaques.

Methods: Serum samples (n=272) were collected from 55 clinically healthy male rhesus macaques. One observation was randomly chosen from each individual for generation of the reference interval. Measurements of SDMA concentrations were obtained using a validated proprietary immunoassay. Creatinine and urea concentrations were determined by a modified Jaffe method and a urease-based method, respectively. Reference intervals were estimated following the guidance of the Clinical and Laboratory Standards Institute (CLSI). The “robust” method for reference interval estimation was used. The uncertainty of the reference limits was estimated by bootstrap resampling (n=1000).

Results: The 2-sided, upper and lower, 95% reference intervals were estimated as (5.4, 14.2 µg/dL) for SDMA, (0.47, 1.37 mg/dL) for creatinine, and (3.0, 28.3 mg/dL) for urea.

Conclusions: Reference values for serum SDMA were generated for rhesus macaques. Additional investigative work for determining intra-individual variation and serum SDMA concentrations in rhesus macaques with compromised kidney functions are currently underway.

Sunday, October 29

4:15 PM – 4:30 PM

DEVELOPMENT AND VALIDATION OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY TO MEASURE REGENERATING ISLAND-DERIVED PROTEIN 3E IN CANINE BLOOD

Laureen Peters¹, Judith Howard¹, Luca Giori², Meike Mevissen¹, Rolf Graf³, Theresia Reding Graf³

¹Vetsuisse Faculty, University of Bern, Bern, Switzerland, ²College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA, ³University Hospital Zürich, University of Zürich, Zürich, Switzerland

Background: Regenerating islet-derived proteins (REG) are upregulated in humans with sepsis, pancreatitis, and gastrointestinal diseases. We recently identified one member of the REG family, namely REG3E, in pancreatic tissue and plasma of dogs, with high expression in pancreatitis and sepsis.

Objectives: To develop and validate an ELISA to measure REG3E concentrations in canine blood and establish reference intervals for healthy dogs.

Methods: An indirect sandwich ELISA was developed using recombinant canine REG3E protein and polyclonal anti-canine REG3E antibodies raised in guinea pigs and rabbits. Assay validation included dilutional linearity and parallelism, spiking recovery, repeatability and reproducibility, stability, interferences, and comparison of serum and heparinized plasma. Right-sided reference intervals were calculated from plasma samples of 80 clinically healthy dogs, using the robust method after Box-Cox transformation.

Results: Limit of detection of the assay was 15ng/ml and lower limit of quantification was 30ng/ml. The assay demonstrated good linearity, parallelism, and recovery, with mean observed-to-expected ratios of 104%, 107%, and 92%, respectively, and no evidence of hook effect. Coefficients of variation were <10.4% for repeatability and <12% for reproducibility at three different levels. Measurements were not significantly influenced by different storage conditions, freeze-thawing cycles, or hemolysis, lipemia, and icterus. There was no significant difference between heparinized plasma and serum samples. Clinically healthy dogs have REG3E concentrations of up to 217ng/ml (95% CI: 156 – 289).

Conclusions: The canine REG3E ELISA has adequate precision, accuracy, linearity, and reproducibility for measurement of REG3E in canine plasma and serum.

Sunday, October 29

4:30 PM – 5:00 PM

MULTICENTER PROSPECTIVE STUDY OF CANINE SPECIFIC S100A12 AS NON-INVASIVE BIOMARKER OF ACUTE ACALCULUS CHOLECYSTITIS IN DOGS

Ahmed El-Sebaey¹, Sergey Pozyabin², Seidfatima Borunova³, Pavel Abramov³

¹Department of Clinical Pathology, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt, ²Department of Veterinary Surgery, Moscow State Academy of

Veterinary Medicine and Biotechnology – MVA by K. I., Skryabin, house 23, Academician Scriabin str., Moscow, Russian Federation, ³Department of Diagnostics of Diseases, Therapy, Obstetrics and Animal Reproduction, Moscow State Academy of Veterinary Medicine and Biotechnology – MVA by K. I., Skryabin, house 23, Academician Scriabin str., Moscow, Russian Federation

Introduction: Acute acalculous cholecystitis (AAC) is a major life-threatening complication in the critically ill and hypovolemic shocked dogs. The initial diagnosis is based on non-specific inflammatory markers and the limited imaging modality criteria.

Objective: We aimed to assess the diagnostic efficacy of S100A12 versus WBC count and CRP in identification of AAC and prognosis of its severity. **Methods:** This was a multicentric prospective study. Over a five-year period, 105 dogs diagnosed with mild and moderate to severe AAC, and healthy control dogs, were included in the study (35 each) based on the hemato-biochemical, ultrasonographic, and histopathologic findings. The serum levels of canine specific S100A12 and CRP were measured by ELISA.

Results: S100A12 concentration was significantly elevated in dogs with mild ($P<0.01$) or moderate to severe ($P<0.001$) AAC compared to the control, and at area under the curve (AUC) of 0.91 and 0.96, respectively, exhibited potential roles in discriminating diseased groups from the control. WBC count and CRP exceed the reference interval and only increased ($P<0.01$) in moderate to severe AAC group and at AUC of 0.81 and 0.87 showed a moderate diagnostic performance in differentiating this group from the healthy one. **Conclusions:** The discriminative potential of S100A12 was superior to that of WBC count and CRP for diagnosing different grades of AAC severity. S100A12 can be considered as a reliable biomarker for AAC at early stages which could help the clinician to initiate a tier one priority medical intervention to increase animal survivability.

Keywords: dogs, cholecystitis, S100A12, WBC, CRP, AUC.

Tuesday, October 31

9:00 AM – 9:15 AM

DIFFERENCES IN PREPARATION METHODS OF CANINE PLATELET LYSATE AFFECT GROWTH FACTOR RELEASE

Thaina Lunardon, Melikasadat Mollabashi, Nikolia Darzenta, Scarlett Sumner, Maria Naskou

Auburn University College of Veterinary Medicine, Auburn, AL, USA

Background: Wound healing is a multicellular dynamic process, modulated by cytokines and growth factors due to enhancement of tissue regeneration. Platelets play a special role during the inflammatory phase of tissue regeneration because of the release of alpha granules. The alpha granules contain several growth factors and cytokines that are responsible for the recruitment and activation of other inflammatory cells. Thus, platelet-derived products such as platelet lysate (PL), have been proposed as an alternative therapeutic for the management of chronic wound healing. Recent evidence suggests that the preparation method and concentration of white blood cells can affect the release of growth factors from platelets.

Objective: To optimize the manufacturing process and evaluate growth factors and cytokines release in canine platelet lysate.

Methods: Blood from six healthy dogs was collected, and platelet-rich plasma were generated by two protocols (leukocyte rich versus leukocyte poor method). A portion of the lysates was exposed to heat inactivation and/or plasma depletion. Subsequently, platelet lysate was generated via five freeze/thaw cycles and pooled from various donors. Quantification of PDGF, TGF- β , VEGF, HGF, and TNF- α was performed by ELISA.

Results: TGF- β and PDGF concentration appeared to be higher when plasma was depleted from platelet lysate. However, VEGF concentration tended to increase following heat inactivation of platelet lysate. To the contrary, HGF concentration tended to be higher in canine platelet lysate without plasma depletion.

Conclusions: Differences in preparation methods of canine platelet lysate appear to impact the final product's growth factor and cytokine concentration.

Tuesday, October 31

9:15 AM – 9:30 AM

OPTIMIZATION OF PLATELET-RICH PLASMA PREPARATION WITH PLATELET FUNCTIONAL ANALYSIS FOR THE USE IN REGENERATIVE MEDICINE

Preeti Chaudhary, Sanggu Kim, Soochong Kim

Chungbuk National University, Cheongju, Republic of Korea

Background

Platelet-rich plasma (PRP) has recently been used in regenerative medicine due to its abundant content of growth factors (GFs) and cytokines. Therefore, we determined to optimize the PRP preparation to achieve the highest platelet recovery rate and platelet function.

Methods

Whole blood from healthy dogs was centrifuged at different run conditions (800, 1200, 1500, and 2000 rpm) for 10 min single-spin or 5 min dual-spin to obtain PRP, and the platelet recovery rate of each condition was compared with the commercial PRP preparation kit. Agonists-induced platelet aggregation and dense granule secretion, TxA₂ generation, and PDGF-BB release were measured to determine the platelet function of each PRP.

Results

The PRP obtained by dual-spin centrifugation at 1500 rpm for 5 min demonstrated the highest platelet recovery rate without WBC contamination. Using platelets obtained from this PRP, 2-MeSADP- and thrombin-induced platelet aggregation and dense granule secretion were maximized, suggesting that optimizing the centrifugal speed and time is indispensable for the utmost PRP collection with optimal platelet function. Additionally,

2-MeSADP- and thrombin-induced TxA_2 generation which causes a positive-feedback effect on stabilizing platelet aggregation, was significantly potentiated at dual-spin 1500 rpm run condition. Interestingly, PDGF-BB was released time-dependently and its release was dramatically increased in PRP adjusted at 1×10^9 platelets/ml indicating the importance of platelet concentration in PRP for maximum GF release.

Conclusion

We standardized the PRP preparation with the optimal platelet function, TxA_2 generation, and GF release that will maximize the effectiveness and efficacy of PRP application in regenerative medicine.

Tuesday, October 31

9:30 AM – 9:45 AM

COMPREHENSIVE ANALYSIS OF PLATELET FUNCTION IN DOGS WITH HYPERADRENOCORTISISM

Sanggu Kim¹, Dohee Lee², Preeti Chaudhary¹, Hakhyun Kim², Byeong-Teck Kang², Soochong Kim¹

¹Laboratory of Veterinary Pathology and Platelet Signaling, College of Veterinary Medicine, Chungbuk National University, Cheongju, Republic of Korea, ²Laboratory of Veterinary Internal Medicine, College of Veterinary Medicine, Chungbuk National University, Cheongju, Republic of Korea

Background

Hyperadrenocorticism (HAC) is a condition induced by high cortisol levels that is related to an increased risk of hypercoagulation, which can lead to catastrophic cardiovascular illnesses in both humans and dogs. Although studies on coagulation factors in HAC patients are well known, platelets and their function, which is crucial in thrombosis, are much less clear. Thus, the effect of HAC on platelet function was determined.

Methods

HAC was diagnosed using adrenocorticotrophic hormone stimulation test and low dose dexamethasone suppression test, and agonist-induced platelet aggregation, dense-granule secretion, and thromboxane A_2 (TxA_2) generation were characterized using washed platelets from normal and HAC dogs.

Results

Although platelet count and MPV were shown to be increased in HAC dogs, 2-MeSADP- and low concentration of thrombin-induced platelet aggregation and secretion were significantly inhibited in HAC dogs. Furthermore, the pre-incubation of prednisolone, a corticosteroid, inhibited 2-MeSADP and thrombin-induced platelet aggregation and secretion only in normal dogs, whereas no additional inhibitory effect was shown in HAC dogs. Moreover, 2-MeSADP- and thrombin-induced platelet aggregation and post-cortisol levels showed a negative correlation, indicating that post-

cortisol levels reflect platelet function in dogs with HAC. Finally, 2-MeSADP- and thrombin-induced TxA₂ generation was significantly inhibited in dogs with HAC, suggesting a significant role for TxA₂ in regulation of platelet function by HAC because positive-feedback effect of generated TxA₂ contributes to agonist-induced platelet responses.

Conclusion

Excessive cortisol in dogs with HAC plays an important role in platelet function through the regulation of TxA₂ generation.

Tuesday, October 31

10:15 AM – 10:30 AM

EVALUATION OF THE EFFECTS OF AN ANTIAPOPTOSIS SOLUTION (Q-VD-OPH) ON PRESERVING NEUTROPHIL VIABILITY AND FUNCTION AT 4°C

Calandra Chuback¹, Ryan Dickinson¹, Khawaja Ahmad², Nicole Fernandez¹, Melissa Meachem¹

¹Western College of Veterinary Medicine, Saskatoon, SK, Canada, ²British Columbia Ministry of Agriculture and Food, Animal Health Center, Abbotsford, BC, Canada

Background: A universal obstacle of research in neutrophil function, is the limited viability of neutrophils following blood sample collection, leaving a narrow time-window from sample collection to evaluation. A preservative solution (Q-VD-OPh) has been shown to stabilize leukocyte viability in humans over a 72-hour period at ambient temperatures. However, there remains limited information regarding how well the preservative maintains neutrophil viability and function in canine patients.

Objectives: Our first objective was to determine the optimal concentration of Q-VD-OPh to add to whole blood samples. Using this concentration, our second objective was to measure and compare neutrophil function (respiratory burst and phagocytosis) via flow cytometric evaluation in samples of normal canine blood (with and without Q-VD-OPh added) on the day of sample collection, as well as 24, 48, 72 and 96 hour intervals at 4 °C.

Methods: Heparinized blood was collected from 20 healthy dogs. Viability was investigated by multi-color flow cytometry using Zombie Green™ to identify cells with compromised membranes and PerCP/Cy5.5 Annexin V staining to identify apoptotic cells. Neutrophil function was investigated using commercial kits designed for flow cytometric assessment of neutrophil respiratory burst and phagocytic ability.

Results: Neutrophil viability was optimized at 80µm Q-VD-OPh as seen by a significant decrease in cells positive for Zombie Green and Annexin V. Final FlowJo and statistical analyses of neutrophil function data is ongoing.

Conclusions: Q-VD-OPh preserves neutrophil viability and likely neutrophil function in canine patients, allowing for increased patient follow-up evaluation and enrolment in studies examining neutrophil function.

Tuesday, October 31

10:30 AM – 10:45 AM

EFFECT OF FIXATION AND DECALCIFICATION OF CANINE BONE MARROW ON DNA AMPLIFICATION

Gabriella Diamantino¹, William Vernau², Janet Beeler-Marfisi¹, Robert Foster¹, Dorothee Bienzle¹

¹University of Guelph, Guelph, ON, Canada, ²University of California, Davis, CA, USA

Background: Bone marrow (BM) cores contain mineralized hard bone and soft hematopoietic tissue, and therefore require fixation and decalcification prior to processing. An optimal approach to generate BM sections suitable for histopathologic assessment and subsequent DNA amplification remains elusive.

Objective: This study aimed to assess the effects of BM fixation and decalcification on lymphocyte antigen receptor gene rearrangement amplification.

Methods/Results: Six replicate samples of sternal BM from six dogs euthanized for various reasons were fixed overnight in either acid-zinc-formalin (AZF) or 10% neutral buffered formalin (NBF). Samples were then decalcified in either fast decalcifier (10% HCl), slow decalcifier (formaldehyde/formic acid) or EDTA for 1, 12, or 24 hours, respectively. Tissues were then routinely paraffin-embedded and processed for histology. One ~3 mm³ section, 10 µm thick, was collected into a microtube, DNA was isolated, and DNA concentration and quality were measured. Next, DNA (50 ng) was amplified with primers to conserved regions of the T cell receptor gamma genes and two segments of the immunoglobulin heavy chain genes to generate amplicons of 75-170, 150-210 and 210-300 base pairs, respectively. Amplification efficiency was graded from 1 to 4 based on review of capillary electrophoretograms. Statistical analysis (Wilcoxon matched-pairs signed rank test) showed that EDTA decalcification yielded better amplification than either slow or fast decalcification, regardless of fixative. However, fixation with NBF resulted in higher amplification scores than fixation with AZF ($p=0.031$).

Conclusions: Fixation with NBF followed by EDTA decalcification resulted in superior DNA amplification compared to fixation with AZF or acid-based decalcification.

Tuesday, October 31

10:45 AM – 11:00 AM

EFFECT OF FUROSEMIDE ADMINISTRATION ON PLASMA ANALYTES AND URINE OSMOLALITY IN RED-EARED SLIDERS (TRACHEMYS SCRIPTA ELEGANS)

Katie Metcalf¹, Maria Aguilar¹, Shannon Dehghanpir¹, Mark Acierno², Mark Mitchell¹

¹Louisiana State University, Baton Rouge, LA, USA, ²Midwestern University, Glendale, AZ, USA

Background: Renal disease is a common ailment of captive reptiles and is closely linked to chronic, subclinical states of dehydration. The diagnosis of renal disease in reptiles is poorly characterized and often relies on invasive diagnostic techniques (e.g., renal biopsy) for definitive diagnosis. Induction of dehydration via furosemide administration may lead to further characterization of renal disease in this species. While the effects of furosemide in reptiles are poorly characterized, the clinical utility of this medication has been documented in various reptilian species.

Objectives: Confirm the utility of furosemide administration and better characterize the diagnostic findings of dehydration in red-eared sliders.

Methods: Twelve adult, male, red-eared slider turtles were randomly assigned to three treatment groups in a complete-crossover study: 10 mg/kg furosemide every 12-hours; 5 mg/kg furosemide every 12-hours; and a control group. For baseline, a PCV, non-mammalian chemistry panel, USG, and plasma and urine osmolality via freezing point osmometry were measured, then water and food were withheld for 48-hours while the treatment was administered. Plasma and urine samples were re-collected, and they were reintroduced to water and weighed over the next 48-hours.

Results: Furosemide-treated turtles exhibited prominent physical evidence of dehydration (e.g., sunken eyes, drying of scales), increased urine production and urine osmolality, and hypochloremia. Other significant indicators of dehydration were also identified between groups including decreased weight and increased USG, plasma and urine osmolality, BUN, phosphorus, magnesium, and total protein.

Conclusions: This study supports the clinical utility of furosemide in the red-eared slider to induce diuresis and dehydration.

Tuesday, October 31

11:00 AM – 11:15 AM

HEMATOLOGIC ABNORMALITIES AND DISEASES ASSOCIATED WITH MODERATE TO MARKED BASOPHILIA IN A LARGE COHORT OF DOGS

Elizabeth Held, Hiroyuki Mochizuki

North Carolina State University, Raleigh, NC, USA

Background:

Basophilia is an uncommon hematologic finding. There are limited studies that have evaluated concurrent hematologic abnormalities and diseases associated with basophilia in dogs.

Objective:

Describe the hematologic and clinical characteristics of dogs with moderate to marked basophilia.

Materials and **Methods:**

CBC reports with blood smear examinations from dogs presented to North Carolina State University veterinary teaching hospital between 1/1/10 and 1/1/20 were retrospectively reviewed for basophilia (> 193 cells/ μL). Basophil counts ≥ 500 cells/ μL and $\geq 1,000$ cells/ μL were defined as moderate and marked basophilia, respectively. Hematologic, clinical, and select biochemical data from patients with moderate to marked basophilia were compared to those of control dogs without basophilia using Fischer's Exact Test and Wilcoxon rank-sum test.

Results:

Of 64,156 canine CBCs, 1,205 (1.9%) had a basophilia. After excluding multiple CBCs from the same patients, moderate basophilia was identified in 159 dogs and marked basophilia in 65 dogs. Common co-occurring hematologic abnormalities included leukocytosis (83%), monocytosis (66%), neutrophilia (65%), anemia (57%), and eosinophilia (37%). Diseases associated with moderate and marked basophilia included eosinophilic lung disease ($p < 0.0001$), leukemia ($p = 0.0036$), mast cell tumor ($p = 0.0157$), and inflammatory bowel disease ($p = 0.0191$). Concurrent eosinophilia, or the severity of the basophilia, was not significantly associated with specific diseases.

Conclusions:

Common conditions associated with basophilia in this large cohort of dogs were hypersensitivity and neoplasia. This study found no difference in disease association in dogs with moderate versus marked basophilia or in dogs with or without concurrent eosinophilia.

Tuesday, October 31

11:15 AM – 11:30 AM

BASOPHILIA IN DOGS, CATS AND HORSES - AN INTEGRATED CLINICAL AND HEMATOLOGIC APPROACH

Alex Mau, Stefan Keller, Amir Kol

University of California - Davis, Davis, CA, USA

Background: Basophils are the rarest blood leukocyte in most healthy domestic mammals and the clinical significance of basophilia is poorly understood.

Objective: To empirically determine magnitude thresholds for basophilia, identify hematologic correlates with basophilia and, identify associations between breeds, specific diseases, disease categories, organ systems and basophilia in dogs, cats and horses.

Methods: All complete blood counts (CBC) from the UC Davis Veterinary Medical Teaching Hospital between 2000 and 2019 were collected and CBCs with basophilia identified by manual differential counts. Magnitude thresholds were determined by evaluating the distribution of basophil counts. For severe cases of basophilia, the concurrent clinical diagnoses were recorded with each being categorized according to

the organ system affected (e.g., integumentary) and the underlying pathomechanism (e.g., neoplastic). Basophilia groups were compared to a time-matched, randomly selected control group and chi-square analyses were performed to evaluate associations with specific diseases, disease groups and organ systems affected.

Results: A total of 143,841, 32,576 and 44,887 CBCs were collected from dogs, cats and horses respectively. Descriptive statistics were determined and breed predispositions identified. Correlation between basophil concentration and 19 other CBC parameters were evaluated. Basophilia was associated with respiratory disease in dogs and cats. Basophilia was associated with skin disease and hypersensitivity in dogs. Basophilia was associated with mast cell neoplasia in dogs and lymphoma in both dogs and horses.

Conclusion: Our study provides an evidence-based approach for clinical interpretation of basophilia in these 3 major veterinary species.

Tuesday, October 31

11:30 AM – 11:45 AM

PERIPHERAL BLOOD EOSINOPHILIA IN DOGS AND IN CATS: IS IT ASSOCIATED WITH ENDOPARASITES?

Maria Chiara Sabetti¹, Serena Crosara¹, Laura Helen Kramer¹, Andrea Corsini¹, Benedetto Morandi², Chiara Cattabiani¹, Linda Danieli¹, Cecilia Quintavalla¹

¹University of Parma, Parma, Italy, ²Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Tolentino, Italy

BACKGROUND

Parasite infections are commonly reported as cause of eosinophilia in dogs and cats, but prospective studies assessing the prevalence of parasitic disease in eosinophilic patients are lacking.

OBJECTIVE

To evaluate the association between endoparasite infections and blood eosinophilia in dogs and cats.

METHODS

Preliminary cross-sectional study. Twenty-eight dogs and 8 cats showing eosinophilia (eosinophil concentration $>750 \times 10^9/L$ and $>1700 \times 10^9/L$, respectively) were prospectively included between November 2022 and June 2023. Fecal flotation and Baermann technique were performed in all animals within 15 days after complete blood count and before anthelmintic treatment. Clinical diagnosis was noted in each case.

RESULTS

Two out of 28 dogs and 3/8 cats with eosinophilia were positive for endoparasites. One dog was positive for hookworms and one for ascarids and whipworms. Among cats 2/3 were positive for *Cystoisospora* spp. and 1/3 for *Cystoisospora* spp. and hookworm spp.

The clinical diagnoses of the dogs with eosinophilia were gastrointestinal disorders (6/28), neoplasia (5/28), infection-inflammatory disease (one positive for ascarids and whipworms) (3/28), renal disease (2/28), megaesophagus (1/28), respiratory disease (positive for hookworms) (1/28), immune-mediated hemolytic anemia (1/28), cardiac disease (1/28). Among cats, the clinical diagnoses were renal disease (3/8), endocrine disorder (1/8), respiratory disease (1/8), infection-inflammatory disease (1/8). 8/28 dogs and 3/8 cats (including all three positives for endoparasites) were assessed during routine health checks.

CONCLUSIONS

Endoparasite infections were uncommon in dogs with eosinophilia. While parasitic infections were described in nearly half of eosinophilic cats despite, they showed no clinical signs.

Tuesday, October 31

11:45 AM – 12:00 PM

CYTOLOGIC FINDINGS, DIAGNOSTIC YIELD, AND ALKALINE PHOSPHATASE STAINING OF CANINE PULMONARY CYTOLOGY (84 CASES)

Sarah Bosch¹, Christopher Lanier¹, Leslie Sharkey²

¹University of Florida, Gainesville, FL, USA, ²Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA, USA

Pulmonary lesions in dogs can represent inflammatory or neoplastic disease; prognosis can vary greatly. Canine bronchoalveolar carcinomas have been shown to exhibit alkaline phosphatase (ALP) stain uptake, and in humans, ALP is sensitive for the detection of primary pulmonary adenocarcinoma in neoplastic effusions. The diagnostic value of pulmonary cytology samples in dogs was assessed using histopathology as a reference standard. ALP staining was performed to characterize expression in the range of samples included in the study. Medical records review identified canine pulmonary cytology with concurrent pulmonary or primary (non-pulmonary) neoplastic histopathology between January 2010 and July 2022. Samples were categorized as adequate or inadequate for interpretation to calculate diagnostic yield and were reviewed for standardized cytologic interpretation. Sensitivity, specificity, and predictive values of neoplastic and non-neoplastic conditions were calculated. ALP staining (KPL BCIP/NBT 1- Component Phosphatase Substrate) was performed. Diagnostic yield was 84%. Cytology was most sensitive for detection of inflammation (96.36%) and neoplasia (87.5%). Cytology had the highest positive predictive value (PPV) for neoplasia (96.55%) followed by necrosis (90.63%), inflammation (89.83%), and hemorrhage (77.78%). Pulmonary carcinoma, osteosarcoma, and eosinophils stained positive for ALP. Hemangiosarcoma, histiocytic sarcoma, melanoma, macrophages, and mesothelial cells stained negative. Clinicians can have a high degree of confidence when a cytologic diagnosis of neoplasia and inflammation is given. Cytology is less

reliable for excluding the potential for neoplasia. ALP may be helpful to aid in the identification of pulmonary epithelial cells; however, the presence of ALP positive epithelial cells does not confirm pulmonary carcinoma is present.

1: EFFICACY OF URINALYSIS TUBES CONTAINING PRESERVATIVES AT ROOM TEMPERATURE VERSUS REFRIGERATED PLAIN GLASS TUBES FOR THE EVALUATION OF CANINE AND FELINE ABNORMAL URINE

Charles Lemieux, Bérénice Conversy, Tristan Juette, Carolyn Gara-Boivin
Faculty of Veterinary Medicine, University of Montreal, Saint-Hyacinthe, QC, Canada

Background: Refrigeration is currently considered the best urine preservation method in veterinary medicine, but several in vitro modifications, notably crystal formation, can occur over time. Preservative-containing tubes (PCT) are widely used for human urine preservation, but little information exists about their efficacy in veterinary medicine.

Objective: To compare the efficacy of chlorhexidine-based PCT at room temperature and refrigerated plain glass tube (PGT) for the evaluation of canine and feline abnormal urine.

Methods: A total of forty urine samples from sick dogs and cats presented to a veterinary hospital were collected and stored in PCT at room temperature and refrigerated PGT. A complete urinalysis including a macroscopic and sediment examination, chemical strip and urinary protein-to-creatinine ratio (UPCR) was performed at 0, 24 and 72 hours after urine collection for both methods. Results were compared with immediate post-collection urine sample without additives.

Results: PCT immediately increased urine specific gravity and UPCR causing 19% proteinuria categorization changes. PCT decreased bacteria over time causing up to 29% false-negatives at 72h. Over time, PCT and PGT increased crystals formation causing 16% and 25% false-positives and decreased canine leucocyte counts causing 50% and 40% false-negatives at 72h, respectively. Physical properties and most other biochemical and microscopic variables, including casts, were not significantly changed up to 72h with both methods.

Conclusions: PCT at room temperature do not significantly improve canine and feline urine preservation compared to refrigerated PGT. Despite refrigeration's limitations in urine preservation, this method is still considered the best until other solutions can be found.

2: ASSESSING THE IMPACT OF TRAINING ON KNOWLEDGE RETENTION AND CONFIDENCE LEVELS IN VETERINARY CLINICAL PATHOLOGY LABORATORY: A PILOT STUDY

Adriana Furtado, Mara Varvil
Washington State University, Pullman, WA, USA

Background: Understanding the effectiveness of training interventions can help improve educational strategies and enhance the learning experience for professionals in veterinary environment.

Objective: This pilot study aimed to assess the impact of training on knowledge retention and confidence levels.

Methods: The experimental group consisted of undergraduate students (n=3) and technicians (n=2) with no prior experience in a veterinary clinical pathology laboratory, while the control group was composed of trained laboratory personnel. Quizzes consisting of multiple-choice, short answer, and case-based questions were administered before training, after one month, and after two months, covering hematology, chemistry, transfusion medicine, urinalysis, and case-solving.

Results: Results revealed significant improvements in both confidence levels and performance throughout the training period. The confidence levels are measured on a five-point scale, ranging from 1 (low confidence) to 5 (high confidence). Initially, 40% (n=2) of participants chose level 1, 40% (n=2) level 2, and 20% (n=1) level 3. After one-month, level 1 decreased to 20% (n=1), while level 3 increased to 40% (n=2). By the end of two months 60% (n=3) of participants displayed level 4 confidence and 20% (n=1) reported even higher confidence (level 5). Performance scores increased from 64% pre-training to 68% after one month and to 91% after two months, with notable advancements in chemistry, transfusion medicine and case-solving abilities. This pilot study highlights a positive correlation between self-confidence and performance in the clinical laboratory skills.

Conclusions: Our findings indicate that targeted training interventions can effectively enhance knowledge retention and confidence levels.

3: QUALITY PERFORMANCE OF CYTOLOGICAL EXAMINATION IN AN ACADEMIC VETERINARY CLINICAL PATHOLOGY LABORATORY IN SOUTH AFRICA

Nabeela Tar¹, Emma Hooijberg²

¹Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa,

²Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa

Background: Measures of agreement and diagnostic accuracy between cytology and histopathology are important quality metrics in cytology. Quality goals for the cytologic diagnosis of neoplasia in human medicine are: absolute sensitivity >60%, complete sensitivity >80% and specificity >55%.

Objective: To determine the quality performance of cytology for the identification of neoplasia in the Onderstepoort Veterinary Academic Hospital Clinical Pathology Laboratory, South Africa.

Methods: This retrospective study used corresponding cytology and histopathology reports from canine patients, from 2018-2021. Results for each modality were categorized as non-diagnostic (C1), non-neoplastic (C2), atypical (C3), neoplastic with tissue type unknown (C4) or neoplastic with tissue type known (C5 epithelial, mesenchymal or round cell). Agreement (κ) between the two modalities and measures

of diagnostic accuracy (absolute and complete sensitivity, specificity, predictive values (PV), accuracy) were calculated.

Results: 172 cases were included; 22 were in the C1 category and not further analyzed. Agreement for the diagnosis of neoplasia (C4+C5) was good ($\kappa=0.61$); complete sensitivity was 84.5%, specificity 84.3%, positive PV 89.5%, negative PV 76.8% and accuracy 84.1%. Absolute sensitivity was: C5 epithelial 74.4%, C5 mesenchymal 70.8%, C5 round cell 81.8%. Diagnostic categories were the same in 79.7% of cases from cutaneous and subcutaneous lesions; the highest percentage of discordant cases were from spleen (56.2%) and lung (75%).

Conclusion: Measures exceeded quality goals, and predictive values were high. These results are similar to those reported in other veterinary cytology studies. Regular monitoring of these metrics should form part of a cytology quality assurance program.

4: SUSPECTED PHENOBARBITAL-INDUCED SYSTEMIC LUPUS ERYTHEMATOSUS IN A STANDARD POODLE

Emma Stacey, Matthew Kornya, Erin Phillips, Felipe Reggeti, Dorothee Bienzle
Ontario Veterinary College, Guelph, ON, Canada

A 3-year-old female spayed standard poodle was presented for investigation of seizures. Treatment with oral phenobarbital (60mg q12h) was initiated. One month later, she was represented for hyporexia and lethargy. A CBC revealed a marked neutropenia ($0.99 \times 10^9/L$; reference interval [RI] $2.9-10.6 \times 10^9/L$) and thrombocytopenia ($36 \times 10^9/L$; RI $117-418 \times 10^9/L$). A biochemical profile showed a moderate azotemia with increased urea (32.5 mmol/L ; RI $3.5-9.0 \text{ mmol/L}$) and creatinine (203 mmol/L ; RI $20-150 \text{ mmol/L}$) concentrations, consistent with acute kidney injury, and proteinuria (UPCR 11.4, RI <0.5). Evaluation of a bone marrow aspirate disclosed a markedly cellular sample. A 500-cell differential count revealed a G:E ratio of 3.9, and 3.8% myeloblasts. The presumptive diagnosis was phenobarbital-induced myelotoxicity. Treatment with phenobarbital was discontinued. Four days later, the neutrophil count had decreased further ($0.24 \times 10^9/L$). A second marrow aspirate was also highly cellular, and a differential count enumerated a G:E of 1.6, and 44% myeloblasts. Myeloid leukemia was considered a differential diagnosis, but since recovery from myelotoxicity may take months, the initial presumptive diagnosis was retained. An anti-nuclear antibody testing had a positive result at a 1:640 dilution; therefore, drug-induced systemic lupus erythematosus (SLE) was prioritized. A CBC repeated one month after detection of neutropenia revealed an adequate number of neutrophils ($3.65 \times 10^9/L$). A third marrow aspirate 79 days after initial assessment yielded a G:E of 1.54, and 1.1% myeloblasts, consistent with the resolution of neutropenia. ANA normalized at this time. In humans, SLE has been reported as a rare sequel of phenobarbital treatment, while this is the first reported instance in a dog.

5: 8 YEARS OF EXPERIENCE WITH CLONALITY TESTING IN FELINE LYMPHOMA

Barbara Rütgen, Nicole Luckschander-Zeller, Birgitt Wolfesberger, Daniel Baumgartner, Sandra Groiss, Ilse Schwendenwein, Sabine Hammer
University of Veterinary Medicine Vienna, Vienna, Austria

Background. Clonality testing is a useful complementary tool in lymphoma diagnostics when morphologic techniques fail to discriminate between neoplastic and reactive lymphocyte populations due to either morphologic inconsistencies or preparatory cell destruction.

Objective. This retrospective study summarizes our experiences in a clinical setting.

Methods. In eight years, 933 cytologic samples of feline lymphoid tissue were submitted for clonality testing. All slides were re-evaluated by microscopy and images were taken for documentation.

Results. Out of 933 cytology samples, 532 (57%) showed inconclusive morphologic results. Among the remaining 401 (43%) cases, 221 (55.1%) featured morphologically clear-cut lymphoma, 105 reactive hyperplasia (26.2%), and 13 exhibited no assignable cell lineage (3.2%). 62 (15.5%) were non-diagnostic due to destroyed cells, 16.3% of cases morphologically consistent with lymphoma, showed a false negative result. Within the 105 cases of reactive hyperplasia 7.6% showed a false positive PCR-result. Within the 532 morphologically inconclusive samples, 196 (36.8%) showed a polyclonal result, 106 (19.9%) cases were clonal for T-cell receptor gamma , 43 (8.1%) clonal for immunoglobulin heavy chain, and 7 (1.3%) showed clonality for both. 165 cases (31.1%) were non-diagnostic due to low DNA concentration or poor DNA-quality and 15 (2.8%) were inconclusive, showing no evaluable traces.

Conclusions. Fifty-seven percent of overall submissions were indicated because no morphologic diagnosis could be established. Among morphologically consistent lymphoma 16.3% showed false negative PCR results. Morphological reactive hyperplasia showed 7.6% false positive results. The percentage of 33.9 % of technically invalid results was high probably due to intraabdominal sampling sites.

6: ALMOST A DECADE OF EXPERIENCES WITH CLONALITY TESTING IN CANINE LYMPHOMA

Barbara Rütgen¹, Nicole Luckschander-Zeller¹, Miriam Kleiter¹, Ondrej Skor², Daniel Baumgartner¹, Sandra Groiss¹, Ilse Schwendenwein¹, Sabine Hammer¹

¹University of Veterinary Medicine Vienna, Vienna, Austria, ²Laboklin, Bad Kissingen, Germany

Background

Clonality testing is a complementary tool in lymphoma diagnostics when morphologic techniques fail to discriminate between neoplastic and reactive lymphocyte populations due to either morphologic inconsistencies or preparatory cell destruction.

Objective

Retrospective study summarizing 9 years of experience.

Methods

1627 cytologic samples of canine lymphoid tissue were analyzed starting 2014. All

slides were re-evaluated by microscopy and images were taken for documentation.

Results

717/1627 (44%) showed inconclusive morphologic results. Among the remaining 910 (56%) cases, 752 (83%) featured unequivocally lymphoma, 76 (8%) reactive hyperplasia, in 82 (9%) samples cells were damaged. A false negative PCR result was yielded in 12.5% of morphologically consistent lymphoma. A false positive result was obtained in 18% of reactive hyperplasia. Among 717 morphologically inconclusive samples 250 (34.9%) cases were clonal for T-cell receptor gamma (TRG). 124 (17.3%) were clonal for immune globulin heavy-chain (IGH) and 25 (3.5 %) showed clonality for both. 163 (22.7%), were polyclonal and 4 (0.6%) were pseudoclonal. 124 (17.3%) were non-diagnostic due either to low DNA concentration or poor quality, in 27 (3.7%) cases traces could not be unambiguously interpreted.

Conclusions

In 50.9% of all submissions the test was not indicated as either an unequivocal morphologic diagnosis of lymphoma or reactive hyperplasia was obtainable. The false negative rate for lymphoma was 12.5%, whereas reactive lymphadenopathy yielded a false positive rate of 18%. Among morphologically inconclusive lymphoma cases positivity for TRG (34.9%) was more frequently observed than IGH (17.3%). 17.2% of assays were non-diagnostic due to low DNA yield/quality.

7: MICRORNA PROFILES DETERMINED FROM LYMPH NODE CYTOLOGY SLIDES TO IMMUNOPHENOTYPE AND PROGNOSTICATE CANINE MULTICENTRIC LYMPHOMA

Dante Meza, Geoffrey Wood, Dorothee Bienzle, Anthony Mustsaers, Paul Woods, Darren Wood

Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

Lymphoma is a heterogeneous disease with highly variable prognosis that represents one of the most common neoplasms in dogs, with the multicentric type being the most frequently diagnosed. There are multiple subtypes of lymphoma which makes it difficult to predict which patients will respond well to treatment. MicroRNAs (miRNAs) are short molecules of non-coding RNA with an important role in multiple biological functions. They are implicated in the development of lymphoma and their expression is often correlated with tumor immunophenotype, response to treatment, and overall survival. The goal of this study is to characterize the miRNA profile from lymph node cytology samples collected during the diagnostic workup, and to use this miRNA profile to classify the subtype of lymphoma and to predict treatment response and prognosis. Cytology samples were used from dogs with a diagnosis of untreated multicentric lymphoma that went on to receive the standard CHOP chemotherapy protocol, with documented immunophenotype by flow cytometry, and no concurrent disease. Quantification was performed using real-time RT-qPCR and customized miRNA PCR arrays assessing 27 canine miRNAs previously determined to be important in canine

lymphoma. Our lab has identified a group of miRNAs that are differentially expressed depending on the lymphoma immunophenotype; miR-130b-3p, miR-181a, b, c and miR-182-5p in association with T-cell lymphoma, and miR-18a, miR-19a, b, miR25-5p, miR-29b-3p, miR-34a-5p and miR-99a-5p in association with B-cell lymphoma. Kaplan-Meier survival curves revealed that miR-125a and miR-146a in B-cell lymphoma, and miR-181b in T-cell lymphoma were correlated with progression-free survival and overall survival.

8: MICRORNA EXPRESSION IN SYNOVIAL FLUID OF DOGS AS A PREDICTIVE VARIABLE OF STIFLE OSTEOARTHRITIS

Kerstin Muner, Samuel Clark, Jasmine Aggarwal, Sarah Malek, Andrea Pires dos Santos

Purdue University, Lafayette, IN, USA

Background: Osteoarthritis (OA) is an inflammatory disease affecting up to 20% of the adult dog population in the USA, usually occurring after the cranial cruciate ligament rupture (CrCLR). The role of microRNAs in OA has been extensively studied. MicroRNAs regulate gene expression in physiological and pathological processes, making them promising biomarkers and therapeutic targets. **Objective:** This study investigated the use of microRNAs as biomarkers of canine OA by performing small RNA sequencing of synovial fluid of Controls (N=5) versus dogs with unilateral CrCLR tears whose contralateral stifles remained Intact (N=5) or developed CrCLR (N=5) over time. **Methods:** Total RNA was converted into microRNA libraries that were pooled and sequenced (Illumina Inc). Analyses were done using CLC Genomics Server 21.0.4. and reads mapped to miRBase. **Results:** Three microRNAs were differentially expressed (DE) comparing Control with Intact samples using the Bonferroni corrected p-value (CI: 99%; FDR < 0.01), where miR-451 and miR-486 were upregulated and miR-184 downregulated in the Intact group compared to Controls. Significant DE was not observed between Intact versus CrCLR nor CrCLR versus Control groups. One-way ANOVA, followed by the Bonferroni corrected p-value (CI: 95%; FDR < 0.05), also revealed that miR-184 was downregulated and miR-339 was upregulated due to OA. **Conclusion:** The functions of these microRNAs in OA need further investigation, especially miR-339, which has not been associated with OA. Based on sRNA-Seq, the DE microRNAs suggest they may be used as diagnostic biomarkers for OA; however, we could not predict OA disease progression in contralateral stable stifles.

9: APPLICATION OF A SANDELL-KOLTHOFF REACTION-BASED TECHNIQUE TO MEASURE URINARY IODINE OF DAIRY CATTLE: A PILOT STUDY

Laura Quishpe Contreras¹, Jerbeson Hoffmann², Carlos Bondan², Tania Weber Furlanetto¹, Félix Díaz González¹, Stella de Faria Valle¹

¹Federal University of Rio Grande do Sul, Porto Alegre, Brazil, ²University of Passo Fundo, Passo Fundo, Brazil

Background: Limited information is available regarding the iodine status of cattle in Brazil. Sandell-Kolthoff reaction-based techniques are an alternative to more expensive methods.

Objective: To apply a Sandell-Kolthoff reaction-based technique to assess the urinary iodine (UI) concentration in dairy cattle samples.

Methods: The UI was measured using the Sandell-Kolthoff colorimetric method, based on the reduction of ammonium cerium sulphate (yellow), using ammonium persulfate as the oxidizing agent. The accuracy of the technique using two different oxidation times (30 and 60 minutes) was assessed by performing a recovery test, where varying volumes of a solution with a known iodine concentration were added to urine samples from cows. A human sample with a known iodine concentration was used as a control. The intra and inter-assay coefficients of variation (CV) were evaluated using three samples, with low (s1), medium (s2) and high (s3) concentrations. For the intra-assay CV, 10 measurements were performed using s1 and s2 and 7 using s3. For the inter-assay CV, 3 measurements were carried out for s1 and s2, while having 4 measurements for s3.

Results: At the recovery test, better results were obtained using 60 minutes, with a recovery rate ranging from 101.96% and 106.14%. The intra-assay CVs for s1, s2 and s3 were 7.5, 6.4 and 4.1%. For the inter-assay test, CV of 4.3%, 1.2% and 2.3% were obtained, respectively.

Conclusion: This pilot study demonstrates the potential applicability of an affordable technique to evaluate the urinary iodine levels in dairy cattle samples.

10: ISOLATION OF BOVINE CD34+ BONE MARROW STEM AND PROGENITOR CELLS USING A MONOCLONAL ANTIBODY AGAINST CD34 PROTEIN

Quentin Leroy¹, Delphine Le Roux^{1,2}, Clotilde Rouxel², Anne-Claire Lagree^{1,2}, Nadia Haddad², Lisa Le Dortz², Mathias Brunet-Manquat^{3,4}, Laurence Guyonneau-Harmand^{3,4}, Yves Millemann⁵, Bérangère Ravary⁵, Pierre Deshuillers^{1,2}

¹Ecole Nationale Vétérinaire d'Alfort, Biopôle, Maisons-Alfort, F-94700, France, ²Anses, INRAE, Ecole Nationale Vétérinaire d'Alfort, Laboratoire de Santé Animale, UMR BIPAR, Maisons-Alfort, F-94700, France, ³Sorbonne Université, UMR_S938 CDR Saint-Antoine, Prolifération et Différenciation des Cellules Souches, F-75012 Paris, France, ⁴EFS Ile-de-France, Unité d'Ingénierie et de Thérapie Cellulaire, Créteil, F-94017, France, ⁵Ecole Nationale Vétérinaire d'Alfort, CHUVA - Animaux de Production, Maisons-Alfort, F-94700, France

Background: There is currently no commercially available bovine anti-CD34 monoclonal antibody. Bovine Hematopoietic Stem and Progenitor Cells (BHSPC) isolation *in vitro* have had mixed results.

Objective: To identify an anti-CD34 antibody recognizing BHSPC and isolate them from bovine bone marrow.

Methods: CD34 protein sequences from multiple species were compared *in silico* to the bovine sequence (identity, Bit score and E-value). A suitable antibody was selected and coupled to phycoerythrin (PE). Bone marrow samples from 7 Prim'Holstein calves were collected in EDTA and mononuclear cells were isolated using a density gradient.

Antibody binding to BHSPC was monitored by flow cytometry. Labeled BHSPC were separated with anti-PE magnetic beads. Enrichment was evaluated by flow cytometry.

Results and discussion: A monoclonal antibody against ovine CD34 was selected based on the high homology (Identity = 84.9 %, Bit Score = 1574, E value = 0) between bovine and ovine CD34. BHSPC accounted for 4.4 to 27.6 % of bone marrow mononuclear cells. Variation in BHSPC proportion may relate to an individual variation in the amount of CD34⁺ cells in the marrow; however, a polymorphism in the coding region of the protein is possible, as previously described. Using magnetic beads, we increased CD34⁺ cells concentration by a 1.96-fold, but with a weak recovery rate of 1.54 %.

Conclusions: This experiment describes a new potential commercial anti-CD34 monoclonal antibody for isolation or identification of BHSPC. Although the enrichment may appear low, the increase in cell purity is sufficient for culture and immortalization of these cells.

11: B CHRONIC LYMPHOCYTIC LEUKEMIA IN AN ADULT CAT

James Andre Mori¹, Emma Stacey¹, Yelda El Helou², Dorothee Bienzle¹

¹Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, ²Complexe Animalier Vétérinaire Accès Vet Blainville, Blainville, QC, Canada

Chronic lymphocytic leukemia (CLL) is an uncommon neoplasm in cats that is usually due to CD4⁺ T lymphocytosis (T-CLL). Affected cats are typically older, and disease progression is slow. Other immunophenotypes of CLL in cats are rarely reported. Here, we describe a case of feline B-CLL.

The patient is an 8.8-year-old male neutered Persian cat that presented for routine annual examination. Mild mucous membrane pallor, hyporexia, lethargy, and weight loss were noted. A CBC showed an extreme leukocytosis (200.5 x10⁹/L, reference interval [RI]: 3.9-19.0x10⁹/L) composed almost exclusively of lymphocytes, and a microcytic (MCV 32.4 fL, RI: 39.0-56.0 fL) regenerative (reticulocytes 54.0x10³/μL, RI: 3.0-50.0x10³/μL) anemia (HCT 0.22 L/L, RI: 0.29-0.45 L/L). Splenomegaly and enlargement of multiple lymph nodes were evident on ultrasound. Blood film review showed mostly small and fewer large lymphocytes with scant pale basophilic cytoplasm and round or convoluted nuclei with coarse to finely clumped chromatin. Aspirates of the right mandibular lymph node showed lymphocytes with similar morphology. Flow cytometric analysis of blood lymphocytes identified exclusively CD18, CD21 and MHC II positive small lymphocytes, consistent with B-CLL. A second CBC 10 days later had similar findings. Serum biochemistry and urinalysis showed mild hyperglobulinemia (48 g/L, RI: 23-42 g/L) and 2+ proteinuria, respectively. Serum iron was low (10 μmol/L, RI: 11-27 μmol/L), and serum electrophoresis identified polyclonal gamma globulins, including a possible restricted peak. The cat is clinically well. Feline B-CLL appears to be rare, and clinical features in this case may differ from those of canine B-CLL.

12: HEMOPARASITE LOADS IN ANURANS EXPOSED TO PESTICIDE-CONTAMINATED ENVIRONMENTS

Lina Crespo Bilhalva¹, Laura Sander Peres¹, João Fabio Soares¹, Andrea Pires dos Santos², Stella de Faria Valle¹

¹Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, ²Purdue University, Lafayette, IN, USA

Background: Over a third of amphibians experienced population reductions or extinction due to various causes, including environmental contaminants and infectious diseases. Understanding these factors is crucial for implementing effective conservation measures to mitigate their effects on native species. Since the proportion of parasitized erythrocytes can influence the prognosis of certain hemoparasitic infections, it could serve as a valuable indicator of disease in these animals.

Objective: This study aimed to assess whether amphibians inhabiting pesticide-contaminated environments exhibit higher parasitemia than pesticide-free environments.

Methods: Blood samples were collected from 39 *Leptodactylus luctator* (Anura: Leptodactylidae) from conventional (n=24) and organic (n=15) rice crops in Southern Brazil. Fresh-whole blood smears were stained with Wright-Giemsa and examined by microscopy. Parasitemia was determined by counting the number of parasitized erythrocytes by protozoa per 2000 RBCs. Shapiro-Wilk test was used to assess the normal distribution, and a non-parametric Mann-Whitney U-test was performed to compare parasitemia between groups ($p < 0.05$).

Results: In the conventional rice crop, the range of parasitized RBCs was 0 to 100, with a median of 12. In contrast, in the organic rice crop, the range of parasitized RBCs was 0 to 19, with a median of 3. Normality was not supported, and significant differences between groups were not observed ($p = 0.06$).

Conclusion: Although with no statistical difference, our findings show higher hemoparasite loads in anurans inhabiting pesticide-contaminated environments, suggesting the potential negative impact of pesticides on amphibians' health. In subsequent stages of this study, molecular techniques should be applied to stratify the parasite species.

13: BILIARY CANDIDIASIS DUE TO CANDIDA PARAPSILOSIS IN A CAT

Suzanne Bussey, Marina Domingues, Nicholas Goody, Jennifer Harris, Glynn Woods
University of Edinburgh, Edinburgh, United Kingdom

A 6-year-old neutered male domestic shorthaired (DSH) cat was referred to the Hospital for Small Animals at the University of Edinburgh for chronic intermittent vomiting, inappetence, lethargy and abdominal pain of two-years-duration. On presentation the cat was bright with no palpable abdominal abnormalities, although there was mild dehydration. Biochemical and ultrasonographic (U/S) findings were consistent with cholangitis, cholelithiasis, hepatopathy and pancreatitis. There was no evidence of biliary tract obstruction. Additionally, there were duodenal mucosal and subtle jejunal muscularis changes seen on U/S. Cholecystocentesis and liver aspiration were performed and submitted for cytology. In the bile, small ($2 \times 3\text{-}5\mu\text{m}$), dark blue, oval yeast with a thin clear halo were found, admixed with green amorphous material,

bilirubin crystals and rare epithelial cells. Mild neutrophilic inflammation and mild vacuolar change was seen in the liver. Fungal culture of the bile revealed *Candida parapsilosis*. The cat improved on empirical antibiotics as well as symptomatic therapy for presumed triaditis and was then subsequently lost to follow up. As therapy did not include antifungals, the clinical significance of the yeast in the bile remains uncertain. To the author's knowledge, this is the first case of *Candida parapsilosis* reported in bile fluid cytology from a feline.

14: RAT BONE MARROW HISTOLOGY: A DEEP LEARNING APPROACH FOR AUTOMATED BONE MARROW DIFFERENTIAL ANALYSIS IN HEMATOLOGIC TOXICITY ASSESSMENT

Michael Logan, Hossein Foroushani, Samantha Wildeboer, Amanda Krempley, Magali Guffroy, Joshua Decker, Marie Bockenstedt, Bhupinder Bawa
AbbVie Inc., North Chicago, IL, USA

Bone marrow evaluation is integral to assessing hematologic toxicity of candidate therapeutics in non-clinical studies. However, microscopic bone marrow examination beyond overall cellularity and M:E estimate is time-consuming and subject to inter-observer variability. We developed a deep learning workflow to automate differential counts using H&E-stained bone marrow histology sections from rodent toxicity studies. Starting with a bone marrow whole slide image, our workflow rapidly and automatically detected tissue, identified bone marrow region, and classified hematopoietic cells into megakaryocyte, erythroid proliferative, erythroid maturing, myeloid proliferative, and myeloid maturing categories. Active learning and data augmentation were used to develop a robust workflow with minimal annotation, and hematopoietic cells were summarized in a table with statistics including number, percentage ratio, and density of each cell. We tested the workflow on five rat toxicology studies with test items known to induce myeloid hyperplasia, megakaryocytic hyperplasia, or multi-lineage hypoplasia. Sensitivity, precision, and F1 score were calculated for each cell category according to pathologist generated annotations. The workflow achieved high accuracy in tissue detection, cell segmentation, and cell classification (0.92 average sensitivity, 0.95 average precision, and 0.93 average F1-score). The concordance between workflow output, pathologists' qualitative bone marrow evaluation, and circulating blood cell counts was also high. The proposed automated bone marrow differential workflow is a promising interpretive aid which may enhance efficiency and accuracy of bone marrow assessment, reduce variability, and provide additional interpretive context for assessing hematologic toxicity. Further studies are needed to evaluate its utility and optimize the workflow for different sample types.

15: HEMOTROPIC MYCOPLASMA (HEMOPLASMAS) IN SYNANTHROPIC RATS (RATTUS NORVEGICUS) FROM A LOW-INCOME NEIGHBORHOOD OF SOUTHERN BRAZIL

Alexander Biondo^{1,2}, Maysa Pellizzaro^{2,3}, Louise Kmetiuk¹, Nelly Elshafie¹, Andrea Pires dos Santos¹

¹Purdue University, West Lafayette, IN, USA, ²Federal University of Bahia, Salvador, Brazil, ³Federal University of Paraná, Curitiba, Brazil

Background: Hemotropic mycoplasmas (hemoplasmas) are small pleomorphic bacteria that attach to erythrocytes and cause anemia in susceptible hosts. Infection by *Mycoplasma haemomuris* was already reported by our research group in free-range and laboratory *Rattus* spp. in Curitiba city, Southern Brazil, with higher positivity in free-range rats from an urban park and zoo. **Objectives:** The present study assessed hemoplasma infection in synanthropic rats *Rattus norvegicus* from a low-income neighborhood of Curitiba City. **Methods:** DNA was extracted from 50 blood samples and screened with a universal hemoplasma qPCR assay followed by conventional PCR and sequenced (WideSeq/Purdue). **Results:** A total of 30/50 (60.0%) was positive for hemoplasmas by qPCR ($C_t < 34.4$). One sample underwent conventional PCR targeting the 16S ribosomal RNA, and was sequenced, revealing the gene was closely related to the feline hemoplasma, *Mycoplasma turicensis* (94.7% identity). **Conclusions:** there is a high prevalence of hemoplasma infection in rats, which suggests hemoplasmas are likely endemic among rats in the studied area, corroborating other studies. Moreover, we report *M. turicensis* infection in a rat, which is rare and needs further investigation. Further studies are needed to understand transmission between rats or potential interspecies transmission between cats and rodents.

16: HEMOTROPIC MYCOPLASMAS (HEMOPLASMAS) IN FREE-RANGING AGOUTIS (*DASYPROCTA AZARAE*) FROM AN URBAN AREA OF SOUTHERN BRAZIL

Alexander Biondo^{1,2}, Andrea Pires dos Santos², Amanda Haisi³, Francisco Conrado⁴, Joao Pessoa Araujo Junior³, Leila Ullmann³, Louise Kmetiuk², Patricia Weckerlin⁵, Nelly Elshafie², Maysa Pellizzaro^{1,6}

¹Federal University of Bahia, Salvador, Brazil, ²Purdue University, West Lafayette, IN, USA, ³São Paulo State University, Botucatu, Brazil, ⁴Tufts University, North Grafton, MA, USA, ⁵City Secretary of Environment, Curitiba, Brazil, ⁶Federal University of Paraná, Curitiba, Brazil

Background: Hemotropic mycoplasmas (hemoplasmas) are small, pleomorphic, uncultivable bacteria that attach to the surface of erythrocytes of various mammalian species, potentially causing anemia. Rodentia has been considered the most abundant and diverse mammal order worldwide, recognized as essential hosts for several bacterial, viral, and parasitic zoonotic pathogens. Agoutis (*Dasyprocta* spp.) have been classified as wild rodents, reportedly endemic in Central and South America, with 11 species and 29 subspecies described to date. Hemoplasma infection has yet to be surveyed in native agoutis (*Dasyprocta azarae*). Accordingly, the present study aimed to assess hemoplasmas infection in free-ranging agoutis from an urban environmental conservation area in Curitiba, Southern Brazil. **Methods:** Blood samples were collected by venipuncture in EDTA-anticoagulated tubes, following bait trapping and physical restraint. DNA was extracted using commercial kits. Quantitative qPCR using pan-hemoplasma primers was used for screening. Positive samples were subjected to conventional PCR for the 16S RNA gene and sequenced. **Results:** Overall, 11/35 (31.43%) of the agoutis were positive for hemoplasmas by qPCR ($C_t \leq 34.4$). Sequencing indicated the presence of *Mycoplasma haemomuris* infection, closely related to *Mycoplasma haemomuris* subsp. *Ratti*. **Conclusions:** The present study was the first hemoplasma survey and detection in agoutis worldwide. The main transmission

route of *M. haemomuris* in free-ranging agoutis needs further investigation, but the concurrent presence of this species in urban rats raises concern for interspecies transmission. Moreover, further studies must be conducted to establish whether the hemoplasma infection threatens the agoutis' health.

Diagnostic Pathology

Sunday, October 29 | 1:35 PM – 1:45 PM

Session Chair: Arnaud Van Wettere

Sunday, October 29

1:35 PM – 1:45 PM

ACUTE INHALATION TOXICITY IN NINE WHITE IBIS (*EUDOCIMUS ALBUS*) FOLLOWING EXPOSURE TO THEATRICAL FOG CONTAINING TRIETHYLENE GLYCOL AND PROPYLENE GLYCOL

Jayne Ellis¹, Richard Fulton², Andreas Lehner², John Buchweitz²

¹University of California Davis, Davis, CA, USA, ²Michigan State University, Lansing, MI, USA

Nine wild American white ibis (*Eudocimus albus*) at a zoological institution were found deceased or were moribund and euthanized within 24 hours following exposure to glycol-containing theatrical fog. Gross necropsy revealed pulmonary congestion, edema, and hemorrhage in all nine birds. Histologically, all birds exhibited pathologic changes within the trachea and lungs indicative of acute respiratory insult. Pathologic changes of the trachea included moderate segmental to severe diffuse epithelial attenuation with loss of cilia, alternating with regions of mucous cell hyperplasia. In the lungs all birds exhibited peri-bronchial and perivascular edema and degenerative changes to the epithelium lining primary and secondary bronchi. Three birds had a longer interval between exposure and death and exhibited multifocal regions of necrosis of the bronchial epithelium, granulomatous pneumonia, and heterophilic peri-vasculitis. The two glycol compounds within the solution, propylene glycol and triethylene glycol, and their metabolites were detected in the lung and kidney tissue from these birds by gas chromatography-tandem mass spectrometry (GC-MS/MS). While exposure to aerosolized glycols has been shown to cause irritation and minor degenerative changes of the respiratory epithelium in laboratory animals and humans, there are no reports of birds exposed to aerosolized propylene glycol and triethylene glycol. This case suggests that birds, likely in part by their unique respiratory anatomy and physiology, are at risk of acute inhalation toxicity and death following acute exposure to aerosolized propylene glycol and triethylene glycol-containing fogging agents.

Sunday, October 29

1:45 PM – 1:55 PM

DOMESTIC CAT HEPADNAVIRUS PREVALENCE, LOCALISATION AND HISTOPATHOLOGICAL FEATURES IN FORMALIN-FIXED PARAFFIN-EMBEDDED LIVER TISSUE SUBMITTED IN THE USA

Min Chun Chen¹, Joao Pedro Cavares¹, Yan Ru Choi², John Cullen³, Randi Gold⁴, Jörg Steiner¹, Jonathan Lidbury¹, Julia Beatty^{2,5}

¹Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College station, TX, USA, ²Centre for Animal Health and Welfare, Jockey Club College of Veterinary Medicine and Life Sciences (JCC), Hong Kong, China, ³North Carolina State University College of Veterinary Medicine, Raleigh, NC, USA, ⁴Texas Veterinary Medical Diagnostic Laboratory, College station, TX, USA, ⁵Department of Veterinary Clinical Sciences, JCC, City University of Hong Kong, Hong Kong, China

Background: Domestic cat hepadnavirus (DCH), a hepatotropic virus related to hepatitis B virus (HBV), has been detected in cats from Australia, New Zealand, Italy, the United Kingdom, Malaysia, Thailand, Japan, Hong Kong, and the USA with a molecular prevalence ranging from 0.2-18%. The pathologic potential of DCH in cats is under investigation.

Aim: To investigate the prevalence of DCH DNA detection, localisation and histopathological features in feline formalin-fixed paraffin-embedded (FFPE) liver samples submitted for histopathology in the USA.

Materials and Methods: Archived FFPE feline liver (biopsy or necropsy) submitted to two diagnostic laboratories in Texas between 2019-2022 were studied. DNA was extracted and tested with DCH-specific conventional polymerase chain reaction (cPCR). In DCH cPCR-positive sections, *in situ* hybridization (ISH) was performed (RNAScope) and histopathology was reviewed.

Result: Four of 186 (2.2% (95% CI: 0.6–5.4) samples tested positive on cPCR. Among DCH-positive cases, histological changes included interface lymphoplasmacytic hepatitis (1/4), portal lymphoplasmacytic hepatitis (3/4), stellate cell hyperplasia and hypertrophy (2/4), individual necrotic hepatocytes (1/4), and microvesicular lipid vacuolation of centrilobular hepatocytes (1/4). Nuclear and cytoplasmic signaling on ISH (3/4) [JB1] was most prominent in periportal areas but did not colocalize with inflammatory infiltrates.

Conclusion: Our preliminary findings support previous studies demonstrating DCH infection in cats in the USA. Further investigation comparing histological features of DCH positive and negative livers is indicated.

Sunday, October 29
1:55 PM – 2:05 PM

DISSEMINATED TUBERCULOSIS IN A GROUP OF CYNOMOLGUS MACAQUES

Allison Gerras¹, Steven Bolin¹, Rinosh Mani², Dodd Sledge²

¹Michigan State University Department of Pathobiology and Diagnostic Investigation, East Lansing, MI, USA, ²Michigan State University Veterinary Diagnostic Laboratory, Lansing, MI, USA

Federal regulation requires all imported non-human primates (NHPs) undergo a 31-day quarantine with routine monitoring for *Mycobacterium sp.* infection via three negative

tuberculin skin tests (TSTs). Facilities often impose additional testing. Four wild-caught, Mauritius-origin cynomolgus macaques tested negative on CDC-mandated TSTs and on non-mandated *Mycobacterium* sp. testing including six additional TSTs, serum IFN γ release assay, two independent serum antibody tests, and thoracic radiographs over 17 months. Seven months after the most recent TST, one macaque presented with lethargy, inappetence, and rapidly worsening respiratory disease. Three others were asymptomatic, but exhibited positive TSTs. All macaques were euthanized and necropsies were performed. Gross and microscopic examination of each macaque revealed hundreds of caseogranulomas variably throughout lungs, liver, spleen, and multiple lymph nodes. Ziehl-Neelson and Fite's acid fast stains did not reveal bacterial organisms. PCR on lung and lymph node in 3 of 4 macaques yielded amplicons with identity to *M. tuberculosis* complex. Culture isolation was successful in 1 PCR positive macaque. Whole genome sequencing performed at the Michigan Department of Health and Human Services identified *Mycobacterium bovis*. Tuberculosis (TB) in NHPs is commonly caused by *M. tuberculosis* and less frequently, *M. bovis*. Cynomolgus macaques often exhibit clinical disease resembling that of latent TB in humans, hampering the sensitivity of surveillance testing. Multimodal surveillance testing for TB is considered the gold standard and was performed for these individuals. This case series highlights the unexpected development of symptomatic and asymptomatic TB in imported cynomolgus macaques in the face of extensive quarantining and rigorous screening.

Sunday, October 29

2:05 PM – 2:15 PM

**FIRST REPORT OF THE EMERGING INFECTIOUS ROSETTE AGENT
(SPHAEROTHECUM DESTRUENS) IN A NORTH AMERICAN CYPRINID FISH**

Bridgette Gunn, Al Camus, John Leary III, Mary Ard

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

The rosette agent (*Sphaerothecum destruens*) is an emerging, highly infectious, obligate intracellular mesomycetozoan parasite that sits at the animal-fungal phylogenetic boundary. It is noted to cause significant disease and mortality in North American (NA) salmonid and, more recently, European cyprinid fishes. Its increasing presence in Europe is partially attributed to the introduction of the invasive Asian topmouth gudgeon (*Pseudorasbora parva*). Tissues from an aquarium-housed warpaint shiner (*Luxilus coccogenis*), a cyprinid indigenous to the Tennessee River drainage, contained widely distributed parasites suggestive of *S. destruens*. Histopathologic features included intra- and extracellular clusters of round, eosinophilic, 2-4 μ m spores located primarily within hepatocytes and hepatic granulomas, but were also seen in biliary epithelial cells, endothelial cells, and within vascular lumens. Consistent with the reported literature, spores stained Gram, periodic acid-Schiff, and Gomori's methenamine silver positive, and acid-fast (Ziehl-Neelsen) negative. Ultrastructurally, isolated parasite spores at various developmental stages with osmiophilic and lipid cytoplasmic structures were contained within hepatocyte and macrophage cytoplasm. Molecular diagnostics using a unique probe targeting an 18S rRNA *S. destruens* gene segment is in development for *in situ* hybridization with RNAscope technology. This is the first report of a natural infection in a native NA cyprinid. Questions surrounding

pathogen origin and transmission in this case prompt concerns for exposure risks and species introductions in native NA cyprinid populations. This report highlights the intersectional role of diagnostic pathology between emerging infectious diseases and ecology.

Sunday, October 29

2:15 PM – 2:25 PM

CLADOGONIUM SP. INFECTION IN A DWARF CHERRY SHRIMP (NEOCARIDINA DAVIDI)

Naomi Falconnier, Mariano Carossino

Louisiana State University, Baton Rouge, LA, USA

Background: *Cladogonium ogishimae* is a parasitic algal epibiont of ornamental dwarf freshwater shrimp, *Neocaridina davidi*, which colonizes the pleopodal subcutis and can cause death due to debilitation, molting complications, and/or secondary infections. These native southeast Asian dwarf shrimp have become exceedingly popular in the aquaculture pet trade which has resulted in the global spread of this parasitic epibiont.

Objective: The goal of this study is to describe the histologic characteristics of a *Cladogonium* sp. infection in dwarf shrimp.

Methods: Gross evaluation was performed as well as routine and Grocott's methenamine silver histologic staining.

Results: One month following a 100% water change, one of ten captive bred 3-month-old dwarf cherry shrimp was found dead with no premonitory signs. Along the interpleopodal spaces were numerous fine light green elongated club-shaped structures. Histologically, the interpleopodal subcuticular tissue was expanded by hemolymph, infiltrating granulated hemocytes, and a branching network of infiltrating rhizoids. The rhizoids culminated into erect algal filaments composed of basal cells that multifocally perforated through the overlying cuticle and branched exteriorly with terminal maturing zoosporangia that when fully sporulated had numerous peripherally located zoospores. The rhizoids and inflammatory infiltrates surrounded the ventral nerve cord and minimally invaded the adjacent musculature. Similar rhizoids were circulating within the hemolymph throughout the cephalothorax and around the midgut with increased numbers of circulating granulated hemocytes.

Conclusions: These microscopic findings were consistent with previous descriptions of parasitic green algae infections belonging to the genus *Cladogonium*. This is the first known report of a systemic *Cladogonium* sp. infection.

Sunday, October 29

2:25 PM – 2:35 PM

DIAGNOSTIC INVESTIGATION OF MYCOPLASMA HYORHINIS AS A POTENTIAL PATHOGEN ASSOCIATED WITH NEUROLOGICAL CLINICAL SIGNS AND CENTRAL NERVOUS SYSTEM LESIONS IN PIGS

Calvin Ko¹, Maria Merodio¹, Ethan Spronk², James Lehman³, Huigang Shen¹, Ganwu Li¹, Rachel Derscheid¹, Pablo Piñeyro¹

¹Iowa State University, Ames, IA, USA, ²Swine Vet Center P.A., St. Peter, MN, USA,

³Merck Animal Health, Lenexa, KS, USA

Mycoplasma hyorhinis (*M. hyorhinis*) is an upper respiratory tract commensal in swine with a typical clinical presentation of arthritis and/or polyserositis in postweaning pigs. However, it has also been associated with conjunctivitis and otitis media, and recently has been isolated from meningeal swabs and/or cerebrospinal fluid of piglets with neurological signs. The objective of this study is to evaluate the role of *M. hyorhinis* as a potential pathogen associated with neurological signs and central nervous system lesions in pigs. The presence of *M. hyorhinis* was evaluated in an outbreak of neurologic disease and a six-year retrospective study by PCR detection, bacteriological culture, *in situ* hybridization (RNAscope®), phylogenetic analysis, and immunohistochemistry characterization of the inflammatory response associated with lesions. *M. hyorhinis* was confirmed by culture isolation and within CNS lesions by RNAscope®. The isolates from the brain had close genetic similarities to those previously reported and isolated from eye, lung, or fibrin. Phylogenetic analysis also detected gene clusters encoding putative capsule polysaccharide, a homolog of hemolysin, and variable lipoproteins among three isolated strains. The retrospective study confirmed by PCR the presence of *M. hyorhinis* in 9.9% of cases reported with neurological clinical signs and histological lesions of encephalitis or meningoencephalitis of unknown etiology. *M. hyorhinis* mRNA was confirmed within cerebrum, cerebellum, and choroid plexus lesions by RNAscope® with a positive rate of 72.7% of the PCR-positive cases. Here we present strong evidence that *M. hyorhinis* should be included as a differential etiology in pigs with neurological signs and CNS inflammatory lesions.

Sunday, October 29

2:35 PM – 2:45 PM

EXTRAINTESTINAL PATHOGENIC E. COLI ASSOCIATED PNEUMONIA DIAGNOSED BY WHOLE GENOME SEQUENCING AND HISTOPATHOLOGY IN SIX CANINE AND TWO FELINE CASES

Alexandra Frankovich, Mario Sola, Rebecca Wilkes, Jobin Kattoor
Purdue University, West Lafayette, IN, USA

Extraintestinal pathogenic *E. coli* (ExPEC) is an uncommon cause of acute fatal pneumonia in canines and felines. A review of ExPEC associated pneumonia cases at the Purdue Animal Disease Diagnostic Laboratory between 2018 and 2023 revealed ten cases including six canines and two felines confirmed with whole genome sequencing (WGS) and two cases of suspected ExPEC that did not have WGS for confirmation; *E. coli* was isolated as a pure culture from the lung for 7/10 cases, with a single additional

organism isolated from the other three cases. The clinical histories ranged from acute respiratory distress, prolonged respiratory signs refractory to medical treatment, and sudden death with no known medical history. One (feline) case had a recent surgical procedure and another (canine) had a clinical diagnosis of myasthenia gravis. Histologic lesions varied in distribution and severity and included hemorrhage, focal and diffuse, alveolar necrosis, fibrin, and mixed inflammation. Intralesional bacterial colonies were observed in 3/8 cases. Necrotizing pneumonia was the predominating histopathologic diagnosis, however; two cases had a diagnosis of suspected aspiration pneumonia and one had hemorrhage without accompanying inflammation. WGS analysis identified cytotoxic necrotizing factor (CNF) 1 or 2 virulence factors in six of the eight confirmed cases. Serotype, phylogroup, and sequence type varied with a predominance of B2 phylogroup (5/8). These findings suggest that ExPEC can have a variety of clinical signs and corresponding histologic findings and that WGS may be useful in cases that lack the commonly reported clinical and histologic findings.

Sunday, October 29

2:45 PM – 2:55 PM

PHAEOPHYCOMYCOSIS IN THE CENTRAL NERVOUS SYSTEM OF CATS

Bianca de Cecco¹, Christine Walsh¹, Jeongha Lee¹, Naomi Falconnier¹, Rudy Bauer¹, Dawn Evans¹, Kristin Vyhna¹, Maria Mitchell¹, Fabio Del Piero¹, Daniel Rissi², Jey Koehler³, Amanda Anderson⁴, Brian Porter⁵, Aline Hoffmann⁶, Emi Sasaki¹

¹Louisiana State University, Baton Rouge, LA, USA, ²Athens Veterinary Diagnostic Laboratory, Athens, GA, USA, ³Auburn University, Auburn, AL, USA, ⁴The University of Texas, MD Anderson Cancer Center, Houston, TX, USA, ⁵Texas A&M University, College Station, TX, USA, ⁶University of Florida, Gainesville, FL, USA

Background: Phaeohiphomycosis is an uncommon, opportunistic infection caused by dematiaceous fungi that are defined by the presence of melanin in their cell walls.

Objective: The study aims to characterize the lesions and distribution of phaeohiphomycosis involving the central nervous system (CNS) of cats.

Methods: A retrospective study was conducted from January 1994 to December 2022 identifying cases of CNS phaeohiphomycosis in domesticated cats from four diagnostic laboratories. Fungal culture had been previously performed in 3 cases. Slides from formalin-fixed paraffin-embedded tissues from selected cases were stained with hematoxylin and eosin and periodic acid Schiff or Grocott's methenamine stain. In six cases, scrolls from the paraffin block were used for DNA extraction, panfungal PCR, and sequencing. Obtained sequences were compared to fungal databases.

Results: Thirteen cases of CNS phaeohiphomycosis in cats were retrieved. The cats ranged from 8 months old to 12 years old. The clinical signs depended on the location of the lesions in the CNS and included ataxia, anisocoria, recumbency, and seizures. All cats were submitted for postmortem evaluation, and presented with gross lesions of dark-green to gray-to-black soft areas randomly distributed throughout the CNS. Histologically, severe pyogranulomatous meningoencephalitis (12), and myelitis (1) with septate pigmented hyphae were observed. Fungal organisms identified with culture

and/or panfungal PCR and sequencing included *Cladophialophora bantiana* (3), *Aureobasidium pullulans* (1), and *Cladosporium* sp. (1).

Conclusions: Phaeohyphomycosis is uncommonly documented in the CNS of cats. Here we provide a more comprehensive clinicopathological description of CNS phaeohyphomycosis in thirteen cats from the southeastern United States.

Sunday, October 29

3:30 PM – 3:40 PM

APPLYING SHOTGUN MASS SPECTROMETRY PROTEOMICS FOR DIAGNOSTIC AMYLOID TYPING IN A CHIMPANZEE (*PAN TROGLODYTES*)

Samantha Polk¹, Stanislaw Stanisheuski², Rachael Gruenwald¹, Carlos Sanchez³, Diana Canetti⁴, Duncan Russell¹

¹Department of Biomedical Sciences, Carlson College of Veterinary Medicine, Oregon State University, Corvallis, OR, USA, ²Department of Chemistry, Oregon State University, Corvallis, OR, USA, ³Oregon Zoo, Portland, OR, USA, ⁴Centre for Amyloidosis, Division of Medicine, University College London, London, United Kingdom

Veterinary amyloid diagnostics have traditionally relied on immunohistochemistry to type the fibril from which the amyloid is derived. However, this method can be associated with technical limitations that negatively influence sensitivity and specificity. Mass spectrometry (MS) shotgun-based proteomics is now widely adopted for human amyloid typing, with the added capability of detecting amyloid variants and pathogenic amino acid substitutions. Herein we test the feasibility of shotgun proteomics to type histologically confirmed hepatic amyloidosis in a 25-year-old captive chimpanzee (*Pan troglodytes*) with hepatomegaly. Four fresh tissue biopsies were digested using ProteaseMAX Surfactant and trypsin. A Bradford assay measured the amount of digested protein, 1.0 to 1.4 mg/ml. Samples were analyzed by liquid chromatography-tandem MS on a Thermo Orbitrap Fusion Lumos coupled with M-class Acquity UPLC. Thermo Proteome Discoverer and Mascot identified 4,228 human proteins and 4,402 matching *Pan troglodytes* proteins using Swissprot databases. Twenty-seven amyloid-associated sequences of interest were downloaded from the UniProt database and eighteen amyloid-associated proteins identical to humans were identified. A universal amyloidogenic signature was confirmed by the presence of ApoE, SAP, and ApoA4, which each had Mascot scores >650. Serum amyloid A was identified as the predominant fibril, with Mascot scores exceeding 1885 (maximum score 4023) along with severely elevated complement C3. Chimpanzee SAA had 84% coverage with human SAA. Herein we demonstrate the feasibility of shotgun proteomics for diagnostic amyloid typing in veterinary species. Routine diagnostic applications in other veterinary species may require laser capture microdissection and a validated algorithm to correctly identify the amyloid type.

Sunday, October 29

3:40 PM – 3:50 PM

A RETROSPECTIVE STUDY OF CONGENITAL CARDIAC MALFORMATIONS IN 29 GOATS

Samantha Kovacs¹, Christine Haake², Eunju Choi¹

¹University of California–Davis, Davis, CA, USA, ²Washington State University, Pullman, WA, USA

Background: Congenital heart defects (CHDs) are sporadically diagnosed in domestic species. Little literature is available for these developmental anomalies in goats. A large study in Saanen goats did not identify any malformations, and limited sporadic case reports of caprine CHDs are available.

Objective: Our objective is to determine the prevalence of caprine CHDs and describe the corresponding gross lesions to aid in the diagnosis of these lesions in goats.

Methods: A retrospective study was performed to catalog CHDs in goats submitted to the University of California–Davis, Veterinary Medical Teaching Hospital, Anatomic Pathology Autopsy Service.

Results: From 2000 to 2021, of 1886 goat autopsies, 29 cases of cardiac malformations were identified (1.5 percent). Thirteen were less than two weeks old, eight were one to six months old, and eight were adults. The most common malformations were ventricular septal defect (VSD; 21 of 29), atrial septal defect or persistent foramen ovale (10 of 29), and double-outlet right ventricle (3 of 29). Nine cases had more than one malformation, typically including a VSD. Conditions that had not been previously reported in goats included double-outlet right ventricle (3), tetralogy of Fallot (1), cor triatriatum sinister (1), and mitral valve dysplasia (1). Two adult cases were incidental and not suspected clinically.

Conclusion: A diversity of cardiac malformations occur not uncommonly in goats. Some are incidental and occur in a wide age range. We recommend a thorough cardiac examination in a goat autopsy, as with other species, with or without a history of cardiac disease.

Sunday, October 29

3:50 PM – 4:00 PM

IDIOPATHIC EOSINOPHILIC MENINGOENCEPHALITIS IN A DOG

Hailee West, Victoria Watson

Michigan State University Veterinary Diagnostic Laboratory, Lansing, MI, USA

A 3-year-old male castrated mixed breed dog with progressive behavioral changes and increasing aggression was euthanized and submitted for autopsy. Grossly, the brain was semi-firm, lacked distinct sulci, and meninges were diffusely expanded by dark brown exudate. This exudate extended into and replaced the outer layers of the cerebral cortex. Brain was submitted for direct fluorescent antibody for rabies virus and polymerase chain reaction (PCR) for Apicomplexan genera. Microscopically, meninges were expanded by numerous eosinophils and hemosiderin-laden macrophages which extended into the cerebral grey matter and surrounded large regions of necrosis and rarefaction. The adjacent white matter contained scattered spheroids and Virchow-Robin spaces were expanded by small mononuclear inflammatory cells. No infectious agents, infarction, or neoplastic cells were observed on routine or special stains. The

brain was rabies negative, and PCR did not detect Apicomplexan genera. Given these findings, idiopathic eosinophilic meningoencephalitis (EME) was considered most likely. Eosinophilic meningoencephalitis is a neurologic disease uncommonly reported in humans and animals. In dogs, this condition has been associated with toxoplasmosis, neosporosis, cryptococcosis, protothecosis, canine distemper virus, rabies virus, bacterial encephalitis, and aberrant migration of parasites. Other non-infectious causes of EME include infarction, trauma, or neoplasia. EME is designated as idiopathic if an underlying disease process cannot be identified. This case represents a classic yet uncommon condition with unknown pathogenesis. An immune-mediated or hypersensitivity process is suspected given these patients may improve with immunosuppressive treatment. In addition, golden retrievers and rottweilers are predisposed, suggesting a possible breed predisposition.

Sunday, October 29

4:00 PM – 4:10 PM

APPLICATION OF IN VIVO LASER CONFOCAL MICROSCOPY AND ITS FEASIBILITY IN ROUTINE DIAGNOSIS OF SURGICAL BIOPSY SPECIMENS

Yea Ji Jeong¹, Gabrielle Fontes², Lindsey Bussau³, Laura Selmic², Christopher Premanandan¹

¹Department of Veterinary Biosciences, The Ohio State University College of Veterinary Medicine, Columbus, OH, USA, ²Department of Veterinary Clinical Sciences, The Ohio State University College of Veterinary Medicine, Columbus, OH, USA, ³Optiscan Imaging Ltd, Mulgrave, Australia

Introduction: The need for intraoperative diagnosis and margin assessments is growing in veterinary surgical pathology. Hand-held laser confocal microscopy enables a real-time assessment of the cellular morphology of the tumor and margin assessment *in vivo* and *ex vivo*. Here, we describe the correlation between confocal laser microscopy images and histopathology and the feasibility of incorporating this technology in assessing surgical biopsy specimens.

Methods: A real-time laser confocal microscope (Optiscan, ViewnVivo®) was used to acquire high-definition confocal images of routine surgical biopsy cases. A group of anatomic pathologists and trainees were asked to provide an assessment of each case with minimal training on the imaging modality. The participants were asked to provide an assessment of each case without and with a clinical history.

Results: Real-time laser confocal microscopy captured detailed cellular morphology and tissue microarchitecture in various lesions encountered with routine surgical biopsy cases. Study participants were able to distinguish abnormal tissue (85.29%), neoplasia from inflammation (69.30%), and the cellular morphology of neoplasia (64.42%). Across all questionnaires, there was a statistically significant improvement in the rate of correct identification when clinical history was provided (p-value: 3.07E-07 to 0.005, paired t-test).

Conclusion: Confocal laser endomicroscopy enables the capture of the high-resolution microarchitecture of tissue and can be readily correlated to histopathology images. We

demonstrated that with little training, anatomic pathologists could evaluate surgical biopsy specimens that can provide a preliminary diagnosis and an assessment of margins when necessary.

Sunday, October 29

4:10 PM – 4:20 PM

EQUINE SARCOIDS: A CLINICOPATHOLOGIC STUDY OF 49 CASES, WITH MITOTIC COUNT AND CLINICAL TYPE PREDICTIVE OF RECURRENCE.

Wilson Karalus¹, John Munday¹, Supatsak Subharat¹, Geoff Orbell², Bernie Vaatstra²

¹Massey University, Palmerston North, New Zealand, ²Gribbles Veterinary Ltd, Palmerston North, New Zealand

Sarcoids are common mesenchymal neoplasms of horses. Although there are few studies in which large numbers of sarcoids have been followed over a long period of time, sarcoids are considered locally invasive and to frequently recur following surgical excision. There are currently no histological features which have been identified to predict which sarcoids will recur after excision. The present study comprised 49 sarcoids for which histology sections were available and in which the outcome of the case was known. Each sarcoid was excised from a different horse. Overall, 12 of the 49 (24.4%) sarcoids recurred after surgical excision. Mitotic count (MC), cellularity, inflammation, necrosis, and cell pleomorphism were evaluated histologically in the sarcoids. Of these, MC predicted prognosis with 4 of 5 (80%) sarcoids with a MC ≥ 20 in a 2.37mm² field recurring, which was a significantly higher recurrence rate than that of sarcoids with a MC <20 (8 of 44 cases (18%), $p=0.0051$). Clinical type was also found to predict recurrence with 3 of 4 (75%) fibroblastic types recurring, which was a significantly higher recurrence rate than that of sarcoids with other clinical types (9 of 45 cases (18%), $p<0.001$). In addition, multivariable analysis adjusted for the effects of age, gender, and breed confirmed fibroblastic type and MC ≥ 20 as significant predictors for recurrence ($p=0.006$ and $p=0.023$, respectively). To the authors' knowledge this is the first large study examining recurrence rates in sarcoids, and the first time that histological features have been used to predict recurrence.

Sunday, October 29

4:20 PM – 4:30 PM

DDPCR AS A NOVEL DETECTION METHOD FOR PTPN11 DRIVER MUTATIONS IN CANINE HISTIOCYTIC SARCOMA

Patrice Witschen, Robert Burnett, Cora Rutledge, Anne Avery, Kelly Hughes
Department of Microbiology, Immunology and Pathology, College of Veterinary
Medicine and Biomedical Sciences, Fort Collins, CO, USA

Background: Canine histiocytic sarcoma (HS) is an aggressive neoplasm in which the definitive diagnosis by histopathology can be challenging due to cellular pleomorphism and secondary inflammation. A targeted diagnostic tool may be useful to support diagnosis of this disease. Previous investigation of driver mutations E76K and G503V within the *PTPN11* gene have shown that these mutations are found in ~40% of Bernese mountain dogs and >25% of golden retrievers (GR) with HS.

Objective: To develop an efficient and sensitive method for detection of E76K and G503V mutations in formalin-fixed paraffin embedded (FFPE) HS samples.

Methods: Dogs diagnosed with HS from the Morris Animal Foundation Golden Retriever Lifetime Study were utilized for this study. FFPE tumor samples (n=20) were obtained from GRs (n=18) diagnosed with HS by two board-certified pathologists. Digital droplet PCR (ddPCR) was performed on extracted DNA using competitive probes to detect wild-type and mutant alleles (E76K and G503V) of *PTPN11* at a detection sensitivity of 1 mutation/3000 cells.

Results: 50% HS cases were positive for one of two mutations, with 39% positive for E76K and 11% positive for G503V. Mutations were mutually exclusive, including one case in which two different tumor sites (spleen and lung) were positive for E76K.

Conclusions: The frequency of *PTPN11* driver mutations in HS was higher in GRs than previously reported. In agreement with other studies, the E76K mutation was more prevalent than G503V. Overall, ddPCR is a promising targeted diagnostic tool to aid in the definitive diagnosis of canine HS.

Sunday, October 29
4:30 PM – 4:40 PM

MIXED EXOCRINE AND NEUROENDOCRINE CARCINOMA OF THE PANCREAS IN DOMESTIC ANIMALS: INCIDENCE AND DIAGNOSIS

Latticha Pluemhathaikij¹, Kerry Lewis², Tuddow Thaiwong-Nebelung¹

¹Michigan State University, East Lansing, MI, USA, ²Riverside Cat Hospital, Okemos, MI, USA

Mixed exocrine and neuroendocrine carcinoma of the pancreas is rare malignant tumor reported in humans and domestic animals. A search of the electronic archive generated at the VDL from 2017-2023 was performed. Thirty-eight pancreatic carcinomas (29 in dogs and 9 in cats) were identified. In dogs, over 80% (24/29 cases) of pancreatic tumors was diagnosed as islet cell carcinomas, suggestive of insulinoma based on hypoglycemia. Two cases of canine pancreatic tumors were pancreatic exocrine carcinoma, whereas almost all cases in cats were diagnosed as exocrine pancreatic carcinomas. In addition, two cases from dogs and one case from cat were diagnosed as mixed exocrine and neuroendocrine carcinoma. In this study, histopathology and immunohistochemistry for pancreatic exocrine and endocrine markers were examined on these cases. In all three cases, the histomorphology and immunohistochemistry confirmed two neoplastic populations that expressed different proportions of epithelial and neuroendocrine markers. In all cases, there were variable numbers of neoplastic pancreatic exocrine epithelial cells labeled by MUC-1, and diverse expression of pancreatic endocrine markers. Both dogs had a clinical history of hypoglycemia, and majority of neoplastic cells were immunoreactive for insulin labeling, consistent with an insulinoma. In one cat, the neoplastic cells involved the liver and lymph node, and the neoplasm exhibited immunoreactivity for vasoactive intestinal polypeptide (VIP), supportive of a diagnosis of Vasoactive Intestinal Peptide-Secreting Tumor. Although histomorphology is helpful to make a diagnosis, immunohistochemistry is required to

confirm the mixed neoplastic components. Further investigation is necessary to determine prognosis and develop appropriate therapeutics.

Sunday, October 29

4:40 PM – 4:50 PM

EXPLORATORY SCREENING FOR MICRO-RNA BIOMARKERS IN CANINE MULTICENTRIC LYMPHOMA

Sabine Hammer¹, Julia Sprung¹, Stefanie Burger², Martin Hofer², Ilse Schwendenwein³, Barbara Ruetgen³

¹University of Veterinary Medicine Vienna, Institute of Immunology, Vienna, Austria,

²University of Veterinary Medicine Vienna, Genomics Core Facility, VetCore, Vienna,

Austria, ³University of Veterinary Medicine Vienna, Clinical Pathology Unit, Vienna, Austria

Background: MicroRNAs (miRNA, small non-coding RNAs) are pivotal in gene regulation fine tuning and their aberrant expression is expected in cancer. miRNAs might serve as diagnostic biomarkers and assist predicting therapeutic response and clinical outcome.

Objective: Aim of this study was to investigate dysregulated miRNAs in lymphomatous lymph node tissues in comparison to normal lymph node material and PBMCs from healthy control dogs. Differences in miRNA expression between four lymphoma entities were assessed.

Methods: 89 canine target miRNAs were profiled by a customized PCR array. Quantification was performed by quantitative real time PCR and relative expression was determined by the delta-delta Ct method using the GeneGlobe Data Analysis Center (Qiagen, www.qiagen.com). Fold changes were evaluated by student's t-test and *p*-values less than 0.05 were considered significant.

Results: In total, 85 out of 89 miRNAs were successfully amplified and many were differently expressed in the lymphoma entities. In the 14 diffuse large B-cell lymphoma (DLBCL) patients, 28 and 24 different miRNAs were significantly dysregulated compared to lymph node material or PBMCs. The six peripheral T-cell lymphoma (PTCL) samples showed 24 and 25 dysregulated miRNAs when compared to the healthy controls. A combined analysis of DLBCL and PTCL samples revealed seven shared and 19 differently expressed miRNAs.

Conclusions: The miRNA-17-92 cluster, miRNA-34a, miRNA-150, and miRNA-181-family might serve as biomarkers in canine T- and B-cell lymphoma. A panel of 26 significantly dysregulated miRNAs will be applied to confirm and validate these miRNAs together with those with unknown function and still missing literature record.

Sunday, October 29

4:50 PM – 5:00 PM

CROWN CELLS IN CANINE PERIVASCULAR WALL TUMORS: WHAT ARE THEY?

Esdras Correa Dos Santos, Elizabeth W. Howerth

University of Georgia, Athens, GA, USA

Background

Perivascular wall tumors (PWT) are spindle cell neoplasms characterized by whorling growth and variable pericytic- myoid differentiation. Tumors often have bi- and multinucleated cells with peripheralized nuclei (insect head and crown cells [CrCs], respectively). Studies characterizing the ultrastructural morphology of these cells are lacking and could help establish their identity and formation.

Objective

To characterize perivascular wall tumor CrCs via ultrastructure microscopy.

Methods

One formal-fixed PWT rich in CrCs from a 13-yr-old canine was processed and routinely stained with hematoxylin and eosin and immunolabeled for desmin, smooth muscle actin (SMA), vimentin, pan-cytokeratin (P-CK), and Iba1. From areas rich in CrCs, 2-mm cores were extracted from paraffin blocks and processed routinely for transmission electron microscopy.

Results

CrCs were positive for vimentin, weakly positive for P-CK, and negative for desmin, SMA, and Iba1. Ultrastructurally, bi- and multinucleated cells had peripheralized pleomorphic nuclei of variable size, dispersed or dense chromatin, and single or absent nucleoli. Cell bodies were irregular, with multiple elongations with branching and bridging. Elongations often surrounded neighboring cells in a slight whorling pattern reminiscent of whorling seen by light microscopy. Cells contained large numbers of intracytoplasmic interlacing filaments with occasional whorling, often associated with circular or rodlike dense bodies.

Conclusions

Ultrastructurally, CrCs resemble normal pericytes and represent a multinucleated version of the PWT cells with abnormal accumulations of cytoplasmic filaments. Whether these cells derive from fusion or acytokinetic division was not determined, but aberrant cytoskeletal structure may be involved. The mechanism of multinucleation and function remains to be determined.

Monday, October 30

8:05 AM – 8:15 AM

COELOMIC COCCIDIA INFECTION IN TWO COWNOSE RAYS (RHINOPTERA BONASUS)

Naomi Falconnier¹, Virginia Brookings², Emi Sasaki¹

¹Louisiana State University, Baton Rouge, LA, USA, ²Shreveport Aquarium, Shreveport, LA, USA

Background: *Eimeria southwelli* is an important cause of chronic progressive wasting disease in cownose rays (*Rhinoptera bonasus*). Heavy parasitism in captive cownose rays is associated with a high mortality although commonly identified in free-ranging populations with unknown morbidity and mortality. A direct lifecycle has been proposed although the route of shedding remains undetermined.

Objective: The goal of this study is to describe the histologic characteristics of coelomic coccidiosis in two cownose rays.

Methods: Gross evaluation was performed as well as routine histologic staining.

Results: Over the course of six weeks, four captive cownose rays exhibited sudden erratic swimming patterns. Two rays responded well to florfenicol and meloxicam administration while the other two did not respond to treatment, one of which was euthanized and the other was found dead. Gross findings included pallor of the skin and coelomic hemorrhage in one ray. In both rays, the splenic capsule was multifocally infiltrated by aggregating lymphocytes, and the visceral mesothelium was hypertrophied with rare intracytoplasmic sporulating oocysts. In one ray, the epigonal organ and epididymis were expanded by a large aggregate of sporulated oocysts measuring 12.5 x 8 micrometers that contained 2 to 4 sporozoites. Other histologic findings included lymphoid cuffing of sporadic cerebral and cranial nerve blood vessels in one ray and astrocyte swelling with vesiculate nuclei in the other ray.

Conclusions: These microscopic findings were consistent with previous descriptions of coelomic *Eimeria southwelli* infections. Coelomic coccidiosis is an important differential in cownose stingrays exhibiting emaciation, lethargy, inappetence, and erratic swimming patterns.

Monday, October 30

8:15 AM – 8:25 AM

THERMAL INJURIES CAUSED BY MICROWAVE RADIATION IN A FERRET

Hilary Ann Lakin^{1,2}, Christiane Löhr¹

¹Oregon State University, Corvallis, OR, USA, ²Purdue University, West Lafayette, IN, USA

The body of a 1 kg, adult, male, neutered domestic ferret was submitted to the Oregon Veterinary Diagnostic Laboratory with a history of having been removed from its cage, placed into a microwave, and removed from microwave deceased.

Bilaterally, corneas were dry and carpi hyperflexed. The skin was fragile and easy to cut and tear. Burned skin or flesh was not noted. The thoracic cavity contained 7mL of serosanguinous fluid. Both lungs were heavy, wet, and dark red, with abundant red-tinged fluid within the main stem bronchi. Kidneys and liver were pale tan, friable, with a dry appearing capsular surface. The wall of the stomach tore easily upon manipulation. The brain was diffusely dark pink. Exposed fat and bones were within expected limits.

Histologically, tissues were hypereosinophilic and had a coagulated to autolyzed appearance with shrunken, dense (hyperchromatic) nuclei, most prominently in the

lung, liver and spleen. Diffusely, alveolar lumens were filled with fine-granular proteinaceous residue. Collagen in lung (periarterial, peribronchial, interstitium), diaphragm, and intestine was intensely amphophilic and smudged; resembling collagenous connective tissue in cauterized sample margins. Skin (pinna) had occasional shrunken keratinocytes and endotheliocytes and fragmented apocrine cells with hypereosinophilic cytoplasm and amphophilic, smudged, dense nuclei. Virchow-Robin space was expanded (empty); nuclei of cerebral neurons were deeply amphophilic, smudged, and shrunken.

Microwave induced thermal injury has not been thoroughly categorized. Fragile skin splitting with well-defined edges, absence of singed/scorched hair and charring, and contracture of limbs ("boxer's attitude") more prominent in flexors than extensors have been reported previously.

Monday, October 30

8:25 AM – 8:35 AM

CHARACTERIZATION OF CRYSTALLINE NEPHROSIS IN MULTIPLE CAPTIVE SHARK SPECIES

Emily Garrison¹, Aimee Berliner², John Trupkiewicz¹

¹Johns Hopkins University, Baltimore, MD, USA, ²National Aquarium, Baltimore, MD, USA

Intratubular and glomerular renal crystals were identified at necropsy in six captive sharks representing five different species with variable associated renal damage. Crystalline nephrosis has not been previously described in Elasmobranchs, although there are rare reports of urolithiasis without associated intratubular crystals. The five shark species represented include four requiem sharks (*Carcharhinus melanopterus*, *Carcharhinus plumbeus*, *Carcharhinus acronotus*, and *Triaenodon obesus*) and one nurse shark (*Ginglymostoma cirratum*). Severe tubular damage associated with a large number of crystals was identified in one black tip reef shark (*Carcharhinus melanopterus*), while the other animals exhibited fewer crystals and mild associated lesions. On H&E, renal crystals are colorless, refractile, forming overlapping plates and rosettes, and are brightly birefringent under polarized light, most consistent with oxalate crystals. Crystals stain positive with Von Kossa, confirming a calcium component. Microspectroscopy is in progress for definitive identification of the chemical composition of these crystals. No consistent concurrent disease process or history was noted among affected sharks, and multiple cohoused conspecifics presented for necropsy without similar crystals. This study suggests that a variety of sharks are prone to crystalline nephrosis of unknown cause. The wide range in the severity of associated renal damage suggests the pathogenesis may be related to chronic exposure with the potential for accumulative disease. Further investigation into diet, medications, environment, and comparison with wild sharks is necessary to understand the pathogenesis and future implications of this finding.

Monday, October 30

8:35 AM – 8:45 AM

CANINE DRACUNULUS INSIGNIS INFECTION IN SOUTHEASTERN WISCONSIN

Brittney Moore, David Gasper

University of Wisconsin - Madison, Madison, WI, USA

Dracunulus insignis is a common parasitic infection of wild carnivores. However, this parasite is infrequently reported in domestic dogs. Herein, we describe clinical, cytologic, and histopathologic findings in a case of *D. insignis* in a 10-year-old female spayed Redbone Coonhound from Southeastern Wisconsin.

The patient presented in November 2022 for forelimb swelling with an associated draining tract that oozed serosanguinous fluid. The patient was treated for suspect cutaneous larval migrans with fenbendazole, antibiotic therapy, and pain management until late December when symptoms subsided. In March 2023, the owner removed a nematode measuring approximately 20 cm from a swollen area of the lateral left hindlimb.

Fine needle aspirate of the left hindlimb swelling revealed numerous long, thin-tailed, curled, nematode larvae with numerous neutrophils and macrophages.

Biopsy of the subcutaneous draining tract revealed chronic suppurative panniculitis with subcutaneous sinus tract formation and intralesional larval nematodes. The overlying epidermis exhibited chronic lymphoplasmacytic and eosinophilic dermatitis with severe edema, mild acanthosis, and moderate orthokeratotic hyperkeratosis.

The organism removed from the hindlimb was submitted to the Kansas State Veterinary Diagnostic Laboratory parasitology service and confirmed to be an adult female *Dracunculus insignis*.

Draining cutaneous tracts can result from infection with hookworm, as was initially suspected clinically, *Dirofilaria immitis*, or less likely, *D. insignis*, as reported here. To our knowledge, canine *D. insignis* infection has not been reported in Wisconsin. Recent literature suggests that canine infection occurs throughout the eastern United States, and that most diagnoses occur in the winter months, as reported here.

Monday, October 30

8:45 AM – 8:55 AM

FORENSIC NECROPSY OF A SHOT DOG.... HOW A HEARTWORM TELLS THE STORY!

Nanny Wenzlow, Bianca de Cecco

LSU-LADDL, Baton Rouge, LA, USA

A 3-year-old male mixed-breed dog was found dead in the owners' backyard and poisoning by neighbor was suspected. Large amounts of blood oozed from the mouth and contained one heartworm. Full body radiographs identified a projectile in the left intercostal muscles. Whole body skinning revealed the entrance of the fatal gunshot to the right forelimb. The projectile traveled through the right scapula, the 5-6th intercostal

space, entering the chest cavity, the right cranial lung lobe, then perforated the pulmonary artery and the adjacent right bronchus, traveling through the left caudal lung lobe and the diaphragm. In the abdominal cavity, the projectile perforated the liver before re-entering the diaphragm adjacent to the rib cage and finally lodging in the perforated intercostal muscle between the distal ends of the left 12-13th rib, where the projectile was recovered. Associated with the wound pathway is bilateral severe hemothorax. The projectiles' trajectory was front to back, right to left, and downwards. The perforation of the pulmonary artery and the adjacent right bronchus allowed the heartworms to leave the heart through these communicating defects and migrate up the trachea before reaching the oral cavity. The manner of death was non-accidental killing, the equivalent of homicide in humans. Forensic veterinary pathology is a growing discipline, that focuses on the meticulous examination of an animal corpse with the aim to determine cause and manner of death. This is a crucial step in identifying and determining if an animal was or was not the victim of acts of cruelty.

Monday, October 30

8:55 AM – 9:05 AM

MULTI-STATE CLOSTRIDIUM BOTULINUM TYPE C OUTBREAK IN HORSES FROM ALFALFA CUBES

David Rotstein¹, April Hodges¹, Mark Glover¹, Jackie Queen¹, Sayeeda Hdabe¹, Lee Anne Palmer¹, Lauren Carey¹, Lloyd Payne¹, Justin Henson¹, Lindsay Bertling², Holly Miller², James Yee², Sumit Sarkar³, Rebecca Wilkes⁴, Emi Sasaki⁵, Mariano Carrosino⁵, Rose Baker⁵, Nanny Wenzlow⁵, Fabio Del Piero⁵, Jeongha Lee⁵, Laura Guarneri⁵, Naomi Falconnier⁵, Andrea Aghaian⁶, Gabriel Gomez⁷, Terry Hensley⁷

¹FDA Center For Veterinarinary Medicine Office of Surveillance and Compliance, Rockville, MD, USA, ²FDA Office of Regulatory Affairs, Silver Spring, MD, USA, ³FDA National Center for Toxicological Research, Jefferson, AR, USA, ⁴Purdue University College of Veterinary Medicine, Lafayette, IN, USA, ⁵LSU School of Veterinary Medicine, Baton Rouge, LA, USA, ⁶CSU College of Veterinary Medicine and Biomedical Science, Fort Collins, CO, USA, ⁷Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, TX, USA

Background: In December 2022, 20 horses from a farm in Louisiana presented to the Louisiana State University School of Veterinary Medicine with neurologic signs (flaccid paralysis, dysphagia, glossal extrusion), mild colic, and respiratory arrest. Similarly presenting cases from Texas, Colorado, and New Mexico were reported, totaling 98 cases with a mortality rate of 58% (57/98). The FDA and state partners initiated an investigation.

Objectives: Provide findings from a multi-state outbreak investigation

Methods: Horses shared exposure to bagged alfalfa cubes from a single manufacturer with common lot codes. Diagnostic laboratories performed necropsies on ten horses and viral encephalitides and toxicologic screening) on select horses. Alfalfa cubes were submitted for *Clostridium botulinum* toxin testing (mouse bioassay). Filth analysis, microscopic evaluation, and a targeted next generation sequencing PCR panel for speciation were conducted on animal remnants from an alfalfa cube.

Results: Botulism was suspected, clinically; some horses were treated and responded to treatment with botulinum antitoxin. Necropsy findings included non-specific lesions: mild, acute hepatitis (5/10) and pulmonary edema (6/10). Significant nervous system lesions were not observed. Botulinum toxin Type C was identified in an antemortem intestinal and alfalfa cube sample. The tissue remnant was of goat origin and consisted of skeletal muscle. No other pathogens or toxic agents were identified. The firm conducted a voluntary recall.

Conclusions: The rapid outbreak response was the result of coordinated efforts by federal and state officials. Botulism in horses is characterized by absence of significant lesions. The mild acute hepatitis identified was possibly associated with terminal sepsis.

Monday, October 30

9:05 AM – 9:15 AM

A CASE OF ACUTE SECONDARY BILIARY CIRRHOSIS: EXAMINING THE TIMECOURSE OF DUCTULAR REACTION

Maya Schlesinger, April Choi

University of California - Davis, Davis, CA, USA

A 6-year-old, female, spayed, Jack Russell terrier dog presented for vomiting and lethargy with markedly elevated but fluctuating biochemical liver values compatible with a mixed but predominantly hepatocellular hepatopathy. A liver biopsy and gall bladder aspirate were obtained via laparotomy, at which time the liver was pale with rounded borders. This initial biopsy revealed a mild lymphoplasmacytic and neutrophilic portal cholangiohepatitis with few increased profiles of bile ducts, mild hepatocellular necrosis, intracellular pigment, and a vacuolar hepatopathy.

The patient declined while in hospital with liver values continuing to fluctuate but remained elevated overall. Seven days following the initial biopsy and gall bladder aspirate, an atypical mucocele was identified via ultrasound and a cholecystectomy and repeat liver biopsy was performed. This second biopsy revealed a cystic hemobilia with some mucinosis and moderate lymphoplasmacytic cholecystitis. The liver biopsies differed remarkably from those obtained seven days earlier in that both the inflammation, profiles of bile ducts, and biliary metaplasia (ductular reaction) were markedly increased.

Ductular reaction has often been thought of as a chronic response to liver injury in which injury induces increased, tortuous profiles of bile ducts and/or proliferation of hepatic stem cells (HPCs) over weeks to months. This case demonstrates that fulminant ductular reaction can manifest in as little as 7 days from the time of biliary injury (likely the gall bladder aspiration). Nonetheless, ductular reaction is important to recognize as a response to hepatobiliary injury and understanding the temporality and mechanisms may aid in better categorizing and prognosticating liver injuries.

Monday, October 30

9:15 AM – 9:25 AM

IDENTIFICATION OF APOLIPOPROTEIN E-DERIVED AMYLOID WITHIN XANTHOMATOSIS LESIONS OF LEOPARD GECKOS (EUBLEPHARIS MACULARIUS)

Mitsuhiro Ikeda¹, Hirotaka Kondo¹, Tomoaki Murakami², Yoshiyuki Itoh², Hisashi Shibuya¹

¹Nihon University, Kanagawa, Japan, ²Tokyo University of Agriculture and Technology, Tokyo, Japan

Background and Objective: Only two reports have documented amyloidosis in reptiles, and amyloid precursor proteins have not been identified. For the first time, amyloid deposits were identified within xanthomatosis lesions in leopard geckos in this study, which aimed to characterize amyloid precursor protein by proteomic analysis and clarify its pathogenesis in reptilian amyloidosis.

Materials and Methods: Seven cases of xanthomatosis in leopard geckos, including four biopsy cases and three necropsy cases, were selected for histopathological evaluation and/or liquid chromatography-tandem mass spectrometry (LC-MS/MS). LC-MS/MS was performed on the foci of amyloid deposits, collected by laser microdissection or 30-G needles. The MS/MS data were collated with theoretical fragmentation patterns of peptide sequences in the NCBI database via the Mascot server.

Results: Amyloid deposition localized within the lesions was found in both biopsy (1/4 cases, 25%) and necropsy (3/3 cases, 100%) cases. The amyloid was positive for Congo red, with yellow to green birefringence under polarized light. On LC-MS/MS analysis, apolipoprotein E (ApoE) had the highest score in all cases. Of the full length of the 274 amino acid residues, Gln₁₈₃-Arg₂₃₀ was frequently detected.

Conclusions: Although ApoE is co-deposited with amyloid in various mammalian amyloidoses, it can also be an amyloidogenic protein in leopard geckos. In particular, Gln₁₈₃-Arg₂₃₀ is responsible for amyloidogenesis because of its high detection rates. It is known that ApoE is involved in cholesterol transport among cells, and the lack of ApoE results in xanthomatosis. It is possible that misfolding of ApoE leads to xanthomatosis in leopard geckos.

Monday, October 30

9:25 AM – 9:35 AM

SELECTED FORENSIC CASES AND THEIR 3-DIMENSIONAL ILLUSTRATIONS

Nanny Wenzlow, Michelle Osborn, Gwyneth Miller
LSU-LADDL, BATON ROUGE, LA, USA

Forensic necropsies require extensive photographic and radiographic documentation with body diagrams summarizing and illustrating the distribution of lesions. Three-dimensional (3D) reconstructions are beneficial for criminal investigations where the demonstration of complex falls, shootings, or stabbings are necessary to clarify

postmortem findings contributing to death and possible acts of cruelty. 3D reconstructions based on necropsy findings were generated for the following cases to clearly illustrate the events.

Case 1: A mare and her rider were found dead on a dirt road. Distribution of lesions suggested that a loss of foot control during the witnessed high-speed gallop caused the horse to fall, completing a forward rotational movement from the left lateral side to the right side, over the back, also damaging severely the horn of the saddle.

Case 2: A male dog had two gunshot wounds to the back and chest with two projectiles lodged within the respective trajectories. The deadly shot lacerated the aorta causing hemothorax. Both trajectories were front to back, minimally right to left, and steeply downwards, suggesting that the gun was angled almost perpendicular to the back, directly above the animal.

Case 3: A male dog was stabbed 7 times to the left and right chest and abdomen under the pretext of self-defense by the perpetrator. All stab wounds were back to front and downwards indicating that the animal was facing away from the perpetrator, suggesting cruelty and not self-defense.

Monday, October 30
10:15 AM – 10:25 AM

DIAGNOSTIC PERFORMANCE OF FROZEN SECTION HISTOPATHOLOGY IN METASTATIC CANINE CUTANEOUS MAST CELL TUMORS

Alejandro Alvarez-Sanchez¹, Katy Townsend², Elena Gorman², Milan Milovancev³, Duncan Russell²

¹Boundary Bay Veterinary Specialty Hospital, Surrey, BC, Canada, ²Carlson College of Veterinary Medicine, Oregon State University, Corvallis, OR, USA, ³Summit Veterinary Referral Center, Tacoma, WA, USA

Intra-operative staging of canine mast cell tumor (MCT) currently relies on routine cytology to determine nodal metastasis. While frozen section (FS) lymph node histopathology is commonly used in humans, its applicability to veterinary settings is poorly documented. The goal of this study was to determine the diagnostic performance of FS histopathology for diagnosing metastatic MCT, as compared to a routine histopathologic gold standard. Performances of imprint cytology (IC), and fine needle aspirates (FNA) were also evaluated. Forty one lymph nodes from twenty dogs with MCT were collected and stained with hematoxylin and eosin (HE) and giemsa (formalin-fixed and frozen tissues), and Wright giemsa and toluidine blue (IC and FNA). Nineteen out of twenty primary tumors were low grade. Frozen HE sections had poor agreement as compared to routine histopathology ($\kappa = 0.15$), however diagnostic performance increased to a good level of agreement when interpretation was accompanied by giemsa ($\kappa = 0.45$). FNA and IC stained with Wright giemsa had agreement comparable to combined FS histopathology ($\kappa = 0.42$ and 0.35 , respectively). FS interpreted with both stains had a sensitivity of 74% and specificity of 75%, as compared to 61% and 92% for FNA. Challenges in morphologic interpretation included inadequate sectioning quality, architectural nodal disruption, ruptured cells, and background metachromatic

granular staining. These data provide support for FS histopathology as a feasible strategy for intra-operative detection of metastatic MCT, with diagnostic agreement similar to conventional cytology. Performance of FS histopathology is conditional upon a metachromatic stain evaluated in conjunction with HE.

Monday, October 30

10:25 AM – 10:35 AM

MULTISYSTEMIC ANGIOMATOSIS AND CARDIAC HEMANGIOSARCOMA IN A DOBERMAN PINSCHER DOG

Kelsey Brown, Nataly Mamaliger, Alexander Saver
University of Wisconsin-Madison, Madison, WI, USA

A 3.3-year-old castrated male Doberman Pinscher presented for euthanasia and necropsy due to acute onset blindness and a 2-year history of bilateral epistaxis. Prior antemortem coagulation parameters were unremarkable, and extensive infectious disease testing was negative. Advanced imaging and nasal biopsies revealed multifocal proliferative vascular lesions suggestive for multisystemic angiomas. On necropsy, gross findings included bilateral epistaxis with turbinate bone loss and multiple dark red, flat to nodular foci disseminated throughout the dermis, brain, spinal cord, lungs, heart, and spleen. These foci histologically corresponded to atypical vascular proliferations. Atypical vessels ranged from small to large-caliber, and recapitulated all layers of the vessel wall, as demonstrated by Jones basement membrane stain, Masson's trichrome stain, and immunohistochemistry for CD31 and smooth muscle actin. Additionally, there was a population of infiltrative CD31-positive spindle cells forming blood-filled vascular channels within the left ventricular myocardium. In the absence of other corresponding vascular structures, these cells were deemed to be a neoplastic population. Multisystemic angiomas and focal intramyocardial hemangiosarcoma were therefore diagnosed. Although found affecting the skin in dogs, angiomas of multisystemic distribution has been rarely reported. The extent of this dog's angiomas lesions across body systems has not been reported in the veterinary literature. The precise origin of these proliferative vessels is unclear, however a genetic etiology is favored. Angiomas may have increased the risk of hemangiosarcoma formation via malignant transformation of the non-neoplastic proliferative vessels.

Monday, October 30

10:35 AM – 10:45 AM

COMPARATIVE PATHOLOGY OF WEST NILE VIRUS AND EASTERN EQUINE ENCEPHALITIS VIRUS INFECTIONS IN FREE-RANGING RAPTORS IN THE SOUTHEASTERN UNITED STATES

Xuan Hui Teo¹, Nicole Nemeth¹, Heather Fenton^{1,2}, Kevin Niedringhaus^{1,3}, Daniel Mead¹, Kayla Adcock¹, Kevin Keel^{1,4}

¹University of Georgia, Athens, GA, USA, ²Australian Registry of Wildlife Health, Taronga Conservation Society, Mosman, Australia, ³University of Pennsylvania, Philadelphia, PA, USA, ⁴University of California, Davis, Davis, CA, USA

Background: West Nile virus (WNV) and eastern equine encephalitis virus (EEEV) are broadly considered causes of encephalitides in wild birds, but differences in associated lesion patterns are poorly documented.

Objective: To guide diagnostic assessment by critically evaluating and comparing the lesions of WNV and EEEV infection in free-ranging raptors.

Methods: A review of archival diagnostic data from the Southeastern Cooperative Wildlife Disease Study was conducted to identify necropsy cases of free-ranging raptors from the Southeastern United States diagnosed with either WNV or EEEV. The cases identified had representative histopathology and/or at least one ancillary diagnostic test performed (e.g., virus isolation, polymerase chain reaction, or immunohistochemistry). Seven cases of WNV infection and three cases of EEEV infection were included. Raptor species represented included the bald eagle (*Haliaeetus leucocephalus*), broad-winged hawk (*Buteo platypterus*), red-tailed hawk (*Buteo jamaicensis*), red-shouldered hawk (*Buteo lineatus*), osprey (*Pandion haliaetus*), and barn owl (*Tyto alba*).

Results: Lesions in EEEV-infected raptors included mononuclear epimyocarditis and mixed cell (mononuclear and heterophilic) meningoencephalitis. Lesions in WNV-infected raptors included mononuclear or mixed cell meningoencephalitis (with variable microgliosis and neuronal necrosis), myocarditis or pancarditis, pancreatitis, and peripheral neuritis. In one EEEV-infected osprey and one WNV-infected bald eagle without overt microscopic lesions, positive immunolabeling with viral antigen was observed in at least one organ.

Conclusion: The lesion patterns in EEEV- and WNV-infected raptors are similar, although the latter appears more likely to manifest extracerebral and extracardiac lesions. Immunohistochemistry can assist to determine viral involvement in cases where compatible inflammatory lesions are subtle or absent.

Monday, October 30
10:45 AM – 10:55 AM

HELICOSPORIDIUM SP. INFECTION IN AN ALBINO CALIFORNIA KINGSNAKE (LAMPROPELTIS GETULA CALIFORNIAE)

Javier Asin¹, April Childress², Eva Dervas³, Michael Garner⁴, Francisco Uzal¹, James Wellehan Jr², Eileen Henderson¹, Anibal Armien¹

¹California Animal Health and Food Safety laboratory, Davis and San Bernardino, CA, USA, ²Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine; University of Florida, Gainesville, FL, USA, ³Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zürich, Zürich, Switzerland, ⁴Northwest ZooPath, Monroe, WA, USA

Background: *Helicosporidium* is a genus of non-photosynthetic, green algae in the family *Chlorellaceae* closely related to *Prototheca*. It has been described as a pathogen

of invertebrate hosts in the orders Diptera, Coleoptera, and Lepidoptera, but never in a vertebrate animal.

Objective: To describe the diagnostic workup in a case of *Helicosporidium* sp. infection in a snake with an emphasis on the histologic and ultrastructural features of the organism.

Methods: A captive, 10-month-old, male albino California kingsnake (*Lampropeltis getula californiae*) was submitted for necropsy with a history of being found dead in the cage. Necropsy, histology (hematoxylin and eosin and special stains), bacteriology, transmission electron microscopy, and molecular investigations were performed.

Results: Grossly, focal hemorrhagic laryngitis and hepatic pallor were visible. Histologically, there were myriads of intravascular, intrahistiocytic and extracellular, unicellular organisms in multiple tissues with large numbers in the liver, lungs and kidneys. Organisms were positive with Gomori methenamine silver and periodic acid-Schiff stains, and variably acid-fast and gram-positive. Ultrastructurally, there were approximately 4 µm cysts and vegetative multiplication cells; the latter had up to 7 daughter cells. Features were consistent with an alga of the family *Chlorellaceae*. *Prototheca* spp. PCR was positive on liver and kidney and sequencing of the obtained amplicon confirmed the presence of *Helicosporidium* sp. with a 99.6% homology to an available sequence (JN869293.1).

Conclusions: This is the first description of *Helicosporidium* sp. infection in a vertebrate host. The mechanism of infection in this case remains unknown, however immunosuppression is suspected to represent a favoring factor.

Monday, October 30
10:55 AM – 11:05 AM

PYOGRANULOMATOUS DERMATITIS IN 2 HORSES FOLLOWING SARCOID TREATMENT WITH MYCOLICIBACTERIAL EXTRACTS

Randall Walker¹, Diego De Gasperi¹, Jennifer Ward², Aline Rodrigues Hoffmann¹

¹University of Florida, Gainesville, FL, USA, ²Specialty Vet Path, Seattle, WA, USA

Two horses were initially presented with cutaneous sarcoids, and then subsequently treated with mycobacterial cell wall extracts (Immunocidin®). The first was a 10 year old Quarter horse gelding, presented for a nodular lesion in the left axillary region. The second was an 8-year-old, gelding presented with a 1-month history of mass on right cranioventral aspect of neck. Following sarcoid excision, both horses were treated with Immunocidin® injections. In both horses, the lesions increased in size after multiple injections within the region. Histologically, both excised masses were characterized by multifocal pygranulomas extending from the dermis to the skeletal muscle. The center of these pygranulomas contained areas of necrosis and lipid with scattered aggregates of acid fast positive short rods. The previously diagnosed sarcoid was still observed in the first horse. Immunocidin®, a non-specific immunotherapeutic consisting of Mycolicibacterium phlei cell wall fraction reformulation, has been reported to be used as a therapy for cutaneous tumors in horses, squamous cell carcinoma in

equids/bovids/caprids, and canine splenic hemangiosarcoma. In a previous study, two horses developed draining tracts and tissue inflammation associated with Immunocidin® injection to treat cutaneous squamous cell carcinoma. The findings described in this current report were concerning for an immune reaction to Immunocidin® therapy used to reduce the size of the sarcoids. Targeted next generation sequencing performed on extracted DNA from the paraffin block identified *Mycolicibacterium phlei* in both biopsies, indicating this is likely the underlying cause of the inflammatory response in both patients.

Monday, October 30

11:05 AM – 11:15 AM

DEMATIACEOUS FUNGAL PLACENTITIS IN TWO HORSES

Brianne Taylor¹, Alex Wittorff¹, Sai Narayanan¹, A Cino Ozuna¹, Craig Miller¹, Timothy Snider¹, Aline Rodrigues Hoffmann², Reed Holyoak¹

¹Oklahoma State University, Stillwater, OK, USA, ²University of Florida, Gainesville, FL, USA

Two unrelated horses presented for premature lactation and parturition. The first mare foaled two months early, and the foal was humanely euthanized due to poor prognosis. Grossly the chorioallantois was thickened, and numerous sharply demarcated, raised, red foci ranging from 4 to 21 cm in diameter were randomly scattered on the chorionic surface. These foci were rimmed by a 1 cm thick yellow, friable border. The amnion was nearly diffusely thickened, yellow, and leathery. A severe fibrinonecrotizing, neutrophilic, and histiocytic placentitis with intralesional pigmented fungal hyphae was diagnosed histologically. Panfungal PCR and sequencing matched *Curvularia/Bipolaris* sp. with 100% identity. The second mare foaled at 323 days gestation and delivered a small but viable foal. Grossly, there was a large, sharply demarcated, light brown region adhered with fibrin. Histologically, there was a similar fibrinonecrotizing placentitis with granulation tissue and fungal hyphae. Panfungal PCR and sequencing matched *Curvularia* sp. with 99.38% identity.

The vast majority of equine placentitis cases are due to ascending infection, most often from bacterial etiologies. Fungal placentitis is less common, with most cases involving *Aspergillus* sp. Dematiaceous fungi are worldwide saprophytes. Dematiaceous fungal infections in horses (phaeohyphomycosis) are most often cutaneous, though central nervous system, upper respiratory tract, and disseminated infections are reported. Placental phaeohyphomycosis is rarely reported in horses. These two unrelated cases highlight the importance of histopathology in the diagnosis of fungal placentitis. Additionally, as pigmentation can vary with these fungal agents, panfungal PCR remains an important diagnostic tool.

Monday, October 30

11:15 AM – 11:25 AM

REAL-TIME PCR FOR DETECTION OF MYCOPLASMA BOVIS AND VIRAL PATHOGENS OF CALF PNEUMONIA IN SOUTHWEST VIRGINIA BASED ON FORMALIN-FIXED PARAFFIN EMBEDDED SPECIMENS

Francisco Carvalho¹, Michelle Todd¹, Christopher Halsey², Kevin Lahmers¹, Tessa LeCuyer¹, Francisco Uzal³

¹Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, USA, ²Virginia Department of agriculture and Consumer Services, Office of Laboratory Services, Richmond, VA, USA, ³California Animal Health and Food Safety Laboratory, San Bernardino, CA, USA

Bovine respiratory disease complex (BRDC) is a major cause of economic losses to the cattle industry in the United States. While molecular tests for BRDC pathogens are available for fresh tissues in numerous laboratories, the use of formalin fixed paraffin embedded (FFPE) lung samples has not been fully investigated. Dead calves (N=27) between 1-month and twelve-months of age with a presumptive diagnosis of pneumonia were received for necropsy for a period of one year. Samples of pneumonic lung of each animal were collected for aerobic culture and routine histopathology. The PCR panel included *Mycoplasma bovis*, bovine respiratory syncytial virus (BRSV), parainfluenza-3 virus (PI-3), bovine viral diarrhea virus (BVD), bovine herpesvirus 1 (BHV-1), bovine coronavirus (BCoV) and influenza D virus (FluD). The most commonly detected agent by PCR was *Mycoplasma bovis* (n=17, 63%), alone or in combination with BCoV (n=8, 30%), BRSV (n=3, 11%) and BVD (n=2, 7%). In one animal, Flu D virus was detected, together with *M. bovis* and BVD virus. In cases in which *M. bovis* was cultured, this agent was also detected with PCR. The most common bacteria isolated on aerobic culture was *Pasteurella multocida* (n=12, 44%), alone or in combination with *Trueperella pyogenes*, *Mannheimia hemolytica* or *Histophilus somni*. The use of FFPE samples resulted in reasonable detection of agents associated with the BRDC. The implementation of this technique greatly expands the uses of FFPE for retrospective epidemiologic studies and facilitates international research collaborations using FFPE specimen banks.

Monday, October 30

11:25 AM – 11:35 AM

SYSTEMIC CHAETOMIUM GLOBOSUM INFECTION IN A COW

Elizabeth Majette, Denae LoBato

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

A 23-month-old polled Hereford cow was euthanized after an eight-month history of weight loss. At necropsy, most mesenteric lymph nodes were firm to hard and enlarged from 6 to 18 cm in diameter; there were few similar nodules throughout the walls of the forestomachs, abomasum, and intestine. There was extensive mineralization of the endocardium of all heart chambers, the endothelium of large elastic arteries, and the pulmonary interstitium. Histologically, there was marked extensive granulomatous and eosinophilic mesenteric lymphadenitis, ruminitis, abomasitis, jejunitis, and pneumonia with bulbous, branching hyphae surrounded by Splendore-Hoeppli-like material. Acid

fast stains were negative for mycobacteria. Panfungal PCR performed on formalin fixed, paraffin embedded lymphoid tissue yielded forward and reverse sequences with 98.42% and 96.14% similarity to several *Chaetomium globosum* strains; fungal culture was not possible. *C. globosum* is a saprophytic ascomycete which has been rarely identified in cutaneous and systemic infections in humans and a dog, but it has not been previously reported in cattle. The distribution of lesions in this case suggests that the fungus entered via the alimentary tract. Dissemination to adjacent lymph nodes with chronic granulomatous inflammation likely led to production of PTHrp and subsequent extensive endothelial and endocardial mineralization. *C. globosum* was an unexpected finding in this case, which highlights the expanding role of molecular diagnostic methods in the identification of fungal pathogens.

Monday, October 30

11:35 AM – 11:45 AM

DECOMPOSED FORENSIC CASES: NO PROBLEM... CAUSE AND MANNER OF DEATH COULD STILL BE DETERMINED!

Nanny Wenzlow

LSU-LADDL, Baton Rouge, LA, USA

Animal bodies in advanced stages of soft tissue decomposition are infrequently submitted for forensic necropsies and represent a diagnostic challenge. Pathologists should not be discouraged, as in most cases, a thorough examination can still reveal the cause and manner of death, confirming, or refuting suspected acts of cruelty.

Necropsy findings of decomposed bodies are documented for the following cases.

Case 1: An old intact bichon-poodle bitch was exhumed three weeks postmortem and submitted for forensic necropsy with the suspicion of acts of bestiality. Postmortem examination refuted acts of sexual abuse, instead revealed neglect with severely matted fur, emaciation with serous atrophy of fat, chronic mandibular fractures and symphyseal luxation, chronic cystitis with very large uroliths, gastric foreign bodies including fentanyl laced pennies and toxic levels of Zinc. The cause of death was septic shock with fentanyl and zinc toxicity; the manner of death was non-accidental killing.

Case 2: A male neutered Yorkshire terrier was exhumed four weeks postmortem and partially dissected by a shelter veterinarian before being submitted for forensic necropsy. Gross findings included a very small perforating trauma to the skull with associated hemorrhages in the calvarium, on the associated meninges and the brain. The cause of death was perforating trauma to the skull; the manner of death was non-accidental killing.

Case 3: Three dachshunds in identical state of decomposition were exhumed three weeks postmortem and acts of cruelty were suspected. Postmortem findings revealed no pathology but crystallized clotted blood in the hearts of all three dogs, suggesting euthanasia.

Monday, October 30
11:45 AM – 11:55 AM

EQUINE INTERVERTEBRAL DISC DISEASE WITHIN THE CERVICAL VERTEBRAL COLUMN OF 25 HORSES FROM 2017-2022

Rebecca Ruby¹, Kindra Orr², Katherine Garrett², Steve Reed², Jennifer Janes¹

¹University of Kentucky Veterinary Diagnostic Laboratory, Lexington, KY, USA, ²Rood and Riddle Equine Hospital, Lexington, KY, USA

Background: Equine ataxia is a common clinical presentation with frequent neurolocalization to the cervical spinal cord. Compressive myelopathy can be caused by a variety of conditions; the most common is cervical vertebral stenotic myelopathy. Equine intervertebral disc disease is rarely reported and may result in pain or neurologic dysfunction.

Objective: The objective was to describe the pathology observed in intervertebral discs within the cervical spinal column in relation to clinical signs and evidence of spinal cord compression.

Methods: A retrospective database search of the electronic records system at the University of Kentucky Veterinary Diagnostic Laboratory identified 25 horses with intervertebral disc disease from 2017-2022.

Results: Horses ranged from 1 to 31 years of age (mean 11.3 years). Breeds included 12 Thoroughbreds, 6 American Saddlebreds, 3 Quarter Horses, and 2 Warmbloods. In all cases abnormal disc material was observed at macroscopic examination. Changes ranged from yellow discoloration and/or fibrillation to loss of disc material and disc herniation. One case of discospondylitis was identified. In 12 cases, evidence of spinal cord compression at the site of disc disease was observed microscopically, characterized by Wallerian degeneration. The history provided indicated neurologic dysfunction present in 17 of 25 horses. Two cases were reported as unable to stand, 1 case was a sudden death and in 5 cases no history was provided. In 3 cases pain was also reported as a clinical sign.

Conclusion: Intervertebral disc disease in horses may occur in any age of horse and may cause neurologic dysfunction or neck pain.

18: FIRST MERKEL CELL CARCINOMA IN A 9-YEAR-OLD MALE CAT IN THE CARIBBEAN

Talía Maldonado Payano^{1,2}, Juan Bisso^{1,3}

¹Universidad Nacional Pedro Henríquez Ureña, Santo Domingo, Dominican Republic,

²PatovetRD, Santo Domingo, Dominican Republic, ³Centro de Especialidades Médicas Veterinarias, Santo Domingo, Dominican Republic

Introduction

Merkel cell tumor is a rare entity in cats, with less than 15 cases reported worldwide. It has been reported in canines and humans, with a benign behavior in dogs and a malignant behavior in humans and cats. There is not much research on cats since it is a

rare diagnosis that must be confirmed with immunohistochemistry for neuroendocrine cells.

Case

A 9-year-old neutered male cat presents with three large, irregularly raised, reddish masses measuring 3 cm in diameter on his left side, which occupies an area of 19 x 14cm. Cytology was inconclusive, surgery for complete resection of the mass was performed and sent to histopathology. The cat died days after surgery, with pulmonary metastasis. Histopathology showed round cells infiltrating the dermis and deep subcutaneous tissue, invading the muscular layer with dense cellular nests. The lesion was circumscribed, non-encapsulated with clean margins. Cells vary from small round cells with scant cytoplasm to polyhedral with clear cytoplasm. Nuclei have multiple nucleoli, with fine granular chromatin. There are areas of central necrosis. The mitotic count and apoptotic bodies were high. The diagnosis was Merkel cell carcinoma. The neoplasm was positive for CK20 and synaptophysin in immunomarkers, confirming the diagnosis.

Discussion

Merkel cell tumors have not been diagnosed in cats in the Caribbean, to the author's knowledge. All reported cases have been in non-tropical countries. That's why we believe reporting would be beneficial so tropical veterinarians include Merkel cell tumors in their differential diagnosis for irregular, raised skin lesions in cats.

19: A RARE CASE OF SCLEROSING ENCAPSULATING PERITONITIS ASSOCIATED WITH PANCREATIC DUCTAL ADENOCARCINOMA IN A CAT

Sanggu Kim¹, Chaelin Kim², Sungin Lee³, Soochong Kim¹

¹Laboratory of Veterinary Pathology, College of Veterinary Medicine, Chungbuk National University, Cheongju, Republic of Korea, ²Laboratory of Veterinary Internal Medicine, College of Veterinary Medicine, Chungbuk National University, Cheongju, Republic of Korea, ³Laboratory of Veterinary Surgery, College of Veterinary Medicine, Chungbuk National University, Cheongju, Republic of Korea

Background

Sclerosing encapsulating peritonitis (SEP) is a rare irreversible peritoneal sclerosis characterized by the formation of thick sheets of fibrous tissue accompanied by inflammatory aggregates which may lead to intestinal obstruction. A few cases have been linked to canine and human malignancies, but histopathological characterization is yet to be determined. Here, we present the first case of SEP with pancreatic ductal adenocarcinoma (PDAC) in a cat.

Case Report

The cat was presented with complaints of lethargy, hyporexia, and recurrent ascites of unknown origins. Physical examination revealed 5% dehydration and abdominal

distension. Radiography showed decreased serosal detail in the middle abdomen. During necropsy, the large and small intestine showed adhesive sclerosis with numerous disseminated seeding nodules ranging from 1 to 5 mm in diameter on the surface of the serosa. Additionally, a multi-lobulated tumor mass, 10 cm x 10 cm in size, was tightly attached to the stomach and intestine. Microscopically, the mass was composed of extensive adipose tissue, locally extensive inflammatory infiltrates, fibrous connective tissue, and invasive proliferating small to medium-sized irregular glands lined by anaplastic ductal epithelial cells. Furthermore, these irregular glands were surrounded by abundant desmoplastic stroma with metastasis to multifocal sites including the liver, stomach, peritoneum, mesentery, mesenteric lymph node, urinary bladder, and lung. Finally, immunohistochemistry against pan-cytokeratin revealed positive expression in normal pancreatic ducts as well as neoplastic glands, confirming the ductal origin of this tumor.

Conclusion

We demonstrated for the first time histopathological features of SEP with PDAC in a cat.

20: CALCITONIN RECEPTOR-STIMULATING PEPTIDE 1-DERIVED AMYLOID DEPOSITION IN A FELINE C-CELL CARCINOMA.

Tomoaki Murakami¹, Natsumi Kobayashi¹, Susumu Iwaide¹, Yoshiyuki Itoh¹, Miki Hisada¹, Takeshi Izawa², Mitsuru Kuwamura²

¹Tokyo University of Agriculture and Technology, Tokyo, Japan, ²Osaka Metropolitan University, Osaka, Japan

Background and Objective: More than 80% of human C-cell carcinomas show amyloid deposition, and calcitonin has been identified as a precursor protein. We veterinary pathologists know that animal C-cell tumors are sporadically associated with amyloid deposition, but the amyloid precursor protein is unknown. This study aims to identify the amyloid precursor protein in a feline C-cell carcinoma.

Materials and Methods: A case of C-cell carcinoma with amyloid deposition, removed from a 15-year-old neutered male American shorthair cat in 2006, was used for this study. Since the formalin-fixed wet samples and paraffin blocks had been disposed of, only a few Congo red-stained sections were available and subjected to the following analyses: transmission electron microscopy of the amyloid lesion; LC/MS/MS of microdissected amyloid deposits; anti-calcitonin receptor-stimulating peptide 1 (CRSP1) immunohistochemistry.

Results and Discussion: Ultrastructurally, an accumulation of fine fibrils, approximately 9 nm in diameter, was observed. LC/MS/MS detected CRSP1 at prominently high values. No previously reported amyloid precursor proteins, such as serum amyloid A and calcitonin, were detected. Amyloid deposits were positive for CRSP1 by immunohistochemistry. Based on these results, we conclude that CRSP1 is the amyloid precursor protein. Interestingly, despite the same underlying tumor, different protein forms amyloid between cats and humans. LC/MS/MS detected a substantial amount of Leu93-Lys116-derived peptides, a partial region within mature CRSP1, and *in silico* analysis predicted an amyloidogenic propensity of this region, suggesting that mature

CRSP1 is involved in the amyloid formation. CRSP1, over-expressed in C-cell carcinoma, is expected to acquire amyloidogenicity through proteolysis.

21: CANDIDA TROPICALIS EMBEDDED IN A PULMONARY ARTERY MURAL THROMBUS IN A PUPPY WITH PDA

Alexis Carpenter¹, Alix Saavedra², Kelly Wigger², Tamara Gull¹, Rosalie Ierardi¹

¹University of Missouri Veterinary Medical Diagnostic Laboratory, Columbia, MO, USA,

²University of Missouri Veterinary Health Center, Columbia, MO, USA

Background: A 6-month-old intact male miniature Australian Shepherd puppy was presented to the Cardiology Service of the University of Missouri (MU) Veterinary Health Center for evaluation of a patent ductus arteriosus (PDA) two months after treatment for parvoviral enteritis. On presentation, the dog was febrile and had a grade 5/6 left axillary continuous murmur. Echocardiogram revealed a high velocity left-to-right shunting PDA with marked cardiomegaly and an oscillating lesion within the pulmonary artery. Blood cultures grew *Candida tropicalis*. One month later, the patient was hypothermic, weak, had multiple episodes of collapse, and had elevated liver enzymes. Humane euthanasia was elected due to grave prognosis and lack of response to therapy. The patient was submitted to the MU Veterinary Medical Diagnostic Laboratory for necropsy.

Results: Postmortem examination revealed a 4 x 1 mm cylindrical, dark red, friable structure (thrombus) attached to the wall of the pulmonary artery. The thrombus protruded into the pulmonary valve and into a 1 to 2 mm diameter opening between the pulmonary artery and the aorta (PDA). Microscopically, the thrombus was composed of fibrin with numerous 5-7 µm diameter basophilic to magenta yeasts and rare pseudohyphae consistent with *Candida* spp. Pulmonary intra-alveolar hemosiderophages and chronic passive congestion of the liver supported a diagnosis of heart failure.

Conclusion: This is a report of a rare instance of fatal thrombotic candidemia complicated by concurrent PDA and a history of parvoviral enteritis.

22: HISTOPATHOLOGIC AND ULTRASTRUCTURAL FEATURES OF NATURAL OAK (QUERCUS SPP) TOXICOSIS AT TWO COLORADO CATTLE RANCHES

Jorge Mendieta Calle¹, Kelly Hughes¹, Elizabeth Howerth², Paula Schaffer¹

¹Colorado State University, Fort Collins, CO, USA, ²University of Georgia, Athens, GA, USA

Natural oak toxicosis is a phenomenon sporadically reported in the United States due to consumption of any part of Oak trees (*Quercus spp*). Ruminants, mainly cattle, are most susceptible to oak toxicosis. Toxicity is attributed to degradation of the hydrolysable tannins into absorbable low molecular weight metabolites that are postulated to bind and damage epithelial and endothelial cells by unknown mechanisms. The clinical manifestations of acute toxicosis are non-specific or broadly suggestive of acute kidney injury. This report documents the clinical, gross, histopathological, and novel ultrastructural features of natural acute oak nephrotoxicity in three beef calves at two ranches in Colorado. Gross postmortem findings included perirenal edema, renomegaly

with white cortical streaks, and hemorrhagic gastroenteritis. Histopathology of the kidneys showed severe tubular epithelial necrosis with intratubular hemorrhage and cellular casts. The ultrastructural findings revealed extensive necrosis of proximal and distal convoluted tubules. In addition to tubular epithelial necrosis with intact basement membranes, there was ultrastructural evidence of glomerular and interstitial endothelial injury and necrosis. The ultrastructural details of toxic nephropathy and vasculopathy induced by oak metabolites in natural cases of bovine oak toxicosis have not been previously described.

23: UNDIFFERENTIATED PLEOMORPHIC SARCOMA WITH METASTASIS IN A RHESUS MACAQUE (MACACA MULATTA)

Ahmad Saied¹, Monica Shroyer², Alexandra Blaney³, Avelina Rodgers¹, Victoria Shephard¹, Cecily Midkiff¹, Christopher Maybe¹

¹Tulane National Primate Research Center, Tulane University, Covington, LA, USA,

²Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, OR, USA, ³Massachusetts General Hospital, Boston, MA, USA

Undifferentiated pleomorphic sarcomas (UPS) are high grade neoplasms with no specific line of differentiation. These neoplasms are rare in non-human primates and in humans. The diagnosis is typically made by exclusion, aided by a contingent of immunohistochemical (IHC) tests. In this article, we report a rare case of an undifferentiated pleomorphic sarcoma with metastasis to the lung and inguinal lymph node in a Rhesus macaque (*Macaca mulatta*). A 7-year-old, female Chinese origin Rhesus macaque presented with a mass on the left caudal flank to the breeding colony hospital at the Tulane National Primate Research Center (TNPRC). A biopsy was taken, and the mass excised. The mass grew aggressively within a few days. The biopsy revealed that the mass is a high grade pleomorphic soft tissue sarcoma. Euthanasia was elected due to poor prognosis. On necropsy, the mass measured 7.5 X 6 X 5 cm. The left inguinal lymph node was markedly enlarged measuring 5.3 X 2.7 X 5.7 cm. The lung had several metastatic neoplastic nodules ranging from 0.5 cm to 1.2 cm in diameter. Histologically, the neoplasm is composed of pleomorphic spindle, and polygonal neoplastic cells arranged in streams, bundles, and sheets. A cadre of immunohistochemical tests were utilized to fully characterize the neoplasm. IHC tests used include: CD34, desmin, epithelial membrane antigen, cytokeratin, S100 protein, alpha smooth muscle actin, CD31, CD68, myogenin, and caldesmon. This is a unique case of UPS with metastasis to the lung and inguinal lymph node in a Rhesus macaque (*Macaca mulatta*).

24: MANDIBULAR PERIPHERAL NERVE SHEATH TUMOR IN A 7-YEAR-OLD SIBERIAN HUSKY

Randi Gold¹, Cynthia Bell²

¹Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, TX, USA,

²Specialty Oral Pathology for Animals, Geneseo, IL, USA

Peripheral nerve sheath tumors originate from Schwann cells, perineural cells, or a combination of both. As a group, they can be broken up into four subtypes (i.e., schwannoma, neurofibroma, perineuroma, and the malignant peripheral nerve sheath

tumor). In the dog, they develop most often in the cranial nerves, brachial plexus, or spinal roots. The schwannoma is the most common subtype in dogs but has also been described in cattle, horses, and other domestic species. A 7-year-old, male castrated, Siberian husky dog presented to a veterinarian for a fast-growing, painful, mandibular mass encompassing teeth 401-403 that was suspected to be infiltrating the alveolar bone. Therapy with amoxicillin-clavulanate and prednisone was not beneficial. A biopsy of the mass was collected for histology and sent to the Texas A&M Veterinary Medical Diagnostic Laboratory for evaluation. Histopathology of the section revealed a spindle cell neoplasm that often palisaded, forming short interlacing bundles, streams, rare whorls, and multifocally produced Verocay bodies from the multiple herringbone cellular pattern. Mitotic figures averaged 3 per 40X field. Multifocally throughout were single enlarged vacuolated cells (suspected ganglion cells). Immunohistochemical staining for claudin-1, laminin, neurofilament, glial fibrillary acidic protein, and a melanoma diagnostic panel containing antibodies against melan-A, PNL2, tyrosinase-related protein 1, and tyrosinase-related protein 2 were all negative. Results of the immunohistochemistry and visual appearance of the cells lead to a presumptive diagnosis of a schwannoma. Peripheral nerve sheath tumors should be included as a differential for spindle cell tumors of the oral cavity in dogs.

25: RHABDOMYOSARCOMA IN THE ORAL CAVITY OF AN 8-MONTH-OLD DOG

Alexandra Ford, Brianne Taylor

Oklahoma State University, Stillwater, OK, USA

An 8-month-old, female Bull Terrier dog had an oral mass associated with the left maxillary gingiva for approximately one month. Initial diagnosis by the referring veterinarian was inflamed granulation tissue secondary to a foreign body. The mass was removed and histopathology was not pursued at that time. Within weeks, the mass returned and several fragments were submitted to the Oklahoma Animal Disease Diagnostic Laboratory for histopathology. Microscopically, sections comprised numerous round to polygonal cells arranged in small packets and interlacing streams variably separated by a preexisting fibrous stroma. Neoplastic cells had indistinct cell borders, moderate amounts of eosinophilic cytoplasm, a round to irregular nucleus, and one to two nucleoli. Anisokaryosis and anisocytosis were marked. Mitoses were 37 in 2.37mm^2 and occasionally bizarre. Mixed inflammatory cells and small areas of necrosis were scattered throughout the neoplasm. Neoplastic cells were diffusely and strongly immunoreactive to vimentin and desmin and showed no immunoreactivity to Melan A, PNL2, S-100, pancytokeratin, MUM1, or CD18. While awaiting immunohistochemistry results the dog was euthanized as the mass continued growing resulting in marked facial swelling and severely enlarged mandibular lymph nodes, suggestive of metastases. The histologic features and immunohistochemistry results were most consistent with a rhabdomyosarcoma. Rhabdomyosarcomas have four distinct morphologic types with variable skeletal muscle differentiation. In this case, histologic features were most consistent with the solid variant of alveolar rhabdomyosarcoma. This case illustrates the importance of including rhabdomyosarcoma as a differential diagnosis for a poorly differentiated neoplasm affecting the head of a young dog.

26: JUVENILE DILATED CARDIOMYOPATHY IN A TOY ENGLISH (MANCHESTER) TERRIER DOG – A CASE REPORT

Smitha Rose Georgy, Ron Slocombe

Melbourne Veterinary School, The University of Melbourne, Melbourne, Australia

A 14-month-old, male, toy English (Manchester) terrier was noted to be lethargic, panting, and quiet although eating and drinking normally, 24 hours before death. On gross examination, the heart appeared enlarged and rounded with pale streaks in the right ventricular wall. The liver was enlarged, with round borders, congested and had fragile fibrin tags on the capsular surfaces. The gall bladder wall was diffusely oedematous. The animal was a cryptorchid. Other organs were within normal limits. On histologic examination of the heart, affecting approximately 15% of the myocardium, there were randomly scattered areas of acute myocardial degeneration and necrosis, characterized by hyper-eosinophilic fibers with pyknotic nuclei and loss of cytoplasmic striations. In a few small areas, there was replacement of the myocardium with mature fibrous connective tissue and small infiltrates of adipocytes.

Tissue samples were sent to the University of Minnesota for Juvenile Dilated Cardiomyopathy genetic testing that screens for a specific DNA mutation that has been discovered in a cardiac potassium channel gene. The results confirmed D/D genotype as homozygous recessive for the defect associated with dilated cardiomyopathy of toy English terriers. This gene defect is related to regulation of the cardiac potassium channel and is inherited as an autosomal recessive trait. In this condition, cryptorchidism commonly occurs concurrently. Sudden death was typically associated with acute myodegeneration and necrosis of the ventricles. In Australia, toy English terriers are an uncommon breed and in-breeding in Australia's small population of this breed is likely to be even more so than overseas.

27: THE GLOBAL HEALTH PATHOLOGY NETWORK (GHPN) AND UNIVERSITY OF VETERINARY AND ANIMAL SCIENCE (UVAS), LAHORE, PAKISTAN COLLABORATIVE NODE: A FRAMEWORK FOR SUSTAINABILITY IN INTERNATIONAL HYBRID DIAGNOSTIC PATHOLOGY TRAINING

Sarah Corner¹, Ishtiaq Ahmed², Tanja Zabka³, Derron (Tony) Alves⁴, Dalen Agnew⁵, Kathy Gabrielson⁶, Corrie Brown⁷

¹Michigan State University College of Veterinary Medicine, Veterinary Diagnostic Laboratory, Lansing, MI, USA, ²University of Veterinary and Animal Sciences, Lahore, Pakistan, ³Genentech Inc., South San Francisco, CA, USA, ⁴Infectious Disease Pathogenesis Section, Comparative Medicine Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA, ⁵Michigan State University College of Veterinary Medicine, Dept. of Pathobiology and Diagnostic Investigation, East Lansing, MI, USA, ⁶Johns Hopkins University, School of Medicine and Bloomberg School of Public Health, Baltimore, MD, USA, ⁷LifeStock International, Athens, GA, USA

The GHPN, which is under the umbrella of the Davis-Thompson Foundation, conducts workshops in resource-constrained settings focused on enhancing veterinary diagnostic abilities using interactive educational modalities and adult learning principles. Since

2017, the GHPN has established a collaborative node with Pakistani veterinary colleagues at UVAS. Both in-person and remote sessions have been very successful, focusing on recognition, reporting, and control of region-specific diseases having economic and public health implications. Workshop and telepathology objectives emphasize basic mechanisms and pathogenesis, necropsy and proper sample collection, lesion descriptions, and generation of morphologic and differential diagnoses based on gross examinations. Participants include field veterinarians, pathologists, para-veterinary workers, and laboratory professionals from universities, government, and the private sector. Discussion facilitates opportunities to strengthen connections – in-country, and internationally. The telepathology sessions include rotating presenters from Pakistan and the USA. Basic equipment requirements for these virtual sessions include a free web-based meeting platform and a stable internet connection. For evaluating microscopic slides in real time, a light microscope and inexpensive universal eyepiece camera (e.g., Accu-Cam 300) are used. Two key factors are necessary for sustainable growth of these programs: (1) Motivated partners committed to improving in-country capacity; and (2) stable funding sources for in-country workshops. This example of a strong node in international pathology training can be used as a framework for veterinary educators seeking to engage in similar programs around the world.

28: A CASE OF SPINAL HEMANGIOBLASTOMA IN A FRENCH BULLDOG

Ji-Hang Yin¹, Luan Henker¹, Larissa Castro², Han Sun³

¹Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA, ²Department of Clinical Sciences, Radiology service, College of Veterinary Medicine, Auburn University, Auburn, AL, USA, ³Department of Clinical Sciences, Neurology and Neurosurgery service, College of Veterinary Medicine, Auburn University, Auburn, AL, USA

Hemangioblastoma is a benign, slow-growing, vascular neoplasm with unique neoplastic components and is rare in dogs. Canine hemangioblastoma arises spontaneously in the central nervous system and appears to have a predilection for the spinal cord. Seven canine cases have been reported to date with five cases in the spinal cord, one in the rostral cerebrum, and one in the brainstem. Treatments include surgical resection and/or a combination of chemotherapy and radiation therapy; however, the prognosis usually varies and is largely unknown.

A 10-year-old female spayed French Bulldog presented to Auburn University Veterinary Hospital for non-ambulatory tetraparesis due to a recurrent spinal mass. Magnetic resonance imaging revealed a well-circumscribed, ovoid, T2-weighted hyperintense, T1-weighted hyperintense, 8-mm x 14-mm, intramedullary mass that occupied approximately 80% of the spinal canal at the 7th cervical to 1st thoracic vertebrae. An estimated half of the intramedullary mass was surgically debulked with C7-T1 dorsal laminectomy and was submitted for histopathology. Histologically, the spinal mass was composed of an abundance of haphazardly arranged capillaries lined by plump endothelial cells and separated by streams and bundles of pleomorphic spindle/stromal cells. Some spindle/stromal cells contained small vacuoles. Immunohistochemically, the lining endothelial cells were strongly and diffusely positive for CD31 antigen and

negative for NSE, GFAP, and S-100. The spindle/stromal cells were strongly and variably immunolabeled with NSE, GFAP, and S-00 antigen and negative for CD31. These findings were consistent with a diagnosis of canine intramedullary hemangioblastoma.

29: IDENTIFICATION OF LITHOSTATHINE-DERIVED AMYLOID IN FELINE PANCREATIC EXOCRINE TUMORS

Niki Sedghi Masoud¹, Susumu Iwaide¹, Yoshiyuki Itoh², Miki Hisada², Tomoyuki Harada³, Noboru Machida⁴, Tomoaki Murakami¹

¹Laboratory of Veterinary Toxicology, Tokyo University of Agriculture and Technology, Tokyo, Japan, ²Smart-Core-Facility Promotion Organization, Tokyo University of Agriculture and Technology, Tokyo, Japan, ³FUJIFILM VET Systems Co., Ltd., Tokyo, Japan, ⁴Laboratory of Veterinary Clinical Oncology, Tokyo University of Agriculture and Technology, Tokyo, Japan

Background: Amyloid deposition in endocrine and exocrine pancreatic tumors is well-studied in humans and the involvement of insulin, amylin, somatostatin, and glucagon as precursor proteins have been extensively studied. However, although amylin and insulin have been identified as amyloid precursor proteins in animals, the extent of amyloid deposition and the identity of precursor proteins in pancreatic tumors remain largely unknown. This study aimed to investigate the presence of amyloid deposition in feline and canine pancreatic exocrine tumors and identify the precursor proteins.

Methods: Paraffin-embedded tissues of 24 cats and 8 dogs with pancreatic exocrine tumors were applied to hematoxylin and eosin (HE) staining and Congo red staining. Congo red-positive regions were dissected from tissue sections of the pancreas, and LC/MS/MS was performed on one of the feline pancreatic exocrine tumors. Immunohistochemistry was performed using anti-lithostathine antibody.

Results: Histologically, amyloid deposits were observed in 8 out of 24 cats (33.3%). In dogs, eosinophilic homogenous deposits were observed in some cases but were determined not to be amyloid because of Congo red negativity. In cats, amyloid deposits were mainly in the corpora amylacea in the lumen and rarely in the interstitium. Mass spectrometry detected lithostathine as a major component of amyloid deposits. Immunohistochemistry results were consistent with proteomic analysis, which showed amyloid deposits that were positive for lithostathine.

Conclusion: This study highlights the potential role of lithostathine as a novel amyloid precursor protein in animals and provides new insights into the pathogenesis of amyloid deposition in pancreatic tumors.

30: HISTOMORPHOLOGIC AND MOLECULAR CHARACTERIZATION OF CANINE DISCOSPONDYLITIS

Carmen Smith¹, Manigandan Lejeune¹, Brittany Cronk¹, Daniel Rissi², Andrew Miller¹, James Hammond³, Gregg Kortz⁴, Rebecca Young¹, Holly White¹, Elena Demeter¹

¹Cornell University College of Veterinary Medicine, Ithaca, NY, USA, ²University of Georgia, Athens, GA, USA, ³Pieper Memorial Veterinary Center, Middletown, CT, USA, ⁴VCA Sacramento Veterinary Referral Center, Sacramento, CA, USA

Background: Canine discospondylitis is commonly attributed to *Staphylococcus spp.*, *Escherichia coli*, and *Brucella canis* bacteremia. Fungal discospondylitis is less common and often results from systemic aspergillosis.

Objective: To characterize the histomorphologic features and etiology of canine discospondylitis.

Methods: Eighteen archived cases of discospondylitis in dogs submitted to the New York State Animal Health Diagnostic Center and University of Georgia over the past fifteen years were identified. Slides were stained with Steiner, Gram, and Grocott-Gömöri's methenamine silver (GMS) stains. PCR for 16S (bacterial) and ITS-2 (fungal) was performed on formalin-fixed paraffin-embedded (FFPE) scrolls. MinION Nanopore and Sanger sequencing were performed, and Sanger sequences were analyzed using NCBI BLAST.

Results: Histomorphologic features associated with fungal discospondylitis included granulomatous inflammation, multinucleated giant cells, vasculitis, and fungal organisms identified with H&E and GMS stains. Features not significantly associated with a bacterial or fungal etiologic agent included the degree of inflammation, presence of necrosis, hemorrhage, bone remodeling, and the anatomic extent of the inflammation. Preliminary sequencing results identified Streptococcal, Staphylococcal, and other bacterial agents, but fungal identification was nonspecific.

Conclusions: The histomorphologic features of canine discospondylitis, including the predominant leukocyte population, multinucleated giant cells, and vasculitis, can inform the most useful molecular diagnostic methods to identify the causative agent. Sequencing and analysis for bacteria is more successful when targeted at specific agents, and fungal environmental contamination complicates interpretation of fungal sequencing results from genome sequencing data. To identify infectious agents from decalcified FFPE samples in archived cases, histopathology must accompany Sanger and MinION Nanopore sequencing.

31: VETERINARY FORENSIC INVESTIGATION OF DOG AND CAT ATTACKS IN WILD ANIMALS BY INJURY PATTERN ANALYSIS

Ya-Wen Yang, Wei-Hsiang Huang, Fu-Hua Yang, Ann-Nee Lee, Chia-Lin Hsiou, Yi-Hsiang Huang, Chia-Wei Lin, Ching-Yun Hsu

Graduate Institute of Molecular and Comparative Pathobiology, School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan

Background: Feral dogs and cats are increasingly preying on native wildlife, posing a significant threat to the ecosystem in Taiwan. The adverse impact of dog and cat attacks on wild animals is evident, as it remains the primary cause for their admission to rescue centers. However, identifying the attacking species relies solely on clinician

experience, lacking validated methods. Studies have highlighted the effectiveness of DNA analysis of saliva from wound swabs in accurately determining the species responsible. Furthermore, analysis of injury patterns can serve as an additional means of identification.

Objective: This study aims to examine the injury patterns resulting from dog and cat bites in various wildlife species.

Methods: Forensic necropsies and wound swabs were conducted on animals exhibiting signs of attacks by other animals. The data collected encompassed signalments, attack history, anatomical locations, and injury patterns.

Results: Among 44 carcasses collected that tested positive for dog or cat DNA, thirty-five were attributed to dog DNA, primarily affecting Formosan muntjac, while nine were attributed to cat DNA, primarily affecting birds. Cat bites display a higher occurrence of lacerations and bruises on the dorsal and lateral flanks, and dog-inflicted injuries were characterized by puncture wounds and lacerations, predominantly found in the hip, tail, and perineum regions of Formosan muntjacs.

Conclusions: The integration of injury patterns and DNA analysis facilitates the determination of trauma causes in wildlife. This study provides valuable insights into injury patterns observed in various endemic mammals and birds in Taiwan.

32: IMMUNOHISTOCHEMICAL DETECTION OF CORTICOTROPIN-RELEASING FACTOR (CRF) FOR EVALUATING AGONAL STRESS IN CATS

Chia-Wei Lin, Fu-Hua Yang, Pei-Wen Liao, Yi-Hsiang Huang, Ann Nee Lee, Chia-Lin Hsiou, Chih-Chin Hsu, Wei-Hsiang Huang

Graduate Institute of Molecular and Comparative Pathobiology, School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan

Background:

The duration of the death process and agonal stress exhibits variation, which may indicate the degree of animal suffering. Researchers have explored immunochemical markers to estimate the agonal period, but a well-established marker in veterinary forensics is still needed. Corticotropin-releasing factor (CRF), a critical regulator of the hypothalamic-pituitary-adrenal axis, is considered a potential marker for assessing agonal stress.

Objective:

This study aims to establish an immunohistochemical marker by analyzing CRF expression in the formalin-fixed paraffin-embedded feline brain tissues to correlate to the agonal stress.

Methods:

Feline necropsy cases were grouped by the body condition score and the agonal duration (determined based on the cause of death and significant conditions). Immunohistochemistry for CRF expression was conducted in feline brain tissues, and the positive signals were quantified.

Results:

A total of 44 cases were collected and analyzed, revealing varying patterns of CRF immunoreactivity across multiple brain regions with various patterns, including the paraventricular nucleus, hypothalamus, thalamus, cerebral cortex, white matter, and hippocampus. Comparatively, the refrigerated group exhibited significantly greater CRF immunoreactivity than the frozen group. The emaciated group displayed significantly higher immunoreactivity in comparison to the non-emaciated group. The short agony group demonstrated significantly elevated CRF immunoreactivity compared to the prolonged agony group.

Conclusion:

The results provide evidence supporting the hypothesis that an extended duration of suffering, such as starvation or cachexia, is associated with heightened stress and secretion of CRF. These findings propose CRF as a promising marker for estimating the duration of agonal stress in veterinary forensics.

33: VALIDATION OF RAS Q61R-SPECIFIC RABBIT MONOCLONAL ANTIBODY (SP174) FOR CONFIRMATION OF CANINE ACANTHOMATOUS AMELOBLASTOMA BY IMMUNOHISTOCHEMICAL STAINING

Magdalena Marcinczyk, Gerald Duhamel, Santiago Peralta
Cornell University College of Veterinary Medicine, Ithaca, NY, USA

Differentiating canine acanthomatous ameloblastoma (CAA) from oral squamous cell carcinoma (OSCC) based on routine histopathology can be challenging. We have previously shown that more than 95% of CAAs (n=21) harbor an HRAS Q61R somatic mutation, while OSCCs carry either wild-type (WT) alleles or other somatic mutations (e.g., BRAF V595E, HRAS Q61L). Given that HRAS Q61R mutations are highly prevalent in CAA, we hypothesized that RAS Q61R-specific rabbit monoclonal antibody (Mab-SP174) may be a useful tool for confirmation of CAA by immunohistochemical (IHC) staining. In the present study, we assessed IHC staining of archived formalin-fixed and paraffin-embedded biopsy samples obtained between 2013 and 2021 with an initial diagnosis of CAA and known to harbor an HRAS Q61R mutation (n=17). Negative controls consisted of HRAS Q61R mutation negative OSCC (n= 6) with either a known HRAS Q61L mutation (n=1), BRAF V595E mutation (n=2), or WT corresponding alleles (n=3) using RAS Q61R-specific rabbit Mab and an automated IHC stainer (Leica Bond-Max). We found that all 17 CAAs showed diffuse and strong membranous RAS Q61R immunoreactivity (100% specificity), while none of the OSCCs showed immunoreactivity (100% specificity). Critical IHC parameters included: (i) heat epitope retrieval (AR9640, Bond epitope retrieval solution 2) for 30-40 minutes, (ii) 1:80 or 1:100 primary antibody

dilution for 60 minutes, and (iii) Bound Polymer Refine Detection (DS9800, peroxide block, post primary polymer reagent, DAB chromogen and hematoxylin counterstain) for 30 minutes. The data supports RAS Q61R-specific rabbit Mab for diagnostic IHC confirmation of CAA and ruling out OSCC in dogs.

34: HEMANGIOSARCOMA OF BONE WITH HEPATIC & PULMONARY METASTASIS IN A RHESUS MACAQUE (MACACA MULATTA)

Ahmad Saied, Christopher Brown, Peter Didier

Tulane National Primate Research Center, Tulane University, Covington, LA, USA

Hemangiosarcoma is a malignant neoplasm of vascular endothelial cell origin. They are rare in non-human primates and humans. Approximately 5-year-old, female Indian origin Rhesus macaque presented to the Tulane National Primate Research Center Clinic with right limb lameness and poor body condition. On physical exam, significant muscle atrophy of the right leg and pelvis were present. Radiographs revealed severe bone degeneration and lysis of the right tibia with pulmonary nodules. On necropsy, the right tibia was markedly thickened up to 3 times normal. Multifocal, 1 mm to 7 mm diameter dark red nodules were present in the periosteum, compact cortical bone, and medullary cavity. Multifocal dark red nodules measuring 1-7 mm were present in the liver and lung. Histologically, the nodules are composed of neoplastic endothelial cells forming irregular vascular clefts and anastomosing vascular channels. Neoplastic cells stained positive with CD31, and von Willebrand factor immunohistochemical stains. Hemangiosarcoma in nonhuman primates is exceptionally rare. This represents the first case of hemangiosarcoma of bone with hepatic & pulmonary metastasis in a Rhesus macaque.

36: LOCALIZED COLOPROCTITIS CAUSED BY NOVEL BASIDILOBOLUS ARIZONENSIS IN A DOG

Annalise Black, Sylvia Ferguson, Laura Rayhel, Kathryn Wycislo

Midwestern University, Glendale, AZ, USA

Background: A 6-year-old male neutered Boxer mixed breed dog exhibited a 3.5-month, progressive course of dyschezia, hematochezia, and constipation. Colonoscopy with endoscopic biopsies revealed non-specific eosinophilic-lymphoplasmacytic colitis. Signs were refractory to multiple therapies including a hypoallergenic diet, antibiotics, prokinetics, laxatives, and anti-inflammatory glucocorticoids. The patient then developed severe circumferential anorectal swelling with draining tracts and obstipation, and was euthanized. Cytology and postmortem examination revealed pyogranulomatous coloproctitis with intralesional fungal hyphae and zygospores. MALDI-TOF mass spectrometry was unable to identify the organism.

Objective: To characterize and identify the etiologic fungus through cytology, histopathology, culture, and DNA sequence analysis.

Methods: Cytologic preparations of the draining anal fluid were stained with a Wright-Giemsa technique. Sections of the colon, rectum, and anus were fixed in 10% neutral-buffered formalin, routinely processed, and stained with hematoxylin and eosin (H&E),

Periodic-acid Schiff (PAS) and Grocott methenamine silver (GMS) stains. The fungal isolate was subcultured onto potato flakes agar (PFA) and Sabouraud dextrose agar (SDA). DNA sequence analysis of the ITS and partial LSU of the nuclear ribosomal RNA genes was performed on mycelia harvested from 1-week old PFA culture.

Results: Cytology revealed globose, beaked zygosporangia with variably granular contents. Histopathology revealed similar zygosporangia and many 30-40 µm in diameter, PAS-positive, GMS-negative, hyaline, broad, and irregular fungal hyphae. DNA sequence analysis confirmed a novel fungal species, designated as *Basidiobolus arizonensis*.

Conclusions: Histopathology, culture, and DNA sequence analysis of the fungus support this case as the first documented infection with *B. arizonensis* in the human and veterinary medical literature.

37: HEMOPERICARDIUM AND CARDIAC-ASSOCIATED HEMANGIOSARCOMA IN AN INLAND BEARDED DRAGON

Laine Feller¹, Andrea Aplasca², John Flanders², John Trupkiewicz¹

¹Johns Hopkins University, Baltimore, MD, USA, ²Maryland Zoo in Baltimore, Baltimore, MD, USA

Compared to infectious or husbandry-related disease, neoplasia has historically been considered an uncommon occurrence in reptiles. However, recent surveys suggest that the incidence in captive snakes and lizards may be higher than previously thought, with Bearded Dragons being over-represented in both number of submissions and neoplastic prevalence. Post-mortem examination was performed on a 9-year-old, male, Inland Bearded Dragon that was found dead following a two-week history of lethargy, anorexia, increased respiratory effort, and bi-cavitary effusion that was mildly responsive to furosemide diuresis. Gross necropsy revealed serosanguinous coelomic effusion (14 ml); marked hemopericardium (9 ml); and a large, firm, tan to dark red tumor at the heart base measuring 2.5 cm x 1.2 cm x 1.0 cm that enveloped the great vessels. Histology revealed a poorly demarcated, invasive neoplasm composed of variably-sized, tortuous and anastomosing, thin-walled vascular channels embedded in collagenous to myxoid stroma. Abortive vascular channels were lined by a single layer of spindle-shaped cells with indistinct cell borders, a small amount of eosinophilic cytoplasm, and plump elongate nuclei that protruded into lumens. The entrapped aorta and multiple large muscular arteries demonstrated moderate to severe segmental intimal thickening by subendothelial fibrosis and angiogenesis (remodeled chronic thrombi), hemorrhage and necrosis, and invasion by neoplastic spindle cells. Tumor histomorphology was consistent with hemangiosarcoma, and immunohistochemistry for factor VIII was pursued for definitive diagnosis. To our knowledge, this represents one of the only reports of hemangiosarcoma in a lizard and the first case of cardiac-associated hemangiosarcoma in reptiles at large.

38: CANINE GLIOMA IN THE FIRST YEAR OF LIFE: 4 CASES

Esdras Correa dos Santos¹, Brian A. Summers², Jessica A. Elbert¹, Doris M. Miller^{1,3}, Daniel R. Rissi^{1,3}

¹University of Georgia, Athens, GA, USA, ²Melbourne Veterinary School - University of Melbourne, Werribee, Australia, ³Athens Veterinary Diagnostic Laboratory - University of Georgia, Athens, GA, USA

Background

Gliomas account for nearly 35% of all primary brain tumors in dogs and typically occur in adult to older dogs. Gliomas in dogs less than 12 months of age are rarely described.

Objective

To describe the occurrence of gliomas in dogs less than 12 months of age.

Methods

Cases were searched from the Athens Veterinary Diagnostic Laboratory and the private archives of one author (BAS). Clinical and pathology reports as well as archived glass slides were reviewed. When available, tissue sections were subjected to immunohistochemistry (IHC) for OLIG2 and GFAP.

Results

Cases consisted of a 4-month-old male American Pit Bull Terrier (case 1), 4-month-old male Borzoi (case 2), 10-month-old male Labrador Retriever (case 3), and 12-month-old male mixed breed dog (case 4). Clinical signs consisted of dullness (cases 1, 4) and seizures (cases 2, 3). All patients were euthanized. Neoplasms occurred in the right parietotemporal lobe (case 1), telencephalic and spinal leptomeninges (case 2), basal nuclei and internal capsule (case 3), and periventricular neuroparenchyma and lateral ventricles (case 4). Histologically, case 1 was diagnosed as a high-grade oligodendroglioma, case 2 as low-grade oligodendroglioma (leptomeningeal oligodendrogliomatosis), case 3 as low-grade astrocytoma, and case 4 as high-grade astrocytoma. IHC findings consisted of robust nuclear immunolabeling for OLIG2 (cases 1, 4) and moderate cytoplasmic immunolabeling for GFAP (cases 3, 4).

Conclusions

Our findings confirm that gliomas are exceptionally rare in young dogs and that such neoplasms are morphologically and immunohistochemically similar to gliomas in adult and older dogs.

39: HALICEPHALOBUS GINGIVALIS IN A QUARTER HORSE MARE

Amelia Andersson, Kaylin McNulty

Department of Pathobiology and Population Medicine, Mississippi State University
College of Veterinary Medicine, Starkville, MS, USA

A 20-year-old Quarter Horse mare presented to Mississippi State University College of Veterinary Medicine Equine Services for being “off” in the hind end, described as swaying and difficulty rising. During hospitalization, this horse rapidly progressed to

lateral recumbency. Humane euthanasia was elected. On postmortem examination, there were two large, firm, tan masses on the right kidney that measured 10cm x 13.8cm x 7cm and 8cm x 6.6cm x 5cm and expanded the renal parenchyma, from the renal cortex to the renal pelvis. Several lymph nodes were moderately to markedly enlarged with widely disseminated, small, slightly raised and smoothly margined, tan nodules. On histopathologic examination, the renal parenchyma was expanded by abundant mixed inflammation with numerous intralesional nematode larvae and adults. The adult nematodes were characterized by platymyarian-meromyarian musculature, a rhabditiform esophagus, and a smooth cuticle. Larval and adult nematodes were also found in low to large numbers throughout the brain, spinal cord and lymph nodes. Mitochondrial sequencing revealed 99.85% (687/688 basepairs) similarity at *cox1* when compared to published whole mitochondrial genome of *Halicephalobus gingivalis* (previously named *Micronema deletrix*). *H. gingivalis* is typically diagnosed postmortem as disease progression is fatal given the neurotropism for the central nervous system and predilection for the kidneys. The complete life cycle is poorly understood; however, research is suggestive of parthenogenesis as only females have been found in host tissue. Infection by *H. gingivalis* is infrequently reported in equids and rarely in humans, with few treatments proven successful after identification of the parasite in host tissue.

40: A CASE OF AMELOBLASTOMA IN A MALE RHESUS MACAQUE (MACACA MULATTA)

Karelma Frontera-Acevedo¹, Armando Burgos²

¹University of West Indies, St. Augustine, Trinidad and Tobago, ²University of Puerto Rico, San Juan, Puerto Rico

Methods: An 18-year-old male rhesus macaque was submitted for necropsy after spontaneous death. The main gross lesions consisted of poor dentition, gingival hemorrhages, ulcers, and a large gingival mass around the left canine and incisors. Sections of this mass were fixed in 10% neutral buffered formalin, routinely processed, and stained with hematoxylin and eosin.

Results: The mass consisted of a hyperplastic gingival mucosa with numerous rete ridges and multifocal, sometimes anastomosing infiltrating islands of polygonal odontogenic epithelial cells with prominent intercellular bridges and peripheral palisading. Epithelial cells contained moderate amounts of eosinophilic cytoplasm and a single round to oval nucleus with finely stippled chromatin pattern. No mitotic figures were noted in 2.37 mm². The stroma consisted of large amounts of collagen with scattered stellate odontogenous mesenchyme, and multiple small empty vessels. Occasionally, the islands differentiated into squamous epithelium with cores of concentric cornified cells. The mass was multifocally infiltrated by neutrophils, lymphocytes, and plasma cells.

Conclusion: This mass histologically resembled an ameloblastoma, a gingival tumor most commonly associated with dogs in veterinary medicine, but also occasionally reported in other species, although very rarely in non-human primates.

41: A RETROSPECTIVE STUDY OF NEOPLASTIC TONGUE LESIONS IN 368 DOGS AND 169 CATS (2010-2020)

Jesse Riker, Daniel Rissi

Athens Veterinary Diagnostic Lab, University of Georgia, Athens, GA, USA

Background: Tongue biopsy is a common type of surgical biopsy submission at the Athens Veterinary Diagnostic Laboratory (AVDL).

Objectives: To retrospectively describe the type and frequency of tongue neoplasms of dogs and cats diagnosed at the AVDL from January 2010 to January 2020.

Methods: Cases were retrospectively searched from the AVDL web-based archive system.

Results: Out of 823 dogs and 413 cats with tongue biopsy submissions during the studied period, 368 (44.7%) and 169 (40.9%) of the lesions were neoplastic, respectively. No sex or breed predisposition was evident. The mean age of affected dogs was 9.8 years. Most canine neoplasms were melanocytic (29.3%), mesenchymal (29.3%), or epithelial (27.7%), with melanomas (91/368, 24.7%), papillomas (54/368, 14.7%), and squamous cell carcinomas (47/368, 12.8%) being the most commonly diagnosed. The mean age of affected cats was 12.3 years. Most neoplasms were epithelial (93.5%) with squamous cell carcinomas (148/169, 87%) being the most commonly diagnosed.

Conclusions: Although the percentage of tongue biopsies that had a neoplastic diagnosis was roughly the same between species, the diversity of neoplasms was much greater in dogs whereas feline tongue neoplasms were almost exclusively squamous cell carcinomas.

42: AMOEBIASIS IN A MARGINATED TORTOISE (TESTUDO MARGINATA)

Yen-Chi Chang¹, Chu-Yung Weng², Yung-Wei Chen², Cheng-Hsin Shih³, Ying-Chen Wu¹, Ter-Hsin Chen¹

¹Graduate Institute of Veterinary Pathology, National Chung-Hsing University, Taichung City, Taiwan, ²Mystery Exotic Animal Hospital, Hsinchu County, Taiwan, ³Graduate Institute of Molecular and Comparative Pathobiology, National Taiwan University, Taipei City, Taiwan

Background: Amoebiasis is one of the most common and fatal protozoal diseases of reptiles, but has rarely been reported in tortoises and has not been reported in marginated tortoise

Case presentation: A marginated tortoise was brought to the Mystery Exotic Animal Hospital with a history of flagellate enteritis, anorexia, and soft shell. After treatment for

a few months, the tortoise was found dead. Necropsy and tissue sample collection for histopathology were performed.

Results: The liver was enlarged and contained yellowish, firm nodules measuring 1-8 millimeters in diameter, which accounted for over 75% of the section. Liver imprints revealed the presence of large protozoa measuring 12-15 micrometers in diameter, with one or more round to oval nuclei consistent with amoebic trophozoites. Acid-fast stain results were negative. Histopathological examination revealed multifocal to coalescing necrogranulomatous inflammation, which replaced the normal hepatic tissue. Numerous amoebic trophozoites were observed at the peripheral area of the necrogranuloma. The remaining hepatic cords were atrophic, and the sinusoids were dilated. In the colon, diffuse erosion to ulceration with a low number of amoebic trophozoites was noted. Microbiological examination showed the growth of multiple bacterial species from the liver lesions. The result of the amoebic PCR was positive, and sequencing showed 98% similarity with the sequence of *Iodamoeba* species in the GenBank database.

Conclusion: This report presents a case of amoebiasis in a marginated tortoise. The treatment and prevention of amoebiasis should be considered in testudines, especially those experiencing predisposing conditions such as temperature changes.

43: A CASE REPORT: SPONTANEOUS BRAIN TUMOR IN A PET RABBIT (ORYCTOLAGUS CUNICULUS)

Katti Crakes¹, Charles Eberhart¹, Ellen Bronson², John Trupkiewicz¹

¹Johns Hopkins University School of Medicine, Baltimore, MD, USA, ²Maryland Zoo in Baltimore, Baltimore, MD, USA

Background: A 10-year-old male domestic lionhead rabbit was euthanized due to progressive hind limb weakness and progressive right-sided neurologic deficits. Necropsy revealed a focal extra-tentorial mass attached to the surface cerebrum compressing the adjacent occipital lobe and cerebellum. To our knowledge, spontaneous brain tumors have not been characterized in pet and laboratory rabbits due to their extremely low prevalence.

Objective: To define histologic and immunophenotypic features of a spontaneous brain tumor in a geriatric rabbit.

Methods: Post-mortem gross, histologic, and immunohistochemical evaluation were performed. Select mouse-derived immunohistochemical markers were utilized due to cross-reactivity with rabbit-derived primary antibodies.

Results: Attached to the surface of the cerebrum, affecting both telencephalic hemispheres, and compressing the occipital lobe is an expansile, well demarcated non-invasive, encapsulated, densely cellular neoplasm composed of small nests and packets of polygonal cells with variably distinct cell margins and moderate amounts of eosinophilic granular cytoplasm. Nuclear atypia is mild and mitosis is 0-2/2.37mm². Neoplastic cells are vimentin-, GFAP-, neurofilament- and CD31-. Synaptophysin and NSE staining are inconclusive.

Conclusions: Anatomic, histologic, and molecular features of this neoplasm resemble a primary neuroendocrine tumor, likely of pineal origin. This diagnostically challenging case showcases a unique neoplastic entity that has not been previously reported in rabbits.

44: RECURRENT BLOAT SECONDARY TO PYLORIC LIPOMATOSIS IN A JERSEY COW

Anna Foley, Joseph Smith, Karen McCormick, Wesley Sheley
University of Tennessee, College of Veterinary Medicine, Knoxville, TN, USA

A 6-year-old, miniature Jersey cow presented for a 2-week history of intermittent bloat. The cow had calved within the past six months and had no significant medical history other than a right pelvic limb lameness. On physical examination, rumen contractions were absent, the abdomen was "papple" shaped, and doughy contents were felt in the rumen on rectal palpation. Abdominal ultrasound revealed reticular contractions, with no evidence of adhesions or free abdominal fluid. A serum biochemistry panel was within normal limits. An exploratory flank laparotomy was performed and a distended omasum and a mildly thickened pylorus were palpated. Dietary restriction and management were attempted without resolution of bloating, and due to poor prognosis, the cow was humanely euthanized.

On necropsy, the rumen and omasum were distended with dry feed material, and the pyloric wall was thickened and appeared to be infiltrated by adipose tissue. Histologic examination confirmed infiltration of adipocytes into the wall of the pylorus extending transmurally from the serosal surface to the muscularis mucosa and dissecting between muscle fibers. No additional significant findings were identified. Therefore, it was concluded that this fatty infiltration of the pylorus was the likely cause of recurrent bloat.

While this kind of infiltrative obstruction is undocumented in cattle, a similar condition known as gastric lipomatosis has been rarely reported in humans as well as other veterinary species. Since this condition has not yet been reported in cattle, it could be a new differential for chronic, recurrent bloat.

45: TWO CASES OF FELINE INTESTINAL HEMANGIOSARCOMA AND A RETROSPECTIVE EVALUATION OF FELINE HEMANGIOSARCOMA FROM 2002-2023

Allysa Cole, Ryan Jennings, Megan Schreeg
Ohio State University, Columbus, OH, USA

Hemangiosarcoma is a rare neoplasm in cats that accounts for less than 2% of all feline neoplasms and is classified as either dermal or visceral. Herein we present two cases of feline intestinal hemangiosarcoma and perform a retrospective evaluation of additional cases of feline hemangiosarcoma retrieved from the Ohio State University College of Veterinary Medicine pathology archives from January 2002 through February 2023. Case 1 was a 12-year-old neutered male domestic shorthair cat that had hemangiosarcoma originating on the serosal surface of the jejunum which perforated the bowel and metastasized to the liver. Case 2 was a 16-year-old neutered male

domestic shorthair cat that similarly had an invasive serosal hemangiosarcoma on the jejunum, with metastasis to the liver, mesentery, and mesenteric lymph nodes. Retrospective search identified a total of 21 cases of feline hemangiosarcoma, including both autopsy (n=19) and biopsy (n=2) submissions. Of these 17 were visceral, 2 dermal, and 2 cases were unknown in origin. Seven cases out of 21 (33%) affected the intestines, including the jejunum (4), ileocecolic junction (4), and ileum (1). Thirteen of the 19 autopsy cases showed evidence of metastatic disease with all but one case being visceral hemangiosarcoma. The diagnosis in 12 cases was supported with immunohistochemical staining of CD31 or Factor VIII. Our data supports the existing literature on the low incidence of hemangiosarcoma and the aggressive nature of visceral hemangiosarcoma in domestic cats, but highlights the relatively high frequency of intestinal involvement in visceral hemangiosarcoma, which appears unique to cats.

46: COLONIC ADENOCARCINOMA IN A CENTRAL ASIAN TORTOISE (TESTUDO HORSFIELDII)

Ya-Ju Chuang, Wei-Hsiang Huang, Hui-Wen Chang, Chian-Ren Jeng, Yen-Chen Chang

Graduate Institute of Molecular and Comparative Pathobiology, School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan

Background

A captive female central Asian tortoise presented with weight loss and anorexia, and received medical treatment for mycoplasma infection. The turtle was found dead about three weeks later.

Objective

Necropsy and histopathological examination were performed to investigate the cause of death in the central Asian tortoise.

Methods

Representative samples were fixed in 10% neutral buffered formalin, and subjected to slide preparation following routine protocols for histopathological examination. Special stains, including Alcian blue and Periodic-acid Schiff (PAS), were performed to rule in the diagnosis.

Results

Gross examination revealed a focal exophytic mass, which was about 3 cm in diameter and bulged toward the lumen of the colon close to the cloaca. Histologically, the mass showed an invasive transmural neoplastic growth and was composed of numerous polygonal cells arranged in large lobules and sheets. These cells contained abundant intracytoplasmic mucin, which was highlighted under Alcian blue and PAS stains, and peripheralized nuclei. Multinucleation of the neoplastic cells was observed. Based on the histopathological findings, the present case was diagnosed as a colonic adenocarcinoma favoring signet-ring cell carcinoma.

Conclusions

Spontaneous neoplasms of the gastrointestinal tract in reptiles are uncommonly described in comparison to those in mammals and birds. Herein, we report a case of colonic adenocarcinoma favoring signet-ring cell carcinoma in a captive central Asian tortoise.

Keyword: signet-ring cell carcinoma, central Asian tortoise, *Testudo horsfieldii*, colon neoplasia

47: ACUTE MYELOID LEUKEMIA IN A CAPTIVE TIGER (PANTHERA TIGRIS)

Alexis Johnson, Debra Miller

University of Tennessee, Knoxville, TN, USA

An 18-year-old, castrated male, captive tiger (*Panthera tigris*) was euthanized following a history of refractory regenerative anemia. Cytology of the bone marrow showed low to moderate numbers of myeloid precursors, appropriate numbers of megakaryocytes, and a paucity of erythroid precursors. Gross findings from all long bones examined were consistent with bone marrow hyperplasia. Histologic examination of the bone marrow showed diffuse effacement by neoplastic myeloid cells, the majority of which had a blast morphology with no distinguishing histologic features indicating a specific cell lineage. There was also a concurrent megakaryocyte hyperplasia. Cells throughout the bone marrow exhibited strong and robust cytoplasmic labeling with myeloperoxidase, and PARR for B- and T- cell receptors was negative. Neoplastic cells were also present in the liver and within pulmonary vessels. PCR testing for feline leukemia virus and feline immunodeficiency virus was negative. All of these findings were consistent with a diagnosis of undifferentiated, acute myeloid leukemia.

48: LIVER LOBE TORSION IN RABBITS: A HISTOPATHOLOGIC REVIEW OF 41 CASES FROM 1985 TO 2021

Alene Pohly, Elliott Chiu, Kevin Keel

University of California, Davis, Davis, CA, USA

Liver lobe torsion is an uncommon condition in veterinary species, but has been reported in various domestic and exotic species. Dogs and rabbits are reported to be the most common companion animal species that present with liver lobe torsions. The medical records of rabbits diagnosed with liver lobe torsions via biopsy or necropsy examination between 1985 and 2021 were reviewed for signalment, clinical signs, and co-morbidities. The results of postmortem examinations were recorded and analyzed for the lobe involved, chronicity, and nutritional state. Histopathology was reviewed and the most common histopathologic findings in torsed lobes were recorded. Forty-one rabbits were included in this study. Twenty-five (25/41; 61%) were diagnosed at necropsy and 16 (16/41; 39%) were diagnosed on biopsy. The caudate lobe was the most common hepatic lobe affected (21/41; 51%). Rabbits between the ages 1 and 5 years old were over-represented (31/41; 75.6%). A slight majority of affected rabbits (22/41; 53.6%) were castrated. By histopathology, 33 cases were diagnosed as “acute” and 8 were diagnosed as “chronic.” In almost all cases (37/41; 90.2%), the affected liver lobe

showed regionally extensive to diffuse coagulative necrosis. Other commonly identified histopathologic features present within torsed lobes included fibrinous serositis, heterophilic inflammation, and ductular reaction. This study aimed to elucidate the histopathologic findings of acute and chronic liver lobe torsions in rabbits and to assess submissions for any possible predispositions. The diagnosis of liver lobe torsions among our accessions have recently increased dramatically, but the causes are still uncertain.

49: RHODOCOCCAL (PRESCOTELLA EQUI) PNEUMONIA AND LYMPHADENITIS IN A LLAMA

Cassie Powers, Ladislav Novotny
Purdue University, West Lafayette, IN, USA

CASE DESCRIPTION: A 1.5-year-old female intact llama was evaluated due to weakness and an inability to stand up. This was the second llama from this farm to have died suddenly. Horses and donkeys were also kept at this farm with no clinically obvious disease. On necropsy there was a yellow, firm, nodular mass composed of yellow to tan material with a friable green central core in the middle left lung lobe. Additional multifocal to coalescing masses were scattered throughout the lungs. Associated tracheobronchial lymph nodes were also affected. On histological evaluation the affected areas were infiltrated and effaced by multifocal, random, variably sized nodules of pyogranulomatous inflammation with abundant intracytoplasmic bacteria (coccobacilli).

LABORATORY Results: Laboratory results for PCR evaluation confirmed *Rhodococcus equi* (recently reclassified as *Prescottella equi*)

DISCUSSION: Nomenclature for *Rhodococcus equi* has recently become rather perplexing, with reclassification attempts including: *Prescottia equi*, *Corynebacterium hoagii*, and *Rhodococcus hoagii*. Presently, *Prescottella equi* is the most up to date nomenclature for this bacterium as of 2022. *Prescottella equi* is a gram-positive zoonotic bacterium primarily associated with multinodular suppurative to pyogranulomatous bronchopneumonia in foals. There has been an increase in reported cases of this bacterium and other equine-related diseases in agricultural-related Old World Camelids as climate change and socio-economic struggles has forced these animals to come into closer contact with equids. In comparison, cases of this disease in New World Camelids are vastly under-reported and should be considered more as a viable differential in the future.

50: HYALINIZING PANCREATIC ADENOCARCINOMA IN THREE DOGS

Stephanie Fuetsch^{1,2}, Danielle Meritet¹

¹North Carolina State University, Raleigh, NC, USA, ²University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Background:

The hyalinizing variant of exocrine pancreatic adenocarcinoma (HPA) has been infrequently reported in dogs.

Objective:

Our objectives were to identify cases in our archives, characterize lesion location, metastasis, and features of the neoplastic cells and the hyalinized matrix.

Methods:

A search of the North Carolina State University's College of Veterinary Medicine case archives between 2008-2022 identified 19 pancreatic carcinoma cases in dogs, three of which were histologically consistent with HPA. Histologic sections of all HPA cases were evaluated with routine hematoxylin and eosin and with application of special and immunohistochemical stains to some sections. Ultrastructural evaluation of matrix and neoplastic cells was performed via transmission electron microscopy for one case.

Results:

Three cases of HPA were identified in the archives (7-11 years of age; neutered; 2 males, 1 female). Masses affected the right limb, body, and left limb, with metastasis (liver, lymph node, lung, and diaphragm) in one case. Routine hematoxylin and eosin revealed characteristic eosinophilic, hyalinized material and neoplastic epithelial cells. Neoplastic cells from the mass with metastasis were chromogranin A and Synaptophysin immunonegative and pancytokeratin immunopositive. Ultrastructural evaluation revealed neoplastic cells with abundant or rare zymogen granules, disorderly cellular arrangements, and fibrillar to granular extracellular matrix. Evaluation of additional special stains is pending.

Conclusion:

HPA can affect different areas of the pancreas and cases tend to occur in middle to senior aged dogs of either sex. Hyalinized matrix deposition can be variable between individuals. Continued gathering and assessment of case information may allow identification of prognostic features.

51: GASTROINTESTINAL MAST CELL TUMOR IN AN AFRICAN DORMOUSE (GRAPHIURUS SP.)

Yen-Chi Chang¹, Jung-Chin Chang², Jo-Wen Chen², Ying-Chen Wu¹, Ter-Hsin Chen¹

¹Graduate Institute of Veterinary Pathology, National Chung-Hsing University, Taichung City, Taiwan, ²Jong-Shing Animal Hospital, Kaohsiung City, Taiwan

Background: Mast cell tumors (MCTs) are well-known neoplasms derived from either mucosal or connective tissue mast cells. While well studied in several domestic species, MCTs are rarely documented in rodents.

Case presentation: A three-year-old, male African dormouse (*Graphiurus* sp.) presented with a history of vomiting and anorexia for 3 months. Sonography revealed thickened gastric mucosa and hyperperistalsis. Abdominal sonography revealed hypoechoic,

noncircumferential, thickened gastric mucosa. The patient died after receiving symptomatic treatment for 2 months. Necropsy and tissue sample collection for histopathology were performed.

Results: At necropsy, locally extensive, pale, thickened mucosal foci obscuring the first half of the stomach lumen was noted. Histological examination revealed moderately polymorphic, round, oval to spindle cells with amphophilic cytoplasmic granules infiltrating the mucosa to tunica muscularis, with moderate numbers of eosinophils. The mucosa was severely ulcerated with the proliferation of granulation tissue. The granules in most tumor cells exhibited metachromasia with the toluidine blue stain. Neoplastic cells revealed positive membranous immunoreactivity to KIT.

Conclusion: Herein, we report the first case report of MCT in dormouse but also the first gastrointestinal MCT in a rodent species. The diagnosis of Gastrointestinal MCT should be taken into consideration in dormice and other small rodent species with chronic vomit or hematemesis and should be treated with caution because of the disparity between the cell differentiation and clinical outcome.

52: PERIPHERAL GIANT CELL GRANULOMA IN A MEISHAN PIG

Lisa Uhl¹, Drew Gall², Tyler Harm¹

¹Iowa State University, College of Veterinary Medicine, Department of Veterinary Pathology, Ames, IA, USA, ²Blank Park Zoo, Des Moines, IA, USA

Peripheral giant cell granuloma (PGCG) is a hyperplastic lesion that is most frequently observed in the gingiva in association with sites of chronic irritation. This lesion is characterized by multinucleated giant cells and spindle cells embedded in a collagenous matrix with variable osteoid deposition and hemorrhage. PGCG has been reported in the canine, feline, and in humans. Here we report a PGCG in a 16-year-old Meishan pig (*Sus scrofa domesticus*). This patient had an oral mass adjacent to a chronically fractured right mandibular fourth premolar. Grossly, the mass was pink, proliferative, and firm. Histopathologic examination revealed a poorly demarcated mass composed of loosely arranged spindle cells with numerous multinucleated giant cells and multifocal islands of variably mineralized osteoid. On immunohistochemical analysis, multinucleated giant cells were positive for vimentin and Iba-1 and negative for melan A and pancytokeratin. Tartrate-Resistant Acid Phosphatase (TRAP) was conducted to determine osteoclastic origin of the multinucleated giant cells. Histologic and immunohistochemical features are consistent with a diagnosis of peripheral giant cell granuloma. To the author's knowledge, this is the first report of a peripheral giant cell granuloma in a pig. This PGCG demonstrates similar histologic features to those observed in the canine, feline, and in humans.

53: MULTIPLE GLOMUS CELL TUMORS IN A CAT

Marvin Firth, Malcolm Silkstone

Nationwide Laboratories, Poulton, United Kingdom

Glomus tumours are rare, benign neoplasms in humans and have been reported in a variety of species primarily in dogs^{1,2,3}, but also horses^{4,5}, non-human primates⁶, cows⁷, and cats^{8,9}. The tumors originate from cells that compose the glomus body: an arteriovenous anastomosis that has a role in physiologic dermal thermoregulation. As in

humans, the tumors occur in cats often on the lower extremities, digit, and head and most are solid-type glomus tumors. To date, multiple glomus tumours have only been reported in the urinary bladder of a cow.

An 8-year-old, male-neutered Domestic Shorthaired cat where a single, 35mm diameter glomus tumour was detected overlying the right hip, then one month later a further 70mm diameter mass was surgically removed from the neck. More recently (after a further 7 months), there were 5, variably sized but qualitatively similar unencapsulated, multilobulated neoplasms that were at the wing of the ilium (2) and surrounding the body of the pelvis (3). The tumors exhibited typical histological and immunohistochemical reactivity for vimentin and alpha-smooth muscle actin consistent with solid-type glomus tumors¹⁰.

To the author's knowledge, this is the first report of multiple glomus cell tumors in a cat with a variety of anatomic locations. Due to the small number of identified cases in animals, there are no firmly established criteria of malignancy. However, this report suggests the possibility of local invasiveness or recurrence with the possibility of multiple *de novo* tumors in multiple anatomic locations in the cat.

54: QUANTITATIVE ASSESSMENT OF THE PERCENT MOISTURE AND TOTAL SOLIDS IN DOG BONE MARROW

Adam Stern, Courtney Valerio, Daliana Roig
University of Florida, Gainesville, FL, USA

Background: Veterinary forensic pathologists examine emaciated dogs suspected to have died from starvation. As part of the autopsy of an emaciated dog, bone marrow fat (BMF) is examined as it is the final fat reserve to be mobilized for energy in canines. The % BMF can be measured; however, this is a costly test and requires technical expertise.

Objective: We aimed to evaluate the moisture and total solids levels in bone marrow from the femurs of emaciated and non-emaciated dogs using a microwave moisture analyzer. This method is an indirect measure of BMF because total solids equals the mass of the solids after moisture is removed. Total solids include protein, carbohydrates, fat, and ash.

Methods: Diaphyseal bone marrow from the femur of 23 dogs (10 emaciated, 13 non-emaciated) were collected postmortem. A portion of the bone marrow fat was examined microscopically and the % moisture and total solids was measured using approximately 1-2 g of bone marrow in the SMART 6™ microwave moisture analyzer. The % moisture from both groups were compared using the Mann-Whitney U test.

Results: The % moisture (mean, range) from non-emaciated dogs (18.08, 8.12-25.85) was significantly different from emaciated dogs (82.79, 73.24-90.86). The p-value is < 0.00001.

Conclusions: Overall, the percent moisture was low in bone marrow from non-emaciated dogs and high from emaciated dogs. The high % moisture appeared to

correlate with serous atrophy of fat. This supports further investigation of moisture and total solids analysis of bone marrow in cases of suspected starvation.

55: NECROTIZING ENCEPHALITIS IN A GIANT PANDA (AILUROPODA MELANOLEUCA)

Han-Yang Wang¹, Ya-Pei Chang², Chiu-Hung Cheng³, Hui-Wen Chang¹, Yen-Chen Chang¹, Wei-Hsiang Huang¹, Yen-Hsueh Lai³, Chian-Ren Jeng¹

¹Graduate Institute of Molecular and Comparative Pathobiology, School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan, ²Department of Veterinary Medicine, School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan,

³Taipei Zoo, Taipei, Taiwan

Background

An 18-year-old male giant panda was showing intermittent occurrences of seizures and began to behave abnormally, including weakness in its hindlimbs and lost its vision. The animal passed away during anesthesia eventually due to the irreversible brain disease and the reason of animal welfare. The results of magnetic resonance imaging examination revealed hyperattenuating imagines at the left hemisphere of the thalamus level and the bilateral caudate nucleus.

Objective

To determine the brain lesion, we performed necropsy, histopathological examination, and immunohistochemistry (IHC) staining.

Methods

The brain was fixed with neutralizing buffered formalin, serial sectioned of topography examination and subjected to preparation of paraffin-embedded tissue blocks and slices with routine process. The IHC stain of GFAP and Iba-1 antibodies were also performed.

Results

Irregular cavitation was observed from the thalamus to the cranial occipital lobe, and the lesion mostly affected the white matter. However; the horizontal and vertical axes of the brain were still not tilted or changed. Microscopically, the malacic lesion was heavily infiltrated and surrounded by a large number of gitter cells and plump hypertrophic gemistocytes. They were highlighted against the antibodies of GFAP and Iba-1. On the other hand, lymphoplasmacytic perivascular cuffing accompanied by perivascular edema was found multifocally at the level of the caudate nucleus.

Conclusions

By ruling out tumor formation, possible pathogen infection, such as canine distemper and polyomavirus, and other potential etiologies, we concluded the present case to be necrotizing encephalitis under the category of meningoencephalitis of unknown etiology.

56: FORENSIC INVESTIGATION INTO THE MASS DEATH OF FERAL WATER BUFFALOES IN A NATIONAL PARK IN TAIWAN

Ching-Yun Hsu¹, Wei-Hsiang Huang¹, Pin-Huan Yu², Pin-Yu Chen²

¹Graduate Institute of Molecular and Comparative Pathobiology, School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan, ²Institute of Veterinary Clinical Science, School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan

Background: Water buffaloes (*Bubalus bubalis*) were introduced to Taiwan in the 1600s and formed a feral population at Yangmingshan National Park. Increased human-buffalo conflicts led to the installation of barbed-wire fencing in 2019. An unprecedented increase in buffalo deaths in the winter of 2020 prompted a multidisciplinary investigation to determine the cause.

Objective: We aim to determine the underlying cause of death of the water buffaloes and associated factors to implement preventive measures to avoid future occurrences.

Methods: Multiple investigations, including necropsies, histological examinations, pathogen detection, toxicological analysis, and blood tests were conducted on 12 carcasses. Medical examination of the surviving herds and evaluation of the ecosystem and management were also performed.

Results: The deceased buffaloes were underconditioned, with rumens containing digesta, and one had foreign bodies. Microscopic examination revealed serous atrophy of visceral fat and bone marrow, along with degeneration, necrosis, and fibrosis of Purkinje fibers. Some buffaloes showed signs of infection, suggesting potential immunosuppressive conditions. Extensive investigations ruled out outbreaks of severe endemic infectious disease and malicious poisoning. Based on comprehensive multidisciplinary investigations, it has been determined that the demise of the buffaloes can be attributed to long-term malnutrition resulting from unfavorable climate, inadequate quality of forage, and limited ranch resources.

Conclusions: This high-profile incident has sparked significant public conversations regarding the applicability of animal protection laws and wildlife conservation laws to specific feral animals (e.g., water buffaloes), as well as raising concerns about the welfare of wild animals.

57: EOSINOPHILIC NODULAR LARYNGITIS AND TRACHEITIS: A UNIQUE PRESENTATION OF FELINE HERPESVIRUS-1 INFECTION IN AN ADULT IMMUNOSUPPRESSED CAT

Seunghee Cho¹, Kristina Vu², Eunju Choi²

¹Green Vet, Kyonggi-do, Republic of Korea, ²Departments of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California–Davis, Davis, CA, USA

Background: An 11-year-old, male castrated, Domestic Shorthaired cat who had been on prednisolone for a year with a long-term history of chronic sneezing and nasal discharge presented to the emergency service with an acute onset of respiratory

distress. The patient was diagnosed with pneumomediastinum, a distal laryngeal mass, a tracheal carinal mass, and multiple pulmonary masses by radiograph. Debulking was attempted but the masses were firmly adhered with a broad base, and the cat was euthanized due to clinical progression.

Objective: The goal of this study is to describe a unique case of in an adult cat, characterized by the presence of nodular lesions.

Methods: Postmortem samples were collected and processed routinely for histologic evaluation. Immunohistochemistry against feline herpesvirus-1 also performed.

Results: On gross examination, the distal laryngeal and carinal masses were confirmed, and the pulmonary masses were interpreted to be bronchiectasis leading to obstructive atelectasis. Histologically, the masses were composed of sheets of eosinophils expanding the mucosa into a nodule covered with elongated and metaplastic respiratory epithelium. Occasionally within these epithelial cells were eosinophilic intranuclear inclusion bodies suggestive of feline herpesvirus-1 infection. Immunohistochemical staining against feline herpesvirus-1 confirmed feline herpesvirus-1 involvement in the formation of these lesions.

Conclusions: This case presents an exceedingly uncommon manifestation of FHV-1 infection which, we speculate, may be associated with long-term steroid therapy. The inclusion bodies were subtle; hence, a thorough search for inclusions or IHC staining for FHV-1 should be warranted with respiratory eosinophilic masses of the cat.

58: FELINE LEUKEMIA VIRUS-ASSOCIATED MYELOPATHY IN A CAT

Yi-Hsiang Huang¹, Ann Nee Lee¹, Hsiang-Ju Chen², Wei-Hsiang Huang¹

¹Graduate Institute of Molecular and Comparative Pathobiology, School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan, ²LUMI Animal Medical Center, Taipei, Taiwan

Background: A 5-year-old spayed female cat presented with chronic progressive lameness in the right hindlimb. The cat had been feline leukemia virus (FeLV) positive since a young age. Radiological examination revealed a narrow disc space at the L4/5 spine. Despite multiple trial therapies (prednisolone, NSAIDs, and opioids), the cat's condition worsened, resulting in non-ambulatory tetraparesis, hyperesthesia of the spine, vestibular signs, and littering issues. Eventually, due to a guarded neurological prognosis and the sudden development of respiratory distress, the cat had to be euthanized one year later.

Objective: We present a rare case of feline degenerative myelopathy associated with FeLV.

Methods: Necropsy was performed, followed by histopathology on representative tissue. Serial sections of the brain and entire spinal cord were carefully examined. Immunohistochemistry (IHC) was conducted to detect FeLV p27 antigen in the central nervous system and other organs. The IHC results were compared with the histopathological findings.

Results: Axonal degeneration, astrogliosis, astrocytosis, intramyelinic edema, and demyelination were observed in the spinal cord and brain. The IHC staining for FeLV p27 antigen showed intracytoplasmic positive signals in the neurons, glial cells, ependymal cells, and choroid plexus, as well as in many other organs without any pathological changes.

Conclusions: The presence of CNS lesions and FeLV p27 IHC signals suggests an association between FeLV infection and degenerative myelopathy. However, the presence of FeLV p27 antigens alone may not be sufficient to cause cell degeneration in other organs.

59: CEREBRAL COENUROSIS IN A DOMESTIC CAT

Juan Velasco Montes de Ocpaa¹, Jonathan Lopez San Vicente², Julio Cesar Hernandez²

¹Diagnostico Veterinario Del Valle, Mexico City, Mexico, ²Hospital Veterinario Marlovet, Mexico City, Mexico

A 6-year-old cat in Mexico City with progressive anorexia and lethargy that started at Marruecos, Africa (where she was from), presented for neurological examination that included encephalic magnetic resonance imaging, showing a telencephalic cyst of 1.2 cm, involving the parietal lobe from the left hemisphere. Necropsy was preformed. At gross examination, a thin-walled cyst was visible from the cortex from the sylvian and ectosylvian gyrus; the cut surface revealed translucent fluid and several well-attached white-yellowish structures. Atrophy of both adjacent white and grey matter, with lateral and third ventriculus compression were noted. Microscopically cortical gray matter and white matter were loose and displaced by a parasitic vesicle, surrounded by a pyogranulomatous infiltrate; inside, multiple larval cestodes were observed, consistent with *Coenurus* spp. Such larval organisms had an invaginated scolex with one to two rostellum suckers and chitinized hooks, along with an integumentary wall and multiple hyperbasophilic calcareous corpuscles. In the rest of the neuropil, mild lymphocytic vasculitis was identified, as well as in the pia mater and arachnoid mater. In the areas adjacent to the lesion, the neuropil exhibits extensive rarefaction and vacuolation. Cerebral coenurosis represents an aberrant brain infection by the metacestode larval stage of *Taenia* sp. tapeworms, which in cats has been documented in rare, isolated cases and shares clinicopathologic features to ovine and human cerebral coenurosis.

60: MULTI-AGENT MYCOTIC INFECTION IN A FREE-RANGING SPECKLED KINGSNAKE (LAMPROPELTIS HOLBROOKI)

Clare Brown, Natalie Stilwell, Larry Ballard, Brittany Baughman
Department of Pathobiology and Population Medicine, Mississippi State University
College of Veterinary Medicine, Starkville, MS, USA

A wild juvenile female speckled kingsnake (*Lampropeltis holbrooki*) presented for postmortem examination to the Mississippi State CVM Diagnostic Lab. The snake was found emaciated and dehydrated with skin lesions suggesting snake fungal disease, a term given for infection with *Ophidiomyces ophiodiicola*. During rehabilitation the animal

died with no premonitory signs. On postmortem examination, the snake was severely emaciated with depleted adipose stores. Multifocal to coalescing, pinpoint to 1 x 0.3 x 0.2 cm, tan to red-brown, firm, raised to slightly sunken, irregular, crusting to ulcerative skin lesions were present, predominantly along the dorsum and with the largest lesion expanding and disfiguring the skin caudal to the left mandible. Histopathology revealed moderate to severe heterophilic and granulomatous epidermitis and dermatitis with serocellular crusting and intralesional fungal profiles. Fungal hyphae were approximately 3-10 µm diameter, negatively staining or occasionally pigmented, and typically aseptate with non-parallel walls and frequent acute or right-angle branching. Yeasts were also present, which were approximately 6-10 µm diameter and irregularly round with a pigmented capsule and basophilic or optically clear, occasionally septate, internal contents. Additionally, two small heterophilic granulomas with intralesional fungal hyphae were present within the tracheoesophageal fascia and the gastric serosa. Fungal culture of the skin lesions yielded growth of three morphologically distinct organisms, with molecular confirmation of identification pending. This case highlights the importance of mycotic infections, including those classified as snake fungal disease due to *Ophidiomyces ophiodiicola*, as an important cause of dermatitis in captive and free-ranging snakes in the eastern United States.

61: MYOCARDIAL PLASMA CELL TUMOR WITH INTRATUMORAL AMYLOIDOSIS IN A DOG

Clara Cole¹, Kayla Alessandrino², Sonja Tjostheim², Kelly Flynn², Sophie Aschenbroich³, Stephani Ruppert¹

¹Department of Pathobiological Sciences, University of Wisconsin-Madison School of Veterinary Medicine, Madison, WI, USA, ²Department of Medical Sciences, University of Wisconsin-Madison School of Veterinary Medicine, Madison, WI, USA, ³Veterinary Diagnostic Laboratory, Colorado State University College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO, USA

Plasma cell tumors are rare neoplasms reported in multiple tissues in dogs; however, this is the first case reported of an amyloid-producing plasma cell tumor involving the heart. A 12-year-old mixed breed dog was evaluated for marked hypercalcemia identified during assessment for acute polydipsia and polyuria. Physical examination identified a new grade II/VI left apical systolic murmur. Masses involving the left ventricular posterior wall and left atrium were identified by echocardiography, suggesting multifocal myocardial neoplasia. Due to concern for quality of life, the patient was humanely euthanized, and limited post-mortem evaluation of the heart was elected. Gross examination identified 10-20, multifocal to coalescing, smooth, pale tan, soft to firm, 0.1-0.3 cm diameter, minimally raised foci to 2.0 cm diameter nodules expanding 10% of the left and right ventricular myocardium as well as the right atrial epicardium. The largest mass (4.0 x 2.0 x 2.0 cm) encompassed the left ventricular posterior wall endocardium and myocardium and extended across the mitral valve annulus into the left atrial myocardium. Transmural samples of the left and right ventricular nodules were submitted for histologic evaluation and revealed a neoplasm with cellular morphology consistent with a plasma cell tumor, with Mott cell differentiation. Immunoreactivity to multiple myeloma 1 (MUM1) immunohistochemical marker confirmed a plasma cell tumor. This myocardial plasma cell tumor had numerous intratumoral pools of amyloid

confirmed with Congo Red stain and displayed characteristic apple-green birefringence. In conclusion, plasma cell tumors should be included in the list of differentials for intracardiac masses in dogs.

62: T CELL LYMPHOMA IN AN AFRICAN SPURRED TORTOISE (*CENTROCHELYS SULCATA*)

Jennifer Hu¹, Jung Lee¹, Mary White¹, Nicholas Haley¹, Adam Riley²

¹Midwestern University, Glendale, AZ, USA, ²AZetVet, Peoria, AZ, USA

A 5 year old female African spurred tortoise (*Centrochelys sulcata*) presented to Midwestern University Diagnostic Pathology Center for necropsy with a history of progressive worsening of hindlimb and forelimb paresis. Bloodwork revealed elevated total calcium, phosphorus, and blood urea nitrogen. Computerized tomography scan revealed multifocal osteolysis of vertebral bodies and widening of intervertebral disc spaces. Necropsy revealed an enlarged thymus, hepatic lipidosis, urolithiasis, and a focal area of epidural hemorrhage overlying the dorsal spinal cord along the caudal thoracolumbar region. Cytologic evaluation of liver impressions revealed a homogeneous lymphoid population composed of intermediate lymphocytes (diameter: 12-13 µm). The lymphocytes had a high N:C ratio with a scant amount of moderately basophilic cytoplasm. The irregularly round, occasionally lobulated, nucleus had finely stippled chromatin with indistinct nucleoli. On histopathology, there was infiltration of neoplastic round cells throughout liver, spleen, heart, skeletal muscle, adrenal gland, thyroid gland, and truncal vertebral bodies. Neoplastic round cells were immunopositive for CD3, and immunonegative for CD79a and CD20. Altogether, these findings confirmed multicentric T-cell lymphoma. Neoplasia in tortoises is rare, with sporadic reports of lymphoproliferative disease in tortoises. Paresis in this tortoise was multifactorial with neoplastic lymphocytes dissecting between sciatic nerves and osteolysis. While similar lymphoproliferative disease in other species can be induced by herpesvirus, it was not detected in this tortoise. The multicentric distribution is consistent with previous reports of lymphoid neoplasms in reptiles.

63: SPONTANEOUS ACUTE PANCREATIC FAT NECROSIS IN AN ELDERLY MALE RHESUS MACAQUE (*MACACA MULATTA*)

Karelma Frontera-Acevedo¹, Armando Burgos²

¹University of West Indies, St. Augustine, Trinidad and Tobago, ²University of Puerto Rico, San Juan, Puerto Rico

Background: Acute pancreatic fat necrosis is a condition more commonly found in dogs in veterinary medicine. In contrast, adenoviral acute pancreatitis has been associated with SIV infected rhesus macaques.

Objective: To characterize and diagnose case of spontaneous acute pancreatic fat necrosis in a rhesus macaque

Methods: A 21 year old male normal to slightly obese rhesus macaque was submitted for necropsy after spontaneous death. One of the main findings was a stiff pancreas with multiple masses and adhesions to the adjacent peripancreatic fat. The omental

and mesenteric adipose tissue was thick and yellow with grainy white speckles throughout. A general collection of tissues was taken and fixed on 10% neutral buffered formalin, processed routinely, and stained with hematoxylin and eosin.

Results: In multiple lobules of the pancreas, the external acini (closest to the fat) have undergone lytic to coagulative necrosis, and these areas are multifocally hemorrhagic and infiltrated by moderate numbers of neutrophils. The neutrophils and hemorrhages extend to the interlobular septa, expanding it. Peripancreatic and multifocal fat necrosis is also present, with hypereosinophilia, and random basophilic granular appearance (mineralization, saponification). No organisms nor intranuclear inclusion bodies are noted.

Conclusion: This macaque presented with a spontaneous (and fatal) case of acute pancreatic fat necrosis, a condition typically seen in dogs in veterinary medicine. No evidence of adenoviral pancreatitis was seen histologically. Possible causes of enzyme activation could be ingestion of toxins or drugs affecting acinar cells, or something causing an obstruction of the pancreatic ducts.

64: ANOMALOUS MULTISYSTEMIC PROLIFERATIVE VASCULAR MALFORMATION IN A CANINE

Brian LaMendola, Ryan Taylor
Cornell University, Ithaca, NY, USA

Background: A six-year-old, spayed female, canid (*Canis familiaris*) was submitted for postmortem examination following euthanasia due to an aortic valve mass leading to right sided heart failure, as well as, severe melena resulting in anemia.

Objective: To characterize an anomalous multisystemic proliferative vascular malformation discovered in a canid necropsy specimen submitted through the Cornell University Animal Health Diagnostic Center.

Methods: A complete post-mortem exam was performed and appropriate tissues were fixed in 10% formalin, routinely processed, and stained with hematoxylin and eosin (H&E). Verhoeff elastin, Masson's trichrome, and Alcian blue histochemical staining and CD31, smooth muscle actin, and Prox 1 immunohistochemistry (IHC) were applied on representative organs.

Results: Histologic examination of the heart, small intestine, large intestine, brain, and left eye reveals multisystemic, nodular proliferations of CD31 positive endothelial cells. These nodular proliferations are both intravascular and perivascular and are often associated pre-existing vessels. The most prominent lesion is a papilliferous mass arising from the aortic valve composed of sheets and streams of endothelial cells supported by a myxoid and collagenous stroma. Proliferative endothelial cells exhibit

mild cellular atypia with absent mitosis.

Conclusions: Histochemical and immunohistochemical characterization of the described lesions are consistent with an anomalous multisystemic proliferative vascular malformation.

65: SARS COV-2 VACCINATION RESPONSE TRIAL IN NON-DOMESTIC SPECIES HOUSED AT THE TORONTO ZOO

Sara Pagliarani¹, Jaime Tuling¹, Phuc Pham¹, Pauline Delnatte¹, Brandon Lillie¹, Nic Masters², Shawn Babiuk³, Sarah Wootton¹, Leonardo Susta¹

¹Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, ²Toronto Zoo, Scarborough, ON, Canada, ³Canadian Food Inspection Agency, National Centre for Foreign Animal Disease, Winnipeg, MB, Canada

Introduction: Since the inception of the SARS CoV-2 pandemic, multiple reports have documented mild to severe clinical disease in non-domestic species worldwide. While recombinant vaccines are generally effective across species, there is very little information regarding the safety or efficacy of SARS CoV-2 vaccination implemented in zoological collections.

Materials and Methods: A Zoetis® Mink coronavirus subunit vaccine was administered following a two-dose regimen to susceptible species at the Toronto Zoo starting in Spring 2022. Opportunistically collected sera (n=56) were obtained from 19 animals across the *Cervidae*, *Felidae*, *Ursidae*, and *Hyaenidae* families. Response to vaccination was assessed using a SARS-CoV-2 surrogate virus neutralization test (sVNT). Serological data were assessed at the species level, and only species with at least one pre-vaccination sample were enrolled.

Results: Samples with > 30% inhibition were considered neutralizing, and at least a 2-fold increase compared to the pre-vaccination sera were considered indicative of seroconversion. Some felids (jaguar, lions, and cheetahs) and the spotted hyaenas showed marked neutralizing activity after boost, with an average increase ranging between 40-fold (jaguar), 68-fold (lions), and 86-fold (hyenas). Most felids and hyenas showed over 50% decline in neutralizing activity after 4 months post-boost. Polar bears (n=3) did not show neutralization.

Conclusions: This preliminary study indicates that different species have varied responses to the Zoetis® SARS CoV-2 vaccine. This could result from species-specific immunological differences, serum incompatibility with the test, or variable efficiency of the vaccination procedure. Confirmation of these data will be sought through other virus neutralization methods.

66: HISTOLOGIC AND CLINICAL STUDY OF HORSES WITH ENDOSCOPIC DIGESTIVE BIOPSIES: 36 CASES (2017-2021)

Daniel Jean¹, Guillaume St-Jean¹, Emmie Vuillier¹, Nanny Wenzlow²

¹University of Montreal, St-Hyacinthe, QC, Canada, ²Louisiana State University, Baton Rouge, LA, USA

Endoscopic digestive biopsies are commonly taken in horses with a typical clinical context of weight loss, malabsorption, hypoproteinemia and/or recurrent abdominal pain.

The objectives are to describe the clinical and histological findings in horses that had endoscopic duodenal and rectal biopsies taken. This retrospective study evaluated 36 horses with digestive biopsies. Retrospective clinical and histologic study includes horses with complete medical records that also had digestives biopsies taken at the Veterinary Medical Teaching Hospital (University of Montreal) between 2017-2021. Over the past 5 years, we observed an increasing trend of endoscopic duodenal/rectal sampling at our Equine Hospital and identified 36 horses with such biopsy samples.

Ten of those horses had no significant histological changes and 26 had increased immune cell infiltrations. Duodenitis and proctitis were most often described as lymphoplasmacytic (54% and 37% from duodenal and rectal tissues respectively). Neutrophilic enteritis was described in 5 duodenal (5/26 for 19%) and 8 rectal biopsies (8/21 for 38%), more frequent than previously reported. The infiltration intensity ranged in most cases from minimal to mild (82%) with low correlation between duodenal and rectal changes (28%) in the same patient.

The clinical usefulness for endoscopic digestive biopsies in horses is likely more important than previously described in the literature and lymphoplasmacytic enteritis is the most frequent histological finding. The histological interpretation of both duodenal and rectal biopsies in equine patient provides a more complete evaluation of the infiltrating immune cells and their intensity.

67: DISSEMINATED MYCOBACTERIUM ULCERANS INFECTION IN A RED-STRIPED EARTHEATER (GEOPHAGUS SURINAMENSIS)

Ya-Ju Chuang, Wei-Hsiang Huang

Graduate Institute of Molecular and Comparative Pathobiology, School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan

Background

A captive red-striped eartheater was found with buoyancy disorder. Analgesic and antibiotic treatments were prescribed and the patient was found dead the next day.

Objective

To determine the cause of the present death, histopathological and molecular examinations were performed.

Methods

The fish was necropsied, and representative samples were fixed in 10% buffered formalin and processed by standard methods for histopathological examination. DNA extraction from the affected organs was performed for polymerase chain reaction (PCR).

Results

Gross examination revealed multifocal ulceration at the skin of the head and multifocal white nodules measuring smaller than 0.1 mm at the epicardium and myocardium of the heart and the surface of the gills. The brain was soft, white, and swollen. Histopathological evaluation of the brain, spinal cord, heart, spleen, gills, liver, and skin revealed disseminated necro-granulomas in variable sizes and centered on clusters of extracellular acid-fast-positive bacilli. Based on the PCR results, *Mycobacterium ulcerans* infection is diagnosed.

Conclusions

Mycobacterium spp. neurologic involvement is relatively rare in animals and is reported in zebrafish (*Danio rerio*) and a leatherback sea turtle (*Dermochelys coriacea*). To the best of our knowledge, the present case is the first confirmative disseminated *Mycobacterium ulcerans* infection in a captive red-striped eartheater.

Keyword: *Mycobacterium ulcerans*, *Geophagus Surinamensis*, central nervous system infection

68: HISTOLOGICAL FINDINGS SUGGESTIVE OF HYPERTROPHIC CARDIOMYOPATHY IN RHESUS MACAQUES (MACACA MULATTA)

Karelma Frontera-Acevedo¹, Armando Burgos²

¹University of West Indies, St. Augustine, Trinidad and Tobago, ²University of Puerto Rico, San Juan, Puerto Rico

Background: Hypertrophic cardiomyopathy (HCM) is the most common primary cardiac disease in cats, although it has been diagnosed in other species. More recently, the California National Primate Research Center (CNPRC) has reported cases of left ventricular hypertrophy (LVH) with features resembling HCM in humans.

Objective: To describe and characterize histologic lesions suggestive of hypertrophic cardiomyopathy in rhesus macaques at the Caribbean Primate Research Center.

Methods: A sample of necropsy cases submitted between 2017 to 2022 to the Caribbean Primate Research Center was examined microscopically to confirm cause of death based on gross presumptive diagnosis. Each animal had data including age, sex, clinical history, and matrilineal descent amongst others.

Results: Seven cases of moderate to severe cardiac lesions were observed. The ages ranged from 8 to 22 years. Some were sudden unexplained deaths, some were trauma cases (fighting), and others had been suspected of having encephalomyocarditis virus, based on the gross changes. The microscopic lesions consisted of variable fibrosis,

edema, myofiber disarray, hypertrophied myocytes, and karyomegaly. Inflammatory cells were not present or present in small numbers.

Conclusion: HCM has so far been reported and studied at the CNPRC. However, it is likely that macaques at other primate research centers also have this condition, and it may go unnoticed. This condition should be considered a differential in sudden unexplained death of elderly macaques, along other infectious causes of myocarditis such as encephalomyocarditis virus.

69: CRIBRIFORM CARCINOMA OF THE MAMMARY GLAND IN A COMMON RACCOON (PROCYON LOTOR)

YANET VELAZQUEZ-JIMENEZ¹, JOSE MIGUEL LOPEZ-RAMIREZ², MIREYA JUAREZ-RAMIREZ³, TOMAS VALDEZ-OCHOA⁴

¹Akilab Servicio Diagnostico Veterinario, Puebla, Mexico, ²Centro Medico de Especies Exóticas y Animales de Compañía Exotic Vet, Puebla, Mexico, ³Universidad Nacional Autonoma de México, México, Mexico, ⁴Clinica Veterinaria Nordica, Xalapa, Mexico

Background: Mammary neoplasms, primarily malignant, occur rarely in nondomestic carnivores. **Objective:** To describe the pathological features of a cribriform carcinoma in a common raccoon (*Procyon lotor*). **Methods:** Postmortem samples were collected and processed for histologic evaluation. Cytologic specimens were stained with Diff-Quick solution and Cytokeratin AE1/AE3, progesterone receptor, estrogen receptor and Ki67 immunohistochemistry was also performed. **Results:** A 7-year-old, female, common raccoon (*Procyon lotor*) had a clinical history of recurrent mammary gland tumor after two surgical procedures with no histopathologic diagnosis. The animal died before the third surgery. The animal was submitted to the Akilab laboratory for postmortem examination. Grossly, the right caudal abdominal mammary gland had a 2.6 cm x 2.2 cm, firm, well-demarcated, multilobulated, yellow-white mass with hemorrhages. Multiple metastatic nodules were throughout the lung and cranial mediastinum. The mass is composed of an encapsulated, multilobulated, densely cellular neoplasm comprised of epithelial cells arranged in a sieve-like pattern, sustained by fibrovascular stroma. Neoplastic cells are polygonal and have scant amounts of eosinophilic cytoplasm with indistinct borders. Nuclei are round to oval with finely stippled chromatin and 1-3 distinct nucleoli. Anisokaryosis and anisocytosis are moderate and there are 4 mitotic figures per 2.37 mm². Cytologic findings were consistent with a malignant epithelial neoplasm and neoplastic cells stained positive for Cytokeratin AE1/AE3 and Ki67. **Conclusions:** The mammary gland neoplasm is a cribriform carcinoma with metastasis to the lung and mediastinum. There are very few reports of mammary gland carcinomas in non-domestic carnivores.

70: GANGLIOGLIOMA IN THE SPINAL CORD OF A DOG: CASE REPORT AND LITERATURE REVIEW

Emily Swan¹, Richard Brooksby², Robert Jones¹, Iren Horkayne-Szakaly¹, Jey Koehler³, Whitney Vickery², Thomas R Burdette², Elise LaDouceur¹

¹Joint Pathology Center, Silver Spring, MD, USA, ²Department of Defense Military Working Dog Veterinary Service, Lackland Air Force Base, TX, USA, ³Aburn University College of Veterinary Medicine, Aburn, AL, USA

Background: A 7.5-year-old male neutered German shepherd dog presented with a one-month history of progressive ataxia and tetraparesis. Magnetic resonance imaging revealed a poorly demarcated intramedullary C6-C7 spinal cord thickening characterized as T2w hyperintense, T1w isointense, and non-contrast enhancing. Additional diagnostics including complete blood count, chemistry panel, urinalysis, CSF analysis, whole body computerized tomography scan, and abdominal ultrasound had no significant, related findings. He was humanely euthanized one month after presentation due to severe, progressive neurologic disease that was refractory to care.

Methods: The only substantial gross necropsy finding was a 4 cm x 1 cm x 1 cm swelling of the C6-C7 segments of the spinal cord with loss of normal architecture. Histology with immunohistochemical stains was performed on a complete necropsy tissue set.

Results: Histopathology revealed a poorly demarcated neoplasm composed of a mixture of haphazardly arranged spindle cells (astrocytes) and neurons that effaced both gray and white matter. Neoplastic neurons had dysmorphic features including binucleation, cytomegaly, and peripheralization of Nissl substance and were scattered throughout bland appearing astrocytes. Neoplastic neurons had diffuse, strong nuclear and mild cytoplasmic immunoreactivity for NeuN. Neoplastic spindle cells had diffuse, strong cytoplasmic immunoreactivity for GFAP.

Conclusions: Ganglioglioma is a rare neoplasm in domestic species. When they do occur in dogs, they typically occur in the brain. There is one report of ganglioglioma in the spinal cord of a dog, but no additional information about this case was provided. There are also individual reports of spinal cord gangliogliomas in a hedgehog and calf.

71: HYALINE MEMBRANE DISEASE IN A 2-DAY-OLD FOAL

Maryanna Parker, Ina Mersich, Jonathan Foreman, Wes Baumgartner
University of Illinois Urbana-Champaign, Urbana, IL, USA

Background: Hyaline membrane disease refers to the microscopic manifestation of equine neonatal respiratory distress syndrome (ENRDS). ENRDS is a clinical diagnosis, thought to have similar pathogenesis and lesions as human neonatal respiratory distress syndrome (NRDS); the condition in horses is poorly understood. There are few histologic descriptions in the literature.

Objective: To describe the gross and histologic lung lesions in a foal clinically suspected to have ENRDS.

Methods: A full necropsy was performed on a 2-day-old thoroughbred male who was euthanized due to poor respiratory function following a terminal, pre-term (1 week premature) caesarian section. Tissues were stained with hematoxylin and eosin for microscopic examination. PCR testing for common viral causes of lung disease in neonatal foals was performed on lung tissue.

Results: The lungs were poorly collapsed, dark red, rubbery and heavy, with prominent rib impressions; sections oozed scant fluid and sections remained submerged in formalin. Histologic changes were consistent with descriptions of NRDs in humans. Epithelial cells lining the bronchioles were often sloughed or necrotic, and denuded surfaces had hyaline membranes. Bronchial epithelium was less affected, but plugs of fibrin, mucus, and dead cells filled multiple lumens. Alveoli were atelectatic and contained small amounts of amorphous protein, fibrin, and squames. Lung samples were PCR negative for equine influenza A virus, equine viral arteritis virus, and equine herpesviruses-1 and -4.

Conclusions: This case shows histologic lesions which are similar to those reported in NRDS.

72: OXALATE NEPHROSIS IN A SERVAL (LEPTAILURUS SERVAL)

Alea Agrawal, Sarah Linn-Peirano

University of Tennessee, Knoxville, TN, USA

An 11-year-old female spayed captive serval presented to clinical staff with acute-onset hyporexia and lethargy. Initial blood clinicopathologic findings included severe azotemia (blood urea nitrogen: 350 mg/dL, creatinine: 25.9 mg/dL), hypocalcemia (total calcium: 5.7 mg/dL), and mild hyperkalemia (5.4 mmol/L). Urinalysis obtained via cystocentesis revealed isosthenuria (urine specific gravity 1.010), marked proteinuria (200-300mg/dL), bacterial cocci with mild pyuria, and 1+ calcium oxalate monohydrate crystals. Despite supportive care for acute kidney injury, the serval died following progressive hypothermia, anuria, and hyperkalemia. A necropsy was performed at the referral clinic and select tissues, including both kidneys, were submitted for histopathology. Both formalin-fixed kidneys had numerous, pinpoint to 1.1 cm diameter cortical depressions. Histologically, there was marked chronic-active lymphoplasmacytic to neutrophilic interstitial nephritis with tubular degeneration, loss, protein casts, and interstitial fibrosis. Both kidney sections contained widespread intratubular, clear, sharply fragmented and radiating, birefringent crystals (consistent with calcium oxalate). In the present case, no ethylene glycol or known toxin exposure was reported. Of significance, three years prior, the sibling of this serval was also euthanized secondary to acute kidney injury, and had widespread renal intratubular calcium oxalate crystals. Although typically associated with ethylene glycol toxicity in carnivores, this case highlights a possible genetic predisposition to oxalate nephrosis in servals. While this entity has been investigated in cheetahs, additional work is necessary to elucidate the pathogenesis of this phenomenon in servals.

73: MOLECULAR OWNER-DOG SCREENING OF MULTIPLE ZOONOTIC PATHOGENS IN INDIGENOUS COMMUNITIES OF BRAZIL

Louise Kmetiuk^{1,2}, Jobin Kattoor¹, Fernando Doline², João Henrique dos Santos², Rebecca Wilkes¹, Andrea Pires dos Santos¹, Alexander Biondo^{1,2}

¹Purdue University, West Lafayette, IN, USA, ²Federal University of Paraná, Curitiba, Brazil

Background: The Indigenous population of Brazil represents approximately 0.4% of the general Brazilian population, estimated at approximately 900,000. This population is among the most vulnerable, given a combination of historical, social, and environmental conditions, and thus may be more exposed to factors leading to increased exposure to infectious diseases. The present study aimed to molecularly screen multiple zoonotic pathogens in Indigenous populations and their dogs. **Methods:** Blood sample collections were obtained from nine Indigenous communities from three ethnicities in southern and southeastern Brazil. Pathogen screening was achieved by Targeted Next-Generation Sequencing (tNGS). **Results:** To date, 100/640 indigenous individuals and 149/243 dogs were screened by tNGS, revealing 19/100 (19.0%) positive human samples, including 15/19 (78.9%) for *Cryptococcus* spp., 2/19 for *Toxoplasma gondii* and 2/19 for *Leishmania* spp. In dogs, 126/149 (84.6%) samples were positive, including 46/126 (36.5%) for *Mycoplasma haemocanis*, 14/126 (11.1%) for *Mycoplasma haematoparvum*, 42/126 (33.3%) for *Cryptococcus* spp., 19/126 (15.1%) for *Babesia canis*, 22/126 (17.5%), 55/126 (43.7%) for *Ehrlichia canis*, 2/126 (1.6%) for *Rangelia vitalli*, 7/126 (5.5%) for *Toxoplasma gondii*, 8/126 (6.4%) for *Leishmania* spp., 11/126 (8.7%) for canine parvovirus, 1/126 (0.8%) for canine distemper virus, 1/126 (8.7%) for canine herpesvirus, 7/126 (5.5%) for *Anaplasma platys*. In addition, 71/126 (56.3%) dogs presented co-infection of the above pathogens. **Conclusions:** The One Health owner-dog approach herein in Brazilian indigenous communities has shown multiple co-infections intra and trans-species, highlighting the importance of multiple and concomitant pathogen detection by tNGS for active surveillance, particularly on zoonotic agents.

74: BAD BLOOD: SUSPECT TRANSFUSION-RELATED ACUTE LUNG INJURY IN A NEW ZEALAND WHITE RABBIT

José Vilches-Moure

Stanford University School of Medicine, Stanford, CA, USA

A male New Zealand White rabbit underwent a lateral thoracotomy and pulmonary arterial banding. Due to blood loss during surgery, the patient received a blood transfusion from a donor rabbit and intravenous fluid supplementation. One day post-op, the patient had increased respiratory rate and effort, but thoracic radiographs did not reveal any abnormalities. Three days post-op, radiographs revealed increased pulmonary opacity and pneumothorax; the animal retained normal appetite throughout this time. Four days post-op, there was wound dehiscence and tissue devitalization at the thoracotomy site, and humane euthanasia was elected due to poor prognosis. On diagnostic necropsy, the lungs were wet and mottled red to dark red. Histologically, the alveolar spaces contained abundant eosinophilic fluid, heterophils, and alveolar macrophages. Blood vessels, particularly large caliber arteries, were accentuated by dense heterophilic perivascular infiltrates that were multifocally infiltrating vessel walls where there was occasional cellular debris. Given the history of blood transfusion and the combination of histologic changes, this likely represents a case of transfusion-related acute lung injury (TRALI). This condition results in non-cardiogenic pulmonary edema, and in humans, TRALI is one of the leading causes of transfusion-associated fatalities. The term VetALI (veterinary acute lung injury) is used to refer to acute lung injury in veterinary species, and numerous reports point to poor prognosis for

recovery after onset, which mirrors the low survival rates reported in humans with TRALI.

75: OCULAR PRIMITIVE NEUROECTODERMAL TUMOR IN A DOG

Sebastián Zetina¹, Elisa Maria Morales¹, Hugo Escalante², Adriana Bernal³

¹Centro De Diagnóstico Veterinario Bajñio, Querétaro, Mexico, ²Clinica Veterinaria Refugiadogs, Queretaro, Mexico, ³Universidad Nacional Autonoma de México, Ciudad de México, Mexico

A case of a primitive neuroectodermal tumor in the eyeball of a mixed breed dog was reported; based on clinical observation, macroscopic, microscopic findings, and immunohistochemical evaluation (neuronal specific enolase (NSE++), synaptophysin (SYN+) and glial fibrillar acid protein (GAFP++)) the diagnosis was conclusive although no clinical signs were reported. Primitive neuroectodermal tumors (PNET) in the eye, as in other places, are neoplasms derived from embryonic neural tissue and are frequently observed in young animals. Dogs with PNET often present ocular pain, beflorospasm, epiphora, and photophobia, as well as secondary diseases such as lens luxation, glaucoma, and even vision loss.

76: SEVERE MENINGOENCEPHALITIS IN A DOMESTIC CAT (FELIS DOMESTICUS) NATURALLY INFECTED WITH H5N1 HIGHLY PATHOGENIC AVIAN INFLUENZA

Jacqueline Kurz, Myrna Miller, Elizabeth Wheeler, Elizabeth Butkus, Madison Vance, Jonathan Fox

Department of Veterinary Sciences, University of Wyoming, Laramie, WY, USA

Highly pathogenic avian influenza H5N1 (HPAI-H5N1) has been reported as a cause of severe pneumonia and occasionally encephalitis in felids. The HPAI-H5N1 outbreak of 2022-2023 affected wild birds and multiple species of wild carnivores in Wyoming. In the spring of 2023, a semi-feral, adult, female cat found severely obtunded was euthanized and submitted for necropsy to the Wyoming Veterinary Diagnostic Laboratory. The cat was from a ranch in central Wyoming, where multiple cats and skunks had been found dead or severely obtunded. Histopathology revealed a severe, multifocal, acute meningoencephalitis with prominent neuronal necrosis most severely affecting the brainstem, midbrain, cerebral neocortex, and thalamus. Moderately severe, acute, neutrophilic bronchitis with marked interstitial edema was also present. HPAI was detected in brain tissue by PCR (Ct = 13.6), along with H5 and N1 antigens (Ct = 20.0 and 19.0, respectively). The microscopic findings in the brain are similar to those previously reported in HPAI-associated encephalitis in domestic cats. While immunohistochemistry for HPAI and rabies virus are pending, the findings strongly indicate influenza-viral encephalitis in this case.

77: JUST NOT FEELING HUNGRY: LYMPHOMA AND INTUSSUSCEPTIONS IN A NEW ZEALAND WHITE RABBIT

José Vilches-Moure, Kate Gates

Stanford University School of Medicine, Stanford, CA, USA

A female New Zealand White rabbit presented for hyporexia which resolved the same day with supportive care. Four days later, the animal had decreased fecal output and was given supportive care (fluids and anti-nausea medication). The next day, the animal had a guarded abdomen, increased borborygmi, tachypnea, hyperthermia, and a normal heart rate. More aggressive supportive care was initiated: intravenous fluids, anti-nausea medication, laxatives, and non-steroidal anti-inflammatories. The following day, the animal had not yet produced any feces and was dysphoric with a splayed leg stance. Abdominal palpation revealed gas-filled intestinal loops and multiple firm intra-abdominal foci. Due to a declining state and being an inappropriate surgical candidate, humane euthanasia was elected. Diagnostic necropsy revealed four non-reducible small intestinal intussusceptions, multiple white, soft, raised lesions in the stomach, pale streaks in the heart, and markedly enlarged ovaries with areas of hemorrhage. Histologically, there was lymphoma in the stomach, small and large intestines, mesenteric lymph nodes, ovaries, uterus, heart, liver, and spleen. The intestinal intussusceptions coincided with transmural expansion by neoplastic lymphocytes. Lymphoma is the second most common neoplasm in rabbits and typically involves the gut-associated lymphoid tissue (GALT), mesenteric lymph nodes, spleen, and bone marrow. Renal involvement, particularly of the renal cortices, is considered pathognomonic. This case was unusual given the lack of renal involvement. In rabbits, gastrointestinal (GI) stasis often occurs in the face of illness, and this case underscores the importance of maintaining lymphoma as a differential diagnosis in instances of GI stasis.

78: DUCTAL PLATE MALFORMATION IN A 10-YEAR-OLD DOMESTIC SHORTHAIRED CAT

Ji-Hang Yin¹, Cierra Starbird¹, Emily Graff¹, Cornelius Withers¹, David Sender², Olivia Stephenson²

¹Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA, ²Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL, USA

Ductal plate malformation is a group of complex congenital hepatobiliary diseases that result from incomplete ductal plate remodeling during bile duct morphogenesis. A rescued 10-year-old male castrated domestic shorthair cat presented to Auburn University Veterinary Hospital for dullness and showed extremely cachexic and emaciated at presentation. Biochemistry revealed elevated liver enzymes. The abdominal ultrasound observed irregular heterogeneous masses that completely distorted hepatic parenchyma. Fine needle aspirates from liver nodules revealed normal hepatocytes and cells resembling capsular cells and biliary epithelium. Primary or metastatic hepatic neoplasia was clinically suspected. Euthanasia was elected. At necropsy, the cat had mild peritoneal effusion with strands of fibrin diffusely covering the serosal surface. The liver was 5.7% of body weight and was markedly undulant with multifocal to coalescing, pale tan to brown, firm, solid, 1-mm to 10-mm diameter, nodules. Additionally, two pink to tan, firm, fluid-filled masses were on the caudate (3 x 2.5 x 1-cm) and left medial liver lobe (3 x 2 x 1-cm). Few clusters of 1-mm to 5-mm cystic structures were randomly distributed on the cross-section of the liver nodules. Histologically, severely effacing and replacing the hepatocytes were nodules of

numerous variably sized, irregularly shaped to dilated cysts, lined by cytokeratin 7-positive attenuated to low cuboidal epithelium and supported by variable amounts of collagen bundles. These pathologic findings were consistent with ductal plate malformation. This case demonstrated ductal plate malformation could mimic neoplastic processes both clinically and grossly and should be considered as a differential in elder feline patient.

79: TWO-STEP CONVOLUTIONAL NEURAL NETWORK METHODS IN DIAGNOSIS OF CANINE MAMMARY GLAND TUMOR

Sanggu Kim¹, Byung-Geun Choi², Heejae Yang², Soochong Kim¹

¹Laboratory of Veterinary Pathology and Platelet Signaling, College of Veterinary Medicine, Chungbuk National University, Cheongju, Republic of Korea, ²Cocoon Inc., Cheongju, Republic of Korea

Background

Mammary gland tumor (MGT) has always been an important topic in veterinary medicine. Deep learning (DL) algorithms, including convolutional neural network (CNN), have recently been applied to accommodate the growing demand in diagnostic pathology, but there are still challenges to achieve higher accuracy in reaching diagnoses.

Objective

We aim to establish a two-step diagnostic support system for canine MGT using CNN to increase diagnostic accuracy.

Methods

Whole slide images (WSIs) were created using an Olympus VS200. In contrast to conventional single CNN methods, the diagnostic procedure was divided into two steps, in which a separate CNN learning model was established for each step. During annotation, the classification was set to four classes in each step: i. background, tumor mass, stroma, and parenchyma were classified to differentiate tumor mass from normal tissue, and ii. background, adenoma, adenocarcinoma, and osteoid matrix were classified to diagnose benign versus malignant tumor with morphological subtyping. In addition, WSIs were cropped into multiple patches of smaller dimension images (512*512 pixels). The proposed CNN model has been trained based on the pre-trained model, EfficientNet V2, with transfer learning.

Results

Upon applying our two-step CNN methods, we obtained an overall 94.5% accuracy in the diagnosis of canine MGTs.

Conclusion

We established the feasibility of two-step CNN-based diagnostic systems using WSIs of canine MGTs. Further enhancing the data generation method and expanding the data volume will make our system useful for future precision diagnoses with higher diagnostic accuracy.

Experimental Disease

Tuesday, October 31 | 2:20 PM – 2:35 PM

Session Chair: LaTasha Crawford

Tuesday, October 31

2:20 PM – 2:35 PM

THE ROLE OF MYC IN MEIBOMIAN GLAND DEVELOPMENT

Su Chan Lee¹, Ashley Campbell¹, Charles Eberhart¹, Cornelia Peterson^{1,2}

¹Johns Hopkins University School of Medicine, Baltimore, MD, USA, ²Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA, USA

Background. The protooncogenic roles of *MYC* in epithelial tissues have been well-established; however, evaluation of its function during development of the Meibomian gland (MG) has not been pursued. **Methods.** Human MG epithelial cells (hMGECs) were incubated in pro-proliferative or pro-differentiative conditions, and *MYC* and Ki67 expression were assessed by immunolabeling. A *K14MycER* transgenic (TG) mouse was utilized to induce *MYC* overexpression *in vivo* following transactivation of the ER hormone binding domain by tamoxifen. MG morphology and *MYC* immunolabeling were evaluated in neonatal (P2) *K14MycER* and WT littermates in which tamoxifen (50mg/kg) in a corn oil suspension was applied topically to the eyelids once at P1. MG length was evaluated using a Student's *t* test ($\alpha = 0.05$). **Results.** *MYC* expression was upregulated in hMGECs when cultured in pro-proliferative medium when compared to pro-differentiative culture conditions, corresponding to increased Ki67 immunolabeling. Temporal and spatial induction of *MYC* was achieved in TG mice when compared to treated wildtype controls ($n = 3$ mice/group). The mean MG acinar length was significantly greater ($p = 0.0172$) in induced TG mice ($63.8 \pm 3.5\mu\text{m}$) than in controls ($49.6 \pm 5.2\mu\text{m}$), and the cells comprising the acini were morphologically consistent with basal cells. **Conclusions.** Upregulated *MYC* contributes to hyperplasia of basal cells within acini during development of the murine MG and may play a role in Meibomian oncogenesis in mature animals. *MYC* may represent a novel target for precision therapy of sebaceous carcinomas of the ocular adnexa.

Tuesday, October 31

2:35 PM – 2:50 PM

THE NONCANONICAL NFKB SIGNALING PATHWAY INFLUENCES EOSINOPHILOPOIESIS IN THE MARROW AND SPLEEN IN THE NIK-/- MURINE MODEL

Irving Allen^{1,2}, Rebekah Smith³, Holly Morrison², Khan Imran⁴, Brie Trusiano^{1,2}

¹Virginia-Maryland College of Veterinary Medicine, Christiansburg, VA, USA,

²Department of Biomedical Sciences and Pathobiology, Blacksburg, VA, USA,

³Department of Biological Sciences, Blacksburg, VA, USA, ⁴Translational Biology, Medicine, and Health, Blacksburg, VA, USA

Eosinophils play an important therapeutic role in combating helminthic pathogens. However, aberrancies in eosinophil proliferation, maturation, activation, and chemotaxis can also lead to acute eosinophilic leukemia, paraneoplastic eosinophilia associated with T cell lymphoma and carcinomas, systemic and local hypersensitivity reactions, and Hypereosinophilic Syndrome (HES), respectively. Idiopathic HES is a disease characterized by persistent eosinophilia ($>1,500$ cells/uL) and infiltration of solid organs and tissues leading to increased morbidity and mortality affecting both human and veterinary patients. Previous studies have demonstrated the role of NIK, an upstream regulator of the noncanonical NF- κ B signaling pathway, in development of HES-like syndrome due to aberrant Th2 polarization in the *Nik*^{-/-} murine model. However, the intricacies involved in eosinophilopoiesis and eosinophil/neutrophil plasticity has not been assessed during development of HES-like syndrome in *Nik*^{-/-} mice. Here we show that loss of NIK influences eosinophilopoiesis and potentially plasticity differently in the marrow and spleen. Loss of NIK also enhances eosinophilopoiesis on days 6 and 13 of culture, but decreases proliferation rates at day 10 in an in vitro bone marrow microenvironment. Interestingly, IL-5 appears indispensable for efficient eosinophil proliferation in a *Nik*^{-/-} in vitro bone marrow microenvironment. Taken together, these results suggest that NIK and the noncanonical NF- κ B signaling pathway affects eosinophilopoiesis and the proliferative potential of eosinophils, highlighting a synergistic relationship with Th2 polarization noted in the *Nik*^{-/-} model. Overall, these findings warrant further study to better characterize the synergistic relationship described above to further understand idiopathic HES and potentially eosinophilic leukemias and paraneoplastic eosinophilia.

Tuesday, October 31

3:30 PM – 3:45 PM

AGE-ASSOCIATED INFLAMMATION CONTRIBUTES TO DELAYED RESPIRATORY SYNCYTIAL VIRUS CLEARANCE IN GERIATRIC COTTON RATS

Jonathan Miller, Cameron Leedale, Stefan Niewiesk

The Ohio State University, Columbus, OH, USA

Respiratory syncytial virus (RSV) infection is a leading cause of severe respiratory disease in the elderly. RSV clearance is delayed in adults over 65 years of age, a finding that is mirrored in the geriatric cotton rat model. Treatment with anti-inflammatory inhibitors of cyclooxygenase (COX) eliminates this delay in geriatric cotton rats, implicating the arachidonic acid cascade as a contributor to impaired RSV clearance. Here, we further characterize the effects of elevated COX activity. We find the eicosanoid prostaglandin D₂ (PGD₂) is elevated in airways of RSV-infected geriatric cotton rats. Administration of PGD₂ to adult cotton rats delays RSV clearance, while treatment of geriatric animals with a PGD₂ synthase inhibitor improves clearance, indicating that age-associated PGD₂ prolongs RSV infection. Restoration of clearance in geriatric animals by COX inhibitors is abolished by CD8⁺ T cell depletion. This demonstrates that the therapeutic effect of COX inhibition is related to restoring cell-mediated immunity. We measured RSV-specific CD8⁺ T cell responses in lung and lymphoid tissue, finding that development of a robust response is delayed in geriatric animals. Collectively, these results indicate that age-associated inflammation is an

important and reversible contributor to the prolonged course of RSV infection in the elderly and highlight PGD2 as a key mediator of impaired cell-mediated immunity.

Tuesday, October 31

3:45 PM – 4:00 PM

INVAGINATION IN THE DROSOPHILA SALIVARY GLAND IS MEDIATED BY AN SP1-LIKE TRANSCRIPTION FACTOR THROUGH THE REGULATED SECRETION OF A GPCR-PATHWAY LIGAND

Ashleigh Shoemaker, Ji Hoon Kim, Deborah Andrew
Johns Hopkins School of Medicine, Baltimore, MD, USA

Background: *Drosophila melanogaster* is a valuable model organism for revealing the cellular and molecular basis of congenital diseases. With the sophisticated techniques available for genetic manipulation, the fruit fly has enormous potential for uncovering pathways essential to organ formation. Our laboratory utilizes the embryonic *Drosophila* salivary gland (SG) as a simple model of epithelial tube formation analogous to the mammalian lungs and glandular tissues.

Objective: Whereas the cellular mechanisms contributing to SG invagination have been described, the signaling pathways coordinating this process remain unclear. We hypothesize that the SP1-like transcription factor Hucklebein (Hkb) mediates the localized secretion of the signaling molecule encoded by Folded gastrulation (Fog), thus activating the downstream G-protein-coupled-receptors (GPCRs) critical for cellular invagination.

Methods: Utilizing targeted gene disruption of *hkb* by CRISPR/Cas9 genetic engineering, in combination with tissue-specific misexpression of *hkb*, I test the model that Hkb regulates secretion of Fog.

Results: Our work reveals that high levels of Fog secretion is limited to the SG cells about to invaginate, and that misexpression of Hkb in all SG cells affects the levels and distribution of secreted Fog. Preliminary data suggests that changes in Hkb expression result in aberrant invagination. Next, we will determine the localization of Fog and its corresponding GPCR in the absence of *hkb* and Hkb downstream transcriptional targets linked to vesicle trafficking.

Conclusions: Our findings suggest that Hkb facilitates the localized secretion of Fog from SG cells, which then activates the downstream GPCR-mediated pathway required for the cell shape changes that underlie invagination.

Tuesday, October 31

4:00 PM – 4:15 PM

CHARACTERIZATION OF NIPAH VIRUS-ASSOCIATED NEUROPATHOLOGY IN AFRICAN GREEN MONKEYS

Kerry Goldin¹, Yanling Lui², Meaghan Flagg¹, Rebecca Rosenke³, Emmie de Wit¹

¹Laboratory of Virology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, USA, ²Integrated Data Sciences Section (IDSS),

Research Technologies Branch, Division of Intramural Research, National Institute of Allergy and Infectious Disease, Bethesda, MD, USA, ³Rocky Mountain Veterinary Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, USA

Nipah virus (NiV) is a paramyxovirus, that causes frequent outbreaks in Southeast Asia. Clinical signs include respiratory and neurologic disease, and mortality is typically very high (~70%). In this retrospective study, brains of African green monkeys from three separate NiV studies were analyzed. Fourteen animals had histologic lesions in the CNS, ranging from mild to severe, and distributed throughout the cerebrum, cerebellum, and brainstem. Lesions could be categorized into non-suppurative meningitis, encephalitis, and discrete foci of parenchymal loss, with the former being the most common. Histologic lesions were mapped and compiled to reveal a random distribution throughout the brain. Immunohistochemistry for leukocytes revealed the most common cell types to be CD8+ T cells and CD68+ cells. An artificial neural network was deployed to generate cell counts for leukocyte IHC to quantify inflammatory populations. When comparing the AI cell counts to the semi-quantitative scores assigned by a pathologist, we found that there was a statistically significant correlation in the more numerous cell types (CD3, CD8, CD68), and that correlation was poor in the rare cell types (CD20, CD4). IHC and ISH against NiV detected viral antigen/RNA within quiescent endothelial cells, encephalitic foci, and within neurons in the absence of inflammation. Our findings shed much-needed light on NiV neuropathology and form the foundation for studies into NiV interactions with the immune system within the CNS. This work was funded (supported) by the Intramural Research Program of NIAID, NIH.

Tuesday, October 31

4:20 PM – 4:35 PM

IN VITRO DRUG SCREENING USING ORGANOID CULTURE DERIVED FROM CANINE LUNG CANCER FOR PRECISION MEDICINE

Yukino Machida¹, Yuna Ishizaki¹, So Doge¹, Kazuhiko Ochiai², Daigo Azakami³, Masaki Michishita¹

¹Department of Veterinary Pathology, Nippon Veterinary and Life Science University, Tokyo, Japan, ²Laboratory of Veterinary Hygiene, Nippon Veterinary and Life Science University, Tokyo, Japan, ³Laboratory of Veterinary Clinical Oncology, Tokyo University of Agriculture and Technology, Tokyo, Japan

Background: Organoid culture is a method in which epithelial cells are 3D-cultured in a microenvironment resembling living tissues, and form an organ-like structure (organoid). Cancer organoids are constituted from a heterogeneous cell population similar cancer tissue formed *in vivo*. Therefore, cancer organoid is excellent tool for elucidating carcinogenesis and drug screening. Therapy for canine lung adenocarcinoma is generally surgical resection, and no other effective treatment has been established.

Objective: The purpose of this study is to extract effective drugs for canine lung cancer therapy by *in vitro* drug screening using lung cancer organoid cultures.

Methods: Organoid culture was performed using surgically resected lung adenocarcinoma, and was used in kinase inhibitor library screening to extract candidate inhibitors that inhibit organoid formation.

Results: This assay has extracted several candidate drugs, such as gefitinib. In an *in vitro* sensitivity assay to gefitinib, the number of organoid cells decreased in a dose-dependent manner. In xenograft models, tumor growth was significantly suppressed in gefitinib-treated group compared with control. In tumors formed in mice, morphology and Ki-67 index did not differ between the two groups, but caspase-3 positive areas tended to be higher in the gefitinib-treated group.

Conclusions: Drug screening using canine lung cancer organoids was able to extract several candidate drugs. Furthermore, the anti-tumor effect of gefitinib on canine lung adenocarcinoma may be due to promotion of apoptosis. Therefore, lung cancer organoid culture is considered to be a useful tool for precision medicine.

Tuesday, October 31

4:35 PM – 4:50 PM

MECHANISMS OF HIPPOCAMPAL NEUROTOXICITY IN A MOUSE MODEL OF PRION DISEASE

Joie Lin¹, Julia Callender¹, Daniel McClatchy², Alejandro Sevillano¹, Tim Kurt¹, Helen Khuu¹, Joy Chen¹, Katrin Soldau¹, Donald Pizzo¹, Jin Wang¹, Anne Bang³, Kim Dore⁴, John Yates², Christina Sigurdson^{1,5}

¹Department of Pathology, University of California, San Diego, La Jolla, CA, USA,

²Department of Molecular Medicine and Neurobiology, The Scripps Research Institute, La Jolla, CA, USA, ³Conrad Prebys Center for Chemical Genomics, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA, ⁴Department of Neurobiology, University of California, San Diego, La Jolla, CA, USA, ⁵Department of Pathology, Immunology, and Microbiology, University of California, Davis, Davis, CA, USA

Prion diseases are fatal neurodegenerative disorders caused by prion protein aggregates. The cellular prion protein, PrP^C, binds prion aggregates, PrP^{Sc}, and also reportedly binds to amyloid beta and alpha-synuclein aggregates. The N-terminus of PrP^C has been implicated in neurotoxicity. To investigate whether and how PrP^C drives neurotoxicity, we generated a knock-in mouse with a G92N substitution in PrP^C (*Prnp*^{92N}) that is 100% fatal. This mutation creates an additional glycan at the N-terminus and may disrupt normal protein signaling. By P25-30, *Prnp*^{92N/92N} mice develop neurological signs, including hindlimb clasp and spontaneous seizures. Histologically, late-stage brains show neurodegenerative changes including severe hippocampal pyramidal neuronal necrosis (CA1), neuritic dystrophy, gliosis, and spongiform change. *Prnp*^{92N/92N} brains lack prion protein aggregates or infectivity, thus uncoupling aggregation from PrP-mediated neurotoxicity. To define neurotoxic signaling pathways, we performed phosphoproteomics on whole brain samples at mid-stage disease (P20). *Prnp*^{92N/92N} brains showed higher phosphorylation of GluN2B at S1303, which has been linked with increased excitotoxicity. Furthermore, *Prnp*^{92N/92N} hippocampal neurons have displayed signs of excitotoxic stress (dendritic beading), which can be rescued by MK801, an NMDA antagonist. *Prnp*^{92N/92N} primary cortical neurons have also

demonstrated aberrations in glutamate-mediated calcium signaling, underscoring the role of runaway excitotoxicity in *Prnp*^{92N/92N} disease pathogenesis. Bulk RNA sequencing of the hippocampus showed the downregulated expression of myelin-related genes, which were also reduced at the protein level by terminal disease. Taken together, these data indicate dysregulated GluN2B-linked glutamatergic signaling and demyelination in the *Prnp*^{92N/92N} mice and suggest that PrP^C normally functions to dampen neuronal activity.

1: COMPARISON OF PRO INFLAMMATORY RESPONSES INDUCED BY BARN AIR PARTICULATE MATTER IN BOVINE PULMONARY RESIDENT ALVEOLAR MACROPHAGES AND MONOCYTE-DERIVED MACROPHAGES

Zahra Nikousefat, Jeff Caswell

Department of Pathobiology, OVC, Guelph, ON, Canada

Particulate matter facilitates the spread of bovine respiratory disease upon encountering calves with air particles. Pulmonary alveolar macrophages (PAMs) and monocyte-derived macrophages (MDMs) recognize them and release pro-inflammatory cytokines, which are signalling molecules that regulate immune responses. However, the mechanism by which immune cells advance pro inflammatory reactions is poorly documented in cattle. In this experiment, bovine blood monocytes were cultured in plates with GM-CSF, and PAM were collected from bovine broncho-alveolar lavage fluid. The mature cells were primed with different sizes and doses of particulate matter collected from calf barn air. After 24 hours of exposure, the cytokines IL-1 β , TNF, IL-6 and IL-8 were measured by ELISA. The two cell types produced similar IL-1 β concentrations in response to different concentrations and sizes of particulate matter. IL-8 and TNF values were higher for PAMs than for MDM, while IL-6 was lower. In healthy animals, PAMs are directly exposed to inhaled particulate matter, making them the macrophages initially responsible for recognizing and engulfing these particles in the alveolar spaces. Also, PAMs are known to produce higher levels of IL-8, which is a potent neutrophil chemoattractant. On the other hand, MDMs encounter particles at the site of inflammation for a long time, so MDMs are recruited to and activated in the lung after initial recognition of particles by PAMs. It makes them exhibit a broader range of cytokine production, including IL-1 β and IL-6. Thus, the interplay between particle characteristics, dose, macrophage phenotype, and the microenvironment can all contribute to the observed response.

2: THE POTENTIAL OF PLATELET-RICH PLASMA IN ALLEVIATION OF PAIN ASSOCIATED WITH CHRONIC COMPRESSION OF DORSAL ROOT GANGLION IN RAT

Preeti Chaudhary, Jaisan Islam, Sanggu Kim, Elina K.C, Young Park, Soochong Kim
Chungbuk National University, Cheongju, Republic of Korea

Introduction

Chronic lower back pain with different stinging phenomena is a common symptom of lumbosacral disorders and results in functional impairment along with chronic neuropathic pain (CNP). Although platelet-rich plasma (PRP) plays an important role in

modulating chronic inflammatory pain and diabetic neuropathy, its therapeutic efficacy in CNP is yet to be understood. We have investigated whether the PRP administration has any effect on CNP using a chronic compression of the dorsal root ganglion (CCD) rat model.

Methods

Animals were divided into three groups: CCD, sham, and control. PRP or PBS was injected intrathecally between the L4-L5 spinal cords and alterations of behavioral responses including mechanical withdrawal threshold test, mechanical withdrawal latency test, and thermal latency test were measured. *In vivo* electrophysiology data were obtained from the contralateral ventral posterolateral (VPL) thalamus and expression levels of specific astrocytic markers were observed by immunofluorescence.

Results

CCD group, but not sham and control, developed a decreased pain threshold level from day 7-day 19. The pain-related behavioral responses were improved towards the pre-surgery level only in PRP-treated CCD models suggesting the role of PRP in the improvement of CNP. Consistently, electrophysiology data showed a significant decrease in VPL thalamic activity upon PRP administration. Moreover, pain-transmission-associated astrocyte markers including GFAP, PKM2, and HMGB1 were ablated in PRP-treated CCD models in IF.

Conclusion

PRP attenuates CNP in a CCD rat model by regulating astrocytic activity associated with pain transmission, thereby providing evidence of using PRP as a novel therapeutic approach for chronic neuropathic pain.

3: CHARACTERIZATION OF EBOLA VIRUS AND SUDAN VIRUS INFECTION FOLLOWING INTRAMUSCULAR EXPOSURE IN FERRETS (MUSTELA PUSTORIUS FURO).

Brittany Beavis, Elizabeth Zumbrun, Shannon Hentschel, Samantha Zak, Xiankun Zeng, Andrew Herbert

U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD, USA

Background: Filoviruses cause viral hemorrhagic fevers in humans and are high-consequence pathogens with high case fatality rates and limited options for treatment and prevention. Historically, non-human primates (NHPs) have been the preferred animal model for evaluating filovirus medical countermeasures. It has become difficult and costly to source NHPs for research, necessitating a new animal model for filoviruses. Ferrets were previously reported to have a similar course of disease and clinicopathological findings to both humans and NHPs when infected with ebolaviruses. This study characterizes the natural history of Ebola virus (EBOV) and Sudan virus (SUDV) in ferrets following intramuscular (IM) exposure.

Methods: The study was performed in two iterations: Ebola virus (EBOV) and Sudan virus (SUDV). In both iterations male (n=12) and female (n=12) ferrets were exposed via IM injection with 1000 plaque-forming units alongside mock-exposed male (n=3) and female (n=3) ferrets. Mock and infected animals were housed in separate rooms within the BSL-4 containment suite but otherwise handled similarly. Animals were observed twice daily and complete blood count, biochemical panel, and viremia levels were monitored. Necropsies were performed by a board-certified pathologist and tissues fixed in 10% neutral buffered formalin for routine hematoxylin & eosin processing as well as virus-specific immunohistochemistry and in situ hybridization.

Results: EBOV and SUDV infection in ferrets mirrors the course of disease observed in NHPs, including gross lesions, microscopic lesions, and clinicopathologic findings, with some variation in clinical signs.

Conclusion: Ferrets represent a reasonable intermediate animal model for ebolavirus research and medical countermeasure efficacy testing.

4: THE COMBINATION OF MUTANT IL-7R AND RASGRP1 IS SUFFICIENT FOR LEUKEMOGENESIS

Steven Hsu^{1,2,3}, Gisele Rodrigues¹, Hila Winer¹, Julie Hixon¹, Wenqing Li¹, Scott Durum¹

¹National Institutes of Health, Frederick, MD, USA, ²Michigan State University, East Lansing, MI, USA, ³Comparative Biomedical Scientist Training Program, Bethesda, MD, USA

Acute lymphoblastic leukemia (ALL) is the most common cancer in children; of which, 15% are T-cell ALL (T-ALL). There is a need to develop targeted therapies to lessen side effects. Approximately 10% of T-ALL cases express gain-of-function, mutant IL-7R. Expression of the mutant IL-7R alone is insufficient for leukemogenesis. Sufficiency can be achieved with the addition of a secondary oncogene. Ras guanine nucleotide releasing protein 1 (RasGRP1), an activator of Ras that is overexpressed in 50% of T-ALL cases, was selected as a potential cooperating oncogene. The hypothesis is that the combination of mutant IL-7R and overexpression of RasGRP1 is sufficient for leukemogenesis. The thymocyte-OP9-DL4 co-culture system paired with retroviral transduction was used to test the hypothesis. Thymocytes were harvested from C57BL/6 mice. Retroviral vectors bearing *mutant Il7r-alpha* and *Rasgrp1* were used to transduce thymocytes. Transduced thymocytes were injected into *Rag*^{-/-} mice. Blood, bone marrow, spleen, lymph nodes, and thymus were analyzed by histology and/or flow cytometry at endpoint. Histologically, the lymphoid organs were expanded by large numbers of neoplastic cells, which were CD8⁺, TCR-beta⁺, and pre-TCR-alpha-low, akin to thymocytes at the immature single positive (ISP) stage. There was also a TCR-beta-delta⁺ subpopulation. In conclusion, the combination of mutant IL-7R and overexpression of RasGRP1 in murine thymocytes is sufficient for leukemogenesis, and the neoplastic cells are arrested at the ISP stage with aberrant TCR-beta-delta expression. RasGRP1 is a potential therapeutic target in T-ALL. The mutant IL-7R-RasGRP1 driven leukemia may serve as a model to study the biology of the aberrant TCR-beta-delta.

5: DOPAMINE RECEPTOR D1 SIGNALING DOWNREGULATES MUTANT EGFR SIGNALING AND ENHANCES RESPONSE TO EGFR INHIBITOR OSIMERTINIB IN LUNG CANCER

Amy Flis¹, Adriana Zingone¹, Leila Toulabi², Christopher Grant¹, Bríd Ryan¹

¹Integrative Molecular Epidemiology Unit, Laboratory of Human Carcinogenesis, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA, ²Molecular Genetics and Carcinogenesis Section, Laboratory of Human Carcinogenesis, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA

Background:

In lung cancer, mutation-targeting therapies including EGFR inhibitors such as Osimertinib (Osim) have significantly improved patient outcomes, but resistance develops inevitably. Thus, strategies to enhance these drugs' efficacy are urgently needed. Our lab recently identified dopamine receptor D1 (DRD1) as a novel tumor suppressor in lung cancer and showed that DRD1 signaling modulates wildtype EGFR signaling.

Objective:

Our current aim is to determine whether DRD1 signaling can regulate mutant EGFR signaling and enhance and prolong response to EGFR-targeting therapies.

Methods:

In EGFR-mutant lung adenocarcinoma cells, we re-introduced strong DRD1 expression via transfection and evaluated changes in cell proliferation, EGFR signaling, and response to Osim. To evaluate effects on resistance, we continuously treated DRD1-expressing cells with slowly increasing Osim doses until cells had a 10-fold IC50 increase.

Results:

Compared to vector control cells, DRD1-expressing cells exhibited reduced proliferation and reduced EGFR protein levels and signaling. An IC50-determining viability assay showed DRD1 expression increased sensitivity to Osim. Although DRD1 expression didn't delay onset of resistance, Osim-resistant cells had downregulated DRD1. When we re-introduced strong DRD1 expression in resistant cells, DRD1 signaling partially re-sensitized cells to Osim.

Conclusions:

Our results show DRD1 signaling can regulate mutant EGFR signaling and enhance the response of EGFR-mutant cells to Osim. The findings in Osim-resistant cells suggest DRD1 signaling inhibits Osim resistance development, and its re-introduction impairs resistance. As we continue to investigate how DRD1 signaling can augment the efficacy of EGFR inhibitors, we aim to identify optimal strategies that will improve lung cancer patient outcomes.

6: MICROPHYSIOLOGICAL MODELS OF THE HUMAN RESPIRATORY SYSTEM

Dayananda Siddappa Thimmanahalli¹, Shabnam Ghiasvand¹, Peter Piepenhagen¹, Caroline Morel¹, Robert O'Brien², Nicholas Panzarino², Mack Madison², Joo-Hye Song², David Habel², Ingeborg Langohr¹, Dinesh Bangari¹

¹Global Discovery Pathology, Translational Models Research Platform, Sanofi, Cambridge, MA, USA, ²Immunology and Inflammation Research Therapeutic Area, Sanofi, Cambridge, MA, USA

Background/Objective: Respiratory diseases are a significant health burden worldwide representing a major unmet medical need. New treatments for respiratory diseases often fail in the clinic either due to lack of efficacy or safety risks. Conventional in-vitro and animal models are inadequate in capturing complex human-specific biology. To better predict efficacy and safety of novel drugs for respiratory disease, we set out to explore microphysiological systems (MPS).

Methods: Primary human bronchial epithelial cells and fibroblasts were cocultured in air liquid interface (ALI) configuration to model the upper respiratory tract. Primary human type II alveolar epithelial cells and human fibroblasts were cocultured in matrigel to model the alveoli. Cultures were treated with recombinant human (rh)IL-13 or cigarette smoke condensate (CSC) in culture media. Organoids or conditioned culture media were evaluated for histology, immunohistochemistry, changes in gene or protein expression.

Results/Conclusion: The ALI coculture model was comprised of pseudostratified respiratory epithelium organized in the form of folds/villous like structures which consisted of mainly basal cells, ciliated columnar epithelial cells, and goblet cells. Fibroblasts were arranged in one or two cell layers with secreted collagen. As expected, treatment with rhIL-13 resulted in an increased number of goblet cells. Treatment with CSC resulted in decreased epithelial thickness and squamous metaplasia. Primary human alveolar type 2 epithelial cells and fibroblasts cocultured in matrigel were grown into 3D structures which recapitulated alveolar anatomy. Taken together, we propose these two 3D MPS can be effectively used to model human airway and alveoli in a drug discovery setting.

7: VIRULENCE COMPARISON OF 4 PORCINE CIRCOVIRUS TYPE 2 (PCV-2) GENOTYPES: 2A, 2B, 2D, AND 2E WITH A SINGLE INFECTION AND CO-INFECTION WITH PCV2 AND PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV)

Jeongmin Suh, Sehyeong Ham, Chanhee Chae
Seoul National University, Seoul, Republic of Korea

The aim of this study was to assess the virulence of four porcine circovirus type 2 (PCV2) genotypes (2a, 2b, 2d, and 2e) in pigs, both when infected with a single PCV2 genotype and when co-infected with a combination of one PCV2 genotype and porcine reproductive and respiratory syndrome virus (PRRSV). Virulence was determined based on the levels of PCV2 loads in the blood and tissue of inguinal lymph nodes, as well as the severity of lymphoid microscopic lesions. Among the single PCV2 genotype

infections, PCV2a, 2b, and 2d exhibited comparable levels of virulence to each other, while being more virulent than the PCV2e-infected group. In the context of PCV2 and PRRSV dual infection, the combination of PCV2d and PRRSV demonstrated higher virulence compared to the other three PCV2 genotypes (2a, 2b, and 2e) each in combination with PRRSV. Both the PCV2a-PRRSV dual infection and PCV2b-PRRSV dual infection groups exhibited greater virulence compared to the PCV2e-PRRSV dual infection group. These findings indicate that PCV2d displays heightened virulence compared to the other three PCV2 genotypes (2a, 2b, and 2e). This increased virulence is suggested to be attributed to an additional amino acid (lysine residue) present within the open reading frame 2 (ORF2) of PCV2d. The decreased virulence of PCV2e compared to the other three PCV2 genotypes (2a, 2b, and 2d) can potentially be attributed to the presence of additional amino acids in ORF2. This study demonstrates that virulence is strain-specific, providing valuable insights for identifying virulence determinants through genotypic comparisons.

8: A MOUSE MODEL OF THE ATOPIC MARCH AND FOOD ALLERGIES

Katelin Davis^{1,2,3}, Estefania Claudio-Etienne¹, Derron Alves¹, Lashawna Leak¹, Karen Laky¹, Pamela Frischmeyer-Guerrero¹

¹National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA, ²NIH Comparative Biomedical Scientist Training Program, Bethesda, MD, USA, ³Purdue University, West Lafayette, IN, USA

Background: The epidemiologic link between atopic dermatitis (AD) and the subsequent development of food allergies (FA), known as the atopic march, has long been recognized. Current models posit that exposure to food antigens through a defective skin barrier promotes sensitization. However, not all infants with AD develop FA and oral tolerance mechanisms should prevent innocuous food antigens from being recognized by the immune system. We hypothesized that AD alters the immune environment in the small intestine to disrupt oral tolerance and predispose infants to FA.

Objective: We sought to model the atopic march in mice to evaluate how AD modulates the intestinal epithelial layer and immune system to promote FA development.

Methods: Calcipotriol was applied to the ears of BALB/c mice to model AD followed by application of ovalbumin to the inflamed skin. AD lesions were validated by gross and histologic evaluation. Epithelial and leukocyte alterations in the small intestine were assessed by histology, flow cytometry, and cell culture.

Results: Calcipotriol induced gross and histologic lesions that mimicked human AD. Mice developed ovalbumin-specific IgE antibodies and anaphylaxed to ovalbumin upon challenge, similar to infants with FA. AD-induced alterations in the intestine included goblet cell hyperplasia and hypertrophy, villous shortening, mast cell expansion, and increased numbers of proinflammatory macrophages and neutrophils. Cytokines levels were elevated in multiple tissues in atopic mice.

Conclusions: Using a mouse model of AD, we find that AD induces a proinflammatory state in the intestine that is associated with the development of FA, mimicking the atopic march.

9: THE MOUSE BODY FARM: HISTOPATHOLOGIC EVALUATION OF DECOMPOSITION OVER TIME

Emily Mackey, Monica Paitsel, George Schaaf, Nancy Kock
Wake Forest School of Medicine, Winston-Salem, NC, USA

The mouse (*Mus musculus*) is the species most commonly used in biomedical research. Despite the fundamental role they play in research, little is known about the rates of tissue decomposition in this widely used laboratory species. The aim of this study was to qualitatively identify and score histologic postmortem changes in laboratory mice at varied time intervals to aid in estimation of time of death and to establish autolytic alterations that may interfere with histopathologic diagnosis of tissues harvested during necropsies. The study involved 28 adult mice (17 males and 11 females) on a C57BL/6 background that were euthanized simultaneously and either necropsied immediately or stored at room temperature, and then necropsied at 24h, 72h, and 120h after death. Selected tissues were collected and routinely processed for histology, embedded in paraffin, and stained with hematoxylin and eosin. The slides were evaluated by light microscopy and scored using a semi-quantitative scoring system based on six criteria of autolysis and the percentage of microscopic field involved. As expected, the data showed that immediate harvesting of fresh samples provided the best quality. The gallbladder, pancreas, and intestinal tract were the most sensitive organs to autolysis while the heart, skeletal muscle, and skin were least affected. Postmortem overgrowth of bacteria were present in the heart, lungs, and brain at 120h after death. These findings could be used to estimate the postmortem interval in laboratory mice

10: MECHANISM OF ACTION OF A NOVEL IN VIVO VIRULENCE FACTOR OF EHRLICHIA — EHF0962

Rory Chia-Ching Chien, Mingqun Lin, Yasuko Rikihisa
The Ohio State University, Columbus, OH, USA

Background

Ehrlichiosis is a potentially life-threatening disease caused by infection of *Ehrlichia* spp., blood-borne obligate intracellular bacteria. To overcome the mammalian immune system, establish infection, and cause diseases within the host, *Ehrlichia* needs to utilize additional strategies (e.g., *in vivo* virulence factors) to those required to infect eukaryotic cells in culture. Our laboratory developed a mouse model of ehrlichiosis using *Ehrlichia japonica* (*Eja*) which causes fatal disease in mice in a dose-dependent manner ($LD_{50} = 100$ bacteria). We created a library of *Eja* mutants by using Himar1 transposon mutagenesis system that randomly inserts transposon into *Eja* genome. We found that mutant H59 is the clone that has a Himar1 insertion in *EHF0962*. *EHF0962* encodes a protein EHF0962 (~13.5 kDa) that is conserved among *Ehrlichia* spp.

Objective

To determine the functions of a novel virulence factor of *Ehrlichia*, EHF0962, in a fatal ehrlichiosis mouse model.

Results

We verified native EHF0962 protein production in wild-type (WT) *Eja* and its absence in H59 (*EHF0962* disrupted by Himar1 insertion). *EHF0962* is dispensable for infection of cultured cell lines. Compared to WT *Eja*, H59 IP- or IV-challenged mice showed delayed mortality and had significantly reduced bacterial loads in the blood, liver, and spleen, suggesting that *EHF0962* is a critical *in vivo* virulence factor. We found that host cell-free H59 rapidly lost infectivity in mouse plasma with/without heat inactivation, heat-inactivated fetal bovine serum, or DMEM.

Conclusions

Our findings suggest that *EHF0962* mediates *Ehrlichia* resistance at the extracellular stage to promote *in vivo* infection.

11: CATTLE WITH THE E211K POLYMORPHISM INCUBATE H-BSE FASTER THAN WILD-TYPE CATTLE AFTER INTRACRANIAL INOCULATION

Eric Cassmann, Justin Greenlee

United States Department of Agriculture, Agricultural Research Service, National Animal Disease Center, Ames, IA, USA

In 2006 a case of H-BSE was associated with a germline mutation in the *PRNP* gene resulting in a lysine substitution for glutamic acid at codon 211 (E221K). In the present study, we aimed to determine the effect of the E211K polymorphism on the incubation time in cattle inoculated with the agent of H-BSE. Cattle representing three *PRNP* genotype groups were included in the study: EE211 (n=3), EK211 (n=4), and KK211 (n=1). Cattle were separated into two inoculation groups and intracranially inoculated with 0.1 gram of brain homogenate from cattle with different *PRNP* genotypes that had H-BSE. The inocula originated from a 2006 US case of H-BSE associated with the E211K polymorphism and a 2004 US case of H-BSE in a wild-type EE211 genotype bovid. Previously published incubation periods from nine EE211 steers inoculated with 2004 H-BSE EE211 were used to aid in the comparisons. All cattle developed clinical disease and satisfied clinicopathologic criteria for the diagnosis of H-BSE. There was no difference in incubation period between inocula types ($p=0.7838$); however, the incubation periods were different between recipient genotype regardless of the inoculum that was administered. Collectively, EK211 and KK211 genotype groups had shorter incubation periods than wild-type EE211 recipient cattle ($p<0.0001$). The mean incubation periods were 10.2 months and 17.2 months respectively. Cattle with the K211 polymorphism have shorter incubation times after inoculation with the H-BSE agent compared to cattle with the wild-type EE211 genotype.

12: VIRULENCE COMPARISON OF FOUR PORCINE CIRCOVIRUS TYPE 2 (PCV2) GENOTYPES (2A, 2B, 2D, AND 2E) IN PIGS SINGULARLY INFECTED WITH PCV2

AND PIGS DUALY INFECTED WITH PCV2 AND MYCOPLASMA HYOPNEUMONIAE

Sehyeong Ham, Jeongmin Suh, Chanhee Chae
Seoul national university, Seoul, Republic of Korea

This study aimed to compare the virulence of four porcine circovirus type 2 (PCV2) genotypes (2a, 2b, 2d, and 2e). This study consisted of 10 groups (6 pigs per group). Four of these groups were infected with each of the four genotypes of PCV2, another four groups were dually infected with *Mycoplasma hyopneumoniae* and one of the four PCV2 genotypes, one group was infected with only *M. hyopneumoniae*, and the last group served as a negative control. Virulence was assessed by measuring the amount of PCV2 loads present in the blood and lymph nodes, and by evaluating the severity of lymphoid lesions. Significant differences in virulence were observed among the four PCV2 genotypes. Within the single infection model, PCV2a, PCV2b, and PCV2d proved to be more virulent than PCV2e, but significant differences in virulence were not observed among PCV2a, PCV2b, and PCV2d groups. In the case of dual infection model, PCV2d displayed greater virulence compared to the other three PCV2 genotypes. *M. hyopneumoniae* enhanced the severity of PCV2-associated lymphoid lesions and increased the load of PCV2 in the blood and lymph nodes, independent of the PCV2 genotype. By contrast, PCV2 was not able to potentiate the severity of lung lesions induced by *Mycoplasma*, nor increase the level of *M. hyopneumoniae* in the laryngeal load. The results of this study demonstrated that PCV2d is of major clinical importance, while PCV2e is of minor clinical importance

14: SAFETY AND IMMUNOGENECITY OF A NEWCASTLE DISEASE VIRUS VACCINE AGAINST SARS-COV-2 IN THE FERRET (*MUSTELA PUTORIUS FURO*)

Sara Pagliarani¹, Jaime Tuling¹, Phuc Pham¹, Alexander Leacy¹, Shawn Babiuk², Brandon Lillie¹, Pauline Delnatte¹, Sarah Wootton¹, Leonardo Susta¹

¹Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, ²Canadian Food Inspection Agency, National Centre for Foreign Animal Disease, Winnipeg, MB, Canada

Introduction: Transmission of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) to animals is a public health concern. In particular, mustelids are susceptible to SARS-CoV-2, as documented by infection in domestic ferrets and outbreaks in farmed minks. Newcastle disease virus (NDV) is an avian vaccine vector with a high safety profile. Here, we evaluated the safety and efficacy of an NDV-vectored vaccine against SARS-CoV-2 in a mustelid representative, the ferret.

Materials and Methods: Groups of ferrets were housed separately and administered the vaccine or control intranasally, using a prime-boost regimen 28 days apart. The control group ($n=4$) received a fluorochrome-expressing NDV (NDV-GFP) at 1×10^7 plaque forming units (PFU)/ferret, while the vaccine groups received NDV expressing the prefusion-stabilized version of SARS-CoV-2 spike protein (NDV-PFS) at low dose (1×10^7 PFU/ferret, $n=4$) or high dose (1×10^8 PFU/ferret, $n=5$). Temperature, swabs, and blood were collected to evaluate, respectively, reactogenicity, shedding, and

development of IgG against the spike protein. At necropsy, tissues were collected to assess vaccine biodistribution and microscopic lesions.

Results: No clinical signs or fever were detected following vaccination. All vaccinated ferrets seroconverted against SARS-CoV-2 spike, while control ferrets did not. Kinetics of seroconversion indicated that the high-dose group produced significantly more antibodies compared to the low dose, as shown by the area under the curve (1.791 ± 0.2959 vs. 0.9803 ± 0.1535). Using a surrogate neutralization assay, neutralizing activity was observed in 100% of vaccinated ferrets in both dose groups. Histopathology revealed no lesions.

Conclusions: NDV is a promising vaccine candidate against SARS-CoV-2 in small carnivores.

15: ENHANCED DISEASE AND LUNG PATHOLOGY IN HETEROLOGOUS PRIME-BOOST H1N1 VACCINATES FOLLOWING MISMATCHED H1N2 CHALLENGE IN THE SWINE MODEL

Vasilis Pliasis^{1,2}, Peter Neasham², Maria Naskou², Rachel Neto², Philip Strate², Fletcher North², Stephen Pedroza², Mark Tompkins³, Constantinos Kyriakis^{2,3}

¹Michigan State University, East Lansing, MI, USA, ²Auburn University, Auburn, AL, USA, ³University of Georgia, Athens, GA, USA

Background: Influenza A virus (IAV) is considered an important respiratory pathogen in global public and animal health. To overcome the ever-increasing IAV antigenic diversity, there is a need to develop broadly protective vaccine strategies.

Objective: The goal of this study was to investigate the immunogenicity and protective efficacy of H1N1 heterologous prime-boost vaccination compared to the corresponding homologous prime-boost monovalent and bivalent vaccines following challenge with a mismatched H1N2 influenza virus in the swine model.

Methods: A total of 36 influenza-seronegative piglets were used in the study. Five groups (n=6) were homologous prime-boost vaccinated with either an oil-adjuvanted whole-inactivated virus (WIV) monovalent A/swine/GA/27480/2019 (GA19) H1N2 vaccine, a WIV monovalent A/swine/MN/A02636116/2021 H1N1 (MN21) vaccine, a WIV monovalent A/CA/07/2009 H1N1 (CA09), a WIV bivalent vaccine comprised of CA09 and MN21 or adjuvant only (mock-vaccinated group). A sixth group was vaccinated at prime with CA09 WIV and boosted with MN21 WIV (heterologous prime-boost group). Four weeks post-boost, pigs were challenged with the GA19 H1N2 strain. Vaccine-induced protection was evaluated based on five parameters: humoral responses, clinical scores, virus titers in respiratory tissue homogenates, BALF cytology, and pulmonary pathology.

Results: Heterologous-boosting did not expand the scope of cross-reactive antibody responses against antigenically distinct swine and human IAVs. Furthermore, following challenge with the mismatched GA19 H1N2 virus, heterologous prime-boosted pigs showed prolonged clinical disease and increased pulmonary pathology scores compared to mock-vaccinated pigs.

Conclusions: H1-specific heterologous prime-boost vaccination, rather than enhancing cross-protection, exacerbated the clinical outcome and pathology after challenge with the mismatched GA19 strain.

17: EFFECT OF AGE ON AQUATIC BIRD BORNAVIRUS-1 INFECTION IN TURKEYS

Lisa Gordon¹, Jaime Tuling¹, Antonius Khoury¹, Alexander Leacy¹, Phuc Pham¹, Emily Brouwer², Brandon Lillie¹, Leonardo Susta¹

¹Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, ²Animal Health Laboratory, University of Guelph, Guelph, ON, Canada

Background: Aquatic bird bornavirus-1 (ABBV-1) is an orthobornavirus associated with neurological disease and inflammation of the nervous system in wild waterfowl. Chickens are experimentally permissive to ABBV-1 infection, suggesting that other gallinaceous species may also be susceptible.

Objective: Determine the pathogenesis of ABBV-1 in turkeys (*Meleagris gallopavo*) by assessing development of clinical signs and lesions, seroconversion, and virus RNA load in tissues. The impact of age on these variables was evaluated by comparing birds infected at 6-weeks- and one-day-of-age.

Methods: 6-week-old (*n*, 80) and day-old (*n*, 80) turkeys were divided into 2 groups (40/group), and inoculated intramuscularly with ABBV-1 (6.9×10^6 FFUs (focus forming units)/bird) or carrier only (control). Birds were monitored daily, and at 1, 4, 8, and 12 weeks postinfection (wpi), 10 birds from each group were euthanized for sample collection.

Results: No gross lesions were observed. Four birds in the young group developed neurological signs by 9 wpi. By 12 wpi, most spinal cords (young: 11/11; old: 8/10) and variable proportions of brains (young: 11/11; old: 1/10) from inoculated turkeys were positive for ABBV-1 RNA by RT-qPCR. At 12 wpi, lymphocytic perivascular inflammation was identified in the brain and spinal cord, respectively, of 100% (11/11) and 73% (8/11) of young inoculated turkeys. No inflammation was detected in the old inoculated birds, or controls.

Conclusions: ABBV-1 delivered intramuscularly can infect and cause clinical encephalitis in turkeys, albeit at low rates. Younger turkeys appear more susceptible to infection, although progression of disease may be slower in older birds.

Industrial and Toxicologic Pathology

Monday, October 30 | 2:12 PM – 2:24 PM

Session Chair: Amit Kumar

Monday, October 30

2:12 PM – 2:24 PM

A UNIQUE BILE DUCT TOXICITY IN DOGS

Rebecca Kohnken, Tim Brayman, Junhai Yang, James Kath, Richard Peterson
Abbvie, North Chicago, IL, USA

Biliary toxicity in preclinical species is not uncommonly observed in small molecule drug development, and is characterized by proliferation of small-caliber ducts (ductular reaction) and elevations in clinical pathology measures of cholestasis (bilirubin, alkaline phosphatase). Toxicity of the large-caliber ducts, however, is rare. This work presents a finding in large bile ducts of dogs administered an investigational test-article for 5 days characterized by epithelial (cholangiocyte) necrosis with periductal mixed cell inflammation, in the absence of any changes in clinical chemistry. With longer duration of dosing (2 weeks and 4 weeks), the primary toxicity finding was cholangiocyte hypertrophy and hyperplasia, with periductal mononuclear cell inflammation and fibrosis. Electron microscopy demonstrated increased numbers of enlarged lysosomes and autophagosomes within cholangiocyte cytoplasm vs that of control animals. Further evaluation revealed concentrated levels of test-article in the bile and within cholangiocytes as compared to surrounding hepatic parenchyma and to plasma. Follow-up investigation into the mechanism of toxicity demonstrated that the test article is highly cytotoxic to cholangiocytes in vitro but not to hepatocytes. Further, the test-article results in a massive induction of autophagic flux in an in vitro lysosome tracking assay. We hypothesize that the physicochemical properties of the test-article (which is highly basic and lipophilic) result in accumulation in bile and uptake into cholangiocytes. Once within lysosomes, the basic nature of the molecule prevents acidification, shunting the lysosome to fuse with autophagosomes. To the authors' knowledge, this mechanism of toxicity and the resulting pathologic finding have not been reported in dogs.

Monday, October 30

2:24 PM – 2:36 PM

DEEP LEARNING MODEL FOR ANALYSIS OF XENOBIOTIC-INDUCED SEMINIFEROUS TUBULAR DEGENERATION AND ATROPHY IN RAT TESTES

Madhu Ravi¹, Jogile Kuklyte², Shane Ryan², Laoise Bissett², Daniel Rudmann³

¹Charles River Laboratories International, Laval, QC, Canada, ²Deciphex, Dublin, Ireland, ³Charles River Laboratories, Ashland, OH, USA

Xenobiotics, including pharmaceutical drugs and environmental chemicals, can have detrimental effects on male reproductive health by inducing testicular changes and infertility in humans. The initial safety or hazard assessment for reproductive toxicity of xenobiotics is usually performed in rat non-clinical studies. An analysis of the global histopathology database within Charles River indicated that degeneration and atrophy were the most commonly reported diagnoses in rat testes. We hypothesize that a decision support deep learning artificial intelligence (AI) convolutional neural network (CNN) classifier for these common lesions in rat testes could be developed and would assist increasing pathologists' diagnostic accuracy and efficiency. Annotations for normal testicular structures and abnormal lesions classes (degeneration/atrophy and atrophy) using INHAND criteria were performed on Whole Slide Images (WSI) of rat testes at 10x magnification using Deciphex Patholytix Preclinical Study Browser. A CNN classifier was developed to create AI generated inference probability masks that detected seminiferous tubule degeneration and atrophy. The classifier was qualified at the pixel level using confusion matrices and F1 scores followed by pathologists visual inspection of the classifier output. The CNN classifier successfully discriminated normal and abnormal testicular structures with high accuracy and sensitivity, and the model has

potential to use as a decision-support tool by toxicologic pathologists while assessing xenobiotic-induced changes in rat testes.

Monday, October 30

2:36 PM – 2:48 PM

DEVELOPMENTAL AND NEUROBEHAVIOR ALTERATIONS FOLLOWING THE EARLY-LIFE EXPOSURE TO PERCHLOROETHYLENE (PERC) IN THE ZEBRAFISH MODEL

Taylor Yenrick, Ji-Hang Yin, Katharine Horzmann

Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA

Background: Perchloroethylene (PERC) is routinely used in industrial applications as a chemical solvent. However, improper disposal results in widespread environmental contamination, and reported developmental toxicity and neurotoxicity in human and animal models. **Objective:** To investigate the immediate and long-term effects of early-life exposure to environmentally relevant concentrations of PERC in zebrafish.

Methods: Fertilized embryos were exposed to 0, 5, 50, and 500 ppb PERC for 24 hours post-fertilization (hpf), 120 hpf, or were rinsed at 120 hpf and raised to 6 months with no additional PERC exposure. Embryo survival and hatching percentage were monitored and a photomotor response assay (PMR) was performed at 24 hpf. Morphologic changes, cardiac function, and vision motor behavioral response were evaluated at 120 hpf. Adult neurobehavioral assessment was conducted through novel tank test, open field test, and T maze. **Results:** Embryos exposed to PERC showed no differences in survival and hatching. Larvae at 120 hpf had increased heart rate at 5 and 500 ppb and altered morphology. Although no behavioral alterations were observed at 120 hpf, embryos at 24 hpf exhibited increased activity. This persisted in adult male fish which had increase in time spent in the top zone of the novel tank test at 5 and 50 ppb. Adult zebrafish exposed to PERC did not demonstrate impaired cognition. **Conclusion:** Our results indicated PERC exposure during early life induced developmental toxicity and altered behavior in adults. The robust behavior of PMR may be predictive of adult behavior.

Monday, October 30

2:48 PM – 3:00 PM

TOXICOLOGIC EVALUATION OF TARGETED PROTEIN DEGRADERS

Rebecca Kohnken, Richard Peterson, Lise Loberg

Abbvie, North Chicago, IL, USA

Targeted protein degraders are a relatively new class of small molecule therapeutics that utilize a heterobifunctional platform to engage both the protein of interest as well as an E3 ubiquitin ligase resulting in polyubiquitination and degradation of the target. While there are currently no marketed degraders, the number of investigational new drugs entering clinical trials is growing. This novel approach has the potential to degrade “undruggable” targets in addition to eliminating any non-enzymatic functions of a target protein by virtue of removing the protein entirely from the cell. Along with this promise comes many theoretical risks regarding safety: relating to the novel mechanism, atypical

physiochemical properties, potential for catalytic activity, and perturbation of a critical cellular function (proteasomal degradation) in unintended cell types. Therefore, degraders pose many interesting challenges for safety assessment. Firstly, due to their large size, development of degraders involves unique considerations around drug solubility and formulation and pharmacokinetics. Second, there is potential for novel mechanisms of off-target toxicity related to either the ligase binding or target binding ligands, or both. Third, there are additional considerations around species selection and applicability of many early safety assessment tools commonly employed for discovery and development of more traditional small molecules. Finally, several toxicities have been reported in the literature which may represent platform concerns for degraders, such as effects in the peripheral nervous system and teratogenicity. This work will provide an introduction and overview of development and safety considerations for targeted protein degraders and present a few illustrative case examples.

Monday, October 30

3:30 PM – 3:42 PM

IMMUNOHISTOCHEMISTRY-FREE ENHANCED HISTOPATHOLOGY OF THE RAT SPLEEN USING DEEP LEARNING

Shima Mehrvar, Lauren Himmel, Kevin Maisonave, Wayne Buck, Magali Guffroy, Bhupinder Bawa

Preclinical Safety, AbbVie Inc., North Chicago, IL, USA

Enhanced histopathologic evaluation of lymphoid organs involves microscopic examination with semi-quantitative descriptions of changes in subanatomic compartments within the tissues. Challenges in both accurate lymphoid compartment recognition and cellularity estimation render the process inherently prone to interobserver variability. Immunohistochemistry is required for definitively differentiating T and B cell compartments; however, routine toxicologic assessments are based solely on hematoxylin and eosin-stained (H&E) slides. We hypothesized that a deep learning (DL) model would accurately segment and quantify lymphoid compartments in the spleen from H&E slides, enabling rapid quantitative morphological assessment. A DL model was developed using naïve and vehicle control Sprague-Dawley rats to quantify area and cellularity of periarteriolar lymphoid sheaths (PALS), follicles, germinal centers, marginal zone, and red pulp from H&E slides. The ground truth for training and validation was obtained from pathologist-guided annotations of H&E slides with co-registered dually CD3/CD79a immunostained slides. The model input was limited to H&E-stained slides, and it quantitatively characterized the range of variability found in these normal rat spleens. Performance of the segmentation model was evaluated by dice similarity coefficient (DSC). We found that the DL model could identify splenic compartments with high accuracy (overall DSC=93.3%) directly from the H&E-stained tissue. DSC for follicles was 89.4% and DSC for PALS was 91.8%, compared to pathologists' annotation of H&E slides with DSC of 75.8% and 77.6%, respectively. This level of model performance in abnormal spleens would support the implementation of this DL algorithm in nonclinical toxicity studies to assist pathologists for gains in efficiency and accuracy.

19: DETECTING VARIOUS RAT-LIVER LESIONS WITH HISTOLOGICAL REPRESENTATION LEARNED BY NORMAL-TISSUE IMAGES

Mitsuru Negishi¹, Shunsuke Tominaga¹, Akira Inomata²

¹Imaging Technology Center, FUJIFILM Corporation, Kanagawa, Japan, ²Bio Science & Engineering Laboratories, FUJIFILM Corporation, Kanagawa, Japan

Background: Histopathologic examination is critical in drug discovery and requires the most time and effort to find out various lesions comprehensively. In natural-image domains (e.g. defect detection for industrial products), machine learning models trained only on normal data are used to detect various anomalies. However, its usefulness has not been studied well in toxicologic pathology.

Objective: Quantitative performance evaluation of liver lesions detection by an anomaly detection (AD) model trained only on normal-tissue images.

Methods: An AD model comprised a deep neural network (DNN) for feature extraction and a parametric density estimator that probabilistically represents normal tissues in feature space. The DNN was trained to classify 48 different tissue classes using 384x384-pixels sub-images in whole slide images (WSIs) of control rats. The estimator was fitted on liver sub-images of control rats. The AD model computed anomaly scores of liver sub-images and these scores were aggregated into WSI-level scores. For performance evaluation, several hundred liver WSIs of control and treated rats were curated from 30 independent experiments in Open TG-GATEs database and 15 types of lesions were annotated.

Results: The AD model detected 10 types of lesions with F1-score > 0.7 and this result was clearly superior to a baseline model using a DNN trained on natural images (ImageNet). The WSI-level scores were strongly correlated to histopathologic grading by toxicologic pathologists in 20 experiments (correlation ratio > 0.5).

Conclusions: The AD model trained only on normal-tissue images will assist toxicologic pathologists to find various lesions efficiently.

20: SPATIALOMICS CHARACTERIZATION OF THE NEUROINFLAMMATORY CONSEQUENCES OF EXTRACRANIAL RADIATION THERAPY IN MICE.

Kimberly Demos-Davies¹, Jessica Lawrence^{1,2}, Clara Ferreira³, Davis Seelig^{1,2}

¹University of Minnesota College of Veterinary Medicine, Saint Paul, MN, USA,

²University of Minnesota Masonic Cancer Center, Minneapolis, MN, USA, ³University of Minnesota Medical School, Minneapolis, MN, USA

Background: Cancer treatment-related cognitive impairment (CRCI) is a consequence of chemotherapy and radiation therapy (RT). The underlying mechanisms responsible for treatment-induced CRCI are unknown. Our prior work in a murine model of CRCI demonstrated anatomic variability in histologic responses in the brain following extracranial RT (ECRT) with or without chemotherapy. Understanding the ability of ECRT to induce gliosis is needed to develop effective mitigation strategies.

Objective: To interrogate the activation status of brain glia in mice receiving ECRT using spailalomics.

Methods: Nine- to thirteen-week-old SKH1 mice were irradiated with a single dose of 20Gy or 30Gy to the skin of the right hindlimb. Brains were harvested at 6 hours, 24 hours, 5 days, 12 days, and 25 days post irradiation for spatial proteomics and immunohistochemistry. The striatum, retrosplenial cortex and hippocampus, key anatomic regions involved with memory, were independently evaluated.

Results/Conclusion: In brain glia, there were dose-dependent increases in neuroinflammatory-associated proteins (S100B, CD39, CD40, GFAP,) and neurodegenerative disease associated proteins (GPNMB, SPP1, CD9) following ECRT. Mice treated with 20Gy radiation had significant increases in microglia-associated proteins (MHCII, GPNMB) in the striatum, retrosplenial cortex and hippocampus. Mice treated with 30Gy radiation had significant increases in proteins expressed by microglia (MHCII) and astrocytes (GFAP, Aldh1l1) in the striatum, retrosplenial cortex and hippocampus. Data show a dose- and brain region-dependent impact of ECRT on the brain that further implicates glia in CRCI. Further work to identify if the spinal cord mediates signals to affect the brain when distant sites are irradiated.

21: HISTOLOGIC EVALUATION OF SYSTEMIC TOXICITY OF TISSUE-REACTIVE ANCHORING PHARMACEUTICAL (TRAP) PACLITAXEL IN CD-1 MICE

Rukesh Chinthapatla^{1,2}, Jazz Stephens^{1,2}, Danielle Meritet¹, Annie Oh¹, Yevgeny Brudno^{1,2}

¹North Carolina State University, Raleigh, NC, USA, ²University of North Carolina, Chapel Hill, NC, USA

Abstract: Tissue-Reactive Anchoring Pharmaceuticals (TRAPs) are currently being investigated as an intra-tumoral delivery system for chemotherapeutic drugs. Local drug administration has been shown to effectively provide long-term exposure of cancer cells to the chemotherapeutic drug while minimizing systemic effects. The TRAPs technology creates materials-free local drug depots through chemical labeling of tissue extracellular matrix. TRAPs depots have better perfusion in tumors with dense fibrous stroma - such as carcinomas with a desmoplastic response - compared to strategies that rely on viscous materials (i.e., polymers and hydrogels). Previous studies have shown positive tumor responses in mice with pancreatic adenocarcinomas using TRAP conjugated paclitaxel (TRAP paclitaxel). Before investigating into the effects of TRAP paclitaxel on tumors in other species, this preliminary study focuses on the possible intradermal and systemic toxicity of TRAP paclitaxel in CD-1 mice through histologic evaluation of the liver, skin, kidneys, gastrointestinal tract, and eyes. The skin and liver were the most affected organs, displaying minimal to moderate inflammation in 63.3% to 66.7% of mice, respectively. Cutaneous lesions are considered secondary to the depot injection while the hepatic inflammation may reflect a minimal, non-specific systemic pro-inflammatory response. Individual and rare small clusters of hepatocyte death were observed in 6.6% of mice; this likely reflects homeostatic turnover of hepatocytes and possible handling-related effects. Given the mild and infrequent local and systemic

changes, we propose TRAP paclitaxel may be considered for future studies on local tumor control in other animal species.

22: NOVEL MURINE MODELS TO STUDY THE PATHOGENESIS AND POSSIBLE THERAPIES FOR THE LIVER DISEASE IN NIEMANN-PICK DISEASE TYPE C1

Patricia Titos, Jonathan Marable, Cynthia Hutchison, Russell Cattley, Maninder Sandey
AUBURN UNIVERSITY, AUBURN, AL, USA

Introduction: Niemann-Pick disease type C is a lysosomal storage disorder characterized by disrupted cholesterol transport, leading to their accumulation within lysosomes. NPC is caused by mutations in NPC1 or NPC2 genes. Current mouse strains used for NPC1 studies are useful for evaluating the neurodegenerative phenotype, but its rapid progression limits NPC's development in other organs, including the liver and spleen. This limitation hinders the study of liver disease in these mouse strains. Therefore, our goal was to create a liver-specific knockout mouse models to better understand the liver disease in NPC1 and evaluate possible therapies. **Experimental design:** We developed a liver-specific knockout models using the Cre-Lox tissue-specific knockout system by breeding a hepatocyte-specific or Kupffer cell-specific Cre with a homozygous NPC1 "floxed" mouse. Mice genotype was confirmed by PCRs. Mice were euthanized, and the liver was removed, post-fixed in 4% paraformaldehyde, paraffin embedded, and 4 µm sections were prepared and stained with H&E stain. **Results:** We have successfully developed two NPC1 mouse knockout models: 1) Hepatocyte-specific deletion of NPC1 gene, and 2) Kupffer cell-specific deletion of NPC1 gene. Our preliminary results show that both knockout models have enlarged liver. **Conclusions:** We expect our liver-specific knockout mouse models to provide a valuable tool for studying the liver disease in NPC1. **Impact statement:** The creation of this liver-specific knockout model provides a significant contribution to the field of NPC1 research and could lead to improved understanding of the disease, which may ultimately lead to the development of better therapies for patients.

23: REGULATION OF HTLV-1 VIRAL TRANSCRIPTION BY THE HOST PROTEIN, YBX1

Susan Smith, Jaideep Seth, Amanda Midkiff, Patrick Green, Amanda Panfil
The Ohio State University of Veterinary Biosciences, Columbus, OH, USA

Background: Human T-cell leukemia virus type 1 (HTLV-1) is the only identified oncogenic human retrovirus and is the etiologic agent of the highly aggressive adult T-cell leukemia/lymphoma (ATL) malignancy. Studies have shown two viral genes, *Tax* and *Hbz*, are essential for transformation and disease pathogenesis. Consequently, regulation of HTLV-1 gene expression is a central feature in the viral lifecycle and directly contributes to its pathogenic potential. Our lab has identified that Hbz interacts with the cellular protein YBX1 via mass spectrometry. YBX1 is a transcription factor involved in growth-associated gene expression.

Objective: The goal of this study is to identify the role of Hbz/YBX1 interaction in viral gene expression and HTLV-1 pathobiology.

Methods & Results: Using reporter gene assays, we found YBX1 activates transcription from the viral promoter or LTR (long terminal repeat). Co-transfection of Tax and YBX1 enhances LTR transcriptional activation, while co-transfection of Hbz and YBX1 inhibits transcriptional activation. Hbz has been shown to repress Tax-mediated transcriptional activation, and we found shRNA-mediated knockdown of YBX1 decreases Tax transcriptional activation of the LTR and decreases Hbz inhibition of Tax. Chromatin immunoprecipitation assays revealed that YBX1 associates with the viral LTR in HTLV-1-transformed T-cell lines. Immunoprecipitation experiments confirmed the YBX1/Hbz interaction in HTLV-1-transformed and ATL-derived T-cell lines. We also found that YBX1 is able to interact with Tax in HTLV-1-transformed T-cell lines.

Conclusion: Our data suggests YBX1 facilitates Tax recruitment to the viral LTR and the Hbz/YBX1 interaction prevents YBX1 transcriptional activation. This work will further define HTLV-1 transcriptional regulation.

24: SPECIES TRANSLATABILITY AND DETECTION OF POTENTIAL PATHOLOGICAL OUTCOMES ASSOCIATED WITH OFF-TARGET PHARMACOLOGICAL ACTIVITY

Kayla Frost, Andy Vo, Rebecca Kohnken, Wayne Buck, Jonathon Green, Yevgeniya Koshman, Michael Liguori, Michael Foley, Bruce LeRoy, Prathap Mahalingaiah, Terry Van Vleet

AbbVie Inc., North Chicago, IL, USA

In vitro screening for off-target pharmacological activity is a critical component of the drug discovery optimization process. Previously, our group mined biomedical literature to uncover common safety liabilities associated with pharmacological interactions with a wide range of off-target receptors, transporters, ion channels, and enzymes including kinases. This study expands on this work by examining the translatability of these interactions across four species commonly used for preclinical safety evaluations. Transcriptional expression profiling of 70 pharmacological targets was used to identify tissues with the highest expression levels of each target in both male and female mice, rats, dogs, and non-human primates. Characterization of conserved genes between sex, species, and tissue specificity will help determine the respective relevance of preclinical study results when extrapolating to humans. Identification of species sensitivity to these off-targets will facilitate appropriate species selection and improve the interpretation of pathological outcomes observed in preclinical studies that are otherwise attributed to these pharmacological interactions. This comprehensive review of off-target expression across species will improve the predictive value of toxicity findings from preclinical investigations.

25: LOST IN THE LABYRINTH: A PRIMER ON BASIC COCHLEAR HISTOLOGY FOR ANATOMIC PATHOLOGISTS

Nicola Parry

CBSET, Lexington, MA, USA

The trained and experienced pathologist must be able to recognize and interpret histopathologic abnormalities in tissues. To achieve this, they must first recognize and

understand the basic histology of any specific tissue, to better facilitate identification and interpretation of pathological events.

However, despite the vital roles of the inner ear in hearing and balance, cochlear histology remains a mystery to many pathologists, for a variety of reasons. In particular, the inner ear is a unique organ that cannot be visualized during life, and methods to biopsy or visualize its cellular structures during life are lacking. As a result, the inner ear is not routinely examined during autopsy or targeted for histopathologic evaluation unless, for example, it is either the focus of a toxicologic or experimental study, or clinical signs indicate this need in the diagnostic setting. Additionally, veterinary students and pathology residents rarely receive focused training in this niche area. Consequently, this combination of reasons tends to leave pathologists unprepared and challenged to examine this highly specialized tissue.

This presentation aims to bridge the gap between normal histology and pathologic changes. It highlights some key microscopic features of the cochlea, summarizing the salient structural, architectural, and cellular components that pathologists should know about this tissue. This will help pathologists in all professional settings to more confidently approach histopathologic examination of the inner ear, and to better interpret its microscopic changes.

26: THE RELATIONSHIP BETWEEN DOG OWNER-REPORTED CANINE CANCERS AND HOUSEHOLD ENVIRONMENTAL EXPOSURE TO POTENTIAL CARCINOGENS: RESULTS OF A NATIONWIDE ONLINE SURVEY OF AUSTRALIAN DOG OWNERS

Kei Owada¹, Chiara Palmieri¹, Luke Knibbs^{2,3}, Ricardo Soares Magalhães^{1,4}

¹School of Veterinary Science, The University of Queensland, Gatton, Australia, ²School of Public Health, The University of Sydney, Camperdown, Australia, ³Public Health Unit, Sydney Local Health District, Camperdown, Australia, ⁴Children's Health and Environment Program, The University of Queensland, South Brisbane, Australia

Recent evidence suggests that household environmental pollutants such as insecticides and herbicides, and air pollutants such as smoke and particulate matter produced by combustion-based indoor heating, such as wood fireplaces, may be potential canine carcinogens that contribute to cancer development. To date, only a few studies have explored this association.

This study aims to better understand the level and nature of exposure to potential household carcinogenic substances in dog owners and their dogs and the association with dog owner-reported canine cancer.

An online survey on dog owners' demographics, living environment, and behavior was conducted in Australia between October 2022 and March 2023. A total of 285 dog owners completed the survey. Associations between dog owner-reported canine cancer incidence and exposure to environmental pollutants in their living environment were investigated using a generalized linear Binomial regression model.

Statistically significant and positive associations were found between dogs having been diagnosed with cancer and middle-aged owner ($p=0.008$), the longer duration of owner living at their current address ($p=0.002$), use of wood burning fireplace for heating ($p=0.004$), dog's frequency of contact with carpet flooring ($p=0.017$), dog's increasing age ($p<0.001$), dogs with medium nose length ($p=0.008$), and the use of shampoo products for dog's flea/tick control ($p=0.009$).

The findings of this cross-sectional study highlight increased risk of dog owner-reported canine cancer in dogs potentially living in households with increased likelihood of exposure to carcinogenic substances. Future longitudinal cohort studies should be designed to ascertain the causality of these relationships using a One Health approach.

27: ABNORMALITY DETECTION IN RAT LIVER USING UNSUPERVISED REPRESENTATION LEARNING IN PRECLINICAL TOXICITY STUDIES

Pradeep Babburi, Bhupinder Bawa, Magali Guffroy, Hossein Foroushani
AbbVie Inc., North Chicago, IL, USA

Recent advances in digital pathology and AI have created an immense opportunity to improve workflow efficiency in conducting preclinical safety studies. In toxicological pathology, there is a high volume of tissue slides routinely evaluated by pathologists in order to detect and study the abnormalities caused by test item administration. Integration of AI into the slide review process that can routinely filter normal tissues can allow pathologists to rapidly identify the target organs and spend more time understanding the mechanism of toxicity. However, due to the complexity and diverse nature of tox findings typically seen in toxicology studies, generating labelled examples for targeted (supervised/semi-supervised) learning is not only time consuming but can be unreliable as well. Here we present an unsupervised approach to detect abnormal tissues using deep generative models. We trained a Bi-directional Generative Adversarial Network (BiGAN) on only the normal tissues of rat liver selected from control group. We tested the trained model on a held-out dataset which included normal and abnormal tissues picked from retrospective studies with recorded severity of moderate to severe grades by study pathologists. The abnormality score was calculated by tracking model loss as a combination of reconstruction and discriminator losses. We achieved promising results that showed clear distinction in the abnormality scores between normal and abnormal tissues. The model achieved an accuracy (F1 Score) of 97% on test data which is indicative that our approach holds the potential to be employed for real-world use in triaging abnormal tissues with no supervision from the pathologists.

28: CADMIUM AND GOUT IN PSITTACINE BIRDS

Elizabeth Hines, Laura Bryan
Texas A&M University, College Station, TX, USA

Cadmium is associated with renal disease in several species including humans, rats, and cats. In other species, including dogs and horses, the effects of cadmium exposure are minimal or, in the case of most species, minimally explored. In birds, liver and kidney cadmium concentrations have been shown to reflect levels of environmental

cadmium contamination. High levels of cadmium also slow renal growth in pullets. However, little work has been done on cadmium in psittacine birds. To explore any potential relationship of cadmium and gout (or other kidney disease) in psittacine birds, we collected fresh kidney, liver, or both, from 43 psittacines submitted for diagnostic necropsy to the Texas A&M Department of Veterinary Pathobiology over two years (May 2021- May 2023). Cadmium concentrations in one or both tissues were evaluated, and analyses performed to assess the relationship of cadmium concentration with gout. Ultimately no significant association between cadmium concentration and gout exists in the examined cases; however, small sample sizes limited statistical analysis. Limitations of this study, and further directions of study, will be addressed.

29: PATHOLOGIC FINDINGS IN POST-SURGICAL VASCULAR ACCESS BUTTON (VAB) COMPLICATIONS IN RATS

Lori Bedient, L. Michele Wilkinson, Eleana Sosnowski
Labcorp Drug Development, Somerset, NJ, USA

The introduction of vascular access button (VAB) devices has increased the availability and efficiency of long-term direct vascular access for preclinical research in multiple species. Life-limiting complications from their implantation and usage is rarely reported in the literature. This case series describes post-implantation complications in Wistar-Han rats implanted with dual-vascularized (jugular and femoral vein) VABs by a commercial supplier. In multiple sequential cohorts, up to 69% of rats presented with surgical site dehiscence, ulceration and abscessation within 1 to 30 days of facility arrival, with 27-50% of each cohort presenting within the first 3 days of arrival. Similar clinical outcomes occurred with two different commercially available VAB devices implanted by the same supplier. The presence or absence of surgical grade glue used by one supplier to secure the felt pad anchor had no effect on clinical outcome. Histologically, affected button sites were characterized by severe ulceration and dermal necrosis under the protective cap, necrosis of scaffold tissue in the polyester felt pad used for anchoring, and colonies of mixed bacteria concentrated around the port tract. These changes were compared to button sites observed in rats implanted with the same VAB devices by a different commercial supplier. In these animals, clinical evidence of post-surgical complications was rare and histologic evaluation revealed intact felt pads with minimal to no epidermal or dermal lesions. Differences in vendor surgical technique, immediate post-operative care, and transport conditions are considered the most likely factors in the development of severe post-surgical complications in VAB devices.

30: PSEUDOMONAS AERUGINOSA-DERIVED VOLATILE ORGANIC COMPOUNDS ACTIVATE AHR SIGNALING AND POLARIZE TOWARD M1 MACROPHAGES, NEUTROPHILS, AND TYPE 17 IMMUNE RESPONSES THAT FAVOR MUCUS HYPERSECRETION IN DISEASED LUNGS

Shanny H. Kuo, Jaishree Sharma, Som Nanjappa, Gee Lau
University of Illinois at Urbana-Champaign, Champaign, IL, USA

Background: The aryl hydrocarbon receptor (AhR) has emerged as a regulator of mucosal barrier function, influencing immune responsiveness in lung by modulating

mucin and cytokine production. AhR, as a ligand-activated transcription factor, is known to bind small molecules derived from the microorganisms, pollutants, diets, and metabolism. Highly expressed in immunocytes, AhR signaling plays essential roles in integrating the effects of the environmental stimuli and immune response. We have previously demonstrated *Pseudomonas aeruginosa*-derived volatile organic compounds (VOCs) can cause airway mucus hypersecretion via AhR-mediated FOXA2 degradation that leads to MUC5AC and MUC5B overexpression and goblet cell metaplasia.

Objective: We hypothesize the PA-derived VOCs may activate particular immune cell subsets contributing to disease pathogenesis and mucus hypersecretion in lungs.

Methods: We investigated the immune profiles of mice receiving chronic intranasal VOCs challenge by flow cytometry.

Results: Flow cytometry analysis of immune cells from both bronchoalveolar lavage fluids and homogenized lungs of mice chronically exposed to VOCs reveals predominantly iNOS⁺ M1 macrophages (58%), neutrophils (11%), and Thy1.2⁺ cells (12%), with the majority of Thy1.2⁺ cells being IL-17A⁺secreting T lymphocytes. In C57BL/6 mice, selective depletion of either macrophages (via clodronate liposome) or neutrophils (via anti-mouse Ly6G) decreased mucin production and restored FOXA2 expression in mouse airways exposed to VOCs. Similarly, IL-17a^{-/-} mice lacking IL-17A showed attenuated mucin production and restored FOXA2 activity following VOCs exposure.

Conclusions: Our data herald the significance of PA-derived VOCs on modulating host immunity with immune polarization toward M1 macrophages, neutrophils, and type 17 proinflammatory responses that favor mucus hypersecretion.

Natural Disease

Monday, October 30 | 2:30 PM – 2:45 PM

Session Chair: Elena Alina Demeter

Monday, October 30

2:30 PM – 2:45 PM

MOLECULAR INVESTIGATIONS OF GANGLIONITIS IN PERFORMANCE HORSES WITH CLINICAL SIGNS OF AXIAL SKELETAL PAIN

LaTasha Crawford¹, Brittney Moore¹, Kevin Haussler², Yvette Nout-Lomas², Melinda Story², Tawfik Aboellail²

¹University of Wisconsin-Madison School of Veterinary Medicine, Madison, WI, USA,

²Colorado State University College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO, USA

A syndrome of axial skeletal pain has been identified in a series of 26 performance horses presenting with a recent history of untoward and dangerous behavior. Although diagnostic workup and routine necropsy failed to identify a cause for the pain, thorough evaluation of the sensory nervous system identified segmental to multifocal ganglioneuritis affecting dorsal root ganglia (DRG) corresponding to the regions of pain.

Immunohistochemistry and multiplex immunofluorescence were used to characterize hypercellularity, neuronal subtypes affected, and molecular signatures of neuropathic pain in DRG from affected horses and unaffected controls. Hypercellularity was attributed to satellite glia proliferation, lymphocyte infiltration, and increased numbers of macrophages. The previously described neuronal degeneration and neuronophagia affected immunolabeled nociceptors, proprioceptors, and putative mechanoreceptor neurons. Affected DRG demonstrated key molecular patterns seen in laboratory animal models of neuropathic pain, including increased neuronal expression of calcitonin gene-related peptide and sympathetic baskets surrounding sensory neurons. Because sensory ganglia are not assessed routinely in any species, it is not always clear whether histologic evidence of ganglionitis is associated with clinical signs. Our data provide further evidence that ganglionitis underlies the pain behaviors in this series of patients and that multiple subtypes of sensory neurons are affected. Challenges encountered during this study included a paucity of literature characterizing DRG in horses and limited availability of ganglia from unaffected control horses. Collectively, our findings highlight pain mechanisms that may be shared across species and help bolster future studies that can ultimately improve anti-pain therapies for equine patients.

Monday, October 30

2:45 PM – 3:00 PM

MYCOPLASMA OVIPNEUMONIAE IS A SIGNIFICANT RESPIRATORY PATHOGEN IN WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS)

Annabelle Burnum, Renata Mammone, Solomon Odemuyiwa

University of Missouri Veterinary Medical Diagnostic Laboratory, Columbia, MO, USA

Background: *Mycoplasma ovipneumoniae* is an important respiratory pathogen of domestic lambs and goats as well as Rocky Mountain bighorn sheep, often causing disease in combination with other bacterial or viral infections and environmental stressors. Similarly, respiratory disease accounts for a large number of deaths in juvenile farmed cervids. While there is overlap with the respiratory pathogens of wild and domestic ruminants, white-tailed deer appear particularly susceptible to *M. ovipneumoniae*.

Objective: Our objective is to characterize respiratory *M. ovipneumoniae* infections in white-tailed deer via gross necropsy, histopathology, and infectious disease testing of cases submitted to a veterinary diagnostic laboratory.

Methods: This retrospective clinical case study was conducted by reviewing necropsy reports from 2004-2023. Case inclusion criteria included cervids that had undergone a *Mycoplasma* general PCR test on lung tissue.

Results: *M. ovipneumoniae* was most often detected in conjunction with other bacterial (e.g., *Pasteurella multocida* and *Bibersteinia trehalosi*) and viral (infectious bovine rhinotracheitis and epizootic hemorrhagic disease virus) respiratory pathogens. In PCR-

positive cases of solitary *M. ovipneumoniae* infection, histologic findings resembled those reported previously in lambs and bighorn sheep, including peribronchiolar lymphocytic cuffing, hyperplasia of bronchiolar epithelium, and neutrophilic inflammation in airways and surrounding alveoli.

Conclusions: *M. ovipneumoniae* is a significant respiratory pathogen of deer and should be included as a component of a cervid respiratory panel.

Tuesday, October 31

1:30 PM – 1:45 PM

SARCOMAS OF SYNOVIAL ORIGIN IN DOGS: AN UPDATE

Linden Craig

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

The synovium consists of two cell types, type A and type B. Type A synoviocytes are histiocytes of bone marrow origin that are immunoreactive with typical markers of histiocyte origin, such as CD18 and Iba-1. Malignant tumors of type A histiocytes are histiocytic sarcomas, which can also arise from resident histiocytes in other tissues. Certain breeds and dogs with previous injury to a joint, especially cranial cruciate ligament rupture, are predisposed to histiocytic sarcoma. Histiocytic sarcoma is an aggressive neoplasm with a poor prognosis. Type B synoviocytes are mesenchymal cells that produce synovial fluid. Malignant tumors of type B synoviocytes are called synovial myxosarcomas (previously synovial myxomas). These can infiltrate into surrounding tissues (including bone), but are slow growing and rarely metastasize, and only to regional lymph nodes. Both of these synoviocyte-origin tumors can cause lysis in multiple bones surrounding the joint, but they have different prognoses and require histopathology and sometimes immunohistochemistry to diagnose them. Synovial sarcoma and synovial cell sarcoma are terms used in the human medical literature for tumors that are not of synovial origin; these terms should not be used in veterinary medicine.

Tuesday, October 31

1:45 PM – 2:00 PM

TESTUDINID HERPESVIRUS 3 INFECTION IN ELONGATED TORTOISES (*INDOTESTUDO ELONGATA*)

Han-Yang Wang, Wei-Hsiang Huang, Hui-Wen Chang, Chian-Ren Jeng, Yen-Chen Chang

Graduate Institute of Molecular and Comparative Pathobiology, School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan

Background

A group of eighteen elongated tortoises (*Indotestudo elongata*) showed nasal and ocular discharge, and some caseous substance in mouth. Then, all tortoises were isolated and administered with supportive treatments but fifteen tortoises still died.

Objective

To determine the cause of the present outbreak, histopathological and molecular examinations were performed.

Methods

Fifteen tortoises were necropsied, and the representative samples were fixed with formalin and subjected to routine process of slide preparation for histopathological examination. DNA was extracted from the affected tissues for polymerase chain reaction (PCR). *In situ* hybridization (ISH) was also conducted.

Results

Microscopically, necrosis accompanied with inflammatory cells, eosinophilic intranuclear inclusion bodies, and occasional syncytial cells are observed in multiple affected organs. Based on the results of PCR, Testudinid herpesvirus 3 (TeHV-3) infection is diagnosed. The phylogenetic analyses reveal that the present isolates are identical to each other. Their nucleotide sequence of DNA polymerase share 97.86% similarity to Germany strain 4295/7R and their amino acid sequence of glycoprotein B share 99.9% similarity to strain CH6883/03, which is previously detected from Hermann's tortoises in Switzerland in 2003 and belongs to higher pathogenicity genogroup B. The ISH results also demonstrate positive signals in the inclusion bodies in multiple affected organs.

Conclusions

The present study is the first case report of TeHV-3 infection in *Indotestudo elongata*.

Tuesday, October 31

2:00 PM – 2:15 PM

INVESTIGATING PROTOZOAL AND VIRAL CAUSES OF IDIOPATHIC ENCEPHALITIS IN AMERICAN BLACK BEARS (*URSUS AMERICANUS*) IN CALIFORNIA AND NEVADA

Devinn Sinnott¹, Brandon Munk², Anibal Armien³, Nate LaHue⁴, Patricia Pesavento¹, Kenneth Jackson¹, John Ly², Karen Shapiro¹

¹University of California, Davis, School of Veterinary Medicine, Davis, CA, USA,

²Wildlife Health Laboratory, California Department of Fish and Wildlife, Rancho Cordova, CA, USA, ³California Animal Health and Food Safety Laboratory, Davis, CA, USA, ⁴Nevada Department of Wildlife, Reno, NV, USA

Background: An increase in neurologic disease affecting American black bears (*Ursus americanus*) was first observed in 2014 near Lake Tahoe, California and has since been reported in 15 counties across California and Nevada. These bears have varying degrees and patterns of encephalitis, the cause of which is currently unknown.

Objective: The goal of this study was to investigate potential causes, including protozoa and known neurotropic ursine viruses, of encephalitis in this population of bears.

Methods: Histopathology slides were reviewed for 80 bears to determine the presence and pattern of encephalitis. Brain tissue from bears with and without encephalitis was screened for protozoa via PCR targeting two genes (ITS1 and 18S), as well as tested for ursine circovirus, chaphamaparvovirus, and polyomavirus via PCR.

Results: Two patterns of nonsuppurative encephalitis were observed: a multifocal, random pattern and a regionally extensive pattern affecting the olfactory bulb and frontobasal regions of the brain. Infection with protozoa was significantly associated with encephalitis, with protozoal DNA detected in 48.5% of bears with encephalitis and 14.9% of bears without encephalitis. Detected parasites included *Sarcocystis neurona*, *Sarcocystis canis*, other *Sarcocystis* species, *Toxoplasma gondii*, and an uncharacterized *Cystoisospora*-like species. Infection with any of the three viruses was not significantly associated with encephalitis. The majority of the bears with encephalitis were three years of age or younger (81.8%).

Conclusion: Although the exact causes of encephalitis in this population of bears remain uncertain, our preliminary findings suggest that protozoa, particularly *Sarcocystis* species, contribute to encephalitis in black bears.

Tuesday, October 31

2:15 PM – 2:30 PM

ENCEPHALOMYELITIS IN CAPTIVE NORTH AMERICAN COLUBRID SNAKES ASSOCIATED WITH A NOVEL PICORNA-LIKE VIRUS

Peres Badial¹, Kuttichantran Subramaniam², Robert Ossiboff¹

¹Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA, ²Department of Infectious Diseases and Immunology, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA

Historically, cases of mononuclear central nervous system (CNS) inflammation in reptiles, while often suggestive of a viral etiology, have posed a diagnostic challenge. However, advanced molecular techniques have expanded the field, identifying both known and novel types of viruses associated with CNS disease. Between February 2022 and March 2023, six juvenile captive colubrid snakes (four California kingsnakes [*Lampropeltis californiae*]; one Nuevo Leon kingsnake [*L. leonis*], and one colubrid hybrid [*L. californiae* x *Pantherophis guttatus*]) displaying neurologic signs were submitted for gross, microscopic, and molecular examination. All submitted snakes exhibited neurological signs characterized by varying degrees of absence of righting reflex, head tremors, paresis, and paralysis. Significant lesions were restricted to microscopic changes in the CNS, including lymphocytic and granulocytic polioencephalomyelitis, gliosis, neuronal vacuolation, neuronal necrosis, and Wallerian-like degeneration. As the nature of the lesions was strongly suggestive of a viral infection, MiSeq next-generation sequencing was performed on a sample of fresh, frozen brain. The BLASTX analysis of assembled contigs revealed a novel RNA virus genome showing the highest amino acid sequence identities of RNA-dependent RNA polymerase (<51%) to picorna-like viruses. In addition, phylogenetic analysis supported this virus as a member of the order *Picornavirales*. While the molecular confirmation of

this novel virus in all snakes and *in situ* hybridization studies are pending, the findings suggest that this picorna-like virus is associated with polioencephalomyelitis in these animals. This case series highlights a novel picorna-like virus associated with clinical neurological disease and polioencephalomyelitis in captive colubrid snakes, particularly of the genus *Lampropeltis*.

Tuesday, October 31

2:30 PM – 2:45 PM

INTER-OBSERVER VARIATION IN SUBTYPING OF CANINE SOFT TISSUE MESENCHYMAL TUMORS – A MULTI-INSTITUTIONAL ANALYSIS

Chris Champion, Andrew Miller

Cornell University, Ithaca, NY, USA

Background: Currently, canine soft tissue mesenchymal tumors are graded histologically based on three features - cellular differentiation, mitotic figures per 2.37 mm² (10 HPF), and the degree of necrosis. Clinicians depend on this grading scheme to determine the likelihood of tumor recurrence and assess the necessity of adjunctive treatment.

In the human and veterinary literature, inter-pathologist agreement on tumor grade is poor and the subjective nature of assigning subtypes can lead to grade discrepancies.

Objective: To further investigate inter-observer variation in subtyping of canine soft tissue mesenchymal tumors.

Method: 300 cases of canine soft tissue mesenchymal tumors were reviewed by the authors and subtyped according to a standardized set of criteria. 15 of these tumors were randomly selected from the study group and scanned slides from these tumors were uploaded to an online slide viewer. A survey was constructed using 'Qualtrics' software. The survey included hyperlinks to the scanned slides and questions asking participants to subtype the neoplasm and substantiate why they assigned each subtype.

The survey was distributed to a group of 21 board-certified veterinary pathologists from 16 different academic institutions and diagnostic laboratories.

Results & Conclusions: Inter-pathologist agreement on canine soft tissue mesenchymal tumor subtyping is suboptimal based on histomorphologic features alone. Given that a pathologist's ability to distinguish between histologic subtypes (i.e. assess the degree of cellular differentiation) has a direct impact on grading, standardization of subtyping criteria is imperative. Assigning predictive prognostic significance to different histologic subtypes cannot be achieved until subtyping amongst pathologists is standardized.

Tuesday, October 31

2:45 PM – 3:00 PM

TRANSCRIPTIONAL PROFILING OF CANINE SOFT TISSUE MESENCHYMAL TUMORS

Andrew Miller¹, Christopher Champion¹, Ann Tate², Faraz Ahmed², Jen Grenier²

¹Department of Population Medicine and Diagnostic Sciences, Section of Anatomic Pathology, Cornell University College of Veterinary Medicine, Ithaca, NY, USA,

²Transcriptional Regulation and Expression Facility, Cornell Institute of Biotechnology, Cornell University, Ithaca, NY, USA

Background: Canine soft tissue mesenchymal tumors (CSTMTs) comprise up to 15% of the primary neoplasms of the dermis and subcutis. While a number of different neoplasms are grouped under this umbrella term, subclassification remains difficult based on histomorphologic features alone.

Objective: To characterize the transcriptome in CSTMTs in order to improve classification and outcome success.

Methods: 300 cases of CSTMTs submitted to the Cornell University Diagnostic Center were re-reviewed and diagnoses were standardized. RNA was extracted from tumor-only tissue and following rRNA depletion, Illumina RNAseq libraries were sequenced to an average depth of 60M reads. Principal components analysis was used to identify sample clustering based on the transcriptome signature, which was correlated with histologic phenotypes.

Results: 283/300 cases generated a sufficient transcriptome profile and had agreement on diagnosis. Histologically the following subtypes were included: perivascular wall tumors (n=125), fibrosarcomas (n=17), myxosarcomas (n=6), mesenchymomas (n=2), liposarcomas (n=1), and undifferentiated (n=132). Unsupervised principal component analysis identified three distinct clusters. Cluster A included 96% of perivascular wall tumors, cluster B included 88% of fibrosarcomas, and cluster C included 80% of myxosarcomas. Undifferentiated tumors were present in all three clusters with 76% in cluster A, 22% in cluster B, and 2% in cluster C. The highest differentially expressed genes included MYLI, COL2A1, ACAN, and XIRP2.

Conclusions: Transcriptomic profiling reveals distinct signatures between different CSTMT subtypes. This highlights the pivotal role transcriptional profiling plays in identifying shared and novel differences in mRNA profiles which could help improve diagnostics, better predict patient outcomes, and inform treatment.

Tuesday, October 31

3:30 PM – 3:45 PM

QUANTIFICATION AND PROGNOSTIC SIGNIFICANCE OF CIRCULATING NEUTROPHILS, CIRCULATING LYMPHOCYTES, AND TUMOR-ASSOCIATED

NEUTROPHILS IN CANINE APPENDICULAR OSTEOSAROMA PATIENTS RECEIVING STANDARD-OF-CARE TREATMENT

Rachael Speare¹, Vanessa Huntley², Jared Fischbach¹, Geoffrey Wood¹, Courtney Schott¹, Darren Wood¹, Alicia Vilorio-Petit²

¹Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, ²Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

Background: Osteosarcoma is the most common bone tumor of dogs, yet few useful prognostic tools exist. High circulating neutrophil count correlates with poorer outcome in humans and dogs. High circulating neutrophil to lymphocyte ratio (NLR) correlates with poorer outcome in humans. Tumor infiltrating lymphocytes have been evaluated in both species. Tumor-associated neutrophils (TANs), generally considered to promote tumorigenesis and treatment resistance, have yet to be investigated in osteosarcoma.

Objective: This study aimed to uncover correlations between outcome and circulating lymphocytes and neutrophils, as well as TANs in canine appendicular osteosarcoma patients receiving standard-of-care treatment.

Results: Circulating lymphocyte, segmented neutrophil (SN), and band neutrophil (BN) counts were recorded for 76 pre- and 86 post-amputation patients. TANs were quantified using myeloperoxidase immunohistochemistry in 34 cases. Kaplan-Meier curves for disease-free interval (DFI) and survival time (ST) were compared by log-rank test. High lymphocyte count correlated with shorter DFI pre- and post-amputation ($p < 0.05$). High SN count correlated with shorter DFI and ST pre-amputation ($p < 0.05$). An increase in total neutrophil count (SN+BN) over 2.23 from pre- to post-amputation correlated with longer DFI ($p < 0.05$). BN count and NLR did not correlate with outcome. TAN counts ranged from 0/mm² - 4.86/mm², and TAN count above 0.58/mm² correlated with shorter DFI ($p < 0.05$).

Conclusions: Prognostic significance of circulating immune cells was identified, corroborating previously identified relationships. Quantification and prognostic significance of TANs was described for the first time. Future work can provide insights into mechanisms underlying the role of tumor immunology in osteosarcoma progression, metastasis, and treatment.

Tuesday, October 31

3:45 PM – 4:00 PM

HISTOLOGIC AND IMMUNOHISTOCHEMICAL FEATURES OF CANINE OCULAR GLIOMA

Ryan Taylor¹, Leandro Teixeira², Gillian Shaw², Andrew Miller¹

¹Cornell University College of Veterinary Medicine, Ithaca, NY, USA, ²University of Wisconsin School of Veterinary Medicine, Madison, WI, USA

Background: Canine ocular glioma is an uncommon intraocular neoplasm with oligodendroglioma and astrocytoma reported.

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

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

Results: 7/18 were oligodendrogliomas with round nuclei, nuclear rowing, myxoid/mucinous matrix, and nuclear molding. 8/18 were astrocytomas with oval to elongate nuclei, pleomorphic cells, abundant eosinophilic cytoplasm (gemistocytic), disorganized pattern, spindle cell morphology and fibrillar eosinophilic stroma. 3/18 were undefined glioma with undifferentiated cellular morphology and biphasic phenotype. High-grade features were noted in 16 of the cases including microvascular proliferation (50%), necrosis (93%) and increased mitotic activity (88%). Retinal spread was noted in 2 cases and intraocular seeding in 4 cases. OLIG2 labeling was noted in 7/7 oligodendrogliomas, 3/8 astrocytomas, and 2/3 undefined gliomas. CNPase labeling was noted in 4/7 oligodendrogliomas, 0/8 astrocytomas, and 2/3 undefined gliomas. GFAP labeling was noted in 3/7 oligodendrogliomas, 8/8 astrocytomas, and 2/3 undefined gliomas.

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

Tuesday, October 31

4:00 PM – 4:15 PM

INTESTINAL CRYPTOCOCCOSIS CAUSING INTUSSUSCEPTION AND OBSTRUCTION IN A YOUNG CANINE: A CASE REPORT

Julia Lombardo¹, Jianfa Bai^{1,2}, Brenda Cederberg³, Brandon Plattner^{1,2}

¹Kansas State University, Department of Diagnostic Medicine/Pathobiology, Manhattan, KS, USA, ²Kansas State Veterinary Diagnostic Laboratory, Manhattan, KS, USA,

³Urgent Pet Care, Wichita, KS, USA

A 2-year-old male intact German shorthaired pointer canine presented for a 1-week history of hematochezia and vomiting refractory to symptomatic treatment. Radiographs indicated a small intestinal obstruction. Exploratory surgery revealed mesenteric lymphadenomegaly, and multiple jejunal masses from 1-5 cm diameter; several were pedunculated, and one was within an obstructive jejunojejunal intussusception. Several masses, including the obstructive mass and lymph nodes, were excised. Given the poor

prognosis, owners elected euthanasia. Multiple jejunal masses were confirmed during necropsy.

The nodules were comprised of dense transmural granulomatous to mixed chronic inflammation that extended into the adjacent mesentery and invaded regional lymph nodes. Numerous 8-20-micron diameter refractile round to oval PAS and GMS positive fungal and yeast bodies with a thick clear capsule were observed. Narrow based budding of the organisms was frequently observed. Metagenomic sequencing was performed on tissue sections, and the identity of the agent was confirmed as the saprophytic yeast *Cryptococcus albidus* (recently renamed *Naganishia albida*).

Cryptococcosis is a rare infection of dogs and cats, mostly caused by *C. gatii* and *C. neoformans*, which are both considered primary pathogens. *C. albidus* / *N. albida* has been reported as the cause of systemic disease in only one dog and one cat previously; both patients were presumed to be immunocompromised. This agent is not considered a primary pathogen, likely representing an uncommon opportunistic infection; however, reports indicate increased incidence of systemic infections in immunocompetent humans in recent years so there may be epidemiologic or public health significance of this agent.

Tuesday, October 31

4:15 PM – 4:30 PM

A RETROSPECTIVE STUDY OF NEOPLASIA IN SOUTHERN WHITE RHINOS (CERATOTHERIUM SIMUM SIMUM) FROM THE NORTH CAROLINA ZOO (2004-2023)

Brigid Troan^{1,2}, Mandy Womble³, Larry Minter¹, Timothy Georoff¹

¹North Carolina Zoo, Asheboro, NC, USA, ²North Carolina State University College of Veterinary Medicine, Raleigh, NC, USA, ³University of Illinois at Urbana-Champaign, Brookfield, IL, USA

Background: Neoplasia is considered uncommon in rhinoceros with few published case reports.

Objective: To fully describe neoplastic disease in southern white rhinoceros (*Ceratotherium simum simum*) from the North Carolina Zoo.

Methods: Necropsy and biopsy submissions from southern white rhinoceros at the North Carolina Zoo from 2004-2023 were reviewed for incidences of neoplasia. When warranted, immunohistochemical staining was pursued.

Results: Out of six rhinoceros with autopsy and biopsy reports (3 males, 3 females, 26.8-54.7 years), a total of 15 neoplasms were identified in four rhinos: 14 at autopsy and one from a surgical biopsy. A distinct sex difference was noted. All three females had both benign neoplasms and at least one, and up to five, malignant neoplasms. The oldest male had only benign neoplasms, while no neoplastic lesions were identified in the two younger males. Benign neoplasms included: hepatocellular, thyroid, and adrenal cortical adenomas, vertebral osteoma, acrochordons and fibromas. Malignant

neoplasms contributed significantly to morbidity and included: CD3 positive / CD20 negative subcutaneous panniculitis-like T cell lymphoma, adrenal cortical carcinomas, pheochromocytoma, ovarian leiomyosarcoma, and squamous cell carcinomas arising from papilloma / hyperplastic lesions (negative on PCR using degenerate primers for papillomavirus). Interestingly, endocrine neoplasms were found in all four affected rhinoceros, and all three females had Melan-A positive / Chromogranin-A negative adrenal cortical neoplasms which were morphologically indistinguishable except for evidence of local invasion and metastasis.

Conclusions: Neoplastic disease occurred frequently in this crash of southern white rhinoceros, particularly in endocrine organs and older females.

Tuesday, October 31

4:30 PM – 4:45 PM

PHYLOGENETIC RELATIONSHIPS AMONG SARCOCYSTIS SPP. IN FREE-RANGING ALASKAN MARINE MAMMALS

Elliott Chiu¹, Devinn Sinnott¹, Raphaela Stimmelmayer², David Rotstein³, Karen Shapiro¹

¹University of California, Davis, Davis, CA, USA, ²North Slope Borough, Department of Wildlife Management, Utqiagvik, AK, USA, ³Marine Mammal Pathology Services, Olney, MD, USA

Sarcocystis spp. diversity within Alaskan marine mammals is largely unknown, particularly in a region of the world where subsistence hunting and consumption of wildlife species is common. Muscle tissue from 1 beluga whale (*Delphinapterus leucas*), 9 polar bears (*Ursus maritimus*), 14 bearded seals (*Erignathus barbatus*), 14 ringed seals (*Pusa hispida*), and 2 spotted seals (*Phoca largha*) was collected during necropsy following subsistence harvesting or stranding. Intramuscular sarcocysts were identified histologically in all systemically healthy animals. To confirm the identity of these sarcocysts, DNA was extracted from skeletal, cardiac, or gastrointestinal smooth muscle and PCR was performed targeting four loci (ITS1, cox1, 18S, and RPOb) followed by Sanger sequencing. Maximum-likelihood phylogenetic trees were constructed for each locus. Alaskan marine mammals *Sarcocystis* sequences clustered within four clades. The majority of *Sarcocystis* sequences recovered were identical to previously reported *Sarcocystis pinnipedi*. Few sequences were identical to *Sarcocystis canis* previously reported from polar bears. Sequences recovered from the beluga whale, at least two polar bears, and seven seals were most closely related to uncharacterized *Sarcocystis* spp. previously identified in a sperm whale (*Physeter macrocephalus*) and fur seal (*Arctocephalus pusillus*). The latter sequences represent two separate clades. Despite incidental detection, the true pathogenic potential remains unknown in these species or in aberrant hosts. *Sarcocystis pinnipedi* has been previously associated with mass mortality events in grey seals (*Halichoerus grypus*) and has also been implicated as the cause of fatal hepatitis in captive marine mammals. Identification of potentially new *Sarcocystis* spp. provides the framework for future investigation.

Tuesday, October 31

4:45 PM – 5:00 PM

ANALYSIS OF CELL POPULATIONS IN THE NORMAL RHESUS MACAQUE (MACACA MULATTA) LOWER INTESTINAL TRACT AND DIAGNOSTIC THRESHOLDS FOR CHRONIC ENTEROCOLITIS

Rebecca Bacon¹, Loni Taylor¹, Stanton Gray², Carolyn Hodo²

¹Texas A&M University College of Veterinary Medicine, College Station, TX, USA, ²The University of Texas MD Anderson Cancer Center, Michale E. Keeling Center for Comparative Medicine and Research, Bastrop, TX, USA

Rhesus macaques (*Macaca mulatta*) are used extensively in biomedical research, often with a focus on the gastrointestinal tract, and yet a full characterization of their normal resident intestinal cell populations has not been published. Additionally, chronic enterocolitis (CE), also known as idiopathic chronic diarrhea, affects up to 25% of colony housed rhesus macaques, often requiring euthanasia for welfare concerns and severely limiting their value as a breeding animal or research subject. We aimed to characterize subjective and objective variables in sections of the ileum, cecum, colon, and rectum in 16 healthy rhesus macaques and compare these results to a cohort of 37 animals euthanized for CE to produce relevant diagnostic thresholds and to improve case definitions for future studies. We found rhesus macaques to overall have more resident intestinal leukocytes than typical domestic species and neutrophils to be an infrequent but expected component of the large intestinal leukocyte population. Animals with CE had significantly increased total leukocyte populations between crypts in the cecum, colon, and rectum; variable increases in specific cell populations across all levels of the distal intestinal tract; and significantly increased intraepithelial CD3+ T cells in the colon and rectum. Concentrations of enteroendocrine cells, enterochromaffin cells, and intestinal mast cells were not significantly different between healthy and affected individuals. This study characterizes individual leukocyte populations in the rhesus macaque lower intestinal tract, is the first to evaluate rhesus macaque intestinal mast cells, and provides key diagnostic thresholds for evaluating animals with potential chronic enterocolitis.

32: A COMPARATIVE HISTOLOGIC ANALYSIS OF HUMAN VERSUS CANINE SYNOVIUM ASSOCIATED WITH ANTERIOR CRUCIATE LIGAMENT INJURIES

Kei Kuroki, Richard Ma, Aaron Stoker, James Cook

University of Missouri, Columbia, MO, USA

Anterior (cranial) cruciate ligament (ACL) injury is a common orthopaedic disorder in humans and dogs that often provokes post-traumatic osteoarthritis (PTOA) despite surgical stabilization of the knee (stifle). There is greater recognition that the synovium is a major regulator of the intraarticular joint environment, including its response to injuries. Characterization of the synovial changes may be a key step toward mitigating the PTOA that occurs after ACL injuries. Although the dog has been considered as an important model there is little comparative understanding between human and canine joint response to ACL injury. In this study, we aim to compare the histological changes that occurs with human and canine knee synovium with naturally occurring ACL injuries using two well-known synovitis histologic grading systems (Krenn and HSS). Human

synovial tissues were collected from 13 patients at the time of ACL reconstruction (IRB #2009879), and Krenn scores, HSS scores, age, and sex were analyzed and compared with a historical cohort of clinical dogs (n=30) undergoing treatment for ACL injuries (Kuroki et al. Vet Surg 2021;50:1.32-1041). The present study indicates that most canine synovial samples collected at the time of treatment for ACL injuries were significantly higher in severity of synovitis versus their human counterpart. These observations suggest that the canine stifle response to ACL injuries may be more inflammatory versus the human response. These biological differences may need to be taken into consideration when evaluating the canine knee as a human preclinical model of ACL injury or surgery.

33: AN ETIOPATHOLOGIC INVESTIGATION OF TREPONEME-ASSOCIATED HOOF DISEASE, AN EMERGING HOOF DISEASE IN FREE-RANGING ELK (CERVUS CANADENSIS)

Elizabeth Goldsmith^{1,2}, Kyle Taylor^{1,2}, Devendra Shah³, Margaret Wild¹

¹Washington State University College of Veterinary Medicine, Pullman, WA, USA,

²Washington Animal Disease Diagnostic Laboratory, Pullman, WA, USA, ³Texas Tech University School of Veterinary Medicine, Amarillo, TX, USA

Background: Treponeme-associated hoof disease (TAHD) is an emerging hoof disease of free-ranging elk that causes lameness and debilitation. TAHD was initially identified in Washington in 2008 with subsequent detection in other northwestern states. Lesions are characterized by suppurative inflammation with spirochetes, identified as *Treponema*. Similar hoof diseases of livestock have polybacterial etiologies due to multiple *Treponema* spp. and other associated bacterial operational taxonomic units (OTUs). 16S rRNA gene amplicon sequencing of TAHD lesions from a limited number of free-ranging elk revealed that *Spirochaetaceae*, *Mycoplasmataceae*, *Fusobacteriaceae*, and *Porphyromonadaceae* were overrepresented in TAHD-positive lesions compared to normal hoof tissues, suggesting a polybacterial etiology.

Objective: Our objective is to investigate bacterial OTUs associated with a spectrum of histologic hoof lesions to further elucidate TAHD etiology and pathogenesis.

Methods: Interdigital hoof tissues were collected from grossly normal and abnormal hooves from 149 free-ranging elk from populations across the northwestern and central US. Sections of interdigital epidermis were examined with H&E and Warthin-Starry stains and categorized. 16S amplicon sequencing was performed to detect bacterial OTUs within lesions and normal tissues. Bacterial OTUs from different types of lesions were compared.

Results & Conclusions: Hoof tissues were placed into four categories: (1) intact epidermis without significant inflammation or necrotic cellular debris, (2) moderate intracorneal necrotic cellular debris, (3) pustular dermatitis, and (4) erosive to ulcerative dermatitis. Spirochetes were identified in the majority of pustular dermatitis and erosive to ulcerative dermatitis cases. Preliminary 16S amplicon sequencing identified several OTUs of interest for further investigation.

34: OUTBRAKE OF FIBRINOGEN AA-CHAIN -DERIVED GLOMERULAR AMYLOIDOSIS IN JAPANESE SQUIRRELS

Susumu Iwaide¹, Nanami Ito², Shiori Ogino³, Kaori Kojima¹, Natsumi Kobayashi¹, Satoshi Koyama¹, Shumpei Hisamoto⁴, Hirotaka Kondo², Yoshiyuki Itoh¹, Miki Hisada¹, Yuki Hoshino³, Daisuke Nakagawa⁵, Yuki Matsubara⁶, Shin-ichi Nakamura^{7,8}, Hiroshi Satoh³, Ken-ichi Watanabe⁹, Motoki Sasaki⁹, Hisashi Shibuya², Tomoaki Murakami¹
¹Tokyo University of Agriculture and Technology, Tokyo, Japan, ²Nihon University, Kanagawa, Japan, ³Iwate University, Iwate, Japan, ⁴Meiji Institute for Advanced Study of Mathematical Sciences, Tokyo, Japan, ⁵Kyoto City Zoo, Kyoto, Japan, ⁶Morioka Zoological Park, Iwate, Japan, ⁷Okayama University of Science, Ehime, Japan, ⁸Kyoto Institute of Nutrition & Pathology Inc., Kyoto, Japan, ⁹Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan

Background and **Objective:**

Reports of amyloidosis in the Genus *Sciurus* have been scarce. Recently, we encountered a high incidence of amyloidosis with unique deposition pattern in Japanese squirrels (*Sciurus lis*) and diagnosed fibrinogen A α -chain (AFib) amyloidosis for the first time in animals. Here we will report the pathogenesis in detail.

Materials and **Methods:**

FFPE from captive 38 Japanese squirrels (*Sciurus lis*) in five zoos and 12 Hokkaido squirrels (*Sciurus vulgaris*) were subjected to the following analyses: Congo red staining, immunohistochemistry, LC/MS/MS, RT-PCR and sequencing of *FGA* gene.

Results:

Histologically, massive amyloid was deposited in the glomeruli except in the tubulointerstitium in 29 of 38 Japanese squirrels (76.3%), as well as mildly in the other organs. No amyloid deposition was observed in Hokkaido squirrels. Proteomic analysis revealed AFib as the major component of amyloid deposits, and other amyloidogenic proteins were not detected. Amyloid was positive for AFib by immunohistochemistry. Genetic analysis revealed no mutations in AFib between amyloidosis-positive and negative cases. Statistical analysis revealed a significant association between amyloidosis and the process of aging ($P < 0.001$). Some Japanese squirrels had their roots in wildlife.

Discussion:

Based on these results, we concluded that Japanese squirrels have a species-specific tendency to develop AFib amyloidosis as aging. Japanese squirrels have evolved from Hokkaido squirrels, so it is likely that this pathological trait was acquired post-evolutionarily. Glomerular-specific deposition pattern may be a pathological hallmark of AFib amyloidosis in animals, as the most common amyloidosis, AA amyloidosis, shows prominent amyloid deposition in the tubulointerstitium.

35: CHARACTERIZING THE ROLE OF N-6 METHYLADENOSINE (M6A) IN HTLV-1 PATHOBIOLOGY

Emily King, Amanda Midkiff, Amanda Panfil

The Ohio State University College of Veterinary Medicine, Columbus, OH, USA

Human T-cell leukemia virus type 1 (HTLV-1) is an oncogenic retrovirus infecting 5-10 million people worldwide. Approximately 10% of those infected develop disease (adult T-cell leukemia/lymphoma, myelopathy/spastic paraparesis, inflammatory disease) after a latency period of several decades. Patient prognosis is poor and there are few effective therapies available for patients with disease. Two viral genes, *Tax* and *Hbz*, have been previously identified as critical to viral persistence and pathogenesis. Methylation of the N6 position of adenine (m6A) is the most common post-transcriptional modification, which until now has not been documented in HTLV-1. This modification is interpreted by cellular reader proteins (YTHDF1-3, YTHDC1-2) that can recognize the m6A modifications and regulate target gene expression. Our lab has found both *tax* and *hbz* mRNAs contain m6A modifications and the reader protein YTHDC1 binds viral transcripts *tax* and *hbz*. Sites of m6A modification were mapped within the HTLV-1 genome, identifying 3 major peaks, the largest of which localizes to the region of the genome encoding both *Tax* and *Hbz* genes. Over-expression of YTHDC1 decreases viral transcription and subsequent virion release. Conversely, shRNA-mediated knockdown of YTHDC1 increases viral transcription and virion release. Finally, HTLV-1-infection of primary CD4⁺ T-cells induces a significant increase in total m6A levels within the cell as early as two weeks post-infection. By understanding the role of m6A in the context of HTLV-1, these techniques and knowledge can be used to develop therapeutics for malignancies and enhance our understanding of viral pathogenesis.

36: RETINOSCHISIS: A RETROSPECTIVE STUDY OF AN UNCOMMON RETINAL CHANGE IN CATS AND DOGS

Joel Di Bernardo, Kim Newkirk, Diane Hendrix

University of Tennessee, Knoxville, TN, USA

Background: Retinoschisis is a poorly documented form of retinal degeneration characterized by cyst-like splitting that occurs between the inner nuclear and outer plexiform layers. The pathogenesis of retinoschisis is incompletely understood in domestic species, but congenital, acquired, and secondary etiologies (glaucoma, inflammation, neoplasia) are described in humans

Objective: This retrospective study investigated the prevalence and associated histologic and clinical features of retinoschisis in cats and dogs submitted for biopsy over a ten-year period.

Methods: The University of Tennessee College of Veterinary Medicine pathology database was searched from 2011 to 2021 for biopsy reports containing keywords “retinal vacuolation” and “retinoschisis.” Clinical details including breed, sex, and age were recorded. Slides were reviewed for changes consistent with retinoschisis.

Results: Of 140 samples with documented “retinal vacuolation,” 4 out of 120 (3%) canine samples and 1 out of 20 (5%) feline samples had changes consistent with retinoschisis. In the majority of cases (80%), there was concurrent retinal detachment. In cases with available histories, increased intraocular pressure, proptosis, and retinal detachment were reported clinical findings.

Conclusion: In cats and dogs, retinoschisis is an uncommon change that is generally secondary to other ocular lesions.

37: GROSS AND HISTOLOGIC FINDINGS IN STILLBORN PIGS WITH PULMONARY HYPOPLASIA WITH ANASARCA

Emily Hoskins, Brian Whitlock, Jon Beever, Dhar Madhu, Michael Aimar Rivera Orsini, Meaghan Harley-Troxell, Robert Donnell
University of Tennessee, Knoxville, TN, USA

Over the past decade, an abnormal phenotype colloquially referred to as “waterbelly” has been observed in the Chester White breed of swine. Recently, a nonsynonymous mutation in the ADAM metallopeptidase with thrombospondin type 1 motif 3 (*ADAMTS3*) gene has been associated with this phenotype. In mice and cattle, loss-of-function mutations in *ADAMTS3* have been associated with a recessive condition named pulmonary hypoplasia with anasarca (PHA). PHA is a fatal congenital condition in which bovine and ovine fetuses develop severe subcutaneous edema, have incompletely developed lungs, and lymphatic dysplasia. Increased fetus size due to extensive edema often leads to dystocia if not previously aborted. This condition has been reported in Dexter, Maine-Anjou, Shorthorn, Slovenian Cika, and their associated crosses in addition to various breeds of sheep. *ADAMTS3* knock out mice do not develop lymphatic vessels leading to a severe lymphedema. Additionally, lymph nodes were not observed in Cika calves born with PHA. The purpose of this pilot study is to characterize the gross and histopathological findings in stillborn pigs with PHA to support the hypothesis that lymphatic dysplasia is present in skin histopathology sections suggesting a conserved pathological mechanism for PHA across bovine, ovine, and porcine species. Grossly, affected stillborn pigs had severe subcutaneous edema, decreased lung size, and more prominent lymphatic vessels compared to unaffected animals. Histologically, lymphatic vessels in affected animals were subjectively markedly dilated compared to lymphatic vessels in control animals.

38: EVALUATION OF THE ROLE OF AB BLOOD SYSTEM PHENOTYPES IN FELINE LEISHMANIOSIS

Eva Spada¹, Federica Bruno², Roberta Perego¹, Luciana Baggiani¹, Noemi Cerutti¹, Vito Biondi³, Germano Castelli², Fabrizio Vitale², Daniela Proverbio¹

¹University of Milan, Lodi, Italy, ²Experimental Zooprophyllactic Institute, Palermo, Italy,

³University of Messina, Messina, Italy

Background: In people susceptibility to certain diseases can be influenced by blood type. For example, studies have shown that cutaneous leishmaniosis is more common in people with Rh-negative blood groups with B alleles. Little is known about the role of blood types in feline infections. Feline leishmaniosis is an emergent disease, and rate of infection in cats in endemic regions varies based on different factors.

Objective: To evaluate if AB blood system phenotypes A, B or AB are associated with natural infection by *Leishmania infantum* in cats from Italy, a country in southern Europe with moderate-to-high endemicity for leishmaniosis.

Methods: *L. infantum* infection was investigated in 706 cats using IFAT (n=687) and real-time PCR (qPCR) on blood (n=673) and/or on lymph node aspirates (n=277). Cats with IFAT titer $\geq 1:80$ and/or positive qPCR were considered infected. In the same feline population, the blood phenotypes A, B and AB were determined using agglutination on tube method, with type B and AB samples confirmed by back typing and immunochromatographic techniques.

Results: *L. infantum* infection was found in 67/706 cats (9.5%), while A, B and AB blood phenotypes prevalence was 83.1%, 10.1% and 6.8%, respectively. *L. infantum* infection was demonstrated in cats of all three phenotypes. There was no significant association between general infection and the blood phenotype ($P=0.7294$), neither for *L. infantum* IFAT seropositivity ($P=0.7740$) or only qPCR positivity ($P=0.4584$).

Conclusions: AB feline blood system phenotype antigens and correlated natural alloantibodies do not appear to play a role in feline leishmaniosis infection.

39: MULTISYSTEMIC EFFECTS OF CANINE DISTEMPER VIRUS IN FERRETS

Adrienne Glaser^{1,2}, Stephanie French¹, Roger Maes¹, Victoria Watson¹

¹Veterinary Diagnostic Laboratory, Michigan State University, East Lansing, MI, USA,

²National Institutes of Health Comparative Biomedical Scientist Training Program, Bethesda, MD, USA

Canine distemper virus (CDV) is an established cause of interstitial pneumonia in ferrets; however, the extent of lesions has not been well described. Over the course of 7 months, twenty-eight 1.5- to 8-month-old ferrets with a varied vaccination history for CDV presented to the Michigan State University Veterinary Diagnostic Laboratory for field (19) or in house (9) autopsy. Ferrets had clinical signs including ocular and nasal discharge, lethargy, diarrhea, and signs attributed to neurologic, and/or respiratory disease. Gross examination revealed bilateral nasal discharge, mottled red to purple, rubbery, non-collapsing to firm lungs, and lymphadenomegaly. Histologic examination demonstrated a variety of lesions including interstitial pneumonia with intracytoplasmic inclusion bodies in bronchial epithelium and alveolar macrophages, occasionally concurrent bronchopneumonia, lymphadenitis, lymphoid depletion, and colonic crypt necrosis. Syncytial cells were identified in multiple organs including lymph nodes, lungs, and kidneys. Although gastrointestinal signs are associated with distemper, this is the first report of CDV-induced colonic crypt necrosis in this species. Diagnostic testing included PCR for CDV on lungs (25/28 positive), spleen (8/8 positive), thymus (5/5 positive), trachea (5/5 positive) and/or urinary bladder (5/5 positive). Immunohistochemical labeling for CDV antigen was demonstrated in the lungs (21/28), small/large intestine (8/14), kidney (6/13), and lymph node (8/12). Crypt epithelial cells in regions of crypt necrosis in the colon labeled strongly for CDV. This case series demonstrates the breadth of lesions observed with canine distemper virus in ferrets, including previously undescribed gastrointestinal lesions in this species.

40: INTEGRATING ANATOMIC PATHOLOGY TECHNIQUES IN WILDLIFE DISEASE SURVEILLANCE: EXPERIENCES WITHIN THE ONE HEALTH CONTEXT IN BRAZIL

Juliana Mariotti Guerra^{1,2}, Ticiana Brasil Ervedosa¹, Eduardo Ferreira-Machado^{1,2}, Pedro Enrique Navas-Suárez¹, Alessandra Loureiro Morales dos Santos¹, Isis Paixão de Jesus¹, Julia de Carvalho¹, Rodrigo Albergaria Réssio¹, Cinthya dos Santos Cirqueira¹, Ana Carolina Souza Ramos de Carvalho¹, Ketlyn Bolsachini Figueiredo¹, Camila Santos da Silva Ferreira¹, Roberta Fernandes Spinola³, Leila Del Castillo Saad³, José Luiz Catão-Dias², Natália Coelho Couto de Azevedo Fernandes¹

¹Instituto Adolfo Lutz, São Paulo, Brazil, ²Universidade de São Paulo, São Paulo, Brazil,

³Secretaria de Estado da Saúde, São Paulo, Brazil

Background: Understanding and monitoring wildlife diseases are crucial for assessing their impact on public health and conservation.

Objective: This study presents preliminary findings from a laboratory-based surveillance program that combines anatomopathological analysis with ancillary techniques to monitor wildlife diseases.

Methods: From January 2019 to June 2021, a total of 1452 samples were evaluated by the Pathology Center of Adolfo Lutz Institute, São Paulo, Brazil. Histopathological evaluation, complemented by immunohistochemical and molecular tests, was conducted.

Results: The study included 71 animal species, with non-human primates comprising 89.1% (N= 1295) of the samples, followed by other mammals (4.4%, N= 65), birds (5.3%, N= 78), and reptiles (0.9%, N= 14). Viral infections accounted for 2.34% (N= 34) of the cases, including yellow fever virus, human alphaherpesvirus type 1, brazilian porcupine poxvirus 1, canine mastadenovirus A and canine distemper virus. Bacterial infections were detected in 8.05% (N= 117) of the cases, featuring *Leptospira* sp., *Chlamydia* sp., and an outbreak of *Klebsiella pneumoniae*. Protozoal infections represented 2.75% (N= 40), with notable cases of toxoplasmosis. Fungal infections, such as aspergillosis and mucormycosis, were observed in 0.48% (N= 7) of the cases. Metazoan parasitic infections were identified in 4.41% (N= 64) of the samples. Neoplastic conditions accounted for 0.96% (N= 14) of the cases, while hepatic iron accumulation was observed in 0.76% (N= 11) of the cases.

Conclusions: Anatomopathological techniques, in conjunction with ancillary tests, provide valuable insights for understanding wildlife diseases and enhancing surveillance efforts within the One Health context.

41: GENOTYPIC VARIANTS BETWEEN FATAL AND NON-FATAL SARCOCYSTIS NEURONA INFECTIONS IN SOUTHERN SEA OTTERS (ENHYDRA LUTRIS NEREIS) IN CALIFORNIA

Devinn Sinnott¹, Melissa Miller², Francesca Batac², Katherine Greenwald², Colleen Young², Pdraig Duignan³, Margaret Martinez³, Michael Harris², Barbie Halaska³, Karen Shapiro¹

¹University of California, Davis, School of Veterinary Medicine, Davis, CA, USA, ²Marine Wildlife Veterinary Care and Research Center, California Department of Fish and Wildlife, Santa Cruz, CA, USA, ³The Marine Mammal Center, Sausalito, CA, USA

Background: Southern sea otters (*Enhydra lutris nereis*) are commonly infected with the protozoal parasites *Toxoplasma gondii* and *Sarcocystis neurona*. Specific strains of *T. gondii* have been associated with fatal infections in sea otters. Comparatively little is known about the relationship between parasite genotype and disease outcome for *S. neurona* in sea otters.

Objective: The goal of this study is to compare the diversity of *S. neurona* genotypes in fatal vs. non-fatal infections in sea otters in California.

Methods: Histopathology slides were reviewed to identify sea otters with fatal and non-fatal infections. Parasite DNA was extracted from brain tissue or cultured isolates (fatal cases) or tongue tissue (non-fatal cases). The *S. neurona* genotype for each infection was characterized using a multilocus sequence typing (MLST) approach targeting six loci including three surface antigens and three microsatellite markers.

Results: Nineteen genotypes were identified among the fatal infections (n=76) and nine genotypes were identified among the non-fatal infections (n=19). Only three genotypes overlapped between the fatal and non-fatal infection groups. One genotype (Ib/c/d/gg) was significantly associated with sea otters that died during large *S. neurona* outbreak events in 2004 and 2021.

Conclusion: Although data in this study is skewed towards fatal infections, these results suggest that differences exist in the *S. neurona* genotype diversity between fatal and non-fatal infections in sea otters. Sarcocystosis is likely a heterogeneous disease in sea otters that is influenced by parasite, host, and environmental factors.

43: FIBROBLAST GROWTH FACTOR 21 EXPRESSION PATTERNS IN HEALTH AND IN SPONTANEOUS FELINE PANCREATITIS

Emily Brinker^{1,2}, Heather Hamilton², Rie Watanabe², Emily Graff²

¹Tufts University, North Grafton, MA, USA, ²Auburn University, Auburn, AL, USA

Background: Fibroblast Growth Factor 21 (FGF21) is an endocrine regulator of the fat-liver axis. Activation of the FGF21 pathway induces weight loss and improves glucose homeostasis in humans, primates, and rodent models of obesity. Additionally, preclinical and retrospective human studies of FGF21 suggest that pancreatitis is an FGF21 deficient state, and administration of FGF21 ameliorates pancreatitis in rodent models. In cats exogenous administration of FGF21 only induced weight loss without altering circulating metabolic parameters, and there are no studies that investigate FGF21 in feline pancreatitis.

Objective: We aimed to investigate the FGF21 pathway, including its co-receptors β -klotho and FGFR1c, in healthy cats, and changes in FGF21 protein expression in

spontaneous cases of feline pancreatitis.

Methods: RT-PCR of FGF21, β -klotho, and FGFR1c was performed on frozen tissues from healthy cats. FGF21 IHC was performed on FFPE tissues from archived healthy cats and necropsy cases of feline pancreatitis.

Results: FGF21 mRNA is highly expressed in the liver and pancreas but not detected in the falciform or inguinal subcutaneous adipose tissue. Feline adipose tissues did express the receptors FGFR1c and β -klotho for FGF21 signaling. In cases of pancreatitis, a subset of macrophages have robust cytoplasmic immunoreactivity to FGF21.

Conclusions: The feline FGF21 signaling pathway is unique in that it does not express FGF21 in adipose tissues, suggesting absence of autocrine or paracrine action in these tissues. Additionally, feline pancreatitis does not appear to be an FGF21-deficient state. Macrophage FGF21 immunoreactivity may represent a potential innate reparative process, as FGF21 can induce M2 polarity.

44: GRANULOMATOUS ARTERITIS/AORITIS ASSOCIATED WITH MYCOBACTERIUM GENAVENSE IN ZEBRA FINCHES (TAENIOPYGIA GUTTATA)

Rachel Howie, Tzushan Yang, Katherine Gibson-Corley, Nicholas Tataryn
Vanderbilt University Medical Center, Nashville, TN, USA

Mycobacteriosis affects a variety of avian species but is rarely investigated in a laboratory animal research setting. Specifically in zebra finches, *Mycobacterium* spp. has been scarcely documented with non-specific systemic lesions. Six zebra finches in a research colony of approximately 150 birds were euthanized or died over a two-year period. Clinical signs were variable and included respiratory distress, lethargy, feather loss, and cloacal prolapse. No apparent gross findings were noted on necropsy. Histopathological examination in all six birds revealed lymphohistiocytic inflammation at the base of the heart that surrounded or infiltrated the aorta and/or other great vessels. The tunica media of these vessels were expanded by a moderate number of foamy macrophages mixed with flocculent eosinophilic material that contained aggregates of acid-fast bacilli. The myocardium also had multifocal necrosis and cardiomyocyte vacuolation with small numbers of macrophages. Twenty-one other zebra finches over the same two-year period displayed similar cardiac lesions but were negative for acid fast staining. *Mycobacterium* genus PCR of pooled fecal samples from sentinel animals and further sequencing confirmed the presence of *Mycobacterium genavense* within the colony. Several finches also displayed noncardiac lesions including amyloidosis in the heart, liver, and spleen, myositis, interstitial nephritis, lymphoplasmacytic dermatitis, subretinal hemorrhage, and pneumonia. To our knowledge, mycobacterial aortitis/arteritis has not been thoroughly described in zebra finches and should be considered a lesion of latent or active mycobacterial infection.

45: DIFFERENTIAL EXPRESSION OF MICRORNAS IN SERUM AND TISSUE FROM DOGS WITH SPLENIC MASSES

Latasha Ludwig¹, Heather Treleaven¹, Arlene Khachadoorian¹, Brigitte Degasper², Ingrid Walter², R. Darren Wood¹, Geoffrey A. Wood¹

¹University of Guelph, Guelph, ON, Canada, ²University of Veterinary Medicine, Vienna, Austria

Background: Splenic hemangiosarcoma (sHSA) is a highly aggressive tumor in dogs that frequently presents as collapse from hemoabdomen. Non-neoplastic splenic masses can have a similar presentation. Even with splenectomy, hemorrhage within sHSAs can make an accurate histopathologic diagnosis challenging. MicroRNAs are small non-coding RNAs in tissues and blood that are dysregulated in cancer.

Objective: Evaluate 59 microRNAs in serum and tissue from healthy dogs and dogs with splenic masses.

Methods: We obtained serum (n=64) and tissue (n=84) from the Ontario Veterinary College Veterinary Biobank and Vetmeduni VetBiobank, Vienna from healthy dogs and dogs with sHSA, and other malignant and benign splenic masses. MicroRNAs were isolated using QIAGEN miRNeasy kits and underwent RT-qPCR using MiRCURY Custom PCR arrays. Samples were normalized and fold changes were determined using $2^{-\Delta\Delta Ct}$. MicroRNAs were considered dysregulated if the fold change was >2 and $p < 0.05$ using a Mann-Whitney test.

Results: Multiple microRNAs were dysregulated in serum and tissue of sHSA compared to controls and other splenic masses. In both serum and tissue, miR-135a-5p was lower and miR-542-3p was higher in sHSA compared to controls. In serum, miR-135a-5p was lower in sHSA across all other mass types. Two microRNAs were lower and five were higher in sHSA tissue compared to benign splenic masses.

Conclusions: Serum and tissue microRNAs are differentially expressed in dogs with sHSA compared to other splenic masses, serving as a potential diagnostic tool. Two microRNAs were similarly expressed in serum and tissue, and thus are potentially produced by and released from neoplastic cells.

46: HISTOPATHOLOGIC CHARACTERIZATION AND MOLECULAR EPIDEMIOLOGY OF CLINICAL CANINE DISTEMPER VIRUS CASES IN PENNSYLVANIA MESOCARNIVORES

Taylor Chan, Madison Stevens, Amanda Barnard, Anna Hakey, Eman Anis, Kevin Niedringhaus

University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA, USA

Background:

Canine distemper virus (CDV) is an important cause of morbidity and mortality in a wide variety of wildlife species and domestic dogs across the globe. Recent studies show multiple CDV lineages circulating among American mesocarnivores and evidence

suggests the current canine vaccines may not fully protect against these emerging CDV strains.

Objective:

To review key histopathological lesions associated with CDV infection and compare the lesions in free-ranging mesocarnivores (raccoons, grey foxes, skunks and mink) and a domestic dog, as well as between CDV lineages in Pennsylvania, USA.

Methods:

Full necropsies, light microscopy (H&E), and immunohistochemistry for CDV were performed. PCR and sequencing of the matrix-fusion (M-F) intergenic region of the CDV were performed to determine the CDV lineages circulating among these clinical canine distemper cases.

Results:

Canine distemper was diagnosed in 28 free ranging animals from four species (19 raccoons, 6 gray foxes, 2 striped skunks, and 1 mink) and one domestic dog from September 2022 – June 2023. Microscopic findings in the wildlife cases ranged from mild mononuclear encephalitis to demyelination without overt inflammation, interstitial pneumonia in the absence of encephalitis and overall strong immunoreactivity. In comparison, the lesion patterns in the dog were more consistent with those described in the current literature and demonstrated a lack of respiratory lesions with significantly more cerebral inflammation and necrosis than the wildlife cases.

Conclusion:

Despite a relatively small sample size, differences in microscopic lesions of CDV infection may reflect disease progression, host susceptibility, and pathogenicity differences between viral lineages.

47: FIRST REPORT OF ABORTIONS IN CATTLE DUE TO BOVINE VIRAL DIARRHEA VIRUS (BVDV) IN SOUTHERN BRAZIL INVOLVING AN ATYPICAL SUB-GENOTYPE IN THE AMERICAN CONTINENT

Renata Casagrande¹, Lucas Marian¹, Jéssica Withoeft¹, Lucas Quevedo¹, Gustavo Pandolfo¹, Jennyfer Julia Sá¹, Maria Augusta Fornara¹, Letícia Baumbach², Claudio Canal²

¹Laboratório de Patologia Animal, Centro de Ciências Agroveterinárias, Universidade do Estado de Santa Catarina (UDESC), Lages, Brazil, ²Laboratório de Virologia Veterinária, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil

Background: Bovine viral diarrhea virus (BVDV) is an important pathogen associated with reproductive failure in cattle.

Objective: The objective of this study was to characterize BVDV-related abortions in cattle and identify the species and sub-genotypes present in south of Brazil.

Methods: Necropsy and histopathological examination of all organs were performed in 87 cattle fetuses. Spleen and thymus fetuses samples were analyzed to BVDV RT-PCR from the 5'UTR region of the *Pestivirus* genome. For gene sequencing, amplification products were sequenced by the Sanger method. *Pestivirus* reference strain sequences and strains obtained were aligned for phylogenetic analysis using the CLUSTAL W™ software.

Results: RT-PCR was positive for BVDV in 5.7% (5/87) of fetuses. Gross analysis showed fetal mummification (1/5), hepatomegaly (1/5), subcutaneous edema (1/5), and perirenal edema (1/5). Histopathological lesions included mild to moderate multifocal lymphoplasmacytic epicarditis and myocarditis (5/5), lymphoplasmacytic interlobular pneumonia (4/5), tubular nephrosis associated with interstitial nephritis (1/5), lymphoplasmacytic necrotic hepatitis (1/5), and lymphoplasmacytic meningitis (1/5). It was possible to sequence four amplification products from the *Pestivirus*, which revealed 96.3-100% similarity between fetal strains and the reference BVDV-1 and BVDV-2 isolates. Three strains clustered in the BVDV-1 branch sub-genotype 1e, atypical in the American and one strain clustered in the BVDV-2b, frequent in Brazil. In this study, the GPV0122-SC-Brazil and GPV0222-SC-Brazil strains classified as 1e were similar, but with an evolutionary ancestry distant from the GPV0522-SC-Brazil strain.

Conclusions: The results detail the first reported BVDV abortions in cattle in Brazil, involving a rare sub-genotype in the American continent.

48: ANATOMOPATHOLOGICAL, SEROLOGICAL AND MOLECULAR ASPECTS OF INFECTIOUS LARYNGOTRACHEITIS CAUSED BY CHICKEN EMBRYO ORIGIN (CEO) VACCINE STRAIN IN BRAZIL

Renata Casagrande¹, Jéssica Withoeft¹, Carolina Bolsanello², Anderson Bonamigo², Maurício Cantão³, Caroline Pisetti⁴, Jennyfer Julia Sá¹, Lucas Quevedo¹, Luizinho Caron³

¹Laboratório de Patologia Animal, Centro de Ciências Agroveterinárias, Universidade do Estado de Santa Catarina (UDESC), Lages, Brazil, ²Companhia Integrada de Desenvolvimento Agrícola de Santa Catarina (CIDASC), Florianópolis, Brazil, ³Empresa Brasileira de Pesquisa Agropecuária – Suínos e Aves (Embrapa), Concórdia, Brazil, ⁴Centro de Diagnóstico de Sanidade Animal (Cedisa), Concórdia, Brazil

Background: Infectious laryngotracheitis of chickens (ILT) was an exotic disease in the state of Santa Catarina, southern Brazil, until September 2020, when it was diagnosed on commercial laying farm in the county of São Ludgero.

Objective: To determine the prevalence of ILT in the municipalities of the São Ludgero region (SL) and in the entire territory of Santa Catarina state (SC).

Methods: Forty-four (SL) and 49 (SC) commercial laying farms were sampled. Twenty chickens were selected per farm for serological (ILT-ELISA®Biocheck). Ten of these hens were necropsied for histopathological analysis. Conjunctiva, larynx, trachea, and trigeminal ganglion were subjected to qPCR amplifying the virus gC gene. Positive

samples were selected for ICP4 and TK gene sequencing. The construction of phylogenetic trees was conducted in MEGA11 software.

Results: In the SL, the seropositivity was 95.4% (42/44) and in the SC 65.3% (32/49). Microscopically, classic ILT lesions were found in three farms (SL), with moderate lymphoplasmacytic and fibrinous laryngitis, tracheitis, bronchitis and rhinitis associated with syncytial cells and basophilic intranuclear inclusion bodies. There was molecular detection of the virus in 88.1% (SL-37/42) and 21.9% (SC-7/32) of seropositive farms. The phylogenetic analysis demonstrated that the sequences obtained in both geographical areas were arranged in the same branches of CEO vaccine-origin strains. There was no T252M mutation in TK gene, demonstrate the occurrence of a low-virulence strain of vaccine origin in southern Brazil.

Conclusions: This is the first documented occurrence vaccine strain of the CEO type causing ILT in Brazil, and despite its low virulence.

49: TOXOPLASMOSIS OUTBREAK IN BLACK-TUFTED MARMOSETS (*CALLITHRIX PENICILLATA*) KEPT IN CAPTIVITY IN SOUTHERN BRAZIL

Renata Casagrande¹, Lucas Marian¹, Jéssica Withoeft¹, Maria Augusta Fornara¹, Gustavo Pandolfo¹, Fagner D'ambroso Fernandes², Milene Pugliese Zapala³, Fernanda Silveira Flores Vogel Silveira Flores Vogel²

¹Laboratório de Patologia Animal, Centro de Ciências Agroveterinárias, Universidade do Estado de Santa Catarina (UDESC), Lages, Brazil, ²Laboratório de Doenças Parasitárias, Centro de Ciências Rurais, Universidade Federal de Santa Maria, Santa Maria, Brazil, ³Zoológico de Brusque, Brusque, Brazil

Background: Toxoplasmosis is a worldwide zoonotic disease that can cause high lethality in New World primates.

Objective: The objective of this study was describing an outbreak of toxoplasmosis in black-tufted marmosets (*Callithrix penicillata*) at a zoo in the state of Santa Catarina, Southern Brazil.

Methods: Four marmosets were found dead, one of whom exhibited prostration and respiratory difficulty, leading to death in 12 hours. The animals underwent necropsy and histopathological examination. Fragments of the liver, spleen and lungs were frozen and sent for PCR and nested-PCR-restriction fragment length polymorphism (RFLP) genotyping of *T. gondii*.

Results: At necropsy was observed liver mild lobular pattern, along with diffusely reddened lungs, spleen, and kidneys (5/5), and hemorrhage in the mesenteric lymph nodes (1/5). Mild to moderate multifocal necrotic hepatitis (4/5) with moderate diffuse hepatocellular degeneration (4/5), mild to moderate multifocal necrotic splenitis (5/5), moderate diffuse pulmonary congestion and edema (5/5), mild multifocal interstitial lymphoplasmacytic pneumonia (1/5), mild multifocal lymphoplasmacytic myocarditis (1/5), and moderate diffuse necrohemorrhagic lymphadenitis (1/5) were observed. Intralesional *T. gondii* tachyzoites were observed in liver, mesenteric lymph nodes,

spleen, and heart. The isolated strains of *T. gondii* were consistent with the isolates TgWtdUs10, TgSoUs39, and TgShUs2, which were originally found in a white-tailed deer (*Odocoileus virginianus*), California sea otter (*Enhydra lutris nereis*), and sheep (*Ovis aries*), respectively, all at the United States of America.

Conclusions: This is an unprecedented report of North American strains of *T. gondii*, as the cause of acute mortality in five captive black-tufted marmosets.

50: PATHOLOGY AND QUANTITATION OF NEURONS IN AUTONOMIC GANGLIA OF DOGS WITH CANINE PANDYSAUTONOMIA

Dennis O'Brien, Renatta Mammone, Gayle Johnson
University of Missouri, COLUMBIA, MO, USA

Canine dysautonomia is an idiopathic disease of young dogs with access to the outdoor environment that is characterized by acute, widespread catastrophic degeneration and loss of autonomic neurons. Retrospective examination of celiac-anterior mesenteric enteric ganglia from 81 cases of canine dysautonomia (1988-2023) determined neuronal density by point/point counts of grid intersects landing on neurons, was expressed as percent intersects with a 400-intersect square grid, measuring 0.243 square mm. Multiple non-overlapping fields were measured for each sample. Median intersects for affected dogs was 1.1 percent, while that for control dogs was 12.2 percent. In 35 cases, paired anterior and posterior mesenteric ganglia from patients were quantitated, and comparison revealed no significant differences (medians 1.9 and 1.2, respectively, $P=0.532$ by Mann-Whitney U statistic. This suggests that the extent of neuronal depletion is similar in multiple ganglia in a single patient. The pathology of dysautonomia varies between neurons and between animals. The diagnosis is often made by identifying angular, brilliantly eosinophilic chromatolytic neurons in affected tissue, but other neurons may be swollen eosinophilic or swollen pale cell bodies that lack Nissl substance and other basophilic cells that still contain Nissl substance with less obvious nuclear damage. Different types of pathology are interspersed or dominate between patients. Neuronal vacuolation, neuronophagia, lymphocytic inflammation are additional microscopic findings present.

51: YERSINIA ENTEROCOLITICA INFECTION IN AFRICAN GREEN MONKEYS (CHLOROCEBUS AETHIOPS SABAEUS) INDUCES INDOLEAMINE DEOXYGENASE 1 EXPRESSION IN THE INTESTINAL MUCOSA

Gayathriy Balamayooran¹, Hannah Atkins², Matthew Jorgensen¹, John Sanders¹, Nancy Kock¹, David Caudell¹

¹Wake Forest University School of Medicine, Winston Salem, NC, USA, ²University of North Carolina, Chapel Hill, NC, USA

Yersinia enterocolitica (YE) causes food borne infections in humans and animals and are often self-limiting infections. However, a YE outbreak in an African green monkey breeding colony at the Wake Forest University School of Medicine was responsible for the death of more than 20 animals. Immunophenotyping of the YE-infected tissues demonstrated robust responses of neutrophils, macrophages, and dendritic cells and poor T and B lymphocyte responses, suggesting failure of the adaptive host immune

response. Indoleamine deoxygenase- 1 (IDO1) is the initial enzyme in tryptophan metabolism that drives the kynurenine pathway. Kynurenine, a metabolite of this pathway, binds to specific receptors and contributes to T regulatory cell differentiation, and suppresses T effector cell function. Furthermore, IDO1 has been extensively studied in oncology and infectious diseases for its role in immunosuppression. We hypothesized that during YE infection, neutrophils and macrophages develop an immunosuppressive phenotype and express IDO1. To test this, we determined the IDO1 expression in the intestinal mucosa and mesenteric lymph nodes of YE-infected and control animals. We observed a marked increase in IDO1 expression in phagocytes and endothelial cells following YE infection in the intestine and mesenteric lymph nodes. These findings suggest YE induced IDO1 plays an immunosuppressive role during YE infection in African green monkeys, accounting for their inability to mount appropriate immune responses.

52: GASTROINTESTINAL TRACT PATHOLOGY OF THE OWL MONKEY (*AOTUS* SPP.)

Martha Hensel¹, Aline Rodrigues Hoffmann², Beth Dray^{1,3}, Gregory Wilkerson^{1,4}, Wallace Baze¹, Carolyn Hodo¹

¹The University of Texas MD Anderson Cancer Center, Bastrop, TX, USA, ²University of Florida, Gainesville, FL, USA, ³Charles River Laboratories, Ashland, OH, USA, ⁴North Carolina State University, Raleigh, NC, USA

Background: Owl monkeys are small, nocturnal, new world primates in the genus *Aotus*, which contains up to eleven different species. Owl monkeys have most commonly been used in biomedical research for study of malaria but are also used for other infectious disease and vision research. The most common and well-described spontaneous diseases contributing to morbidity and mortality are cardiomyopathy and nephropathy, but less is known about lesions affecting the gastrointestinal tract.

Objective: To conduct a retrospective review of gastrointestinal pathology of *Aotus* spp. submitted for necropsy from a captive breeding and research colony.

Methods: Records from breeding colonies of four *Aotus* spp. at the Keeling Center for Comparative Medicine and Research over a 14-year period (2008-2022) were queried to identify instances of spontaneous gastrointestinal disease that directly contributed to cause of death.

Results: Of the 235 adult owl monkeys submitted for necropsy, 10.6% (25/235) had gastrointestinal disease listed as a significant factor that contributed to morbidity and mortality. One of the most common diagnoses was intestinal incarceration and ischemia (7/25), including mesenteric rent (n=3), intestinal torsion (n=1), strangulating lipoma (n=2), and an inguinal hernia (n=1). Neoplasia was identified in 7/25 animals, including jejunal adenocarcinoma (n=4), intestinal lymphoma (n=2), and oral squamous cell carcinoma (n=1). Additional diagnoses included gastric bloat (4/25), intussusception (4/25), and mycotic esophagitis and/or gastroenteritis (3/25).

Conclusions: This report documents spontaneous lesions in the gastrointestinal tract of *Aotus* spp. that contributed to morbidity and mortality, some of which were previously unreported in the literature.

53: PROGNOSTIC SIGNIFICANCE OF COX-2 AND 14-3-3 Σ , AND ASSOCIATION OF E-CADHERIN AND COX-2 EXPRESSION WITH FEATURES OF MALIGNANCY IN VIMENTIN-POSITIVE (UNDIFFERENTIATED) CANINE GASTRIC CARCINOMAS

Bronwen Hulme¹, Alejandro Suárez-Bonnet¹, Kerstin Erles², Simon Priestnall¹, Alexandros Chardas¹

¹The Royal Veterinary College, Hatfield, United Kingdom, ²VPG Histology, Bristol, United Kingdom

Canine gastric carcinoma (CGC) is a rare but aggressive neoplasm affecting dogs most commonly at nine years old. CGC has been previously reported as a naturally occurring model for human disease. Undifferentiated CGC is the most frequently diagnosed histological subtype and is therefore an important target for immunohistochemical investigation.

The expression of vimentin, E-cadherin, COX-2 and 14-3-3 σ was analyzed in 20 full-thickness cases, with clinical follow-up from VPG Histology, in primary tumour and neoplastic emboli (12/20, 60%) along with routine histopathological evaluation to identify features of malignancy.

All tumours showed vimentin-positive neoplastic cells. Assessment of the depth of invasion (DOI) with E-cadherin expression (17/20, 85%) demonstrated that tumours invading as far as the muscularis (9/20) and the serosa (11/20), when compared, showed significant differences ($p=0.0021$) in the staining intensity for those reaching the serosa. A decrease in the expression of E-cadherin and concomitant increase in vimentin expression ($p=0.0052$) highlights the importance of epithelial-mesenchymal transition. COX-2 expression, seen in 18/20 cases, was significantly correlated ($p=0.0175$) with DOI to the level of the serosa (11/20).

8/12 cases showed vimentin-positive emboli, and 10/12 stained positively for at least one marker indicating the preponderance of pathways and proteins that play a part in the intricacies of tumour invasion and metastasis. Increased COX-2 and 14-3-3 σ expression was found to be significantly associated with a shorter median survival time (2 versus 65.5 days, $p=0.0413$ and 1 versus 68 days, $p=0.0005$, respectively) and alongside E-cadherin and vimentin, may serve as future prognostic markers or even therapeutic targets.

54: CYTOKERATIN EXPRESSION BY SARCOMAS DOES NOT INDICATE SYNOVIAL ORIGIN

Linden Craig, Annalisa Wager

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

In this retrospective study, 25 subcutaneous soft tissue sarcomas and 5 articular soft tissue sarcomas were tested by immunohistochemistry for expression of cytokeratin. Of the 25 subcutaneous sarcomas, 8 (32%) expressed cytokeratin in 1-50% of the neoplastic cells. None of the 5 articular sarcomas expressed cytokeratin. Cytokeratin

expression does not depend on synovial origin ($p=0.29$). Survival rates were compared for 24 dogs (19 subcutaneous and 5 articular). The average survival of the dogs with subcutaneous sarcomas was 28.1 months [CI: 17.8, 38.4] and those with articular sarcomas was 30.0 months [CI: 3.7, 56.3]. Of the dogs with subcutaneous sarcomas, those with cytokeratin expression survived an average of 31.2 months [CI: 17.8, 44.6] and those without cytokeratin expression survived an average of 22.0 months [CI: 8.4, 35.6]. Neither location of the sarcoma ($p=0.98$) nor cytokeratin expression ($p=0.53$) affected survival. Cytokeratin expression does not indicate synovial origin and does not predict survival in dogs with subcutaneous sarcomas. Immunohistochemistry for cytokeratin is not recommended for subcutaneous or synovial origin sarcomas in dogs.

55: THYMUS HISTOLOGY FINDINGS IN STILLBORN AFRICAN GREEN MONKEY (CHLOROCEBUS AETHIOPS SABAEUS) INFANTS

Tessa Kell¹, Gayathriy Balamayooran², Matthew Jorgensen², David Caudell²

¹Washington State University, Pullman, WA, USA, ²Wake Forest University School of Medicine, Winston Salem, NC, USA

Many third trimester stillbirths in African green monkey (AGM) infants remain unexplained. Acute thymic involution is described in human stillbirths and neonatal infants and is associated with specific diseases. However, detailed descriptions of thymic pathology are underreported in nonhuman primate stillborn/ perinatal infants. Here we report gross and histological findings of the thymus in AGM stillborn and two C-section derived control infants from Wake Forest School of Medicine breeding colony. Eleven still born infants were identified over a two-year period of 2020-2022, 1/11 was female and 10/11 were males. Among them 4/11 had macrosomia and 2/11 had placentitis; 2/11 infants were from first time dams and 9/11 were from dams that had eight or more pregnancies. The thymus from stillborn infants weighed more than the controls (0.82 vs 0.46g). Digital quantification of the nuclear density of the thymic cortex and medulla were both positively correlated with thymus weight. Histologically there was variable loss of corticomedullary distinction (10/11), and loss of nuclear density (9/11) observed in stillborn infants compared to the controls. In both groups, thymic cysts and macrophage and neutrophil infiltration were observed. Interestingly adrenal glands weights were higher in the stillborn infants compared to the controls (0.25 vs 0.09g). These findings suggest a possible role of glucocorticoid metabolism in AGM stillbirth. Further studies are needed to determine the mechanism involving glucocorticoid metabolism and thymus pathology during pregnancy. This model could offer insight into the role of *in-utero* stress and pituitary-thymic-adrenal axis during fetal development and perinatal health.

56: ASTROVIRUS-ASSOCIATED ENTERITIS IN A RABBIT FARM WITH ONSET OF DIARRHEA

daniel rejmanek, nicolas streitenberger, Beate Crossley, anibal armien, Omar Gonzales Viera

California Animal Health and Food Safety (CAHFS) Laboratory System, Davis Branch, University of California, Davis, Davis, CA, USA

Approximately 20% of 300 rabbits (*Oryctolagus cuniculus*) raised for meat on a farm developed diarrhea. Two females, juvenile rabbits with a history of being lethargic and with epistaxis were studied. Grossly, abundant semi-dry, dark-green fecal material stained the perianal region, tail, and hindlimbs. Jejunum and ileum contain moderate amounts of watery, tan-yellow to yellow-green, slightly frothy digesta. Microscopically, the small intestinal villi were blunted and atrophic with multifocal severe cytoplasmic vacuolation and necrosis of enterocytes with mild epithelial regeneration. Numerous lymphocytes, plasma cells, and lesser macrophages and heterophils expand the lamina propria and submucosa. Rabbit hemorrhagic disease virus -2 real-time PCR was negative. Gram-stained small and large intestinal smears did not reveal gram-positive *Clostridium spiriformes*. *C. perfringens* alpha, beta, and epsilon toxins and toxins A and B of *Clostridioides difficile* in the intestinal content were ruled out by ELISA. Fecal flotation revealed >10 coccidia oocysts per slide. Negatively stained electron microscopy using the feces of both animals detected numerous small round viral particles (28nm in diameter), which were submitted for whole genome sequencing identifying rabbit astrovirus. The microscopic lesions observed in the rabbits are similar to those caused by astroviruses in humans and other animals. Since its first report in 2011, Rabbit astrovirus has been detected in asymptomatic and diarrheic rabbits with more frequency in the diarrheic animals compared to the asymptomatic groups. We described the gross and microscopic lesions of rabbit astrovirus and we believe that it causes enterocytic degeneration and necrosis leading to villous atrophy, enteritis, and diarrhea.

57: FOCAL DYSAUTONOMIA IN THE CRANIAL MESENTERIC GANGLION OF A MISSOURI DONKEY

Renata Mammone, Rosalie Ierardi, Gayle Johnson

University of Missouri Veterinary Medical Diagnostic Laboratory, Columbia, MO, USA

Background: Dysautonomia is a failure of the autonomic nervous system and has been reported in equids, dogs, and cats. In the U.S., canine cases are clustered in the Midwest, particularly in Missouri. The etiology is unknown but is suspected to be an environmental toxin.

Methods: A 5-year-old pregnant donkey jenny was presented to the University of Missouri Veterinary Medical Diagnostic Laboratory for necropsy following celiotomy and persistent ileus.

Results: The most significant gross finding was fecal dehydration and compaction. Microscopic examination revealed markedly decreased neuronal density and chromatolysis in the cranial mesenteric ganglion (CMG). Quantification of viable neuron cell bodies was accomplished by counting grid intersects. A ratio of viable neuron grid intersects to all intersects was calculated for the CMG in a clinically unaffected donkey (0.129) and the affected donkey (0.0230). The autonomic neurons of the brain and the intestinal submucosal and myenteric plexuses were unaffected. Other ganglia were not sampled.

Conclusion: This focal CMG dysautonomia is suspected to be a distinct disease process from equine “grass sickness” and “mal seco”.

58: MICRORNA IMPLICATIONS IN THE PROGNOSIS OF DIFFUSE LARGE B-CELL LYMPHOMA

Ekramy Sayedahmed, Michael Childress, Michael Gribskov, Nathan Lichti, Nelly Elshafie

Purdue University, West Lafayette, IN, USA

Background: Lymphoma is a frequent dog tumor in different forms, like multicentric, thymic, gastrointestinal, cutaneous, and solitary. Canine diffuse large B-cell lymphoma (DLBCL) is a non-Hodgkin's aggressive lymphoma that requires extreme care and treatment similar to its human counterpart. The devastating burden and progression of DLBCL need innovative diagnostic and prognostic markers like microRNA (miRNA) to favor disease outcomes. MicroRNAs are non-coding RNA molecules that regulate downstream gene transcription and are essential in various physiological and pathological processes, including cancer growth and disease progression.

Objective: This study investigates the correlation between microRNA expression levels and progression-free survival (PFS) among dogs diagnosed with DLBCL regarding specific microRNA subsets. **Methods:** The small RNA sequencing data analysis from ten canine fresh-frozen lymph nodes distinguished a set of differentially expressed miRNAs. This set of miRNAs was validated through quantitative PCR using an independent group of 44 archived lymph nodes collected from dogs at the time of diagnosis. **Results:** Significant upregulation of miR-16-5p, miR-125a, miR-192-5p, and miR-187-3p was associated with cases having lower PFS levels. **Conclusions:** These results provide essential information that can be utilized for personalized treatment solutions for the affected dogs using these microRNAs as a disease progression indicator.

59: OUTBREAK OF BACILLUS CEREUS CAUSING ANTHRAX-LIKE DISEASE IN RED KANGAROOS (*MACROPUS RUFUS*)

Bianca de Cecco¹, Naomi Falconnier¹, Weiyi Chen², Yu Young Go², Laura Peak¹, Emi Sasaki¹, Christine Walsh¹, Maria Mitchell¹, Mariano Carossino¹, Fabio Del Piero¹

¹Louisiana State University, Baton Rouge, LA, USA, ²City University of Hong Kong, Kowloon, Hong Kong

Background: *Bacillus cereus* is a gram-positive endospore-forming bacteria frequently associated with foodborne diseases in humans. Anthrax-like disease associated with atypical *B. cereus* infection has been described in humans in the US, and other mammals in Western Africa.

Objective: The goal of this study was to describe the gross, histologic, microbiological, and molecular characteristics of an outbreak of anthrax-like disease caused by *Bacillus cereus* in red kangaroos (*Macropus Rufus*).

Methods: Routine gross examination, histologic processing, bacteriological culture, MALDI-TOF typing, and whole genome sequencing (WGS) were performed.

Results: Three red kangaroos from a wild animal preserve were found dead with no premonitory signs. No changes in husbandry were reported prior to the outbreak.

Grossly, splenomegaly with multiple yellow foci of necrosis was noted in all animals, in addition to dark red intestinal mucosa in two kangaroos, and cutaneous excoriations in one kangaroo. Histologically, sepsis secondary to fibrinonecrotizing splenitis with numerous intralesional capsulated bacilli was diagnosed in all kangaroos. Two kangaroos also had severe necrotizing enteritis and hepatitis and one had marked necrotizing cellulitis and lymphadenitis. Bacterial culture yielded heavy growth of *Bacillus cereus* in splenic samples. WGS from one of the bacterial isolates revealed a close phylogenetic relationship with other *B. cereus* carrying the pBCXO1 virulence plasmid that contains anthrax-associated virulence genes and is an analog plasmid to pXO1 found in *Bacillus anthracis*.

Conclusions: This is the first report of *B. cereus* leading to anthrax-like disease in kangaroos. This disease form carries significant public health risks due to potential zoonotic transmission.

60: PERITONEAL DIALYSIS IN CRIA DIAGNOSED WITH CONGENITAL RENAL DYSPLASTIC CRIA AND FAILURE OF PASSIVE TRANSFER

Rachael Wolters^{1,2}, Emily Leahey², Sarel Van Amstel², Linden Craig²

¹Vanderbilt University, Nashville, TN, USA, ²The University of Tennessee College of Veterinary Medicine, Knoxville, TN, USA

Renal dysplasia is a congenital condition characterized by abnormal kidney development, leading to impaired renal function and potentially life-threatening complications. Concurrently, failure of passive transfer (FPT) can occur in newborn cria, exacerbating health issues and compromising the immune system. This case report presents a unique clinical scenario of a llama cria diagnosed with failure of passive transfer, juvenile nephropathy with hydronephrosis, and subsequent treatment using peritoneal dialysis and supportive care. The aim was to alleviate azotemia; however, complete resolution was not achieved despite reducing its severity. Consequently, the owners elected humane euthanasia. This case marks the first reported instance of juvenile nephropathy in a cria and the initial use of peritoneal dialysis in a young camelid. Postmortem analysis revealed severe bilateral diffuse chronic fibrosing nephritis with glomerular fibrin thrombi. Concurrent findings included adrenocortical and jejunal hemorrhage, along with microvascular fibrin thrombi. These pathological findings underscore the importance of considering juvenile nephropathy as a differential diagnosis in young cria presenting with failure to thrive. Additionally, they support further investigation into the application of peritoneal dialysis as a therapeutic option for these patients. The knowledge gained from this case report contributes to our understanding of renal dysplasia and its management in crias, ultimately striving for improved outcomes and welfare in these vulnerable animals.

61: SURVEILLANCE OF GASTROINTESTINAL VIRUSES IN FREE-RANGING SEA TURTLES IN THE UNITED STATES

Weerapong Laovechprasit¹, Kelsey Young¹, JoAnna Bowers¹, Heather Harris², Allison Tuttle³, Adam Kennedy⁴, Charles Innis⁴, Brian Stacy⁵, Terry Norton^{6,7}, Bette Zirkelbach⁷, James Stanton¹

¹University of Georgia, Athens, GA, USA, ²Upwell, Morro Bay, CA, USA, ³Mystic Aquarium, Mystic, CT, USA, ⁴New England Aquarium, Boston, MA, USA, ⁵NOAA, National Marine Fisheries Service, Office of Protected Resources, University of Florida, Gainesville, FL, USA, ⁶Sea Turtle Center and Jekyll Island Authority, Jekyll Island, GA, USA, ⁷The Turtle Hospital, Marathon, FL, USA

Various forms of gastrointestinal disease are reported as a relatively frequent finding among stranded sea turtles, which are classified as vulnerable, endangered, or critically endangered by the IUCN Red List. In many animals, gastrointestinal viruses are common and can significantly affect the health of hosts; however, little is known about enteric viruses of sea turtles. Determining whether viruses play a role in gastrointestinal diseases first requires a survey to identify viruses found in the digestive tracts of sea turtles. This project analyzed thirty-five gastrointestinal specimens from six species of sea turtle with various conditions, including those that were clinically healthy, cold-stunned, immunocompromised, or deceased. Sampled turtles originated from various locations across the continental United States (West Coast [CA, OR, and WA], Atlantic Coast [MA, GA and FL], and the Gulf of Mexico [FL, AL, MS, and TX]). Random, deep MinION sequencing revealed fourteen viral families and two unclassified viruses in fifteen individuals. Both DNA and RNA viruses were detected. Double-stranded (ds) DNA (adintovirus) and single-stranded (ss) DNA (multiple parvoviruses, adeintovirus, circovirus, cyclovirus, and other CRESS viruses) were identified. RNA viruses included those with dsRNA (totivirus, and partitivirus), positive-sense ssRNA (multiple picornaviruses, hepevirus, weivirus, picobirnavirus, salovirus, and narnavirus) and negative-sense ssRNA (chuviruses). Although the host(s), origin, and clinical significance of these viruses requires further study, these results contribute to the baseline knowledge of viral communities in sea turtles and can serve as a basis for future hypothesis-driven research required to understand the impact of these viruses on sea turtle health.

62: MILIARY OSTEOMA CUTIS IN AN ARBOREAL MANTELLA FROG (MANTELLA LAEVIGATA); A CASE REPORT

Elliott Chiu, Jenessa Gjeltrema, Kevin Woolard, Verena Affolter
University of California, Davis, Davis, CA, USA

A single, adult, female Arboreal Mantella frog (*Mantella laevis*) from a group of 16 cared for at the Sacramento Zoo was noted to have diffusely rough and thickened skin with a miliary appearance most prominent over dorsum and limbs. PCR for *Batrachochytrium dendrobatidis* – Chytridiomycosis - was negative. The frog arrested while recovering from anesthesia for skin biopsy collection. Histology revealed dermal nodules composed of differentiated bone arising from an abnormally thickened Eberth-Katschenko layer, consistent with miliary osteoma cutis. In addition, multifocal skeletal muscle mineralization of the limbs was observed. Etiology for this multifocal dermal ossification and dispersed mineralization remained unknown. Death was attributed to impaired electrolyte imbalance and cutaneous respiration due to the presence of inappropriate dermal bone.

63: GLOMERULAR LIPID EMBOLI IN BLACK AND SPOTTED LEOPARDS (PANTHERA PARDUS)

Hieuhanh Cox, Andrew Cushing, Sarah Linn-Peirano, Michelle Dennis
University of Tennessee College of Veterinary Medicine, Knoxville, TN, USA

This retrospective study aimed to describe clinicopathological findings of black and spotted leopards (*Panthera pardus*) with glomerular lipid emboli. Routinely-processed paraffin-embedded sections of kidney were histologically reviewed for 15 leopards examined by the University of Tennessee College of Veterinary Medicine autopsy service (2013-2022). Glomerular lipid emboli, defined as discrete singular to occasionally clustered non-staining vacuoles within glomerular capillary loops, and rarely within renal arterioles, were identified histologically on routine H&E, Jones methenamine silver, and periodic acid-Schiff stains in 11/15 leopards (73%; mild = 6, moderate = 1, severe = 4). Affected leopards had a median age of 17 (range 9-23) years, and median body condition score 3/5 (range: 2-4). Serum biochemistry was performed in 11 leopards; 2 had hypercholesterolemia (>200mg/dL). Free catch urinalysis was performed in 5 animals and 4 of these were proteinuric using routine dipstick testing. In addition to glomerular lipid emboli, all leopards had histologic evidence of glomerular injury and chronic kidney disease, including glomerular hypertrophy, occasional mesangial expansion and hypercellularity, segmental sclerosis, lymphoplasmacytic interstitial nephritis, fibrosis, and 10/11 had renal tubular protein casts. Comorbidities associated with glomerular lipid emboli in other species include hypercholesterolemia, corticosteroid therapy, trauma, pancreatitis, diabetes mellitus, panniculitis, and emaciation. The former two findings were reported in study leopards. Lipid emboli were not identified in any other tissues. This study highlights the potential for glomerular lipid emboli in felids which has not been previously reported. Further investigation is warranted to determine the association of glomerular lipid emboli with chronic kidney disease and proteinuria.

64: RETROSPECTIVE STUDY OF NEOPLASIA AND CONCURRENT DISEASE IN BEARDED DRAGONS, 2010–2023.

Gbemisola Akingbade, Elizabeth Howerth, Sarah Schneider, James Stanton, Brittany McHale, Rita McManamon, Nicole Gottdenker, Esdras Correa Dos Santos, Megan Corbett
University of Georgia, Athens, GA, USA

Background: While neoplasia is not rare in bearded dragons, few reports specify the most common tumors and association with concurrent diseases.

Objective: To review bearded dragon necropsy and biopsy submissions to three diagnostic services at the University of Georgia from 2010-2013 for neoplasia and concurrent diseases.

Methods: Three databases were searched using key terms neoplasia, neoplasm, adenoma, adenocarcinoma, carcinoma, fibroma, round cell, lymphoma, leukemia, neuroendocrine, spindle cell, sarcoma, tumor, mass, and oma.

Results: Submissions included biopsies or necropsies from 757 bearded dragons, the majority (420 (55.5%)) submitted after 2018. Of these, 73 (9.6%) were diagnosed with neoplasms, 43 (58.9%) reported after 2018. Most neoplasms occurred in animals 4 years and older (n=41, 56.2%); with the youngest affected being 1.5-yr-old. Relatively even numbers of males and females were submitted, but neoplasia was more common in females (n=40 [54.8%]) than in males (n=26 [35.6%]). Six neoplasms were in animals of unknown sex. The most common tumor was squamous cell carcinoma (n=8 [11.0%]), the most common location for the primary occurrence was the skin (n=21 [28.8%]); and the most common metastatic site was liver (n=20 [27.4%]). Out of the 73 cases with neoplasms, 37 had concurrent diseases, the most common of which was hepatic lipidosis (n=21, 28.8%).

Conclusion: Reasons for the increasing numbers of submissions after 2018 and an apparent increase in number of tumor cases is uncertain. Possibilities could include changes in environmental and husbandry conditions, as well as, Increased longevity.

65: CELL COMPOSITION OF NECROTIC LUNG GRANULOMAS IN AUTOPSIED HUMAN PATIENTS: A COMPARISON BETWEEN TUBERCULOSIS AND TUBERCULOSIS WITH HIV-1 COINFECTION

Kerstin Muner¹, Amaro Nunes Duarte Neto², Ana de Sa Guimaraes^{1,2}, Andrea Pires dos Santos¹

¹Purdue University, West Lafayette, IN, USA, ²University of Sao Paulo, Sao Paulo, Brazil

Background: Tuberculosis (TB) is among the deadliest infectious diseases worldwide. In addition, it increases the death rates among HIV-1 patients, characterizing a severe public health concern. The mycobacterial infection can favor the development of acquired immunodeficiency syndrome (AIDS), while HIV-1 infection can lead to active TB. TB primarily affects the lungs, causing chronic inflammation that leads to the development of granulomas. The granuloma is the hallmark of TB and plays a critical role in disease progression. **Objective:** Considering that HIV-1 infection may disrupt granuloma architecture, we compared the cell composition of necrotic granulomas between autopsied human patients with tuberculosis and coinfecting with tuberculosis and HIV-1. **Methods:** H&E and immunohistochemistry were performed in TB⁺/HIV-1⁻ (n=5) and TB⁺/HIV-1⁺ (n=5) samples, for seven markers: CD4, CD8a, CD20, CD56, CD68, FoxP3, and Pax5. We quantified the number of strong positives (Nsp) per area annotated from necrotic granulomas (μm^2) using the Aperio ImageScope, Positive Pixel Count V9 algorithm. **Results:** There were noteworthy differences in granuloma morphology when comparing the two groups. Also, the Nsp per annotated μm^2 of all markers but CD8a was higher in TB⁺/HIV-1⁻ than in TB⁺/HIV-1⁺ patients. Nevertheless, there was no statistical difference between TB⁺/HIV-1⁻ and TB⁺/HIV-1⁺ for any quantified individual markers, but CD8a:CD4 ratio was statistically higher for TB⁺/HIV-1⁺ patients. **Conclusion:** Although granuloma cell composition is essential to understanding the dynamics between the interplay of pathogens and the immune system, further studies are still necessary to enlighten its implications on immune modulation and disease progression.

66: MYOSITIS OR METAMORPHOSIS? RETROSPECTIVE DIAGNOSES IN LARVAL ANURANS

Megan Corbett, Elizabeth Howerth
University of Georgia, Athens, GA, USA

Background: Anuran larvae (tadpoles) present a diagnostic challenge due to small size, rapid decomposition, variation in natural history, and metamorphosis. Additionally, histologic atlases and visual training resources for normal development and disease histopathology are lacking. Metamorphosis, while mechanistically and molecularly well-characterized, presents a unique diagnostic challenge with histologic overlap with decomposition changes, myositis, and myodegeneration.

Objective: To characterize musculoskeletal changes against a background of metamorphosis in tadpoles submitted to the University of Georgia Infectious Disease Laboratory Zoo and Exotic Animal Pathology Service between 2014-2023.

Methods: The web-based laboratory archive system was searched for tadpole cases using keywords “tadpole”, “toadlet”, and “froglet” from January 1, 2014 to June 1, 2023.

Results: Nine individuals from 6 genera ranging from tadpole to froglet were examined. Six individuals (67%) were diagnosed with myofascial changes including edema, degeneration, and necrosis, five of which were in tail and limb skeletal muscle. Of these, one had a folding fracture and serous atrophy of fat, as well as extensive resorption of the tail (metamorphosis) suggesting inadequate nutritional reserves to complete metamorphosis rather than primary myofascial pathology. Four had changes consistent with autolysis and metamorphosis misdiagnosed as myofascial disease. Of the remaining individuals, two were severely autolyzed, one had no specific findings, and one had mild, focal nephritis and mild myodegeneration in the head.

Conclusions: Tadpoles are uncommon submissions and present unique diagnostic challenges, and myofascial pathology may be over diagnosed. Further characterization of decomposition and metamorphosis on myofascial histopathology is needed to ensure accurate diagnoses in these animals.

67: ASSOCIATION OF ANTIBODY AND CELL-MEDIATED IMMUNE RESPONSES WITH NEUTROPHIL RESPONSE IN BRONCHOALVEOLAR LAVAGE OF HIGH-RISK AUCTION CALVES

Seyed Saeid Tabatabaei¹, DeLenn Burrows¹, Monica Baquero², Veronica Fursova¹, Elizabeth Sharron¹, Jeff Caswell¹

¹Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, ²Zoetis Animal Health, Kalamazoo, MI, USA

Bovine Respiratory Disease (BRD) poses significant challenges to the livestock industry due to decreased productivity. Understanding the complex immunological responses to pathogens and environmental factors is necessary to develop effective control and prevention strategies. The interplay between innate and adaptive immunity is crucial for effective host defense, promoting pathogen clearance and subsequent immune

protection. We hypothesized that the ability of calves to raise antibody and cell-mediated immune responses to novel antigens is associated with pulmonary inflammation and neutrophil recruitment to the lungs; this occurs during the high-stress, high BRD risk period after arrival at the feedlot. Fifty-three mixed-breed steers were purchased from an auction barn in Ontario during 2021 and 2022. Bronchoalveolar lavage fluid (BALF) was collected on days 1 and 4 after arrival. Cytology slides were prepared and stained to perform a manual differential count. In the second month, the animals were challenged with Antigen A, and blood was collected on the same day and 14 days later to measure the baseline antibody titer and primary response to Antigen A, respectively. To evaluate delayed type IV hypersensitivity, the skin thickness was measured in the same animals 24 hours after intradermal injection of Antigen B on day 14. Neutrophil percentage in BALF on day 4 was correlated with the antibody-mediated response (Pearson's $r=0.28$, $p=0.042$). No significant association was found between the cell-mediated response and neutrophil percentage on days 1 and 4. This knowledge will be useful for breeding programs to increase the resistance or tolerance of calves to BRD.

68: MULTIPLEX GENE EXPRESSION ANALYSIS IN THE HEART OF BOXER DOGS WITH ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY (ARVC)

Laura Machado Ribas¹, Jasmine Aggarwal¹, Taylor Bailey², Kerstin Muner¹, Andrea Pires dos Santos¹, Suzanne Cunningham³, Luis Neves Dos Santos¹

¹Purdue University, College of Veterinary Medicine, West Lafayette, IN, USA, ²Purdue University, College of Health and Human Sciences, West Lafayette, IN, USA, ³Tufts University, Cummings School of Veterinary Medicine, North Grafton, MA, USA

Background: Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited myocardial disease reported in dogs, cats, and humans. ARVC is characterized by the replacement of normal myocardium by adipose and fibrous tissue, and it manifests as malignant ventricular arrhythmias, cardiac dilation, syncope, and sudden death. Although multiple mechanisms have been implicated in the pathogenesis of the disease, there is no reference standard ante mortem diagnostic test, which is still based on histopathology. Since narrow diagnostic criteria are available to characterize ARVC in Boxers, additional biomarkers for this disease are needed.

Objective: We aimed to identify biomarkers present in the heart of canine patients with ARVC that can aid in the early diagnosis of the disease.

Material and Methods: Total RNA was extracted from formalin-fixed paraffin-embedded (FFPE) cardiac tissue samples from twelve dogs and submitted for nCounter® analysis (NanoString Technologies, Inc., WA, USA). The dogs were classified by the cause of death and ARVC status. The groups consisted of four Boxer dogs presented with sudden cardiac death and a definite histopathological diagnosis of ARVC; four Boxers presented with non-cardiac-related death despite ARVC; and four cardiac tissues from non-Boxer healthy dogs.

Results: 56 genes were significantly ($p < 0.05$) differentially expressed with a mean difference greater than 1.5-fold between patients who experienced sudden and non-cardiac-related deaths.

Conclusion: These findings suggest that gene expression analysis can potentially be a promising tool for investigating sudden death in Boxers with ARVC. Further investigation into the observed genes and validation tests are needed.

