Clinical Pathology Focused Scientific Session I

Sunday, November 10, 2019 | 1:30 p.m. – 4:30 p.m.
Session Chair: Amy L. Warren, BVSc, PhD, DACVP, University of Calgary, Calgary, Alberta, Canada

Sunday, November 10, 2019
1:30 p.m. – 1:45 p.m.

EVALUATION OF THE URINARY MIRNA EXPRESSION PROFILE IN DOGS WITH CHRONIC KIDNEY DISEASE DUE TO X-LINKED HEREDITARY NEPHROPATHY

Sabrina Clark, Candice Chu, George Lees, Mary Nabity
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Background: Traditional biomarkers of chronic kidney disease (CKD) often do not identify early stages of disease and do not reflect the underlying pathogenesis. microRNAs (miRNAs, miRs) show promise as novel biomarkers of disease. They are remarkably stable in bodily fluids and expression can be altered in a disease-specific manner.

Objective: To investigate urinary miRNAs as biomarkers for disease onset and progression in canine CKD.

Methods: Urinary RNA was isolated from five dogs with CKD caused by X-linked hereditary nephropathy (XLHN) at early (T1, proteinuria), middle (T2, azotemia), and late (T3, end-stage) disease and from four age-matched unaffected dogs. Next-generation sequencing followed by RT-qPCR was employed to identify stably expressed miRNAs for endogenous normalization and differentially expressed (DE) miRNAs between unaffected and affected dogs. *In situ* hybridization (ISH) was performed on FFPE kidney tissue to localize expression of miR-21, miR-486, and miR-8890.

Results: Sequencing identified 137 DE urinary miRNAs. miR-16 and miR-30e were identified as endogenous reference miRNAs. Seven DE miRNAs were selected for verification by RT-qPCR; six demonstrated significant differences between unaffected and affected dogs at various time points with miR-486 and miR-8890 elevated at T1. ISH revealed absence of miR-21 in unaffected dogs and increased expression with disease progression. miR-486 and miR-8890 exhibited similar expression between unaffected versus affected dogs early in disease but then increased with progression.

Conclusion: Differences in urinary miRNA expression were identified during XLHN disease progression. The abnormally expressed miRNAs are promising biomarkers for detecting and monitoring CKD and might help elucidate the pathogenesis of XLHN.

Sunday, November 10, 2019
1:45 p.m. – 2:00 p.m.

URINARY MICRORNA PROFILING IN DOGS WITH CHRONIC KIDNEY DISEASE CAUSED BY GLOMERULAR DISEASES

Candice Chu[1], Rachel Cianciolo[2], Jessica Hokamp[2], George Lees[3], Mary Nabity[11]
Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA, [2]Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA, [3]Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA

Background: The majority of proteinuric dogs with chronic kidney disease (CKD) have glomerular disease. Renal biopsy and comprehensive histopathologic examination are currently indispensable to establish diagnosis and guide treatment. Biofluid microRNAs (miRNAs, miRs) could serve as promising non-invasive biomarkers for patients deemed unsuitable for biopsy.

Objective: To correlate miRNA expression in urine with histologic diagnosis and disease stage using small RNA sequencing (RNA-seq) and qRT-PCR.

Methods: MiRNA isolated from urine collected from 18 dogs with amyloidosis, glomerulosclerosis, and immune complex-mediated glomerulonephritis and 6 clinically healthy control dogs was sequenced using small RNA-seq. Stably expressed miRNAs and differentially expressed (DE) miRNAs that were present among the disease groups and disease stages (azotemic vs. non-azotemic) were identified and subsequently evaluated in a second cohort of 32 diseased dogs using qRT-PCR.

Results: MiR-151 and miR-28 were identified as endogenous reference miRNAs. Based on sequencing data, 16 urinary miRNAs were DE in CKD dogs versus controls and three were DE between azotemic and non-azotemic dogs. However, differential expression could not be verified in the five miRNAs that were selected for evaluation by qRT-PCR. In azotemic dogs, the distinctive expression of urinary miR-126, miR-335, and miR-128 grouped the dogs into the three categories of glomerular disease based on sequencing data, and these miRs were statistically different among the categories based on qRT-PCR.

Conclusion: Urinary miRNAs might serve as novel biomarkers to differentiate the type of glomerular disease present in azotemic dogs. Testing of more samples and additional diseases is necessary to verify their diagnostic value.

Sunday, November 10, 2019
3:30 p.m. – 3:45 p.m.

CHARACTERIZING THE PRESENCE OF MELAMED-WOLINSKA BODIES IN CANINE URINE CYTOLOGY

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Background: Melamed-Wolinska (MW) bodies are intracytoplasmic inclusions found microscopically in urothelial cells. In humans, their presence can help identify cells as urothelial in origin but cannot classify a lesion as benign or malignant. The significance of MW bodies in canine urine is unknown.

Objectives: To determine the occurrence of MW bodies in canine urine sediment samples based on patient characteristics, urine collection technique, and the presence of urinary tract (UT) inflammation/infection (I), urothelial neoplasia (N), and diseases unrelated to the distal UT (other, O).

Methods: Cytological examination of urine sediment samples containing urothelial transitional cells from dogs presenting to a veterinary teaching hospital.

Results: Samples from 102 dogs were included and 33, 21, and 48 were classified as I, N, and O, respectively. 20 samples contained MW bodies obtained from 15 dogs (75%) with urothelial carcinoma (N), 4 dogs (20%) with UT inflammation (I) and 1 dog (5%) with *Ehrlichia* sp. infection (O). Dogs with MW bodies were significantly older (10.4 vs. 7.2 years, P = .01). MW bodies per sample ranged from 1-36/100 transitional cells with an average of 11 (I), 10 (N), and 1 (O). Inclusions were more common in voided urine samples (13/20, 65%) without apparent sex and breed predilection.

Conclusion: In this study population, MW bodies occurred more frequently in dogs with urothelial carcinoma. However, high numbers can also be found in dogs with inflammatory UT diseases and the sole presence of MW bodies in urothelial cells cannot be used to support a diagnosis of neoplasia.

Sunday, November 10, 2019
3:45 p.m. – 4:00 p.m.

DETECTION OF SERUM AND URINE FREE LIGHT CHAINS IN VETERINARY PATIENTS

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Background: Free light chains (FLC) should be freely filtered from serum and therefore are not easily detected by serum protein electrophoresis. Serum FLC (sFLC) characterization is recommended in human medicine because increased sFLC is associated with greater risk of renal pathology. Electrophoretic detection of urine FLC (uFLC) in the dog has been described.

Objectives: Determine if sFLC and uFLC can be detected using commercially available human immunofixation reagents.

Methods: Serum and urine samples from three patients submitted to Colorado State University for routine protein electrophoresis and immunofixation and an electrophoretic profile suggestive of uFLCs were used. These included: 1) A cat with a serum and urine IgG monoclonal gammopathy, 2) a dog with a serum and urine IgG/IgM biclonal gammopathy and 3) a dog with an electrophoretic diagnosis of light chain only disease. Non-reduced lithium-dodecyl-sulfate polyacrylamide gel electrophoresis

(nrPAGE) evaluation to confirm FLC and a human immunofixation based FLC kit (Sebia Antisera K & L free light chains (PN 4836)) was used on all samples.

Results: nrPAGE identified FLC in all samples. All uFLC bands failed to label by routine immunofixation but were identified by the anti-human λ LC and anti-human free λ LC reagents. Similar labeling was detected in the serum samples and corresponded to previously uncharacterized low-amplitude peaks in the serum electrophoretograms.

Conclusions: A commercially available human FLC assay labeled serum and urine FLC in 2 canine and 1 feline cases.

Sunday, November 10, 2019
4:00 p.m. – 4:15 p.m.

PRECISION, ACCURACY, AND TOTAL ERROR DETERMINATION FOR SERUM AND URINARY CORTISOL IN DOGS: WHAT IS ACHIEVABLE WITH THE IMMULITE 2000XPI, AND HOW DOES THAT IMPACT RESULTS' INTERPRETATION?

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Background: There is no allowable Total Error (TEa) consensus in veterinary endocrinology. Serum and urinary cortisol in dogs are interpreted with thresholds not taking in account precision and biases between methods. Radioimmunoassays become less available, and urinary cortisol was not previously validated in dogs by chemiluminescence.

Objective: Validate the measurement of cortisol in urine in dogs with the Siemens chemiluminescent assay on the Immulite 2000Xpi; document the linearity, recovery, detection limit, precision, biases (spiking and institution comparison), and observed TE (TEo) in urine and serum as a first step toward a consensus for TEa; determine the performance of various Quality Control (QC) rules in both media.

Methods: Cortisol-free matrices were obtained from 50 pooled sera with undetectable cortisol, and one urine sample with undetectable cortisol from an Addisonian dog. They were spiked with a cortisol concentrate covering the reportable range. Comparison studies with other reference institutions were performed for serum and urine with selected patient samples. Global bias, concentration-targeted bias, Bland-Altman plots, and Passing-Bablok regressions were performed. Sigma metrics and QC rules were determined given various TEa and various Probabilities of Error Detections (Ped) as candidate possibilities; conversely, optimal TEa and Ped were determined given satisfactory sigma for various QC rules.

Results and Conclusions: Canine urinary cortisol measurement was validated on the Immulite 2000Xpi. Serum cortisol interpretation with thresholds is improved by the consideration of corresponding TEo. The choice of TEa influenced acceptable QC rules more than any other parameter, stressing the critical importance of TEa for result interpretation.

Sunday, November 10, 2019
4:15 p.m.-4:30 p.m.

COMPARISON OF SERUM VS. EDTA-PLASMA IN CANINE MAJOR CROSSMATCH REACTIONS

Megan Caudill, Kristina Meichner, Amie Koenig, Roy Berghaus, Bridget Garner
University of Georgia, Athens, GA, USA

Background: Protocols for crossmatch reactions vary in veterinary medicine, particularly in regards to the use of recipient serum versus plasma. Sources suggest that major crossmatch results may differ when recipient plasma is used instead of serum, but there are conflicting reports as to the exact effects on the results.

Objectives: The aim of this study was to determine the rate and degree of discrepancy, if any, between canine major crossmatch reactions using serum versus EDTA-plasma, performed via a standard tube method.

Methods: 100 duplicate canine major crossmatch reactions were performed with both "recipient" serum and EDTA-plasma against a single, healthy "donor". Decreasing concentrations of anti-dog erythrocyte antibody were added to generate strong positive, weak positive, and negative results for each crossmatch reaction. Crossmatch results were followed through the following phases: immediate spin, cold, warm, albumin, and Coombs'. Semi-quantitative results were compared between reactions using serum versus EDTA-plasma.

Results: Weak positive, major crossmatch reactions were significantly more likely to demonstrate stronger agglutination in EDTA-plasma compared to serum in the immediate spin phase, cold phase, warm phase, and albumin phase ($P < 0.001$). No statistically significant difference between serum and EDTA-plasma results was detected in the Coombs' phase ($P = 0.313$).

Conclusions: In this experimental setting, EDTA-plasma and serum were both deemed acceptable for use in canine major crossmatch reactions. EDTA-plasma may be preferable to more sensitively detect weak agglutination and to reduce the volume of recipient blood needed to complete the crossmatch reaction.

Clinical Pathology Focused Scientific Session II

Tuesday, November 12, 2019 | 8:00 a.m. – 12:00 p.m.
Session Chair: Amy L. Warren, BVSc, PhD, DACVP, University of Calgary, Calgary, Alberta, Canada

Tuesday, November 12, 2019
8:00 a.m. – 8:15 a.m.

HEPATIC T CELL-RICH B CELL LYMPHOMA IN A YOUNG GREAT DANE

Gary Lee[1], Janet Beeler-Marfisi[1], Stefan Keller[1], Mei-Hua Hwang[1], Nikos Darzentas[2,3], Haiyang Chang[1], Emily Ratsep[1], Rebecca Egan[1], Dorothee Bienzle[1]
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A 10-month-old male intact Great Dane was presented to the Ontario Veterinary College with marked ascites. Cytologic analysis of abdominal fluid and hepatic aspirates revealed numerous large lymphocytes, consistent with lymphoma. Most large lymphocytes within the abdominal fluid were positive for CD4, CD5, CD18, CD45 and MHC II on flow cytometry, prompting a diagnosis of T cell lymphoma. Due to the presumed poor prognosis, the owners elected euthanasia. Postmortem examination revealed an enlarged, light brown liver with numerous 1-8 mm in diameter, flat to slightly raised, white foci occupying approximately 20% of the organ. Histopathologic analysis showed effacement of the liver by aggregates of large B cells (positive for CD20 and CD79a) surrounded by smaller T cells (positive for CD3) consistent with a hepatic T cell-rich B cell lymphoma. Neoplastic cells were not identified in other organs. Immune repertoire sequencing confirmed the presence of a clonal B cell population within the liver, and a polyclonal T cell population in samples from the liver and abdominal effusion. Clonal B cells were not detected in significant levels among effusion cells and the most abundant T cell clones were found in both samples. This is a report of an unusual lymphoma in a young dog, in which cytologic evaluation of the abdominal effusion was not fully reflective of the complex underlying disease process. This case illustrates the benefit of immune repertoire sequencing to delineate lymphocyte clones in a complex lymphocytic tumor.

Tuesday, November 12, 2019
8:15 a.m. – 8:30 a.m.

RATES AND TYPES OF ERRORS AT A CLINICAL PATHOLOGY LABORATORY IN A VETERINARY TEACHING HOSPITAL

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Background: Many clinical case management decisions are based upon laboratory testing. Information regarding types and frequencies of errors in veterinary laboratories are largely extrapolated from human studies. One veterinary report investigated errors in a commercial laboratory in Europe, but differences in operations between a commercial laboratory and a veterinary teaching hospital warrant an investigation into errors. A laboratory information management system can be used to mitigate error, but is not currently used in our veterinary teaching hospital.

Objective: This study investigated occurrence of errors related to laboratory medicine associated with the clinical pathology laboratory at a veterinary teaching hospital in the US.

Methods: Samples were reviewed for errors over two 2-month periods, the first when new students and house officers begin clinics and the second six months later. Errors were classified by phase of testing, frequency, and six sigma values were assessed as a measure of performance. Rate of hemolysis, icterus, and lipemia were evaluated separately, as these could be pathologic or from error.

Results: 4.7% and 3.5% of samples were affected by error in each period. Frequencies of subtypes of error were similar to previous investigations, but total error rate was

higher. Pre-analytic error predominated. Total error rate and rates of pre-analytic error occurring within and outside of the laboratory were significantly different between the periods. Six sigma showed generally adequate performance.

Conclusions: Higher error rate may relate to the teaching environment. Areas for improvement have been identified and interventions planned. Repeat studies could investigate efficacy of interventions.

Tuesday, November 12, 2019
8:30 a.m. – 8:45 a.m.

SCORING EXERCISE-INDUCED PULMONARY HEMORRHAGE – ACCURACY OF HUMAN OBSERVER AND DEEP-LEARNING BASED ALGORITHMS

Christof A. Bertram[1], Christian Marzahl[2], Marc Aubreville[2], Jason Stayt[3], Anne-Katherine Jasensky[4], Florian Bartenschlager[1], Marco Fragoso-Garcia[1], Samir Jabari[5], Svenja Elsemann[6], Ann K. Barton[7], Andreas Maier[2], Jenny Hill[3], Robert Klopfleisch[11] Institute of Veterinary Pathology, Freie Universität Berlin, Berlin, Germany, [2]Pattern Recognition Lab, Computer Science, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany, [3]VetPath Laboratory Services, Ascot, Australia, [4]Laboklin GmbH und Co. KG, Bad Kissingen, Germany, [5]Institute of Neuropathology, University Hospitals Erlangen- Nürnberg, Friedrich Alexander University Erlangen-Nürnberg, Erlangen, Germany, [6]Department of Neurosurgery, Universitätsklinikum Erlangen, Erlangen, Germany, [7]Equine Clinic, Freie Universität Berlin, Berlin, Germany

Background: Exercise-induced pulmonary hemorrhage (EIPH) is a common disease of racehorses with strenuous exercise. There is good evidence that this disease has negative impact on athletic performance. The gold standard diagnostic method is cytological examination and scoring of pulmonary hemosiderophages (PH). An established scoring system requires grading of 300 PH into five grades depending on the degree of cytoplasmic hemosiderin storage.

Objective: To determine accuracy of cytological scoring of PH by human observers and deep learning-based algorithms.

Methods: A ground truth dataset of 17 completely annotated whole slide images (WSI) of equine bronchoalveolar lavage stained for iron content was developed. Accuracy of eight human observers and the classification algorithm were determined in 2000 randomly-selected PH. Precision of the object detection algorithm was tested in three WSI.

Results: Classification of participants had an intra-observer concordance of 68-88% with Cohen's κ ranging from 0.60–0.84. Mean consistency with the ground truth dataset was 0.73%. The algorithm achieved a κ=1.0 and classified 85% of the 2000 cells in agreement with the dataset.

The object detection algorithm had a mean average precision of 0.66 with an average error in the grade of 0.09. Calculation time of entire whole slide images was <2 minutes.

Conclusion: Manual scoring is not only monotonous and time-consuming, but also exhibits high degree of inter- and intra-observer variability. On the other hand, the algorithmic approach has an accuracy comparable to human experts, 100% reproducibility and short calculation times. Therefore, we consider image analysis as a feasible solution for quantification of EIPH.

Tuesday, November 12, 2019
8:45 a.m. – 9:00 a.m.

DIFFERENTIAL GENE EXPRESSION AND IRON STORAGE IN TUMOR AND NON-TUMOR TISSUE IN CANINE HEPATOCELLULAR CARCINOMA

Klaudia Polak, Paula Schaffer, Madison Zenk, Dillon Donaghy, Christine Olver
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Background: Hepatocellular carcinoma (HCC) is the most common primary liver tumor in dogs. Iron overload is associated with HCC in humans and dogs. One study detected non-regenerative anemia and thrombocytosis in 50% and microcytosis in 30.8% of dogs with HCC. The liver is a major iron storage site and its iron regulatory proteins may play a major role in canine HCC.

Objectives: First, we aimed to determine whether neoplastic hepatocytes exhibit differential expression of iron regulatory genes in normocytic versus microcytic dogs. Next, we compared iron regulatory gene expression and hepatic iron stores between canine HCC tumor tissue, adjacent non-tumor tissue, inflamed non-HCC liver tissue, and normal liver tissue.

Methods: We isolated RNA and used NanoString technologies to compare expression of 94 genes and Prussian blue staining to assess iron stores in 15 tumor and adjacent non-tumor specimens, 8 normal controls, and 10 inflamed tissues. We analyzed the relationships of gene expression with hepatic iron stores and hematologic parameters.

Results: Tumor tissue had reduced expression of the major iron regulatory proteins hepcidin and TFR2 as well as STEAP2, a metalloreductase involved in iron uptake. TFR1, survivin, and STEAP3 were up-regulated in tumor tissue. Hepcidin levels in non-tumor tissue negatively correlated with MCV. Adjacent non-tumor tissue was markedly iron loaded compared to tumor and control tissues.

Conclusions: Significant differential expression of multiple iron-regulatory genes, most notably hepcidin, is present in canine HCC and adjacent iron overloaded, non-tumor tissue. Disruption of iron regulation may be linked to development of canine HCC.

Tuesday, November 12, 2019
10:30 a.m. – 10:45 a.m.

A PANFUNGAL PCR ASSAY PERFORMED DIRECTLY ON STAINED CYTOLOGY SLIDES

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Background: Panfungal PCR has emerged as a method for rapid identification of fungi and oomycetes in sample types such as fresh tissue, culture, and formalin-fixed paraffin-embedded (FFPE) tissue.

Objectives: This study aimed to (1) optimize specific components of a panfungal PCR assay for use on stained cytology slides, and (2) assess the diagnostic accuracy of this assay for identification of fungi and oomyctes.

Methods: Medical records were reviewed for cases where cytologic examination and culture and/or antigen/antibody-based tests for dimorphic fungi and oomycetes were performed on the same tissue or lesion, yielding 40 cases where a fungus was identified by both methods and 30 controls where no fungi were identified by either method. The method for obtaining DNA from cytology slides was optimized. Conventional PCR targeting the internal transcribed spacer region and sequencing were performed on one cytology slide from each case/control.

Results: In the majority of cases, the panfungal PCR assay was able to provide an etiologic diagnosis to at least the genus level consistent with the identification provided by the reference method. Performance of the assay on cytology slides is superior to previously published results using FFPE tissues.

Conclusions: The panfungal PCR assay is capable of providing genus or species level identification of fungi and oomycetes from stained cytology slides without the need to collect additional samples or perform more invasive tests.

Tuesday, November 12, 2019
10:45 a.m. – 11:00 a.m.

EFFICACY OF FOUNTAIN FLOW CYTOMETRY FOR RAPID DETECTION OF BACTERIAL SEPSIS IN BODY CAVITY FLUIDS

Samantha Evans[1,2], Alexandra Bonney[2], Sara Wist[2], Paul Johnson[3], Kelly Santangelo[2]
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Background: Early recognition of bacterial sepsis in body cavity effusions is essential to patient survival. Current diagnostic techniques suffer from either prolonged analysis time (bacterial culture) or poor sensitivity and/or specificity (cytology and blood-fluid glucose differential). Fountain Flow cytometry (FFC), which was originally developed for the detection of very low concentrations (as little as 0.1 CFU/mL) of bacteria in industrial products and water sources, may fill this diagnostic niche.

Objective: To assess the sensitivity and specificity of FFC to detect bacterial sepsis in effusions from veterinary patients.

Methods: Sixty client-owned mammalian patients with thoracic or abdominal effusion presenting to the Colorado State University Veterinary Teaching Hospital were enrolled. Samples were evaluated by routine fluid analysis and cytology, FFC, and the reference method of bacterial culture.

Results: Forty-five dogs, 13 cats, and 2 horses were enrolled, including 28 abdominal and 32 thoracic effusions. Five samples were clinically septic with a positive bacterial culture. The sensitivity of FFC to detect sepsis was 60%, while the specificity was 94.4%. Positive and negative predictive values for this population were 50% and 96.2%, respectively. Factors negatively affecting the performance of FCC are suspected to include chyle, necrotic material, and filamentous bacteria.

Conclusions: Preliminary data indicates that further optimization of FFC for biologic samples is required. Given that FFC offers fast (<30 minutes total analysis time), economical, portable, and adaptable new technology, this initial study suggests that it may have value as a clinically assay for rapid diagnosis of bacterial sepsis in veterinary patients.

Tuesday, November 12, 2019
11:00 a.m.-11:15 a.m.

VARIATION IN BIOCHEMISTRY TEST RESULTS BETWEEN ANNUAL WELLNESS VISITS IN APPARENTLY HEALTHY GOLDEN RETRIEVERS

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[1]Texas A&M University, College Station, TX, USA, [2]Morris Animal Foundation, Denver, CO, USA

Background: Biochemistry testing is recommended as part of annual wellness exams, but distinguishing normal variation from clinically important alterations in analyte concentration can be challenging.

Objective: Define variability in biochemistry results between annual wellness visits for healthy Golden Retrievers.

Methods: Records were reviewed for 2458 participants in the Golden Retriever Lifetime Study, which includes annual biochemistry testing by a reference laboratory. 196 dogs met the inclusion criteria: (1) no diagnoses, clinically important physical exam abnormalities or non-preventative prescription medications for 3 consecutive years; (2) over 1 year old at the first included health check; (3) not pregnant in the first or second included year. For 23 biochemistry analytes, annual changes were calculated by subtracting the second year's result from the first year's result. After outlier detection by the Reed method, non-parametric annual change intervals were constructed. Annual changes were calculated for a validation cohort of 238 dogs that were assessed as systemically healthy for 3 consecutive years, but had minor health problems (e.g. otitis externa) during this period.

Results: Absolute and percentage annual change intervals were constructed for 23 analytes. For all analytes, over 90% of dogs in the validation cohort were within the annual change intervals.

Conclusion: Most dogs that did not develop overt systemic disease in the 12 months following wellness testing were classified as normal by annual change intervals. Further work is needed to determine the sensitivity of annual change intervals to subclinical

disease and transferability to other canine populations and data generated by other laboratories.

Tuesday, November 12, 2019
11:15 a.m. – 11:30 a.m.

VALIDATION OF THE CELLAVISION DM96 FOR MANUAL RETICULOCYTE ENUMERATION

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Background: The reticulocyte count is an important factor used to assess erythropoietic activity of bone marrow. Although reticulocyte enumeration using automated hematology analyzers is routinely performed, sources of interference may result in spurious reticulocyte profiles. In these situations, manual counting using a supravitally-stained blood smear is required. This may be performed at the microscope however utilization of semi-automated digital hematology platforms may be a worthy alternative.

Objective: The primary objective was to compare reticulocyte percentages using digital images from the CellaVision DM96 with percentages obtained using Advia 120 and traditional counting using light microscopy. We hypothesize that the CellaVision will be reliable and accurate for reticulocyte enumeration.

Methods: Fifty-two canine and thirty-four feline blood samples were analyzed using Advia 120. Manual reticulocyte percentages were counted using light microscopy with a Miller disc. Subsequently, the same smears were scanned using the CellaVision DM96 and platelet gallery images were used to obtain a manual reticulocyte percentage.

Results: Reticulocyte percentages from the DM96 showed an R^2 of 0.88 and 0.89 compared to manual counting and an R^2 of 0.83 and 0.93 compared to the automated hematology analyzer in dogs and cats, respectively. Bland-Altman plots showed that both CellaVision and Miller disc manual counts tended to underestimate the reticulocyte percentage relative to the automated analyzer.

Conclusions: This data provides support for the use of the CellaVision digital hematology platform for reticulocyte enumeration.

Tuesday, November 12, 2019
11:30 a.m. – 11:45 a.m.

CHANGES IN INTRAERYTHROCYTIC AND INTRALEUKOCYTIC CELLULAR REACTIVE OXYGEN SPECIES IN LPS-INDUCED SEPTIC HORSES

Priscila Serpa, Andrea Pires dos Santos, Sandra Taylor
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Background: Reactive oxygen species (ROS) are reactive molecules involved in the pathogenesis of numerous diseases. Endotoxemia is a common complication of gastrointestinal and neonatal diseases in horses, resulting in systemic inflammatory

syndrome due to the production of inflammatory mediators and ROS by inflammatory cells. A flow cytometry (FC) assay to measure ROS using 2',7'-dichlorodihydrofluorecein diacetate (DCFH-DA) was developed in this study. Oxidized DCFH-DA produces fluorescence proportional to ROS concentration.

Objective: To evaluate intraerythrocytic and intraleukocytic ROS during LPS-induced sepsis using FC.

Methods: Blood from seven horses was collected at baseline, 30 minutes, one, two, seven, 13, and 24 hours after intravenous infusion of LPS (30 ng/kg). Blood cells were incubated with DCFH-DA, and then left unstimulated or stimulated with hydrogen peroxide for FC analysis. After gating, median fluorescence intensity was calculated and analyzed for variance over time points.

Results: There was significant increase in ROS in neutrophils and monocytes ($p < 0.0001$) after LPS infusion. This difference is seen between two and 13 hours in neutrophils, and as soon as 30 minutes and up to 24 hours in monocytes. No oxidative effect was observed in unstimulated erythrocytes and lymphocytes ($p = 0.0528$ and 0.1734, respectively).

Conclusions: LPS-induced sepsis was an effective experimental model to study the oxidative effects of endotoxemia in blood cells. This model in association with FC detection of ROS can be used in future studies to study the effects of therapeutic agents in the treatment of endotoxemia and other conditions leading to oxidative stress in horses.

Tuesday, November 12, 2019
11:45 a.m. – 12:00 p.m.

THE ROLE OF PKCΔ IN OUTSIDE-IN SIGNALING IN PLATELETS

Preeti Chaudhary, Soochong Kim
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Integrins play a key role in regulating platelet functions, including platelet adhesion, spreading, aggregation, secretion, clot retraction, and platelet procoagulant activity. Engagement of αIIbβ3 promotes platelet-platelet interaction and generates outside-in signals that reinforce activation. The role of protein kinase C δ (PKCδ) in platelet activation has been studied, but its role in outside-in signaling in platelets has not been determined. To determine the role of PKCδ and its signaling pathways in integrin-mediated outside-in signaling in platelets, we used platelets deficient in PKCδ and pharmacological inhibitors of proline-rich tyrosine kinase 2 (Pyk2). Platelet spreading to immobilized fibrinogen resulted in PKCδ (Tyr311) phosphorylation suggesting that integrin αIIbβ3 activation causes PKCδ phosphorylation. Platelet spreading on immobilized fibrinogen was inhibited in PKCδ -/- mice, and integrin αIIbβ3-mediated phosphorylation of Akt and ERK were significantly inhibited in PKCδ -/- platelets indicating a role of PKCδ in outside-in signaling. Blockade of Pyk2 also inhibited platelet spreading on fibrinogen, and integrin αIIbβ3-mediated PKCδ (Tyr311) phosphorylation was inhibited in the presence of Pyk2 inhibitor, suggesting the contribution of Pyk2 in

regulation of PKCδ in outside-in signaling. In addition, integrin-mediated Pyk2 and PKCδ phosphorylation was inhibited in the presence of Src family kinase inhibitor PP2. Finally, clot retraction from PKCδ -/- mice was dramatically delayed, suggesting that PKCδ plays a role in regulating aIIbb3-dependent interactions with elements of the cytoskeleton. PKCδ regulates platelet spreading and clot retraction and plays an important role in outside-in signaling which is regulated by Pyk2 in platelets.

Clinical Pathology Focused Scientific Poster Session A

C-01: COMPARATIVE HEMATOLOGY - INAPPROPRIATE METARUBRICYTOSIS IN CANINE AND HUMAN DISEASES

Claire Andreasen, Elizabeth Hines
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Background: Inappropriate metarubricytosis is associated with syndromes in human medicine, but not well-defined in veterinary medicine. Canine inappropriate metarubricytosis has been reported in lead toxicity, heatstroke, splenic dysfunction, chemotherapy treatment, and bone marrow dysfunction; and suspected to be associated with hypoxia, neoplasia, sepsis/endotoxemia, pancreatitis, and liver disease. Prognostication of metarubricytosis in dogs has been restricted to heatstroke.

Objective: A retrospective study of canine inappropriate metarubricytosis analyzed if similar diseases contribute to the occurrence of nRBCs in canines as in humans, and the correlation of mortality outcomes.

Methods: From 2013-2018, a review of 1759 canine patients with ≥ 1 nRBC/100 WBC and >29% hematocrit resulted in a selection of 72 patients with 119 laboratory reports based on a reported >5 nRBCs/100 WBCs without anemia (reference interval of hematocrit 37-55% for canines >5 months of age and 25-37% for canines <5 months of age).

Results: Analysis determined that canine inappropriate metarubricytosis is associated with lead poisoning, heat stroke, primary splenic dysfunction (non-neoplastic), splenic neoplasia (with or without chemotherapy), non-splenic neoplasia (with or without chemotherapy), thromboemboli/disseminated intravascular coagulation (DIC) with a subcategory of splenic infarct, diabetes (with or without ketosis), primary hepatic dysfunction, hypoxia, sepsis/endotoxemia, intervertebral disc disease, pancreatitis, primary bone marrow dysfunction, inflammation, and miscellaneous diseases. Intervals for nRBC/100 WBCs overlapped between these syndromes. A significant difference was not found for number of nRBC/100 WBCs and mortality outcomes.

Conclusion: Initial findings indicate while humans and canines share similarities in diseases associated with inappropriate metarubricytosis, certain conditions may be unique to canines.

C-02: HEMATOLOGICAL AND CLINICAL CHARACTERISTICS OF DOGS WITH CIRCULATING MACROPHAGES

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Background: Macrophages are rarely found on blood smear evaluation. Clinical significance of circulating macrophages has been poorly characterized.

Objective: To retrospectively describe hematological and clinical characteristics of dogs with circulating macrophages on blood smear evaluation.

Methods: Canine CBC data with blood smear evaluation of a 10-year period were used. Dogs with ≥3 macrophages on a blood smear were included after re-evaluation of blood smears (macrophage group). Clinical and hematological data of the macrophage group were reviewed and compared to dogs without circulating macrophages during the same period (control group).

Results: Of 61,631 CBC records, a total of 39 dogs were identified to have circulating macrophages. Common hematologic abnormalities include neutrophil left shift (79%), anemia (69%), thrombocytopenia (84%), with PCV and platelet, eosinophil and lymphocyte counts being significantly lower compared to the control group. Clinical diagnoses of dogs in the macrophage groups were as follows: 19 inflammatory conditions (5 hepatocellular inflammation/necrosis, 3 rickettsial infections, 2 pneumonia, 2 sepsis and 7 others), 18 neoplastic diseases (6 lymphoma, 5 histiocytic neoplasia and 7 others) and 2 unknown/miscellaneous causes. Median survival time of dogs in the macrophage group was significantly shorter (34 days vs 595 days in the control group) with an increased odd of death at 1 month after diagnosis (OR: 3.90).

Conclusions: Common conditions associated with circulating macrophages are severe inflammation and lymphoid and histiocytic neoplasia. The frequent anemia, thrombocytopenia and inflammatory leukogram may reflect severity of the disease, which results in the poor prognosis of dogs with circulating macrophages.

C-03: CHANGES OF CANINE LEUKOCYTE CONCENTRATIONS AFTER TREATMENT WITH PREDNISOLONE

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Background: Certain blood leukocyte changes are expected in dogs treated with glucocorticoid drugs but expectations are based on very limited research.

Objective: Verify the frequency and magnitude of changes in leukocytes and platelets 0-60 hours after prednisolone treatment in an extensive, well-controlled study.

Methods: Ten beagle dogs were treated intravenously (IV) with 1 mg/kg prednisolone sodium succinate or the same volume of saline in a cross-over study using the dogs as their own controls. Blood was collected at -1, 0, 0.33, 0.67, 1, 2, 3, 4, 6, 9, 12, 24, 28,

32, 36, 48 and 60 hours after treatment. Prednisolone effects were considered clinically detectable if prednisolone results were outside saline control results.

Results: Neutrophilia and leukocytosis occurred in all dogs 2-12 hours after IV prednisolone. Peak neutrophil counts were 10-19 x 10^9/L. Lymphocyte counts decreased 3-9 hours after prednisolone but only 5 of 10 dogs ever had lymphocyte counts below saline controls (1.0 – 4.0 x 10^9/L). Increased monocytes occurred 2-9 hours post-treatment but only 4 of 10 dogs had monocytes greater than saline controls (0.2-0.6 x 10^9/L). No changes in platelet counts were detected.

Conclusion: IV prednisolone induced leukocyte changes were very time dependent. Neutrophilia and lymphopenia were greatest at 6 hours post-treatment. Prednisolone effects had disappeared by 24 hours post-treatment. Eosinopenia and monocytosis were mild and detectable in only 1-4 of 10 dogs. “Steroid leukogram” pattern expected after one prednisolone treatment should be neutrophilia peaking at 6 hours and much less consistently lymphopenia, monocytosis and eosinopenia.

C-04: ASSESSMENT OF NEUTROPHIL FUNCTION IN CANINE CANCER PATIENTS UNDERGOING CHEMOTHERAPY

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Background: Decreased neutrophil function has been reported as a risk factor for development of severe infection in people receiving chemotherapy. Decreased neutrophil oxidative burst following administration of chemotherapy has been reported in dogs with lymphoma.

Objectives: The main objective of the study was to determine if neutrophil function, as measured by oxidative burst and phagocytic activity, is affected by chemotherapy 7-10 days following initiation of treatment in dogs with various tumor types. A secondary objective was to determine if there is a correlation between neutrophil numbers and neutrophil function before or after chemotherapeutic treatment.

Methods: Flow cytometric assessment of neutrophil oxidative burst and phagocytosis following stimulation with *E.coli* was performed in 17 tumor-bearing dogs pre and post initial chemotherapeutic treatment and 8 tumor-free control dogs. Spearman rank correlation was performed to determine if neutrophil numbers and neutrophil function were significantly correlated.

Results: Median neutrophil percentage oxidative burst was significantly lower 7-10 days post initial chemotherapy treatment compared to pre-chemotherapy values ($p=0.0023$). Similarly, oxidative burst as measured by mean fluorescent intensity (MFI) was also significantly lower post chemotherapy compared to pre-treatment values ($p=0.049$). No significant difference in phagocytic activity was noted, however. Neutrophil numbers and function were not significantly correlated.

Conclusion: The results of the study suggest that chemotherapeutic treatment decreases neutrophil oxidative burst, with no significant effect on phagocytic activity 7-10 days post treatment. Furthermore, neutrophil numbers may not be a good reflection/indication of neutrophil function.

C-05: VALIDATION OF THE SYSMEX XN-V HEMATOLOGY ANALYSER IN DOGS

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Background: The Sysmex XN-V has been recently derived from the XN series, the new generation automated hematology systems for human blood analysis. A complete method validation of a new instrument is required before its implementation.

Objective: The purpose of this study was the validation of the hematology analyser XN-V in dogs, and the evaluation of the effects of interfering substances, carryover and storage on hematological variables with canine EDTA-blood specimens.

Methods: Imprecision and linearity were evaluated with canine EDTA-blood specimens and quality control material. Sixty-four dogs were used to compare XN-V results to the XT-2000iV as the reference method and to manual counts. Interference and stability studies were conducted on EDTA-blood specimens after supplementation with increasing concentrations of hemoglobin, intralipid and bilirubin or after storage at 4°C and 24°C up to 72h respectively.

Results: RBC counts, HGB, HCT, MCV, MCH, MCHC, WBC had between- and within-series CVs <2% with quality control material, and within-series CVs <1.73% with canine blood. Platelet counts obtained by impedance, optical and fluorescence methods had within-series CVs <7.7% with quality control material and <3.2% with canine blood. Linearity was excellent for almost all variables. The correlation between the XN-V and the XT-2000iV was high, and there was no significant difference between differential WBC counts. Hemolysis and lipemia had the most significant and concentration-dependent effects. Some hematological variables showed high variations with storage and temperature, especially HCT, MCV and MCHC.

Conclusions: Overall, the performance of the Sysmex XN-V analyser was excellent and compared favourably with the XT-2000iV.

C-06: VALIDATION OF THE SYSMEX XN-V HEMATOLOGY ANALYSER IN HORSES

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Background: The XN-V has been recently derived from the Sysmex XN series, the new generation automated hematology systems for human blood analysis. A validation of a new instrument is required before its implementation, including evaluation of imprecision, linearity and comparison with the reference method.

Objective: The purpose of this study was the validation of the Sysmex XN-V for equine blood.

Methods: EDTA-blood specimens from horses and quality control material were analysed on the Sysmex XN-V to evaluate imprecision and linearity. Fifty-four healthy and sick horses were used to compare results of the new analyser to the Sysmex XT-2000iV as the reference method and to manual NRBC and 100-cell differential WBC counts.

Results: RBC counts, HGB, HCT, MCV, MCH, MCHC, WBC had between- and within-series CVs <2% with quality control material, and within-series CVs <2.22% with equine EDTA blood specimens. The CVs for platelet counts obtained by impedance, optical and fluorescence methods with quality control material between- and within-series were <7.7%, <6.4% and <4.1% respectively and within-series CV with equine blood were respectively 8.54%, 3.17% and 2.18%. By visual inspection, linearity was excellent for all variables except reticulocytes and plateletcrit (PCT), and differences from linearity were low (<10.6%) except for reticulocytes and PCT. The correlation between the Sysmex XN-V and the XT-2000iV was high with Spearman r>0.92 for WBC counts, RBC counts, HGB, HCT, MCV, MCH and r>0.82 for PLT counts.

Conclusion: Overall, the performance of the Sysmex XN-V analyser was excellent and compared favourably with the Sysmex XT-2000iV.

C-07: VALIDATION OF SIEMENS ADVIA 2120 FOR WHITE BLOOD CELL DIFFERENTIAL COUNT IN RABBITS: PRELIMINARY RESULTS

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Background: Siemens Advia 2120 is a laser-based hematology analyzer that is widely used in veterinary medicine; however, it has not been previously validated for determining the 5-part differential white blood cell count (DiffWBCC) in rabbits.

Objective: The aim of this prospective study was to evaluate the performance of Advia 2120 DiffWBCC in rabbits compared to manual DiffWBCC.

Methods: Fifty-nine EDTA-anticoagulated blood samples collected for diagnostic purposes were analyzed within 6h of collection. The Advia 2120 DiffWBCC percentages were compared to the manual DiffWBCCs, performed blindly by two independent observers on modified Wright-stained blood smears by counting 200 cells. Statistical analysis was performed using R statistical language.

Results: After evaluation of the Advia 2120 scattergrams, nine samples were excluded due to suboptimal gating of the population clusters in the PEROX channel, with indistinct differentiation mostly of neutrophils from eosinophils, and lymphocytes from monocytes. Pearson's correlation coefficients for neutrophils and lymphocytes were 0.92 and 0.88, respectively, indicating good to excellent correlation. Spearman's correlation coefficients for monocytes, eosinophils, and basophils were 0.61, 0.36, and 0.85, respectively, indicating fair, poor and good correlation, respectively. Passing Bablok regression analysis revealed a constant error for lymphocytes (intercept: 5.7%, 95% confidence interval [CI]: 2.4-11.0%) and basophils (intercept: 1.2%, 95% CI: 0.51-1.61%). A proportional error was detected for eosinophils (slope: 2.3, 95% CI: 1.4-6.0) and basophils (slope: 0.8, 95% CI: 0.66-0.96).

Conclusions: Based on these preliminary results, Advia 2120 appears to perform generally well for determining the DiffWBCC in rabbits when samples with erroneous scattergrams are excluded.

C-08: VALIDATION OF A NOVEL ASSAY TO DETECT INTRAERYTHROCYTIC REACTIVE OXYGEN SPECIES IN HORSES

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Background: Reactive oxygen species (ROS) are free radicals produced as a byproduct of normal metabolism or cellular stress. Erythrocytes are highly exposed to ROS, which results in their decreased life span and impaired oxygen delivery. Today, assays to detect ROS are expensive, laborious, and usually use indirect markers. One alternative is the use of 2',7'-dichlorodihydrofluorecein diacetate (DCFH-DA). When intracellularly oxidized by ROS, DCFH-DA becomes a fluorochrome, resulting in fluorescence proportional to ROS concentration.

Objective: To validate the use of DCFH-DA for detection of equine intraerythrocytic ROS by flow cytometry.

Methods: Erythrocytes from 31 horses were separated by centrifugation, incubated with DCFH-DA, and then either left unstimulated or stimulated with hydrogen peroxide. For specificity, each cellular component of blood was isolated and plotted according to its size and complexity. For precision, samples were run in triplicates, five consecutive times, for comparison of intra- and inter-assay repeatability. Stability was determined by analysis for up to 48 hours after blood collection. Acceptable coefficient of variation (CV) was set to ≤20%.

Results: Intra-assay CV was 1.7% and 13.3% and inter-assay CV was 4.8% and 17.8% for unstimulated and stimulated samples, respectively. Unstimulated and stimulated samples were stable for up to 48 and 24 hours, respectively. There is a statistical difference ($p < 0.0001$) in fluorescence intensity between unstimulated and stimulated.

Conclusions: The assay was found to have acceptable specificity, precision, and stability. The use of DCFH-DA to detect equine intraerythrocytic ROS is a promising technique with multiple applications for studying oxidative stress in horses.

C-09: EVALUATION OF OXIDATIVE DAMAGE IN ERYTHROCYTES OF RATS EXPOSED TO HIGH DOSES OF BLUEBERRY (POLY) PHENOLS

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Background: Reactive oxidative species (ROS) are unstable molecules generated continuously as byproducts of cell metabolism. When in excess, ROS may cause oxidative damage resulting in cell injury, aging, and cell death. Antioxidants are substances that delay or prevent oxidative damage caused by ROS. (Poly)phenols [(P)P] are rich in blueberries and have beneficial effects when consumed in low doses; however, there is no current safety data of high dose consumption, despite its common use as natural colorants and in functional foods.

Objective: To evaluate the effect of [(P)P] on the erythrocytes of rats exposed to high dose.

Methods: Five-month old, female, Sprague-Dawley rats (n=10/group) were fed blueberry[(P)P](0 or 1000 mg/kg bw/d). Samples were analyzed on days 0, 30, and 90. Directly labeling of intraerythrocytic ROS was achieved using 2'-7'-dichlorodihydrofluorescin diacetate (DCFH-DA). DCFH-DA oxidation by ROS produces highly fluorescent products that are detected by flow cytometry. Peripheral blood smears were stained with Giemsa and New Methylene Blue in order to monitor morphological changes of erythrocytes.

Results: Although there was a significant increase of intraerythrocytic ROS levels overtime, no significant changes were observed between control and treatment groups by flow cytometry and no significant morphologic changes were observed.

Conclusion: Blueberry [(P)P] had no effect on the oxidative status of erythrocytes of rats in high dose measured by flow cytometry. The significant increase in intraerythrocytic ROS overtime is likely related to aging. These preliminary results add to other evidences that high dose of [(P)P]do not cause hormesis.

C-10: REFERENCE INTERVALS AND THE EFFECT OF AGE AND SEX ON SELECTED HEMATOLOGIC AND SERUM BIOCHEMICAL ANALYTES IN 4,809 ENDURANCE-TRAINED SLED DOGS

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Background: Endurance-trained sled dogs are a unique, extremely fit population for which specific reference intervals may be warranted. This population may also provide information on the hematologic and serum biochemical changes that occur based on age and sex.

Objective: To determine reference intervals for selected hematologic and serum biochemical analytes as well as the effect of age and sex in a large endurance-trained population of sled dogs.

Methods: Jugular venous blood was collected from 2012-2018 from 4,809 dogs as part of the pre-race screening for participation in the Iditarod Trail Sled Dog race. Reference intervals were determined. Statistically significant ($p < 0.05$) effects of age and sex were evaluated.

Results: A total of 9,753 individual data points were collected from the 4,809 dogs (61% male) ranging in age from one to twelve years old. Significant effects of age were identified for leukocytes, platelets, and mean corpuscular volume, and serum Glucose, K^+, Ca^{2+}, Cl^-, Phosphorus, Albumin, and Total bilirubin concentrations, and AST and CK activities. Significant differences between males and females were present for platelets, hematocrit, Glucose, Na^+, Albumin, Globulin, SUN, Creatine, and Total bilirubin concentrations, and ALT, ALP, and AST activities.

Conclusions: The most significant observations include decreases in serum $Ca^{2+}$and Phosphorus concentrations with increasing age, lack of evidence for “anemia of aging” and evidence of “inflammaging.” This unique population presents information from extremely fit dogs unique to any information currently published and may be beneficial in the context of canine aging as well as aging in human endurance athletes.

Clinical Pathology Focused Scientific Poster Session C

C-01: EFFECT OF TEMPERATURE AND TIME ON SERUM BIOCHEMICALS PARAMETERS STABILITY FROM CAPYBARAS (HYDROCHOERUS HYDROCHAERIS).

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Background: A commonly problem seen in biochemical analysis routine is the time and temperature used to samples storage as it is not known exactly how these parameters affect the stability of the obtained results. Studying these variations is important, especially in wild animals, where usually there are no nearby laboratories for rapid processing of samples.

Objective: Based on this, the current study was performed upon storage of capybara serum samples at two temperatures and six different times.

Methods: Under anesthesia, blood samples were collected in dry tubes, and sent to the laboratory. At laboratory the blood was centrifuged for serum withdrawal. The biochemical examinations were performed at time 0 and then multiple aliquots separated for storage in 24 and 72-hours, 1-week, 1, 3 and 6-months, and each of the duplicates was stored at temperatures of 4°C and -20°C. An automatic equipment (Cobas C111) were used to analyses: BUN, creatinine, ALP, γ-GT, ALT, AST, TP, albumin and globulin.

Results: ALT and AST showed no difference ($p>0.05$) in temperature. But both showed relevance regarding storage time, with ALT showing instability in time 1 week, and AST in 24-hour time. BUN, ALP, TP and albumin showed significant instability ($p<0.05$) between temperatures regardless of storage time. Creatinine, γ-GT and Globulin showed no differences between the times and the storage temperature.

Conclusions: The samples that showed a difference between the temperatures were more stable at 4°C in relation to time 0. The study showed that time and temperature storage may cause alterations in capybara biochemicals parameters.

C-02: CHANGES IN URINE TEST STRIP ANALYTES WITH STORAGE OR REFRIGERATION

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Introduction: This study evaluates the effects of storage and refrigeration on urine test strip results for pH, protein, glucose, ketones, bilirubin and blood.

Methods: 135 urine samples from hospitalized dogs and cats stored from 7-16 days were evaluated at 4 and 72° F using Chemstrip 10 UA test strips. Results at 72° F were compared to the originally reported values to evaluate effect of storage.

Results: PH: 15/135 (10.6%) refrigerated samples changed, of these 53.3% increased; 30/135 (22.1%) stored samples changed, 73.3% increased. Protein: 23/135 (16.2%) refrigerated samples changed, 52.2% decreased; 33/135 (24.3%) stored samples changed, 75.8% increased. Ketones: 21/135 (14.8%) of refrigerated samples changed, 71.4% decreased; 33/135 (24.3%) stored changed, 78.8% decreased. Blood: 19/135 (13.4%) refrigerated samples changed, 89.5% decreased; 37/135 (27.4%) stored samples changed, 91.9% decreased. Bilirubin: 4/135 (2.8%) refrigerated samples changed, 100% decreased; 12/135 (8.8%) stored samples changed, 58.3% decreased. Glucose: 4/135 (2.9%) refrigerated samples changed, 50% increased; 7/135 (5.0%) stored samples changed, 100% increased. False negative results were most common with refrigeration for ketones (15/135, 10.3%), and protein (6/135, 4.4%) and after storage for ketones (15/135, 11.1%), blood (15/135, 11.1%), bilirubin (5/135, 3.7%) and protein (4/135, 3.0%). False positive results were most common with refrigeration for protein (5/135, 3.7%) and ketones (5/135, 3.7%), and after storage for protein (9/135, 6.7%), glucose (4/135, 3.0%) and ketones (6/135, 4.4%).

Conclusions: Storage has a greater effect on results than temperature. False negative results are more common than false positive results and are more likely with storage vs. refrigeration.

C-03: INFLAMMATORY, REDOX STATUS AND AUTOIMMUNITY BIOMARKERS IN 23 DOGS WITH ARTHRITIS SECONDARY TO LEISHMANIOSIS

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Background: Canine leishmaniosis (CanL) is a multisystemic disease with diverse clinical and clinicopathological manifestations. Inflammatory, redox status, and immunologic biomarkers have been uncommonly investigated in CanL, particularly in CanL-associated arthritis.

Objective: The aim of this study was to describe the profile of inflammatory, redox status and autoimmunity biomarkers in dogs with CanL-associated arthritis.

Methods: Twenty-three adult (5.78±2.32 years) dogs of various breeds, with a confirmed diagnosis of CanL and neutrophilic arthritis (neutrophils >10% of nucleated cells) were included. Concurrent vector-borne infections had been reasonably excluded. Blood samples were collected on admission, and prior to institution of any treatment. Sera were assayed for C-reactive protein (CRP; solid sandwich immunoassay), haptoglobin (colorimetric assay), ferritin (immunoturbidimetry), paraoxonase-1 and total antioxidant capacity (TAC; spectrophotometry), antinuclear antibodies (ANA; immunoperoxidase test) and rheumatoid factor (RF; Rose Waller test).

Results: Of the 23 dogs, 21 (91.3%) showed increased CRP (64.0±40.8 μg/ml, reference interval [RI]: <12 μg/ml) and ferritin (473.7±209.6 ng/ml, RI: 10-190 ng/ml), and 12 (52.2%) had increased haptoglobin (310.6±103.2 mg/dl, RI: <300 mg/dl). Decreased paraoxonase-1 (1.46±0.52 IU/ml, RI: 3-4.3 IU/ml) and TAC (0.47 [0.07-0.57] mmol/l, RI: >0.35 mmol/l) were found in all and in 2 (8.7%) of the dogs, respectively. None of the dogs was positive for ANA (RI: <50), while one (4.4%) was positive for RF (RI: <32; titre regarded as clinically insignificant).

Conclusions: CanL-associated arthritis appears to be related to an exaggerated acute phase response, while compromised antioxidant capacity and autoimmune responses seem to be infrequently present or absent.

C-04: BEAK AND FEATHER DISEASE IN PSITTACINES FROM BRAZIL

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Background: The beak and feather disease virus (PBFDV) infection is enzootic in free cockatoo populations in Australia, being the most common viral disease in parrots in this country. Currently, its distribution is worldwide owing to bird trade, reaching countries in Europe, Asia, Africa, North America and South America. In Brazil, was first detected in 1998 at São Paulo State in a *Cacatua alba*. Some investigations have shown high occurrence of infection in Brazilian wild birds, possibly due to the permanence of these animals in triages centers and zoos captivity, which assign immunosuppressed wild birds, close to exotic carrier birds.

Objective: Determine the occurrence of PBFDV in birds kept at the animal triage center (CETAS-IBAMA-DF) from March 2016 to May 2019 in Brasilia, Brazil.

Methods: Polymerase chain reaction (PCR) for PBFDV screening was used for genome detection in blood samples at the Veterinary Clinical Pathology Laboratory, located at the Veterinary Hospital of Small Animals (University of Brasilia – Brazil).

Results: From 170 birds, 29 (25.0%) were positive, within PBFDV prevalence of 30.6% (26-85) in *Amazona aestiva* specimens, and 9.7% (3-31) *Ara ararauna* specimens. The occurrence rates findings were higher, but similar to those reported by other authors.

Conclusions: These infections may be related to stress, high concentration of birds and poor captivity conditions, in that way providing the PBFDV transmission and maintenance between birds.

C-05: FECAL SACCHAROMYCES CEREVISIAE RESEMBLING OTHER PATHOGENIC YEAST IN TWO KITTENS

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Background: Two eight-week-old kittens from an Illinois shelter were presented to the referring veterinarian for diarrhea. A fecal examination confirmed coccidiosis and fecal smears from both kittens contained yeast-like organisms suspected to be possible *Cryptococcus* sp. The fecal smears were forwarded to Iowa State University for evaluation. The kittens were treated for coccidiosis, and two weeks later the diarrhea resolved, but fecal smears still contained the yeast-like organisms. From the re-check, fecal cytology slides and fresh feces were submitted for further identification. The kittens did not have other signs or lesions consistent with a systemic fungal/yeast infection.

Objective: Identify the fecal yeast-like organisms and determine if pathogenic or non-pathogenic.

Methods: The fecal cytology smears were stained with Wright-Giemsa and GMS stains. Fresh feces were submitted to Purdue University for a pan-fungal PCR followed by DNA sequencing.

Results: On fecal smear cytology, there were mixed bacteria, and numerous 3-6um diameter internal round to ovoid structures, with a thin outer cell wall and a surrounding clear non-staining zone/capsule. These structures had narrow-based budding and small protrusions, similar to pseudo-hyphae. GMS staining was weak. Differential diagnoses included *Cryptococcus* sp., yeast such as *Candida* sp., ingested components, or pollen spores. DNA sequencing identified the organism as *Saccharomyces cerevisiae*.

Conclusion: *Saccharomyces cerevisiae* is a component of some probiotics and Brewer's yeast. The kittens probably received probiotics in the shelter. Since the use of probiotics is becoming widespread in companion animals, it is important to differentiate these yeast components from potential pathogenic yeast.

C-06: PHYSICAL, BIOCHEMICAL AND CYTOLOGICAL FINDINGS OF CHYLOUS AND PSEUDOCHYLOUS EFFUSIONS IN SMALL ANIMALS

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Background: Chylous effusions (ChE) and pseudochylous effusions (PsE) are uncommon in small animals. An adequate recognition of their main characteristics could help into diagnosis and treatment.

Objectives: To evaluate the physical, biochemical and cytological characteristics of ChE and PsE from dogs and cats.

Methods: Data of ChE and PsE recorded between 2013 and 2019 were retrospectively evaluated, including results of 24 patients and main physical, biochemical and cytological (automated total nucleated cell count and manually differential count) parameters were assessed. Chemical analysis included effusion glucose and protein concentration, effusion:serum triglycerides ratio (E:ST ratio) and effusion cholesterol:triglycerides ratio (EC:T ratio).

Results: 22/24 effusions were classified as ChE, and 2/24 as PsE, 6 from canine (5 ChE, 1 PsE) and 18 from feline (17 ChE, 1 PsE) patients. Anatomically, were classified as pleural (21), peritoneal (2) and pericardial (1). Appearance of ChE was described as milky white (16) and pink (6), PsE was milky white (1) and turbid yellowish (1). Mean of chemical parameters obtained in ChE was: protein (5.3 mg/dL), glucose (136.7 mg/dL), triglyceride (1011.4 mg/dL), E:ST ratio (14.1) and EC:T ratio (0.13), while in PsE were: protein (2.4 mg/dL), glucose (133.5 mg/dL), cholesterol (205.5 mg/dL) and EC:T ratio (9.9). Mean TNCC of ChE was 11014/µL with a lymphocyte predominance (63,6%), while TNCC for PsE was 4275/µL.

Conclusion: ChE of pleural origin were the most common in dogs and cats. Although ChE and PsE have physical similarities, biochemical and cytological parameters are essential to the correct classification between them.

C-07: BALLOON CELL MELANOMA IN A MINIATURE PINSCHER

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Case Description: A 15-year-old male castrated Miniature Pinscher presented for increased respiratory effort and unilateral nasal discharge from the right nares with a mass in the right submandibular region.

Clinical Findings: Physical examination revealed a mass in the right submandibular region.

Diagnostic Procedures and Pathologic Findings: Cytologic examination of the right submandibular mass revealed sheets of large, atypical epithelial cells with a large, round nucleus with coarse chromatin and a single prominent nucleolus, and distended, finely vacuolated cytoplasm. Differential diagnoses based on cytomorphology included oncocytoma, sebaceous adenocarcinoma, rhabdomyosarcoma, and balloon-cell melanoma. The resected mass was histologically examined, revealing a malignant neoplasm of undetermined cell type infiltrating the submandibular lymph node. Initial immunohistochemistry was positive for vimentin and negative for cytokeratin and CD18. Additional melanoma panel (MPT blend from Kansas State Diagnostic Laboratory) was positive for Melan A, PNL2, TRP-1, and TRP-2.

Clinical Relevance: The cytologic and histologic features along with the immunohistochemical stains resulted in a diagnosis of metastatic balloon-cell melanoma. This variant of melanoma is rare but should be considered when cytology reveals sheets of large, atypical cells with expanded, finely vacuolated cytoplasm.

C-08: ADENOCARCINOMA IN THE NECK OF A PET RAT

Ilaria Cerchiaro[1], Jane Wardrop[1], Marcie Logsdon[1], Alisha Massa[2], Kyle Taylor[2], Cleverson Souza[1]
[1]Veterinary Teaching Hospital, Washington State University, Pullman, WA, USA, [2]Washington Animal Disease Diagnostic Laboratory, Washington State University, Pullman, WA, USA

Background: Thyroid, salivary and mammary tumors are possible differentials for glandular neoplasms in the neck of pet rats.

Objective: Cytological, histopathological and immunohistochemical description of a neck mass in a pet rat.

Methods: A 1-year old, female, pet rat with progressive weight loss was referred to the WSU Veterinary Teaching Hospital for a 2.5 cm, multilobular mass on the ventral neck. Cytology, histopathology and immunohistochemistry of the mass were performed.

Results: On cytology, clusters of epithelial cells were seen associated with an amorphous, eosinophilic material. Acinar arrangement, dark blue intracytoplasmic granules and occasional atypia (multiple, variably sized and shaped nucleoli, binucleation and moderate anisocytosis and anisokaryosis) were observed. The background was characterized by lakes of an amorphous, lightly basophilic material. A probable follicular thyroid carcinoma was diagnosed. Only mass was submitted for histopathology. The mass was composed of numerous, variably encapsulated lobules of acini lined by cuboidal cells on a scirrhous stroma and frequently filled with homogeneously proteinaceous material. One mitosis in ten 400x fields and mild anisocytosis and anisokaryosis were observed. A well-differentiated follicular thyroid carcinoma was suspected. However, immunohistochemistry for thyroxine and thyroglobulin were negative, necessitating a final diagnosis of adenocarcinoma of unknown tissue origin with salivary gland adenocarcinoma and mammary adenocarcinoma as top differentials. No further clinical information was available.

Conclusions: Cytology of neck masses in pet rats, even when highly suggestive of a thyroid origin, should be supported by histopathology and immunohistochemistry, since lesions resembling thyroid tissue may instead represent other glandular neoplasms.

C-09: LYMPHOPLASMACYTIC LYMPHOMA IN A 7-YEAR-OLD MAINE COON CAT

Jelena Palic[1], Annabelle Heier[1], Annika Lehmbecker[2], Maria Elena Turba[3]
[1]Vet Med Labor GmbH Division of IDEXX Laboratories, Ludwigsburg, Germany, [2]Department of Pathology, University of Veterinary Medicine, Hannover, Germany, [3]Genefast, Forlì, Italy

A 7-year-old male castrated Maine Coon cat presented with edema of the right hindlimb and markedly enlarged right popliteal lymph node. CBC showed neutropenia of 1500/µL. Radiographs and ultrasonographic examination were unremarkable. Cytology of right popliteal lymph node revealed a mixed population of cells, consisting predominantly of medium to large plasmacytoid-appearing lymphocytes, low to moderate numbers of well-differentiated plasma cells and low numbers of small lymphocytes. Plasmacytoid -appearing lymphocytes had round nuclei with finely stippled chromatin and one prominent round nucleolus. Low numbers of binucleated cells and bizarre mitotic figures, and rare multinucleated cells were observed. Histopathological examination of the lymph node showed effacement of the normal lymph node architecture by dense sheets of neoplastic cells. Round to polygonal tumor cells were of intermediate size and had a low to moderate amount of cytoplasm. Round to indented hyperchromatic nuclei were often eccentrically located and contained one distinct nucleolus. Anisocytosis and anisokaryosis were moderate and 21 mitoses/10 HPF were present. Multifocally, neoplastic cells infiltrated into the adjacent adipose tissue. Congo red staining was negative. High numbers of tumor cells were positive for Lambda light chain immunoglobulin; moderate numbers stained positive for MUM-1. Clonal B cell receptor (BCR) gene rearrangement was detected by clonality analysis. Taken together, findings supported a diagnosis of lymphoplasmacytic lymphoma. Lymphoplasmacytic lymphoma is a clonal proliferation of intermediate-sized B-cells with plasmacytic differentiation. It is important to differentiate lymphoplasmacytic lymphoma

from myeloma-related disorders in cats due to different chemotherapy treatment and since lymphoplasmacytic lymphoma carries less favorable prognosis.

C-10: EXTRAMEDULLARY MYELOMONOCYTIC LEUKEMIA WITH LYMPHOCYTE ANTIGEN RECEPTOR REARRANGEMENT IN A DOG

Katherine Morrison, Janet Beeler-Marfisi, Rebecca Egan, Stefan Keller, Samuel Hocker, Dorothee Bienzle
Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

A 6-year-old male castrated Border Collie cross was referred to the Ontario Veterinary College for evaluation of three months of peripheral lymphadenomegaly. Historical hematologic abnormalities included a one-month history of mild, poorly regenerative anemia, inappropriate rubricytosis, and lymphocytosis (19.04×10^9/L, 0.69-4.50). In addition to these abnormalities, a CBC obtained on admission to OVC revealed neutropenia (1.5×10^9/L, 2.9-10.6) and confirmed lymphocytosis (31.1×10^9/L, 0.8-5.1). Predominant cells on the blood film were leukocytes 14-16 µm in diameter with pale basophilic cytoplasm and occasional fine azurophilic granules. Nuclei were round-oval to irregularly lobulated, had fine chromatin and variably prominent large nucleoli. Neutrophils were atypical with hypolobulated nuclei and fine dispersed chromatin. A fine needle biopsy preparation from the left popliteal lymph node contained a similar population of atypical neutrophils (20-30%) and monotypic round cells (>70%). PCR for antigen receptor rearrangement of the same sample showed clonal rearrangement for both B and T cell loci. Flow cytometric analysis of cells aspirated from the right popliteal lymph node revealed a population of cells with high forward and side scatter that was uniformly positive for CD18 and CD45, highly positive for CD14 and CD4, had low positivity for CD34, and lacked CD3, CD5, CD8 and CD21. Since the patient was free of clinical illness, despite bicytopenia and ~20% blasts in circulation, bone marrow was not aspirated. The constellation of antigens expressed on neoplastic cells was typical of myelomonocytic precursor cells, and the neoplasm was considered most consistent with chronic extramedullary myelomonocytic leukemia involving clonal lymphoid antigen receptor rearrangements.

C-11: IDENTIFICATION OF CANINE IGG4 AND A CASE OF CANINE IGG4-RELATED DISEASE

Laura Snyder[1], Lydia Colopy[2], Kai-Biu Shiu[2], Anne Avery[3], Emily Rout[3], Dillon Donaghy[3], A Moore[3]
[1]Marshfield Labs, Veterinary Services, Marshfield, WI, USA, [2]VCA Veterinary Emergency Service, Veterinary Specialty Center, Middleton, WI, USA, [3]Colorado State University, Fort Collins, CO, USA

Background: Canine IgG4 is functionally similar to human IgG4. Human IgG4-related disease (IgG4-RD) is recognized by increased serum IgG4 and IgE, peripheral eosinophilia and IgG4+ plasmacytosis, phlebitis and fibrosis inducing mass-like lesions in the parotid gland and elsewhere. Polyclonal IgG4 increases can appear monoclonal by serum protein electrophoresis, leading to misdiagnosis of a monoclonal gammopathy and multiple myeloma in IgG4-RD patients.

Objective: Evaluate if an immunofixation-based panel can recognize canine IgG4 in clinical samples. Materials: 17 canine monoclonal immunoglobulin proteins characterized by tandem-mass spectrometry as IgA, IgM, IgG2, IgG3 or IgG4 were used to confirm that an immunofixation-based panel selectively identifies canine IgG4. A case of canine IgG4-rich restricted polyclonal gammopathy was reviewed and compared to human IgG4.

Results: Immunofixation accurately differentiated monoclonal IgG4 cases (N=2) from other monoclonal immunoglobulins. The IgG4-rich restricted polyclonal patient had eosinophilia and plasmacytosis of the submandibular lymph node, spleen and bone marrow, parotid gland enlargement and a hyperglobulinemia initially interpreted as a monoclonal gammopathy. Serum IgE concentrations were markedly elevated. Flow cytometry and PARR of the spleen found a polyclonal B-cell/plasma cell population. Infectious disease testing was negative. Treatment was immunosuppressive doses of prednisone, appropriate therapy for human IgG4-RD; the patient responded well with diminished serum IgE and resolution of clinical signs and electrophoretic changes.

Conclusion: This case is the first reported diagnosis, clinical progression, and successful treatment of IgG4-RD in a dog and highlights the added benefit of an expanded immunofixation panel to a broad diagnostic panel when distinguishing IgG4-RD from multiple myeloma.

Diagnostic Pathology Focused Scientific Session I

Sunday, November 10, 2019 | 1:35 p.m. – 5:00 p.m.
Session Chair: Aline Rodrigues Hoffmann, DVM, PhD, DACVP, Texas A&M University, College Station, TX

Sunday, November 10, 2019
1:35 p.m. – 1:45 p.m.

CHARACTERIZATION OF NEUROPATHOLOGIC LESIONS ASSOCIATED WITH AVIAN REOVIRUS INFECTION IN COMMERCIAL BROILER CHICKENS

Tzushan Yang[1], Natalie Armour[2], Martha Pulido-Landinez[2], Alejandro Banda[2], Heidi Rose[2], Brittany Baughman[1]
[1]Mississippi State University College of Veterinary Medicine, Mississippi State, MS, USA, [2]Mississippi Veterinary Research and Diagnostic Laboratory, Pearl, MS, USA

Avian reovirus is an important pathogen within the commercial poultry industry, and is often associated with significant economic impact, especially in broilers. Typical lesions caused by the virus include tenosynovitis/arthritis, myocarditis, multiorgan lymphoid depletion, and enteritis. Neurologic disease linked to reovirus is rarely reported with natural infection in chickens. This report describes histologic brain lesions associated with reovirus infection in commercial broiler chickens in Mississippi. Affected birds ranged from 27-40 days old, and exhibited severe neurologic signs including torticollis and head tremors. Necropsy confirmed severe inflammation in the brain in 26 birds submitted from affected houses. The histologic lesions were most severe in the

brainstem and cerebellar white matter, characterized by robust mononuclear perivascular cuffs, neuronal degeneration and necrosis, sometimes accompanied by microglial nodules, and neuropil vacuolation with axonal degeneration. Similar lesions were found occasionally in the cerebral cortex and spinal cord. Affected birds often had concurrent lesions characteristic for avian reovirus, such as lymphonodular tenosynovitis, pericarditis or myocarditis, and widespread lymphoid aggregates in multiple organs. Fresh brain tissues were PCR positive for avian reovirus and reovirus was isolated from pooled brain samples. Other major causes for neurologic disease in chickens were ruled out based on further diagnostic testing. While the histologic findings from these cases can mimic other common viral encephalitides in chickens, the lesion distribution and inflammatory pattern appeared to be consistent within our cases. These findings suggest that avian reovirus should be considered as a possible cause for chickens with similar neurologic presentations and histologic features.

Sunday, November 10, 2019
1:45 p.m. – 1:55 p.m.

SPONTANEOUS PROLIFERATIVE TYPHLOCOLITIS OF AN UNKNOWN CAUSE IN FVB/N MICE

Lauren Peiffer, Cory Brayton, Karen Sfanos
Johns Hopkins University School of Medicine, Baltimore, MD, USA

Wild type FVB/N mice derived from an in-house breeding colony at Johns Hopkins University developed spontaneous diarrhea prior to experimental use. Pooled fecal samples and serum were sent to Charles River Laboratories for PCR Infectious Agent (PRIA) testing and serology testing, respectively. All PRIA and serology results were negative. Complete necropsies were performed on two affected animals. Partial necropsies were performed on all other animals and included histologic exam of the small intestine, cecum, colon, and mesenteric lymph nodes. 13 of 27 animals (48.1%) had colon walls that were diffusely thick and firm and the lumen contained watery feces with no formed fecal pellets. 5 of 14 (35.7%) males were affected and 8 of 13 (61.5%) females were affected. All affected mice had one to three, 1cm tan, cystic structures in the mesentery which were filled with turbid white fluid. Histologically, there was a chronic-active proliferative typhlocolitis with severe medullary sinus ectasia of mesenteric lymph nodes. Warthin-Starry did not reveal spirochete bacteria. Cecal contents and feces were collected from all animals and frozen colon was collected from eight affected animals. Differentials include a heritable spontaneous mutation (e.g. IL-10 deficiency), an infectious disease caused by a novel pathogen, and an inciting pathogen that was no longer present. Future directions include whole exome sequencing of colonic tissue to identify genetic mutations and 16S rRNA gene sequencing of cecal and fecal contents for bacterial profiling.

Sunday, November 10, 2019
1:55 p.m. – 2:05 p.m.

MYCOBACTERIUM ABSCESSUS PNEUMONIA IN A CAT

Cheng-Hsin Shih[1], Ying-Chen Wu[2], Wei-Hsiang Huang[1]
[1]Graduate Institute of Molecular and Comparative Pathobiology, National Taiwan

University, Taipei, Taiwan, [2]Animal Disease Diagnostic Center, National Chung Hsing University, Taichung, Taiwan

Background: Mycobacterium abscessus, classified among the rapidly growing and nontuberculous mycobacteria, is one of the causative agents of atypical mycobacterioses. These bacteria are ubiquitous in the environment. They can cause opportunistic infection in both healthy and immunocompromised animals or people. Here we report the first case of feline Mycobacterium abscessus pneumonia. Case description: A ten-year-old spayed female domestic shorthair cat presented with chronic cough for three years. Although treatments, including antibiotics and steroids, were administered, the respiratory signs still worsened. CT scan showed extensive lung consolidation except for the right caudal lobe. Pneumonia was suspected, but the etiology was not identified. The cat died at home and the body was submitted for pathological examination.

Results: On necropsy, the cat was obese without any trauma. The lungs were diffusely consolidated, failed to collapse and contained multifocal to coalescent beige, bulging, plaque-like lesions. Microscopically, diffuse granulomatous pneumonia characterized by numerous lipid-laden macrophages, some lymphocytes, and rare neutrophils effaced the pulmonary parenchyma. The alveolar inflammatory infiltrates often surrounded a central lipid vacuole which contained small numbers of acid-fast bacilli. Mycobacterium abscessus was cultured from frozen lung samples.

Conclusions: The current case, which presented with granulomatous pneumonia with intralesional acid-fast bacilli and lipid vacuoles, is consistent with atypical mycobacterial pneumonia caused by ubiquitous nontuberculous Mycobacterium abscessus. To the author's knowledge, this is the first case report of feline Mycobacterium abscessus pneumonia.

Sunday, November 10, 2019
2:05 p.m. – 2:15 p.m.

BRONCHOPULMONARY DYSPLASIA WITH SECONDARY BULLA RUPTURE AND BACTERIAL BRONCHOPNEUMONIA IN A YOUNG SPHYNX CAT

Megan Schreeg[1], Bennett Deddens[1], Zachary Kern[1], Julie Allen[1,2], Devorah Stowe[1], Ian Robertson[1], Adam Birkenheuer[1], Eleanor Hawkins[1], Janice Harvey[1]
[1]North Carolina State University College of Veterinary Medicine, Raleigh, NC, USA, [2]Cornell University College of Veterinary Medicine, Ithaca, NY, USA

Background: Bronchopulmonary dysplasia occurs in human infants and has been reported in one cat previously. A 2.5-year-old spayed female Sphynx cat was managed for two years for chronic cough and exercise-induced tachypnea that was initially responsive to corticosteroids. Serial imaging studies revealed progression from a marked diffuse bronchial and focal alveolar pattern to saccular bronchiectasis and bullous emphysema. Bronchoalveolar lavage revealed mixed inflammation and chronic hemorrhage. The cat ultimately presented for acute pneumothorax with suspected bulla rupture and was subsequently euthanized.

Objective: Our aim was to confirm bulla rupture and further characterize the features of bronchopulmonary dysplasia in this cat.

Methods: Postmortem lung samples were collected, submitted for bacterial culture, and processed routinely for histologic analysis.

Results: Gross evaluation revealed marked multifocal to coalescing bullous emphysema with pleural fibrosis, endogenous lipid pneumonia, and congestion in non-emphysematous parenchyma. A focal ruptured bulla was confirmed. Histopathology revealed marked distortion of pulmonary architecture by thickened, disrupted, and blunted alveolar septae that were lined by hypertrophied type II pneumocytes. Alveolar septae formed large bullae or markedly narrowed air spaces, with frequent consolidation of thickened septae into areas of fibrosis. These findings are consistent with bronchopulmonary dysplasia. A mild neutrophilic bronchopneumonia was also present and aerobic culture grew *Bordetella bronchiseptica*.

Conclusions: Bronchopulmonary dysplasia should be considered as a differential in young feline patients with radiographic evidence of bronchiectasis and bullous emphysema. The pathogenesis of this disease in the cat is unknown, but can be further complicated by secondary bacterial infection and bulla rupture.

Sunday, November 10, 2019
2:15 p.m. – 2:25 p.m.

A PERITONEAL INFLAMMATORY MYOFIBROBLASTIC TUMOR IN AN ARABIAN MARE

Nataly Mamaliger, Alicia Olivier, Amy Lack
Mississippi State University College of Veterinary Medicine, Starkville, MS, USA

This report describes an inflammatory myofibroblastic tumor in a five-year-old Arabian mare who presented for colic signs. At postmortem examination there were multifocal to coalescing variably sized tan nodules on the abdominal serosa, including the abdominal wall, spleen, diaphragm, and large intestine, with the number of nodules on the serosal surface of the colon and cecum. Histologically, the masses are composed of a densely cellular, poorly demarcated and infiltrative mesenchymal neoplasm composed of spindle cells arranged in interlacing bundles or occasional storiform whorls within a collagenous stroma. Neoplastic spindle cells have little atypia arranged in interlacing bundles or storiform whorls with prominent nuclei and no mitotic figures. with Infiltrating the masses, often surrounding vessels, are large numbers of plasma cells, lymphocytes and large foamy macrophages. Neoplastic cells are diffusely immunopositive for smooth muscle actin, variably for vimentin and cytokeratin, rarely for desmin and negative for Factor VIII, CD117 and S100. Based on the histological pattern, staining characteristics and inflammatory cell infiltration, the tumor was classified as an inflammatory myofibroblastic tumor. Inflammatory myofibroblastic tumors are uncommon in the human literature and rarely described in the veterinary literature. Neoplastic cells exhibit a myofibroblastic phenotype which are typically reactive to vimentin, smooth muscle actin and desmin. It is proposed that cytokines produced by the neoplastic cells signal remarkable inflammatory cell infiltration. Inflammatory myofibroblastic tumor is a

diagnosis of exclusion, and the variable appearance has resulted in inconsistent nomenclature.

Sunday, November 10, 2019
2:25 p.m. – 2:35 p.m.

HISTOLOGY GUIDED MASS SPECTROMETRY DISTINGUISHES BETWEEN MAST CELL TUMOR GRADES

Melissa Swan[1], Katy Smoot[2], Matt Powell[2], Shelley Newman[3], Yava Jones-Hall[1], Abigail Cox[1]
[1]Purdue University, West Lafayette, IN, USA, [2]New River Labs, Morgantown, WV, USA, [3]Long Island University CW Post, Brookville, NY, USA

Background: Mast cell tumors are graded using either the Kupiel 2-tiered system, the Patnaik 3-tiered system, or both. Notably, mast cell tumor grade does not always correlate with prognostic outcome.

Objective: The purpose of this study is to identify the proteomic profiles of mast cell tumors and to determine if there are unique signatures that correlate with histologic grade.

Methods: Twenty-five mast cell tumors from each Patnaik tumor grade (n=75) were selected and concurrently graded using the Kiupel 2-tiered system. Proteoscope^{TM}software was used to annotate neoplastic mast cells, tumor stroma, and overlying epithelium in the tumors. Formalin-fixed paraffin-embedded samples were processed by deparaffinization, antigen retrieval, tryptic digestion, and matrix application. Data was acquired using a matrix-assisted laser desorption/ionization time of flight mass spectrometer, and discriminatory peaks were selected for the model analysis.

Results: Statistical software identified 12 discriminatory peaks discerning grade 1 from grade 3 tumors in neoplastic mast cells and 15 discriminatory peaks for tumor stroma. Internal cross validation accuracies were calculated using a leave-20%-out subsampling approach and resulted in 90.7% for neoplastic mast cells and 88.2% for stroma. Analysis of receiver operating characteristic curves identified a total of 18 neoplastic mast cell peaks with a value >0.8 or <0.2, indicating differential expression of the corresponding proteins.

Conclusions: Proteomic signatures from neoplastic mast cells showed more consistent and predictive markers in distinguishing tumor grade than stroma and epithelium. Future studies will include determining if the proteomic test is predicative of prognosis, especially for grade 2 tumors.

Sunday, November 10, 2019
2:35 p.m. – 2:45 p.m.

SYSTEMIC MASTOCYTOSIS PRESENTING AS SUBCUTANEOUS HEMORRHAGE AND EDEMA IN A GREYHOUND

Alexander Aceino, Unity Jeffery, Carolyn Hodo
Department of Veterinary Pathobiology, Texas A&M University, College Station, TX, USA

A 5-year-old spayed female greyhound with a one-month history of progressive ventral cutaneous edema, hemorrhage, and pain was submitted for autopsy. Grossly and histologically, the subcutaneous tissues of the entire ventrum and all limbs were severely expanded by hemorrhage and edema. Superficial to the panniculus carnosus was a dense sheet of neoplastic mast cells. The neoplastic cells contained toluidine blue positive granules and formed aggregates and nodules within several visceral organs including the liver, spleen, heart, kidney, and bone marrow. Systemic mastocytosis, characterized by infiltration of multiple organs by neoplastic mast cells, is a well described entity in human medicine with specific criteria for diagnosis, but is ill defined in veterinary literature. Diffuse edema and hemorrhage is an unusual presentation of mast cell tumors in dogs. Antemortem diagnostics including complete blood count, coagulation profile, and viscoelastic coagulation testing were suggestive of a primary hemostatic defect. Hemostatic disorders are reported in humans affected by systemic mastocytosis but have not been well described in veterinary literature.

Sunday, November 10, 2019
2:45 p.m. – 2:50 p.m.
Veterinary Student Presenter

PROGNOSTIC FEATURES OF CANINE GLIAL TUMORS

Joshua Merickel[1,2], G. Elizabeth Pluhar[1,2], M. Gerard O'Sullivan[1,2]
[1]College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA, [2]Masonic Cancer Center, University of Minnesota, Minneapolis, MN, USA

The dog is proving to be useful as a translational model for human beings with brain tumors. Hitherto, canine glioma histopathologic diagnosis and prognosis have been based on criteria developed for human glioma, an approach which is less than ideal given our increasing awareness of differences in this disease between the two species. Here we report, for the first time, histopathologic features of canine gliomas that correlate with long-term clinical outcome as defined by survival. Histologic sections of tumor biopsies and whole brains (when available) were reviewed for 37 dogs with glioma, all of which had been treated with cytoreductive surgery and immunotherapy. Tumors were diagnosed as astrocytic, oligodendroglial or undefined glioma (using Comparative Brain Tumor Consortium criteria). Putative features of malignancy were evaluated, viz. mitotic counts, glomeruloid vascularization, necrosis, and diffuse infiltration of brain. Mitotic counts were graded on a 0 to 4 basis; other features were noted as present or absent. For biopsies, dogs with astrocytic tumors live longer than those with oligodendroglial or undefined tumor types (median survival 734, 205, 130 days respectively). Low-grade gliomas had better outcomes than high-grade gliomas (median survival 734, 194 days, respectively). Low mitotic counts, absence of

glomeruloid vascularization and of necrosis correlated with increased survival (median 293, 223, 220 days, respectively). High mitotic counts, glomeruloid vascularization, necrosis and diffuse infiltration correlated with poor outcomes (median 190, 170, 154, 212 days, respectively). Whole brain analysis had similar and more robust correlations. These findings will facilitate more accurate prognosis for canine glioma.

2:50 p.m.-2:55 p.m.
Veterinary Student Presenter

UNIQUE CLINICAL PRESENTATION OF MANDIBULAR OSTEOMYELITIS IN A WALLABY

Allison Gerras[1], Kimberly Thompson[2], Victoria Watson[1,3]
[1]Veterinary Diagnostic Laboratory, Michigan State University, Lansing, MI, USA, [2]Binder Park Zoo, Battle Creek, MI, USA, [3]Department of Pathobiology and Diagnostic Investigation, Michigan State University, Lansing, MI, USA

A 4-year-old female red-necked wallaby (*Macropus rufogriseus*) was euthanized after a nine-month history of progressive bilateral enlargement of the mandible. On radiographs there was bilaterally symmetrical smooth osseous proliferation along the ventral surface of the mandible. Repeated bone biopsies had no growth on culture and initial biopsy samples contained only reactive bone. There was no evidence of dental disease on examination and radiographs, and she failed to respond to long-term antibiotic treatment. On postmortem, the left and right mandible were focally expanded by firm, smooth, rounded masses. On cut section, both mandibular masses were white to tan with scattered miliary light brown foci. Histologically, the sections of mandible contained abundant trabeculae of woven bone lined by osteoblasts and numerous reversal lines interspersed with abundant fibrosis and multifocal aggregates of neutrophils, macrophages, multinucleated giant cells, lymphocytes, and plasma cells which often surrounded radiating, eosinophilic, globular material (Splendore-Hoeppli). Bacterial cultures revealed numerous bacteria including *Actinomyces hyovaginalis* and *Clostridium septicum*. Mandibular osteomyelitis is a leading cause of morbidity and mortality in macropods. The terminology of macropod mandibular osteomyelitis has been inconsistent and often implies specific agents such as “lumpy jaw” suggestive of actinomycosis and “oral necrobacillosis” suggestive of *Fusobacterium necrophorum* infection. The most current term, Macropod Progressive Periodontal Disease (MPPD), considers compounding factors like polymicrobial infections, plaque-mediated gingivitis, and abnormal dentition that predispose macropods to this progressive and debilitating disease. This case was further complicated as the clinical presentation was not suggestive of osteomyelitis.

2:55 p.m.-3:00 p.m.
Veterinary Student Presenter

SPECIFIC IN SITU DETECTION OF TRYPANOSOMA CRUZI IN CHAGAS DISEASE

Anna Blick, Raquel Rech
Texas A&M University, College Station, TX, USA

Trypanosoma cruzi is the causative agent of Chagas disease and affects nearly 8 million people worldwide. Cardiac Chagas disease occurs when the parasite changes

from a blood (trypomastigote) to tissue (amastigote) form and infiltrates the heart, forming pseudocysts within cardiomyocytes. The inflammatory response is lymphoplasmacytic myocarditis. However, lymphoplasmacytic myocarditis is sometimes observed without pseudocysts, making the confirmation of a clinical diagnosis via histology difficult. The purpose of this study was to: (1) develop a specific *in situ* technique to identify amastigotes for use as a diagnostic tool, and (2) investigate the presence and distribution of *T. cruzi* amastigotes in the tissue of animals with Chagas disease. We performed a fluorescent in situ hybridization (FISH) assay using an oligonucleotide probe specific to the *T. cruzi* kinetoplast and identified *T. cruzi* DNA within the cardiac tissue of fifteen dogs, a coyote, and a horse previously diagnosed with Chagas disease by histology, PCR, or indirect-fluorescent antibody assay. All dogs had lymphoplasmacytic myocarditis; ten dogs had readily visible amastigotes seen on H&E, and five had no visible amastigotes on H&E. We also determined our probe's specificity for *T. cruzi* by confirming a lack of cross-reactivity against other protozoa including *Leishmania* sp., *Neospora* sp., *Sarcocystis neurona*, *Trypanosoma evansi*, and *Toxoplasma* sp. This study will allow for further exploration of *Trypanosoma cruzi* infections in both domestic and wildlife populations.

Sunday, November 10, 2019
3:30 p.m. – 3:40 p.m.

METASTATIC CERVICAL PARAGANGLIOMAS WITH BONE INVASIONS IN TWO DOGS

Albert Jeon, Lilian Oliveira, Alexis Livacarri, Christina Scanlon, Philip Hamel, Erin Porter, Jeffrey Abbott
University of Florida, Gainesville, FL, USA

Background: A malignant cervical paraganglioma is rarely reported in dogs. Case presentation: Case #1) The computed tomography (CT) of a dog with neck pain demonstrated (1) a right-sided cervical mass with intracalvarial and vertebral canal invasion, and (2) a mass at the heart base. Case #2) A magnetic resonance imaging (MRI) of a dog with tetraparesis demonstrated a left-sided cervical mass with vertebral canal invasion, and resultant extradural spinal cord compression. Post-mortem examination: Case #1) Two multinodular masses were associated with the right common carotid artery. Smaller nodules extending to the atlanto-occipital joint that were seen protruding into the C1 spinal canal and invading into the petrous temporal bone. Of note, there was a nodular mass at the heart base. Case #2) A mass at *THE* left retropharyngeal space near *DELETE the near the* C1 was found with bony invasion into the calvarium involving tympanic bulla and petrous temporal bone, and a mass within the C2 spinal canal was observed compressing the spinal cord. The masses were composed of neuroendocrine cells forming packets and nests. The majority of cells within masses stained positive with Grimelius stain and were immunoreactive with synaptophysin immunohistochemistry (IHC). About 20-25% of cells were variably immunoreactive with neuron specific enolase (NSE) IHC.

Conclusions: Both neoplasms were diagnosed as malignant paragangliomas. In dogs, there are rare reports of these tumors with bone metastasis. In both of the presented

cases, the neoplasms exhibited atypical aggressive bon*Y* invasion into the axial skeleton resulting in neurologic clinical signs.

Sunday, November 10, 2019
3:40 p.m. – 3:50 p.m.

CANINE EXTRA-GASTROINTESTINAL STROMAL TUMORS

Hannah Laurence, Lauren Harris, Juan Muñoz Gutiérrez
Colorado State University, Fort Collins, CO, USA

Background: Gastrointestinal stromal tumors (GISTs) are mesenchymal neoplasms of the gastrointestinal tract that arise from the interstitial cells of Cajal in the submucosa or muscularis of the stomach, small intestine, cecum, and colon. GISTs have been reported in humans, dogs, and horses. In humans, GISTs are rarely found as primary neoplasms of extragastrointestinal tissues such as omentum and mesentery, and are termed extra-gastrointestinal stromal tumors (eGISTs). There are no published reports of canine eGISTs.

Objective: To determine if eGISTs occur in canine submissions received at the Diagnostic Medicine Center (DMC) - Colorado State University.

Methods: Biopsy cases submitted to the DMC from 2013-2018 were considered. Criteria for inclusion were canine cases with a morphologic diagnosis of primary omental/mesenteric mesenchymal neoplasm/sarcoma (without intestinal wall involvement). An immunohistochemical panel was used to identify eGISTs and included desmin, DOG1, and CD117.

Results: 25 cases of primary omental neoplasms were identified. 7 cases were sarcomas. 3 of 7 cases were positive for DOG1 and diagnosed as eGISTs. The excluded cases included true GISTs, fibrosarcomas, liposarcomas, and myxosarcomas. The selected cases are omental masses from an 11-year-old neutered Coonhound, 10 year old neutered Curly Coated Retriever, and a 10-year-old neutered Standard Poodle.

Conclusions: From our small sample size, we conclude that eGISTs occur in older dogs and have a low incidence. In humans, the incidence of eGISTs is reportedly low and omental eGISTs have aggressive behavior. eGIST should be considered as differential diagnosis for omental masses in dogs.

Sunday, November 10, 2019
3:50 p.m. – 4:00 p.m.

HISTOLOGY GUIDED MASS SPECTROMETRY ACCURATELY DISTINGUISHES BENIGN AND MALIGNANT SKIN MELANOMAS IN DOGS

Caitlin Brown[1], Katy Smoot[2], Matthew Powell[2], Yava Jones-Hall[1]
[1]Purdue University, West Lafayette, IN, USA, [2]New River Labs, Morgantown, WV, USA

Background: Canine melanomas are common. Tumor location, pigmentation, and histologic appearance vary, thus, accurately predicting their biological behavior can pose a diagnostic and clinical challenge.

Objective: The objectives of this study are to characterize the proteomic signatures of benign and malignant canine cutaneous melanomas and the overlying epithelium and associated stroma; and to determine if tumor location is a significant predictor.

Methods: One hundred canine melanomas were grouped based on histologic diagnosis (benign or malignant cutaneous, malignant oral, or malignant subungual) with 25 samples per group. Proteoscope™ software was employed to annotate neoplastic melanocytes, tumor stroma, and epithelium. Formalin-fixed paraffin-embedded tissues were subsequently deparaffinized for antigen retrieval, tryptic digestion, and matrix application. Data were collected with a MALDI-TOF mass spectrometer and loaded into SCiLS software to determine discriminatory peaks for model analysis.

Results: Statistical software identified 14 discriminatory peaks differentiating cutaneous malignant from benign lesions in neoplastic melanocytes with a leave-20%-out internal cross validation accuracy of 89.7%. Using the same algorithm, malignant melanomas separated from benign lesions with 82.5% accuracy. Receiver operating characteristic curves identified 19 peaks with values >0.8 or <0.2 that correspond to differentially expressed proteins in cutaneous malignant from benign lesions.

Conclusions: Proteomic signatures from tumor cells discriminate between benign and malignant cutaneous melanomas. Notably, our studies also show that pigmentation may discriminate between skin tumors better than other histopathological features. Future studies will characterize benign vs. malignant oral and subungual melanomas and assess the prognostic predicative value of proteomic signatures in melanomas.

Sunday, November 10, 2019
4:00 p.m. – 4:10 p.m.

PUTATIVE EPIDERMOLYSIS BULLOSA IN A LITTER OF NEONATAL BASSET HOUNDS

Teresa Garcia, Duncan Russell
Oregon State University, Corvallis, OR, USA

Background: Epidermolysis bullosa (EB) is a group of blistering diseases affecting skin and mucus membranes. Three inherited types of EB have been described in dogs, distinguished by the level of splitting at the basement membrane zone. Epidermolysis bullosa simplex (EBS) affects basal keratinocytes, junctional epidermolysis bullosa (JEB) affects the lamina lucida, and dystrophic epidermolysis bullosa (DEB) affects the anchoring filaments between the lamina densa and the superficial dermis. Herein, we describe suspected inherited EB in a single litter of neonatal Basset hounds, in which this condition has not been previously reported.

Case Description: A clinically normal bitch was bred to a relative (grandfather) by artificial insemination. Out of seven puppies, one was stillborn, and another died twelve hours later. Within two days following birth, three male puppies were noted to have blisters variably involving the bridge of the nose, nasal planum, and pawpads. Multiple nails were sloughed and crusts were noted around the ear canals. Two such affected

puppies died naturally and one was humanely euthanized. The two remaining puppies remain clinically normal.

Results: Three puppies were submitted for routine necropsy evaluation. All had severe, subacute to chronic, multifocal, cutaneous bullous dermatopathy with ulceration and superficial infection predominated by gram positive cocci. In addition to bullae/ulcers involving the pawpads and nasal planum, ulcers were also noted in the oral cavity and esophagus. PAS positive material was noted at the roof of the blisters.

Conclusions: An autosomal recessive genodermatosis is suspected. Genetic analysis and ultrastructural evaluation are underway.

Sunday, November 10, 2019
4:10 p.m. – 4:20 p.m.

A NOVEL BURSAL VIRUS, VISCERAL GOUT, AND CEREBRAL VASCULAR URATE DEPOSITION IN A DOUBLE-CRESTED CORMORANT, PHALACROCORAX AURITUS

Bianca Pfisterer, Cheryl Greenacre, Mohamed Abouelkhair, Stephen Kania, Mee-Ja Sula
University of Tennessee, Knoxville, TN, USA

A juvenile, wild caught, great black cormorant, Phalacrocorax carbo, was submitted for necropsy at the University of Tennessee Veterinary Medical Center after a history of neurologic signs and seizures. Gross necropsy findings included marked inanition and severe multifocal disseminated urate deposition (visceral gout). Microscopically, the myocardium, lungs, kidneys, meningeal and cerebral vessels had urate deposition surrounded by epithelioid macrophages and fewer multinucleated giant cells. The gross and microscopic findings of visceral gout, specifically with deposition in the meningeal and cerebral vasculature likely resulted in the neurologic signs and seizures. Additional microscopic findings included lymphocytolysis within the bursa of Fabricius and the spleen. The bursa of Fabricius had large basophilic intranuclear viral inclusions and next generation sequencing was performed. A novel aviadenovirus was sequenced with only 76% homology to the closest related adenovirus, fowl aviadenovirus D. This is the first report of a novel bursal adenovirus and urate deposition in the cerebral vasculature of a cormorant.

Sunday, November 10, 2019
4:20 p.m. – 4:30 p.m.

MENINGEAL GRANULAR CELL TUMOR IN THE CEREBRUM OF A GREEN TREE PYTHON (MORELIA VIRIDIS)

Daniel Finnegan[1], Andrew Cartoceti[2], Amanda Hauck[1], Elise LaDouceur[1]
[1]Joint Pathology Center, Silver Spring, MD, USA, [2]Smithsonian National Zoo, Washington, D. C., DC, USA

Background: Central nervous system (CNS) neoplasia is rarely reported in reptiles, with snake gliomas representing most cases. Granular cell tumors (GCT) are thought to arise from the neural crest and are composed of sheets of round to polygonal cells with

eosinophilic granules that likely represent autophagosomes or autophagolysosomes. Most GCTs manifest as oral neoplasia in people, dogs, and cats, cerebral meningeal neoplasia in dogs, pituitary gland neoplasia in people, and pulmonary neoplasia in people and horses.

Methods: A full set of tissues, including decalcified serial sections of the head, were examined histologically with HE stain. Immunohistochemistry (S100) and special stains (periodic acid-Schiff [PAS] with diastase) were performed on sections of the head.

Results: A 10-year-old female green tree python (*Morelia viridis*) presented for severe constipation and hyporexia. Despite treatment, she was found dead the following morning. Histologically, she had ulcerative colitis with transmural hemorrhage (trauma from constipation). Additionally, a large neoplasm was arising from the ventral meninges and markedly compressing the cerebrum and midbrain. The neoplasm was composed of sheets of round to polygonal cells with cytoplasmic immunoreactivity to S100 and eosinophilic cytoplasmic granules that were PAS positive and diastase resistant. These findings are diagnostic for a meningeal granular cell tumor.

Conclusions: CNS neoplasia is uncommonly reported in snakes. Routine examination of the brain *in situ* via decalcified sections of the head is recommended in reptiles. In this case, neurological deficits secondary to the meningeal GCT may have caused inappetence and led to dehydration and constipation.

Sunday, November 10, 2019
4:30 p.m. – 4:40 p.m.

PSITTACOSIS IN A CAT (FELIS DOMESTICUS)

Hailey Sanderson, Marce Vasquez, Hally Killion, Kerry Sondgeroth, Jonathan Fox
Department of Veterinary Sciences, University of Wyoming, Laramie, WY, USA

Chlamydophila felis (*C. felis*) infection is common in domestic cats and results in conjunctivitis, upper respiratory tract infection, and less frequently pneumonitis. In contrast, *Chlamydophila psittaci* (*C. psittaci*) has not been definitively identified as causing disease in cats but is common in birds and is an important human pathogen. Differentiating between these agents is important. Here we report a fatal case of *C. psittaci* infection in a domestic cat. A litter of five 8-week-old kittens, and the mother, were yielded to a pet rescue center in Riverton, Wyoming in October 2018. Over a period of about 4 weeks all the cats including the mother became sick, thin and icteric prior to death, despite antimicrobial treatments. Post-mortem evaluation of one kitten revealed suppurative and necrotizing hepatitis with intra-hepatocellular bacteria, and non-suppurative pneumonitis and leptomeningitis/ventriculitis. Genomic DNA was extracted from liver and used for 16s rRNA PCR and sequencing which confirmed a single bacterial species with 100% identity to *C. psittaci*. Subsequent PCR on formalin-fixed paraffin embedded tissues also revealed *C. psittaci* in brain, intestine, and tracheobronchial lymph node. Psittacosis should be considered as a differential diagnosis in cats with bacterial hepatitis, and or non-suppurative pneumonitis and meningitis/ventriculitis.

Sunday, November 10, 2019
4:40 p.m. – 4:50 p.m.

HISTOPATHOLOGIC CHARACTERIZATION OF SYNOVIAL BIOPSIES FROM CLINICAL CANINE PATIENTS WITH CRANIAL CRUCIATE LIGAMENT DISEASE

Kei Kuroki[1], Ned Williams[2], James Cook[1]
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Cranial cruciate ligament disease (CCLD) is a common orthopaedic disorder in dogs that often provokes stifle osteoarthritis (OA) despite medical and surgical interventions. It has been recognized that the synovium plays critical roles in the development and progression of OA. As such, further characterizing the roles for synovium in the etiopathogenesis of OA associated with CCLD will help unravel ways to better prevent and treat this highly prevalent disorder. Assessment of synovial biopsies from canine patients with CCLD provides an ethical, clinically relevant method for increasing our understanding of the roles of synovium in OA associated with CCLD. Therefore, this study investigated the histopathologic features of synovium associated with CCLD (n=30) in order to determine characteristic changes that are common in CCLD and that may further elucidate mechanisms of disease in OA associated with CCLD. Synovitis associated with CCLD was often highly inflammatory with rich in cells that are pertaining to humoral immunity (i.e. B cells, plasma cells, Mott cells, bi- and multinucleated plasma cells). Persistent and excessive humoral immune responses may be key mechanisms that perpetuate a local inflammation in the synovium by CCLD.

Sunday, November 10, 2019
4:50 p.m. – 5:00 p.m.

HISTIOCYTIC SARCOMA IN TWO FREE-RANGING FLORIDA MANATEES (TRICHECHUS MANATUS LATIROSTRIS)

David Rotstein[1], Martine deWit[2], Nicole Stacy[3], Mike Kinsel[4]
[1]Marine Mammal Pathology Services, Olney, MD, USA, [2]Florida Fish and Wildlife Conservation Commission, St Petersburg, FL, USA, [3]University of Florida College of Veterinary Medicine, Gainesville, FL, USA, [4]University of Illinois Zoological Pathology Program, Brookfield, IL, USA

Background: The Florida manatee (*Trichechus manatus latirostris*) population has experienced mortalities associated with anthropogenic activity, brevetoxicosis, less commonly pathogens, and rarely neoplasia. Reproductive neoplasia has been reported in female manatees.

Objective: Describe gross and histopathologic findings of histiocytic sarcoma in two Florida manatees.

Methods: Samples from necropsied free-ranging adult females (n =2) from 2011 (Manatee 1) and 2019 (Manatee 2) were processed and stained with hematoxylin and eosin; immunohistochemistry for vimentin, muscle specific actin, and Iba-1 were performed on sections of the neoplasm. Variably-sized tan masses ranging from 1.5 cm in diameter to 6.0 cm X 4.5 cm X 3.2 cm were observed in the liver, spleen and lymph

nodes of Manatee 1 and in the liver, lung, parietal pleura, uterus, ovary, and hemidiaphragm of Manatee 2. In Manatee 1 and 2, there were round to spindyloid cells exhibiting moderate to marked anisocytosis, multinucleation, and reniform nuclei. Manatee 1 also had a second round cell population in the spleen composed of myeloid cells. Immunoreactivity for neoplastic cells and resident macrophages was observed for Iba-1 for Manatee 1. Neoplastic cells in Manatee 2 were immunoreactive for vimentin and Iba-1.

Conclusions: The neoplasms were histiocytic sarcomas based on morphologic characteristics and Iba-1 immunoreactivity. Manatee 1 also had splenic myeloid leukemia. Given the very low incidence of neoplasia in Florida manatees, small sample size, and time period between cases, it is impossible to determine trends in sex, location, or age-class. These are the first report of histiocytic sarcoma in manatees.

Diagnostic Pathology Focused Scientific Session II

Tuesday, November 12, 2019 | 1:30 p.m. – 5:00 p.m.
Session Chair: Aline Rodrigues Hoffmann, DVM, PhD, DACVP, Texas A&M University, College Station, TX

Tuesday, November 12, 2019
1:30 p.m. – 1:40 p.m.
2018 ACVP/AAVLD Award Winner
A UNIQUE IMMUNOPHENOTYPE OF THYMOMA-ASSOCIATED LYMPHOCYTES IN A DOG
Yvonne Wikander, Calli Coffee, Mary Lynn Higginbotham, Nora Springer
Kansas State University, Manhattan, KS, USA

Small cell lymphocytosis is a diagnostic challenge as cytomorphological features of these cells overlap significantly between polyclonal (reactive) versus clonal (neoplastic) populations. In dogs with mediastinal masses, small cell lymphocytosis has been associated with both thymoma and small cell lymphoma. Flow cytometric immunophenotyping is often used as part of the diagnostic work-up for these cases. In a well-cited study, small lymphocytes associated with thymoma were reported to express CD4+CD8+ immunophenotype, and <10% CD4+CD8+ cells was determined to be diagnostic for small cell lymphoma. We report a unique case of thymoma-associated lymphocytosis lacking a CD4+CD8+ immunophenotype. Flow cytometry analysis of the mediastinal mass, diagnosed as a thymoma based on classic cytological findings of heterogeneous lymphocytes, mast cells, and clusters of thymic epithelium, and peripheral blood demonstrated 20-50% CD5+CD4-CD8- T cells respectively, with remaining lymphocytes being CD4+. Less than 1% of all lymphocytes were CD4+CD8+ T cells in both locations. PCR for Antigen Receptor Rearrangement performed on the mediastinal mass and peripheral blood resulted in polyclonal T cell populations in both sites. Collectively, these findings are consistent with a diagnosis of thymoma, not small cell lymphoma, with peripheral T cell lymphocytosis. Based on this case, the immunophenotype of thymoma-associated lymphocytes might be more diverse than previously recognized. Therefore, immunophenotype alone is likely insufficient to differentiate thymoma-associated lymphocytosis versus small cell lymphoma. The

immunophenotype of small lymphocytes in dogs with mediastinal masses and peripheral lymphocytosis should be interpreted in concert with cytological or histological results as well as assessment of clonality.

Tuesday, November 12, 2019
1:40 p.m. – 1:50 p.m.

DIFFERENTIATION OF FELINE INFLAMMATORY BOWEL DISEASE FROM SMALL CELL LYMPHOMA USING HISTOLOGY GUIDED MASS SPECTROMETRY

Sina Marsilio[1,2], Shelly Newman[3], J Estep[4], Paula Giaretta[5], Jonathan Lidbury[1], Emma Warry[6], Andi Flory[7], Paul Morley[8], Katy Smoot[9], Erin Seeley[9], Matt Powell[9], Jan Suchodolski[1], Jörg Steiner[1]
[1]Gastrointestinal Laboratory, Texas A&M University, College Station, TX, USA, [2]Department of Medicine & Epidemiology, University of California, Davis, CA, USA, [3]Long Island University CVM, Brookville, NY, USA, [4]Texas Veterinary Pathology, LCC, San Antonio, TX, USA, [5]Department of Veterinary Pathobiology, Texas A&M University, College Station, TX, USA, [6]Department of Small Animal Clinical Sciences, Texas A&M University, College Station, TX, USA, [7]Veterinary Specialty Hospital, San Diego, CA, USA, [8]Veterinary Education, Research, and Outreach Center, Texas A&M University, Canyon, TX, USA, [9]New River Labs, LLC, Morgantown, WV, USA

The specificity of clonality testing (PARR) for the differentiation of feline IBD from small cell lymphoma (SCL) has recently been called into question with a high rate of false-positive results reported for the T-cell receptor assay. Histology guided mass spectrometry (HGMS) allows for the analysis of endogenous molecules directly in formalin-fixed paraffin embedded (FFPE) tissue sections in targeted cell subpopulations.

HGMS was used to generate *in situ* molecular fingerprinting from FFPE tissue sections from 43 cats with IBD and 51 cats with SCL. Cases were classified as either IBD or SCL by a tumor-board consisting of 6 boarded specialists (internists, anatomic pathologists, and oncologists) based on all available case data. FFPE tissue sections were deparaffinized, underwent antigen retrieval, and were subjected to on-tissue tryptic digestion. Mass spectra were collected from histopathologically preselected areas of monomorphic or pleomorphic lymphocyte populations (50-micron target regions). A linear discriminant analysis classification algorithm was created based on a subset of cases (21 IBD, 25 SCL) using the acquired mass spectral data. A second subset (22 IBD, 26 SCL) was tested against the algorithm to determine the test accuracy.

HGMS correctly classified 42/48 cases (87.5%), while 10.4% were misclassified and 1 case was inconclusive (2%). Diagnostic sensitivity, specificity and accuracy of HGMS were 89%, 86%, and 88%, respectively. In contrast, in our dataset clonality testing showed a sensitivity, specificity and overall accuracy of 81%, 27%, and 54%, respectively.

Results indicate that mass spectrometry may be a powerful tool for accurate differentiation of feline SCL and IBD.

Tuesday, November 12, 2019
1:50 p.m. – 2:00 p.m.

HISTOPATHOLOGIC FEATURES OF A SYNDROME IN BERNESE MOUNTAIN DOGS CHARACTERIZED BY HEPATIC AND CEREBELLAR DEGENERATION

K. Paige Carmichael, Mauricio Seguel, James Stanton
University of Georgia, Athens, GA, USA

Background: In 1996, a syndrome dubbed Canine Hepatocerebellar Syndrome (CHS) was described in sire-related Bernese mountain dogs. We describe gross and histological features of this syndrome in two Bernese Mountain dog puppies (littermates) presenting with clinical signs of ataxia.

Results: Grossly, the cerebellum of both puppies are symmetrically smaller than normal and the folia are flattened. Both livers are micronodular and have scattered pale foci. One puppy has tortuous blood vessels noted in the abdominal cavity. Histologically, cerebellar folia have greatly reduced numbers of Purkinje cells and those remaining are frequently shrunken, rounded, and lack Nissl substance. Purkinje cells are occasionally swollen with vesiculated nuclei and cytoplasm. The granular cell layer is reduced in thickness and contains apoptotic cells. The molecular layer is also reduced in thickness. Liver lesions consist of hepatocellular and hepatic acinar atrophy. Portal areas have multiple vascular profiles due to prominent tortuous arterioles (microvascular dysplasia), biliary hyperplasia and collapse of portal veins. Portal lymphatics are variably dilated. Decreased numbers of ganglion cells are noted in the retina and remaining ganglion cells are shrunken and irregular with loss of Nissl substance.

Conclusions: CHS has been seen throughout the USA and Europe and pedigree analysis suggests it is most likely an inherited autosomal recessive condition. Hepatic and cerebellar lesions are a consistent finding in all cases examined. Ocular lesions have not been previously described. The cause of this syndrome is uncertain although other studies on canine abiotrophy implicate synaptic dysfunction.

Tuesday, November 12, 2019
2:00 p.m. – 2:10 p.m.

OLIG2 EXPRESSION IN FELINE EPENDYMOMA

Andrew Miller[1], Eric Glass[2], Marc Kent[3], Daniel Rissi[4], John Edwards[5]
[1]Cornell University College of Veterinary Medicine, Department of Biomedical Sciences, Section of Anatomic Pathology, Ithaca, NY, USA, [2]Red Bank Veterinary Hospital, Tinton Falls, NJ, USA, [3]University of Georgia College of Veterinary Medicine, Section of Small Animal Medicine and Surgery, Athens, GA, USA, [4]Athens Veterinary Diagnostic Laboratory, Department of Pathology, University of Georgia College of Veterinary Medicine, Athens, GA, USA, [5]Texas A&M University College of Veterinary Medicine, Department of Veterinary Pathobiology, College Station, TX, USA

Background: In domestic animals, ependymomas occur more commonly in the cat compared to other species. They occur mainly in the lateral ventricle and histologically are defined by rosettes and pseudorosettes of polygonal to elongate glial cells that are often positive for glial fibrillary acidic protein (GFAP). Olig2 is a transcription factor that

is predominantly expressed in gliomas; however, its expression pattern in feline ependymomas is unknown.

Objective: This study aims to define the immunolabeling pattern of Olig2 in feline ependymoma.

Methods: We performed a retrospective database search of feline ependymoma cases at three institutions. Seventeen cases were identified and reviewed histologically with immunohistochemistry performed for Olig2 and GFAP.

Results: Patients' average age was 9.5 years with 10/17 domestic shorthair, 4/17 domestic longhair, and 3/17 Siamese. Ten cats were female and seven were male. Neoplasms were located in the lateral ventricles (10/17), third ventricle (2/17), mesencephalic aqueduct (2/17), fourth ventricle (1/17), spinal cord (1/17), or not recorded (1/17). Intranuclear immunolabeling for Olig2 was detected in 16/17 cases. In 3/17, labeling was in >75% of the cells, 2/17 cases had labeling in ~50% of the cells, 6/17 had labeling in ~25% of the cells, and 5/17 had labeling in less than 5% of the cells. One case lacked Olig2 labeling. GFAP immunolabeling was detected in 12/17 cases and absent in 5/17.

Conclusions: As opposed to canine ependymomas where Olig2 immunolabeling is incredibly sparse, Olig2 immunolabeling is present in a majority of feline ependymomas in a widely variable percent of cells.

Tuesday, November 12, 2019
2:10 p.m. – 2:20 p.m.

EVALUATION OF BETA AMYLOID PRECURSOR PROTEIN EXPRESSION IN TRAUMATIC BRAIN INJURY OF DOGS AND CATS

Timothy Wu, Teresa Southard
Cornell University College of Veterinary Medicine, Ithaca, NY, USA

Background: Axonal injury associated with traumatic brain injury (TBI) cannot be detected with hematoxylin and eosin (H&E) staining until 24 hours or more after the injury, making diagnosis of TBI in more acute deaths problematic. Beta-amyloid precursor protein (APP) is a membrane glycoprotein produced in the neuronal cell body, which accumulates proximal to areas of axonal damage, with visualization by immunohistochemistry as early as 35 minutes after injury. APP immunolabelling has been previously demonstrated in humans, sheep, pigs, and rats with TBI, but not dogs or cats.

Objective: Evaluate brain tissue from cats and dogs with known history of TBI and varying post-injury survival times via histopathology and immunohistochemistry for APP.

Methods: Twenty-seven animals (8 cats, 21 dogs) with histories of TBI were necropsied. H&E stained slides were evaluated. APP immunohistochemistry was performed on sections of brain at the level of the corpus callosum and areas with histologic evidence of injury.

Results: Immunohistochemistry revealed strong immunolabelling of axonal spheroids in 6 out of 27 (22.2%) cases, representing 5 dogs and 1 cat. There was no correlation between the degree of immunolabelling and breed, age, sex, location, traumatic incident, or survival time. Some cases had variable amounts of granular immunolabelling within neuron cell bodies, with immunolabeling of all neuron cell bodies in some cases.

Conclusions: The results suggest that APP immunohistochemistry may be valuable in detecting axonal damage in cats and dogs with TBI. The significance of granular staining in cell bodies is uncertain and represents an opportunity for continued research.

Tuesday, November 12, 2019
2:20 p.m. – 2:30 p.m.

NEUTROPHILIC CHOLANGIOHEPATITIS/CHOLECYSTITIS CAUSED BY INTRABILIARY COCCIDIOSIS IN 3 DOGS

Eunju Choi[1,2], Manigandan Lejeune[2], Amira Abdu[2,3]
[1]University of California, Davis, CA, USA, [2]Cornell University, Ithaca, NY, USA, [3]Assiut University, Assiut, Egypt

Background: Neutrophilic cholangiohepatitis/cholecystitis in dogs is generally associated with ascending bacterial infection. Recently, apicomplexan protozoa has been identified in aspirated bile and was molecularly identified as either *Hammondia heydorni* or *H. triffittae*, now with a proposed name as *Heydornia heydorni* and *H. triffittae*, respectively. *Heydornia spp.* are within the Sarcocystidae with close homology to *Neospora caninum*. The final host for *H. heydorni* and *H. triffittae* are the dog and red/artic fox, respectively. While *H. heydorni* is mostly considered nonpathogenic, it has been reported to cause diarrhea in immunosuppressed dogs. In this case series, histologic examination identified three dogs with severe neutrophilic cholangiohepatitis, two with concurrent neutrophilic cholecystitis, associated with intracholangiocytic apicomplexan protozoa.

Objective: To identify the apicomplexan protozoa with biliary tropism.

Methods: Immunohistochemical staining against *Toxoplasma gondii* and *Neospora caninum* and internal transcribed spacer (ITS) PCR with subsequent sequencing were performed.

Results: The intracholangiocytic and free zoites in inflamed portal tracts immunoreacted with the *Toxoplasma gondii* antibody but not with the *Neospora caninum* antibody. ITS PCR performed on the affected gallbladder in one dog resulted in a positive band and, through sequencing, was identified as 99% homologous to *Heydornia spp*.

Conclusion: *Heydornia* spp, occasionally found in the stool of domestic and wild dogs that was deemed nonpathogenic, can, in rare cases, exhibit strong tropism to the biliary system and cause severe neutrophilic cholangiohepatitis/cholecystitis. Apicomplexan protozoa should be considered a potential etiology of neutrophilic biliary tree disease.

Tuesday, November 12, 2019
2:30 p.m. – 2:40 p.m.

FIRST REPORT OF ADENOVIRAL HEMORRHAGIC DISEASE IN THREE MULE DEER (ODOCOILEUS HEMIONUS) IN ARIZONA

Sylvia Ferguson, Jung Keun Lee
Midwestern University College of Veterinary Medicine, Glendale, AZ, USA

This study presents the gross and histopathological findings of three cases of adenoviral hemorrhagic disease (AHD) in mule deer (*Odocoileus hemionus*). These cases represent the first confirmed outbreak of deer adenovirus (*Odocoileus* adenovirus-1) in Arizona. Over the span of a month, three female captive mule deer, consisting of one adult and two juveniles, were submitted to Midwestern University's Diagnostic Pathology Center for post-mortem examination. All of these deer were from the same deer farm and had similar clinical histories consisting of an acute onset of hemorrhagic diarrhea and sudden death. Grossly and microscopically, all cases had severe pulmonary edema and hemorrhagic enteritis. Additionally, 2/3 cases had low numbers of large basophilic intranuclear inclusions expanding endothelial cells within the small intestine and lungs. These tissues were also positive for deer adenovirus on immunohistochemistry. Viral PCR of pooled small intestine, lung, and spleen from each of the cases were positive for deer adenovirus and negative for bluetongue and epizootic hemorrhagic disease.

Tuesday, November 12, 2019
2:40 p.m. – 2:50 p.m.

MOLECULAR AND PATHOLOGIC INVESTIGATION INTO PROTOZOAL ABORTION IN BRITISH COLUMBIA, CANADA

Stephen Raverty[1], Amy Sweeney[2], Elizabeth Zhang[2], Gloria Adedoyin[2], Josh Waddington[3], Theresa Burns[4], John Dick[3], Carl Ribble[4], Tomy Joseph[1], Devon Wilson[5], Michael Grigg[2]
[1]Animal Health Center, Abbotsford, BC, Canada, [2]National Institutes of Health, Bethesda, MD, USA, [3]Greenbelt Veterinary Services, Chilliwack, BC, Canada, [4]Center for Coastal Health, Nanaimo, BC, Canada, [5]University of British Columbia, Vancouver, BC, Canada

Background: Since initial detection and characterization of Neospora caninum, this parasite has emerged as a significant cause of reproductive loss in dairy cattle worldwide. Neosporosis is the most common cause of infectious bovine abortion in British Columbia (BC), with annual losses to the industry in excess of $20,000,000.00 Canadian.

Objective: To better define the etiology and pathology of bovine protozoal abortions in the Fraser Valley, British Columbia. Local dairy producers and practitioners were contacted through on-site visits, clinic newsletters and regional producer groups to solicit fetal submissions. Clinical history, morphometrics and complete necropsies were conducted with tissues harvested for molecular studies, routine bacteriology, molecular studies, serology and histopathology.

Results: Enhanced serology and molecular screening detected N. caninum in 53% of case material and identified an additional 3 protozoa in fetuses, including: Toxoplasma gondii (12.6%), Sarcocystis caninum (3.2%) and S. cruzi (1.8%). **Conclusions:** Protozoa are a significant cause of fetal abortion and financial loss to dairy producers. Despite the availability of commercially validated serology kits and polymerase chain reaction tests, clinical assessment of individual cattle abortions and herd reproductive health continue to be a persistent challenge. Detection of additional Apicomplexan species in fetal tissues, a lack of distinct histopathology features and poor agreement among conventional serology and PCR tests may account for some of this disparity. We will present the application of advanced serology and molecular tools to advance our understanding of natural infection by these parasites in dairy herds.

Tuesday, November 12, 2019
2:50 p.m. – 3:00 p.m.

A HISTOLOGICAL AND IMMUNOHISTOCHEMICAL ASSESSMENT OF ROUND CELL NEOPLASIA IN PSITTACINE BIRDS

Daniel Gibson[1], Nicole Nemeth[2], Hugues Beaufrère[1], Csaba Varga[1,3], Michael Garner[4], Leonardo Susta[1]
[1]University of Guelph, Guelph, ON, Canada, [2]University of Georgia, Athens, GA, USA, [3]Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, ON, Canada, [4]Northwest ZooPath, Monroe, WA, USA

Background: In psittacine birds, tumors originating from lymphocytes, plasma cells, or histiocytes (i.e., round cell neoplasia) are sporadic and poorly documented. Morphologic and immunohistochemical characterization is often lacking, limiting accurate diagnoses and proper characterization of these diseases.

Objectives: To evaluate the histologic features of round cell neoplasia diagnosed in a cohort of psittacine birds, and to assess corresponding cell lineage by immunohistochemistry.

Methods: Cases of psittacine birds diagnosed on postmortem with round cell neoplasia were retrieved from three North American institutions. Demographic data were collected, and tumours were characterized for anatomic distribution, growth pattern, cellular morphology, and immunoreactivity for T (CD3) and B (Pax-5 and MUM-1) cell markers. Linear regression was used to assess the likelihood of immunophenotypes to affect specific organs.

Results: A total of 38 birds with a median age of nine years, representing 14 psittacine species were included. Tumors were mainly infiltrative and multicentric and composed of homogenous sheets of round to polygonal cells with a mitotic index ranging from 0 to 107 per high-power field. The most common immunophenotype was consistent with B-cell lymphoma (*n*=19) based on Pax-5 immunoreactivity and was significantly associated with the gastrointestinal system. Other immunophenotypes included T-cell lymphoma (*n*=3), plasma cell tumours (*n*=3), and cases with double reactivity for both B- and T-cell markers (*n*=3).

Conclusions: This is the first study to describe diagnostic and immunohistochemical features of a large cohort of round cell neoplasia in psittacine birds and provides preliminary information to make specific diagnoses based on cell lineage.

Tuesday, November 12, 2019
3:30 p.m. – 3:40 p.m.

NON-OCULAR MELANOCYTIC NEOPLASIA IN CATS: A RETROSPECTIVE STUDY OF 32 CASES

Stephanie Muller[1], Martina Croci[2]
[1]IDEXX Vet Med Labor, Ludwigsburg, Germany, [2]IDEXX Diavet Labor, Bäch, Switzerland

Background: Non-ocular melanocytic neoplasia is considered rare in cats. Accurate prediction of clinical outcomes is challenging.

Objectives: The aim of the study was to evaluate the association between clinical or pathological parameters and overall survival time of 32 cats with non-ocular melanocytic neoplasias.

Methods: The database of IDEXX (Germany and Switzerland) was retrospectively searched for cases of feline non-ocular melanocytic neoplasias between 2016 and 2019. Clinical data, including location of the primary tumor, were collected from medical records. Histologic samples were reviewed. Achromic tumors were admitted upon immunohistochemical positivity for Melan A, PNL2 and S100. Evaluated parameters included morphological diagnosis, mitotic count (MC), histotype, junctional activity, degree of pigmentation, necrosis, tumor size and vascular invasion. Clinical outcome information was retrieved via telephone interviews with the referring veterinarians.

Results: Twenty-nine tumors located in skin (4 on foot, 2 on lip, 2 on jaw, one on limb, 2 on eyelid, 3 on nose, 2 on back, 8 on pinna, 2 on flank, 3 on tail) and oral mucosae (3) were included. Seven were diagnosed as low grad*E* (22%) and 25 as high grad *E*(78%). All oral tumors were high grad*E*. Follow-up information was available for 21 cats (65%). Seventeen cats treated with surgery survived significantly longer than cats that received only medical treatment. Median survival was 95 days. Variables related with a poor clinical outcome included MC > 4 per ten 40x fields and lack of treatment administration.

Conclusions: According to this study, surgery should be considered as a priority.

Tuesday, November 12, 2019
3:40 p.m. – 3:50 p.m.

ICHTHYOPHONUS INFECTION IN OPALEYE (GIRELLA NIGRICANS)

Elise LaDouceur[1], Judy St. Leger[2], Alexandria Mena[3], Ashley Mackenzie[4], Paul Hershberger[4]
[1]Joint Pathology Center, Silver Spring, MD, USA, [2]Cornell University, Ithaca, NY, USA, [3]SeaWorld, San Diego, CA, USA, [4]U.S. Geological Survey, Nordland, WA, USA

Introduction: *Ichthyophonus* was originally classified as a fungus, but molecular studies have resulted in reclassification as a mesomycetozoan. This parasite has a broad host range of mostly marine fish from across the globe. Pathogenicity varies and may depend on multiple host/environmental/parasite factors. The life cycle is direct and transmission is believed to occur only during ingestion. Opaleye are omnivorous, marine fish native to the Pacific Ocean.

Methods: Wild caught opaleye housed in a public display aquarium were sporadically found dead. The fish were examined grossly and histologically with HE. Special stains (GMS, PAS), tissue explant culture (heart, liver, spleen), and PCR were performed on select cases.

Results: From 2016-2019, 17 opaleye were diagnosed with ichthyophoniasis. Grossly, four animals had pinpoint brown/black foci on coelomic adipose tissue. Histologically, liver, spleen, heart and posterior kidney had mesomycetozoan granulomas in all cases; other organs were less commonly infected. Four opaleye had goiter; additional substantial lesions were not identified. Granulomas surrounded melanized debris, leukocytes, and mesomycetozoa, represented by folded membranes (collapsed schizont wall), intact schizonts (50- to >200-µm-diameter with a multilaminate membrane), plasmodia (budding from shizont or free in tissue), or rarely hyphae (budding from schizont). *Ichthyophonus* was grown from fresh, unfrozen tissues. PCR using 18S rDNA primers returned a 1730 BP region, the sequence of which aligned most closely with *I. hoferi* Clade C.

Conclusion: Ichthyophoniasis is considered the cause for ongoing mortality in these opaleye. *I. hoferi* Clade C is often associated with freshwater fishes and mullet from the Meditereanean Sea.

Tuesday, November 12, 2019
3:50 p.m. – 4:00 p.m.

OROPHARYNGEAL TRICHOMONAS GALLINAE INFECTION IN A WESTERN SCREECH OWL (MEGASCOPS KENNICOTTII)

Devinn Sinnott[1], Mai Mok[2], Michelle Hawkins[1], Eliza Burbank[3], Richard Gerhold[3], Kevin Keel[1]
[1]University of California, Davis, Davis, CA, USA, [2]Necropsy Services Group, Davis, CA, USA, [3]University of Tennessee, Knoxville, Knoxville, TN, USA

Clinical Background: An after hatch year Western screech owl (*Megascops kennicottii*) presented to the UC Davis Veterinary Medical Teaching Hospital after being found down. A caseous plaque was noted within the oral cavity on physical examination. Wet mount preparation of an oral swab yielded numerous motile and encysted trichomonads. Antibiotic, vitamin K, and intravenous fluid therapy was initiated but the owl became dyspneic and died.

Gross and Histologic Findings: On postmortem examination, a plaque-like accumulation of soft, pale tan to white exudate was adhered to the oropharynx, occluded approximately 80% of the tracheal and esophageal lumina, and extended into

the choana. Histologically, the tongue and oropharyngeal mucosa were overlain with a thick layer of fibrinonecrotic debris surrounded by robust granulomatous and heterophilic inflammation. Immunohistochemistry for *Trichomonas* sp. antigen revealed abundant organisms embedded within the fibrinonecrotic exudate. No trichomonads or evidence of infection were seen in any other tissues.

Molecular Diagnostics: Sequencing of the ITS-1 gene from paraffin embedded scrolls yielded 99% homology to *Trichomonas gallinae*. Subtype classification is pending Fe-hydrogenase sequencing.

Conclusions: Infection with *T. gallinae* has been reported in spotted owls in California and is thought to occur due to predation on columbids (e.g. pigeons and doves), the most common carriers of this parasite. However, as a smaller owl species, Western screech owls do not typically prey upon larger birds such as columbids. *Trichomonas gallinae* has been sporadically reported in multiple songbird species, and predation upon songbirds may have served as the source of infection in this case.

Tuesday, November 12, 2019
4:00 p.m. – 4:10 p.m.

GROSS AND HISTOLOGIC LESIONS IN STRANDED DOLPHINS FOLLOWING FRESH WATER INCURSION IN THE MISSISSIPPI SOUND

Timothy Morgan[1], Debra Moore[1], Mystera Samuelson[2], Moby Solangi[2]
[1]Mississippi State University, Starkville, MS, USA, [2]Institute for Marine Mammal Studies, Gulfport, MS, USA

Background: As of July 2019, over 130 bottlenose dolphins (*Tursiops truncatus*) had stranded along the Mississippi Gulf Coast in the Mississippi Sound in the Spring of 2019. This followed two openings of the Bonnet Carre Spillway, which released trillions of gallons of fresh water into Lake Pontchartrain and the Mississippi Sound. Many of the dolphins recovered had severe ulcerative and erosive skin lesions consistent with freshwater exposure.

Objective: Two of the dolphins that died during rescue attempts were necropsied within a few hours of death. Our objective was to document the lesions present and determine the cause of death.

Methods: Necropsies on these two fresh animals were performed. Gross and histologic lesions were evaluated.

Results: The most obvious gross lesions were numerous widely disseminated ulcerative and erosive skin lesions that covered up to 75% of the skin surface. Histologically, there was severe ballooning degeneration of the epidermis that was most severe in the stratum spinosum. Lesions frequently had severe secondary bacterial and sometimes fungal infections, with extension of fungal infections into the underlying dermis. One of the dolphins had bilateral chronic severe osteoarthritis of the shoulder joints and multifocal mild verminous bronchopneumonia. Mild acute tubular necrosis was present in the kidneys.

Conclusions: The lesions seen, particularly the skin lesions, were consistent with freshwater exposure. The dolphins had severe secondary bacterial and fungal infections at the sites of the skin lesions. The cause of death is attributed to fresh water exposure with secondary bacterial and fungal infection and probable endotoxemia.

Tuesday, November 12, 2019
4:10 p.m. – 4:20 p.m.

HISTOLOGIC AND ULTRASTRUCTURAL FINDINGS OF NUCLEOPOLYHEDROVIRUS (BACULOVIRIDAE) AND CYPOVIRUS (REOVIRIDAE) IN EUROPEAN GYPSY MOTH CATERPILLARS (LYMANTRIA DISPAR DISPAR)

Elise LaDouceur
Joint Pathology Center, Silver Spring, MD, USA

Introduction: Nucleopolyhedroviruses (NPV) are the most common viruses to infect and cause disease in insects, particularly lepidopterans. Due to high host specificity, easy host-to-host transmission, and high pathogenicity, NPV is commonly used for biological control of pest species. Cypoviruses (CPV) have a broader host range than NPV to include dipterans and hymenopterans, and less commonly cause epizootics. European gypsy moths are an invasive lepidopteran in North America.

Methods: NPV-infected, CPV-infected, and control caterpillars were dissected and processed for standard HE histology and transmission electron microscopy. Hemolymph of NPV-infected and control caterpillars was examined using phase contrast microscopy.

Results: NPV-infected caterpillars had marked hypertrophy and intranuclear inclusions in cells comprising the fat body, epidermis, and tracheoles. The fat body was atrophic and the exoskeleton was thin with decreased layering. Intranuclear viral particles were identified in nuclei of the fat body, epidermis, and tracheoles, budding from tracheolar nuclei, and entering airways. Phase contrast microscopy revealed intranuclear inclusions in NPV-infected circulating hemocytes. CPV-infected caterpillars had numerous intracytoplasmic inclusions in midgut epithelial cells, and atrophy of the fat body and Malpighian tubules. Intracytoplasmic viral particles were in the midgut epithelial cells in CPV-infected caterpillars.

Conclusions: NPV is ingested, infects midgut epithelial cells, and subsequently infects the tracheolar system, using the respiratory tree to spread throughout the body. Viral proteases and chitinases cause liquefaction of the host, releasing myriad NPV virions into the environment. CPV infection is localized to the midgut in most species and slows growth and development of the host.

Tuesday, November 12, 2019
4:20 p.m. – 4:30 p.m.

PARVOVIRUS INFECTION IN A RESCUED FREE-RANGING TAIWANESE PANGOLIN (MANIS PENTADACTYLA PENTADACTYLA), TAIWAN

Wen-Ta Li[1,2], Li-Hsin Wu[3], Sin-Ling Wang[3], Eric Hsien-Shao Tsao[3]
[1]National Taiwan University, Taipei, Taiwan, [2]Fishhead Lab LLC, Staurt, FL, USA, [3]Taipei Zoo, Taipei, Taiwan

Background: Pangolins are prevalent in Asia and are either endangered or threatened due to habitat loss and human consumption. The susceptibility of pangolins to infectious agents is largely unknown.

Objective: To describe a case of fatal parvovirus infection in a rescued Taiwanese pangolin in Taiwan.

Methods: Blood samples, heart, lung, liver, spleen, kidney, small intestine, and feces were collected and submitted for testing.

Results: Pathological analysis revealed necrotizing and hemorrhagic enteritis. The samples were all positive for the PCR targeting the VP2 gene of parvovirus. The DNA sequence from amplified amplicons of the VP2 gene was completely matched to that of canine parvovirus (CPV)-2c (100% identity with GenBank accession MF347725 and others), which was phylogenetically closely related to the CPV-2c previously identified in Taiwan and China.

Conclusions: The current case demonstrates a new host range of canine parvovirus and a new risk for the conservation of pangolins. From another perspective, the adaptation of CPV-2c in a pangolin population may increase the selection pressure on CPV-2c and thereby contribute to the mutation of CPV-2c and new strain development, which may increase the pathogenicity and cause immunization failure for wildlife and companion animals. A better understanding of parvovirus in wildlife is important to determine its evolution, evaluate the risk of viral transmission among wildlife/companion animals, and advance a strategy for parvovirus control/prevention in wildlife/companion animals.

Tuesday, November 12, 2019
4:30 p.m. – 4:40 p.m.

MELANOPHOROMAS AND PROGRESSIVE CUTANEOUS MELANOSIS IN A GOLDFISH (CARASSIUS AURATUS)

Devinn Sinnott, June Ang, Katherine Watson, Esteban Soto, Denise Imai-Leonard, Verena Affolter
University of California, Davis, Davis, CA, USA

Clinical Background: An adult male goldfish (*Carassius auratus*) presented to the UC Davis Veterinary Medical Teaching Hospital for evaluation. Pigmented masses were noted on the skin caudal to the operculum and on the cornea. Biopsies of both masses were diagnosed as melanophoromas. The skin mass was surgically removed and

treated with cryotherapy. Cryosurgery of the corneal mass was later performed. Two months after removal of the corneal mass, progressive hyperpigmentation of the skin was noted, starting along the dorsum, extending ventrally, and including the fins.

Gross and Histologic Findings: On postmortem examination, approximately 75% of the skin surface of the body and fins was solid black to mottled orange and black. Histologically, hyperpigmented regions corresponded to areas of increased numbers of melanophores in the superficial dermis with abundant finely granular melanin and thin cytoplasmic processes compared to normally pigmented skin. Aggregates of coarsely clumped free melanin and melanin-laden macrophages were occasionally seen within the variably hyperplastic and dysplastic epidermis and within small vessels on the inside of the coelomic body wall. The corneal mass had regrown and was most consistent with a melanophoroma. Granulation tissue was present at the site of the previously removed skin mass. No other melanotic lesions were seen in any other tissues examined.

Conclusions: The cause of the progressive melanosis is uncertain. Genetics, environmental conditions, oncogenic viruses, or non-specific reaction to tissue injury are potential contributing factors, or this may represent a syndrome similar to humans with diffuse cutaneous melanosis secondary to melanoma.

Tuesday, November 12, 2019
4:40 p.m. – 4:50 p.m.

PYOTHORAX AND PLEURITIS ASSOCIATED WITH PORPHYROMONAS SPP IN A BENNETT'S WALLABY

Ahmad Saied, Stephan J. Locke
University of Kentucky Veterinary Diagnostic Laboratory, Lexington, KY, USA

A 10-year-old, male, Bennett's Wallaby (*Macropus rufogriseus*) at a local zoo had a history of periodontal disease and an abscess below the eye. Both issues were treated and deemed resolved. Few months later the wallaby exhibited lethargy and loss of appetite. Despite intensive care, clinical signs progressed to severe lethargy and inappetence; subsequently the animal was euthanized. At necropsy, the thoracic cavity contained > 500 mL of grey to brown viscous fluid, with multiple tan granules. At the level of the thoracic inlet, an approximately 2.5 cm diameter mediastinal abscess was noted. The parietal and visceral pleura on the left side of the thoracic cavity was covered by fibrin and had multifocal to coalescing areas of hemorrhage. Microscopically, multifocal microabscesses / pyogranulomas centered on large numbers of bacterial colonies are noted in the mediastinal abscess. Anaerobic bacterial cultures from the thoracic fluid and the mediastinal abscess yielded *Porphyromonas spp, Filifactor villosus* and *Fusobacterium spp.* Aerobic bacterial cultures did not yield any bacterial growth from the thoracic fluid or the mediastinal abscess swab. *Porphyromonas spp*. are strictly anaerobic, Gram-negative, bacilli that have been associated with oral diseases (gingivitis and periodontitis) in both human beings and animals including wallabies. It is likely that *Porphyromonas spp* had spread from the periodontal disease this wallaby had in the past, and established in the mediastinal area where an abscess had developed. The abscess subsequently ruptured and led to pyothorax with associated pleuritis and pneumonia.

Tuesday, November 12, 2019
4:50 p.m. – 5:00 p.m.

CORYNEBACTERIUM FRENEYI BACTERIAL SEPTICEMIA SECONDARY TO CONTAGIOUS ECTHYMA IN A WILD MUSKOXEN (OVIBOS MOSCHATUS)

Jamie Rothenburger[1], Juliette Di Francesco[1], Krista La Perle[2], Lisa-Marie Leclerc[3], Frank van der Meer[1], Erin Zabek[4], Susan Kutz[1]
[1]Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada, [2]Comparative Pathology & Mouse Phenotyping Shared Resource, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA, [3]Department of Environment, Government of Nunavut, Iqaluit, NU, Canada, [4]Animal Health Centre, British Columbia Ministry of Agriculture, Abbotsford, BC, Canada

Background: Muskoxen (*Ovibos moschatus*) are a keystone species in the Arctic and are valued by local communities for food, income and cultural importance. On Victoria Island, Nunavut, Canada, the muskox population has experienced a severe decline over the last decade, which may be related to multifactorial effects of climate change, low genetic diversity, mineral deficiencies, and an emergence of pathogens such as *Erysipelothrix rhusiopathiae*, orf virus, and *Brucella* spp. Through an ongoing muskox surveillance project, we received the carcass of a juvenile male muskox from Cambridge Bay, Nunavut for autopsy.

Results: Ulcerative and proliferative dermatitis was present on the haired skin, lips and mucocutaneous junction of the muzzle, consistent with contagious pustular dermatitis associated with orf virus infection. There was associated suppurative cellulitis of the head and neck with suppurative lymphadenitis of the regional lymph nodes. Additional lesions suggestive of bacterial septicemia included laminitis, suppurative meningitis and necrosuppurative hepatitis. Bacterial culture yielded *Corynebacterium freneyi* in the muzzle lesion, brain, liver, kidneys and spleen. The orf virus infection was the likely entry point for bacteria, leading to debilitating septicemia.

Conclusions: Community knowledge suggests signs of orf virus infection emerged in this muskox population in the mid-2000s. The virus was first isolated and confirmed in 2014. Causes for orf virus emergence are unknown, but may be linked to cumulative stressors. The association between mortality events and orf virus infection were not previously clear but this case provides evidence that secondary infections may be an important cause of death in orf-affected muskox.

Diagnostic Pathology Focused Scientific Poster Session A

D-01: EGFR AND KRAS IN CANINE INTESTINAL ADENOCARCINOMAS: A POTENTIAL PROGNOSTIC AND THERAPEUTIC TARGET?

Seung-Hee Cho, Byung-Joon Seung, Soo-Hyeon Kim, Ha-Young Lim, Jung-Hyang Sur
Konkuk University, Seoul, Republic of Korea

Background: In humans, epidermal growth factor receptor (EGFR) is overexpressed in many colorectal cancer. Cetuximab, a monoclonal antibody, specifically binds to EGFR, inhibiting the growth-factor binding and receptor activation, and consequently inhibiting

cell proliferation in many human cancer. There is also reports that cetuximab is effective in the treatment of some canine tumors. However, KRAS gene mutation is found in a high percentage of human colorectal cancer, and mutation in KRAS affects the action of cetuximab on EGFR, resulting in resistance to anti-EGFR drug.

Objective: In this study, we evaluated the EGFR gene expression and KRAS mutation in canine intestinal neoplasms and investigated the possibility of applying cetuximab to canine intestinal adenocarcinoma.

Methods: A total of 10 canine intestinal epithelial neoplasms (2 benign neoplasms and 8 carcinomas) were retrospectively evaluated for EGFR gene copy number by in situ hybridization, and KRAS mutational status was evaluated by DNA extraction and sequencing in 5 carcinomas among the 8 carcinomas.

Results: Gene copy number was significantly higher in carcinomas than in benign neoplasms (p=0.00). KRAS gene was wild type in all 5 cases. Unlike human colorectal cancer, kras mutation did not show a high rate in canine intestinal adenocarcinoma, and overexpression of EGFR gene was confirmed.

Conclusions: EGFR may be promising prognostic and therapeutic target in canine intestinal adenocarcinoma.

D-02: THE BODY SHOP: TEST YOUR DIAGNOSTIC ACUMEN

Elizabeth Howerth
University of Georgia, Athens, GA, USA

Pathology is rife with “body” terms for various structures ranging from viral inclusions seen at the light microscopic level to those seen by electron microscopy in storage diseases. Many “bodies” are diagnostic for or associated with specific diseases or conditions, while others are normal findings. Test your “body” knowledge with ten light and electron microscopic images, and help fill in the BODY CHART with a “body” for each letter of the alphabet? You have been challenged.

D-03: CARDIOMEGALY, CARDIAC PATHOLOGY, AND THE PATHOLOGIC DIAGNOSIS OF FELINE HEART DISEASE

Kathleen Kelly
Cornell University College of Veterinary Medicine, Ithaca, NY, USA

The pathologic diagnosis of feline cardiac disease seems straightforward; however, there is clinical and pathologic overlap between several types of cardiomyopathy. Clinically, disease heterogeneity and progression between types of cardiomyopathy is increasingly recognized. Hypertrophic cardiomyopathy (HCM) is characterized by increased cardiac mass; however, the histologic changes have a variable association (~30-100%) with cardiomegaly. Lesions are more frequent in cats with a clinical HCM dying of their cardiac disease; ultimately, the role for HCM without histologic lesions in anesthetic death (AD) and sudden death (SD) is undefined. To understand the association of heart weight (HW) and demographic data with cardiac disease, we

performed a retrospective comparison of necropsy data (death circumstances (SD, AD, euthanasia) body weight (BW), HW) of cats with cardiac disease, HCM, and controls. There was significant differences in BW between HCM, other cardiac disease, and controls (Kruskal-Wallis p=0.0002) and comparing HCM to other cardiac disease (Mann Whitney p=0.02). HW was significantly increased with cardiac disease over controls (KW p=0.0001) but did not distinguish HCM. HCM histologic changes were infrequent (23%) with cardiomegaly and was not associated with HW>20g (Fisher's exact). HW did not differ in SD, AD, or cats with non-cardiac disease. Diverse cardiac lesions not limited to HCM occurred in 10/20 (50%) of SD cases; the remainder had equal proportion of other organ disease or lacked lesions. Similarly, lesions were infrequent in cats with AD (18%). These data reinforce inconsistency between HW and cardiopathology and highlight the need for pathologic consensus criteria for distinguishing significant disease.

D-04: IMMUNOHISTOCHEMICAL EXPRESSION OF ERG ONCOPROTEIN IN CANINE VASCULAR TUMORS: COMPARISON WITH FACTOR-VIII RELATED ANTIGEN AND CD31

José Ramos-Vara, Margaret Miller, Dee DuSold
Purdue University, West Lafayette, IN, USA

Background: ERG oncoprotein induces proliferation and invasiveness of neoplastic cells. It is over-expressed in most human vascular tumors and in many prostatic tumors.

Objective: Immunohistochemical evaluation of ERG expression in benign and malignant canine vascular proliferations and comparison with factor VIII-related antigen (F8) and CD31.

Methods: Sections from 94 formalin-fixed, paraffin-embedded vascular proliferations (hemangioma, 10; hemangiosarcoma, 69; lymphangioma, 10; lymphangiosarcoma, 3; vascular hamartoma, 2) were tested with anti-ERG (monoclonal UMAB76, Origene, Rockville, MD) diluted 1/300 and incubated 60 minutes at room temperature. Heat-induced antigen retrieval (DIVA, Biocare Medical, Pacheco, CA) was used. The percent of cells with nuclear ERG expression was multiplied by labeling intensity (recorded as no labeling, 0; weak, 1+; intermediate, 2+; strong, 3+), resulting in a maximum immunoreactivity score of 300. Serial sections were tested with antibodies to CD31 and F8.

Results: All vascular proliferations and normal endothelial cells expressed ERG with an immunoreactivity score between 200 and 300 (mean, 293; median, 300). Labeling intensity was unrelated to tumor type. Immunoreactivity scores for CD31 and F8 were 70-300 (mean, 256; median, 300) and 70-300 (mean, 269; median, 300), respectively.

Conclusions: Immunohistochemical detection of ERG is a very sensitive method to demonstrate vascular differentiation of canine neoplasms. In humans, ERG is a highly specific marker in both benign and malignant vascular tumors. This might be the case in dogs although the specificity of this marker was not examined in this study.

D-05: MYCOPLASMA BOVIS INFECTION IN NEWBORN CALVES

Fernanda Castillo-Alcala[1], Kelly Buckle[2], Hye Jeong Ha[2], Josepha DeLay[3]
[1]School of Veterinary Science, Massey University, Palmerston North, New Zealand, [2]Ministry for Primary Industries, Upper Hutt, New Zealand, [3]Animal Health Laboratory, University of Guelph, Guelph, ON, Canada

Background: *Mycoplasma bovis* causes various disease syndromes in cattle most importantly mastitis, pneumonia and arthritis. However, there are very few published reports of *M. bovis* natural infections in neonatal calves, and reports of intrauterine infections resulting in pneumonia or delayed occurrence of polyarthritis are infrequent.

Objectives: To 1) morphologically characterise the gross and microscopic lesions present in premature calves born to dams diagnosed with *M. bovis* mastitis and 2) utilize specific *M. bovis* real time PCR (rtPCR) and immunohistochemistry for *M. bovis* detection in examined calves.

Methods: Full postmortem examinations were conducted on seven premature, newborn calves born to dams with *M. bovis* mastitis. Seven fresh tissue samples, including lung, brain, and synovia, and six joint swabs were tested for *M. bovis* by rtPCR. Immunohistochemistry was performed on selected sections of lung, synovium, heart, spleen and brain.

Results: All seven calves had gross lesions mostly confined to the joints and characterised by variable degrees of fibrinous synovitis. Microscopic lesions had a similar tissue distribution and overall morphologic pattern (synovitis, epi- and pericarditis, interstitial pneumonia and meningitis). Swabs obtained from the joints (n=5/6), and fresh tissues including lung (n=1/4) and brain (n=1/2) tested positive for *M. bovis* by rtPCR. *Mycoplasma bovis* antigen was identified in epithelial cells of the bronchioles (n=3/6) and the choroid plexus (n=1/1).

Conclusions: *Mycoplasma bovis* may play a role in natural infections in premature, neonatal calves and should be differentiated from other neonatal bacterial septicaemias such as those observed in calves with failure of passive transfer.

D-06: AN UPDATE ABATTOIR SURVEY STUDY ON THE PREVALENCE OF OVARIAN DISORDES IN NON-PREGNANT SHE-CAMELS (CAMELUS DROMEDARIES) WITH CORRELATION TO BACTERIOLOGICAL ISOLATION.

M Elshazly, Sahar Abd El-Rahman, Dalia Hamza, Merhan Ali
Faculty of Veterinary Medicine, Cairo University, Egypt., Cairo, Egypt

Background: Camels (Camelus dromedaries) are highly valued animals and their successful breeding depends on healthy reproductive organs.

Objectives: The aim of the current study was to identify ovarian pathologies in camels that may interfere with reproduction.

Methods: Bacteriology and histopathology were carried out on 500 camel ovaries collected from three abattoirs in the Giza governorate, Egypt, during the period of January 2016 to January 2018.

Results: A total of 144 (28.8%) camels were found with a variety of ovarian pathologies, which were classified in non-neoplastic and neoplastic lesions. The non-neoplastic lesions (22.6%) included cystic ovary (8.2%), paraovarian cyst (4%), ovariobursal adhesion (1.2%), oophoritis (4%), brown pigmentation of interstitial cells (4.4%) and inactive ovary (1.2%). The neoplastic lesions (6.2%) included papillary cystadenoma (0.4%), granulosa cell tumor (0.8 %), luteoma (0.8%), thecoma and luteinized thecoma (0.4%), teratoma (1.8%), fibroma (0.8%), fibrothecoma (0.4%), fibroadenoma (0.2%) and cavernous hemangioma (0.4%). The most prevalent isolated organisms were; S. aureus (34.8%) and E. coli (30.3%), followed by Klebsiella pneumoniae (9.09%), Enterobacter spp. (9.09%), and Candida albicans (9.09%), while the least prevalent was Pseudomonas aeruginosa (7.57%).

Conclusions: It is important to identify the ovarian pathologies present as they may lead to significant infertility problems. Most of the isolated bacteria are opportunistic zoonotic pathogens that could be acquired during ovarian examination.

D-07: CUTANEOUS SQUAMOUS CELL CARCINOMA IN SIBLING PIGS

Sara Wyckoff, Ogi Okwumabua, Matthew Cuneo, Alexandra Brower
Midwestern University, College of Veterinary Medicine, Glendale, AZ, USA

Background: Porcine neoplasia is uncommonly reported, in large part because of the short lifespan of food producing animals. Reports of skin tumors are rarer yet, and are predominantly breed-associated malignant and benign melanoma. UV radiation, alone or in combination with chronic physical irritation or viral infection, is associated with development of cutaneous squamous cell carcinoma (CSCC) in multiple species.

Objective: Describe the spontaneous development of CSCC in three sibling pigs, and the etiologic risk factors that were investigated.

Methods: Clinical case review followed by humane euthanasia, postmortem examination, histopathologic examination of skin lesions, and PCR testing of affected skin samples using porcine and bovine papillomavirus primer sets.

Results: In a group of four pigs housed together and exposed to intense, natural UV radiation for one year, three siblings simultaneously developed cutaneous squamous cell carcinoma. The unrelated fourth pig did not develop skin lesions. The skin lesions in the siblings were exophytic to pedunculated, and had a characteristic bilateral distribution over predominantly the flank and the base of the convex portion of the ears. In all three pigs, neoplasia was accompanied by chronic-active, lymphoplasmacytic and eosinophilic dermatitis with hyperkeratosis, acanthosis, intra-epidermal pustules and coccoid bacteria. PCR testing of the skin lesions was negative for *Sus scrofa* papillomaviruses and for bovine papilloma virus 1 and 2.

Conclusions: Our conclusion, based on the findings provided, is that a viral etiology is unlikely and that genetic susceptibility, chronic inflammation, and UV radiation were likely factors in the pathogenesis of CSCC in these pigs.

D-08: PHEOCHROMOCYTOMA AND CATECHOLAMINE-INDUCED CARDIOMYOPATHY IN A DOG

Sarah Coe[1], Tereza Stastny[2], Scott D. Fitzgerald[1]
[1]Michigan State University Veterinary Diagnostic Laboratory, Lansing, MI, USA,
[2]Michigan State University Department of Small Animal Clinical Sciences, East Lansing, MI, USA

A five-year-old male neutered mixed breed dog presented to MSU CVM with a two-day history of lethargy, vomiting twice, and increased respiratory rate. On physical exam, the heart rate was 300bpm. On ECG, wide-QRS-tachycardia progressed to ventricular fibrillation. Lidocaine, diltiazem, defibrillation and resuscitation efforts were attempted without success. Upon post-mortem examination, an adrenal mass was discovered, and the epicardial surface of the left ventricle of the heart was diffusely streaked red to white. The adrenal mass was diagnosed as a pheochromocytoma histologically and confirmed with positive immunohistochemical labelling for Beta-endorphin and Metencephalin. The heart had multifocal to coalescing myofiber degeneration, expansion of the interstitium by myxomatous material, and lymphohistiocytic and neutrophilic myocarditis. A bacterial or apixmoplexan cause for myocarditis was ruled out with culture and PCR, respectively. In the absence of other causes for myocarditis and myocardial degeneration, this case is consistent with pheochromocytoma with catecholamine-induced cardiomyopathy. Endogenous catecholamine cardiotoxicity has been documented in humans, non-human primates, rodents, and more recently in dogs. The effects of excess endogenous catecholamine production are similar to those seen experimentally induced in dogs with exogenous catecholamines.

D-09: CHOLEOEIMERIA POGONAE INFECTION IN THE GALLBLADDER IN A GROUP OF BEARDED DRAGONS (POGONA VITTICEPS)

Dawn Evans, Anke Stöhr, Tatiane Watanabe, Nathalie Rademacher, R. Bennett, Jacqueline Elliott, Rudy Bauer, Fabio Del Piero
Louisiana State University, Baton Rouge, LA, USA

Background: Infection with coccidia is common in reptiles and affected sites include intestines and gallbladder. Most recent reports have reclassified coccidia in the gallbladder of reptiles as *Choleoeimeria* sp. based on endogenous developmental stages.

Observation: Thirty-one bearded dragons (*Pogona vitticeps*), as part of a research colony at the Louisiana State University, were housed in a research facility. A cholecystectomy was performed in one animal when an enlarged gallbladder was observed during ovariectomy due to follicular stasis. Four animals developed decreased appetite and lethargy with dehydration and poor body condition noted on physical examination. The complete group was assessed via diagnostic imaging,

parasitologic examination on feces and histopathology on surgically removed gallbladders (n=5), and animals that were euthanized (n=3) or died (n=2).

Results: Alterations on ultrasound in the gallbladders in 25/26 animals included luminal sludge or choleliths and/or thickened walls. Fecal testing on 20 out of 26 animals revealed seven positive with oocyst morphology consistent with *Choleoeimeria pogonae*. One euthanized animal had various developmental stages of coccidia in the intestines most consistent with *Isospora* sp. Gallbadder and bile duct sections had endogenous coccidia development most consistent with *Choleoeimeria pogonae*. Oocysts in the gallbladder were identified in one cholecystectomy specimen and one animal that died.

Summary: Imaging in addition to fecal flotation and histopathology aided in identifying gallbladder coccidiosis in a group of bearded dragons. Organisms were interpreted as *Choleoeimeria pogonae* (reclassified from *Eimeria pogonae* in 2016). There was also microscopic evidence of an intestinal infection with *Isospora* sp in one individual.

D-11: CASE REPORT: CUTEANEOUS PROTOTHECOSIS IN A DOG

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A 4-year-old female Chihuahueño dog presented to a private practice in Mexico City for interdigital ulcerative lesions on the thoracic limbs and excoriation of the lips and nose. Cytologic evaluation showed lymphocyte proliferation without a definitive diagnosis, consequently biopsies of the lips and nose were performed. The tissue sections contained a mixed inflammatory population of foamy macrophages, degenerate neutrophils, lymphocytes and plasma cells that surrounded degenerate collagen fibers. Mixed with the inflammation, were numerous round to oval unicellular microorganisms, varying in size between 6-20 µm with a thick hyaline cell wall (sporangia). These sporangia contained multiple basophilic daughter cells (endospores). The organisms stained strongly with GMS and diffusely with PAS. A histopathological diagnosis of severe pyogranulomatous multifocal dermatitis with numerous algal organisms (presumably Prototheca spp) was made. Further diagnostic tests were not carried out and treatment was started with amphotericin B. After improvement following a six-month treatment, the patient presented again for soft swellings in the hock region of both hindlimbs. Skin fine needle aspiration was performed and submitted for cytologic evaluation. The direct smears contained a mixture of non-degenerate neutrophils and macrophages, as well as numerous algal organisms that consisted of round, approximate 10 µm diameter sporangia with a thick clear cell wall; sporangia contained multiple round to oval dark structures (endospores). Electron microscopy and PCR are pending for a definitive diagnosis.

D-12: NEURONAL INTERMEDIATE FILAMENT ACCUMULATION IN THE CENTRAL NERVOUS SYSTEM NEURONS OF NUDE RATS (F344/NJCL-RNU/RNU)

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Pale eosinophilic material accumulation in the central nervous system (CNS) neurons was observed in one male and two female nude rats (F344/NJcl-*rnu*/*rnu*) aged between 8 and 18 weeks out of 23 animals examined (13%). These animals were housed and cared for normally, had received no prior treatment, and showed no apparent clinical abnormalities or gross findings. In the 3 rats, degenerative neurons were detected in the thalamus, external pyramidal cell layer, Purkinje cell layer, cerebellar medulla, brainstem, and spinal cord, but not in the hippocampus. In the pyramidal cells in the cerebral cortex and Purkinje cells, dendrites were swollen. The pale eosinophilic materials were negative for PAS and Schmorl stains. There were no other histopathological abnormalities including neuronal necrosis or loss, gliosis, or microglial proliferation in any area, or torpedo in Purkinje cell axon. Meanwhile, eosinophilic body (EB) was observed in most of the degenerative neurons. EB was positive for PAS and stained blue with Schmorl stain. Immunohistochemistry confirmed that the pale eosinophilic materials were α-internexin and neurofilaments and that EB was labeled with anti-ubiquitin antibody. Ultrastructurally, accumulated intermediate filaments and aggregates of secondary lysosomes were observed in the degenerative neurons. Neuronal α-internexin accumulation demonstrates diagnostic specificity for human neuronal intermediate filament inclusion disease (NIFID). To our knowledge, this is the first description on α-internexin accumulation in the CNS neurons in animals. Therefore, it was suggested that nude rats (F344/NJcl-*rnu*/*rnu*) can be used as a spontaneous animal model for human NIFID.

D-13: PEROSOMUS ELUMBIS IN A STILLBORN RHESUS MACAQUE (MACACA MULATTA): A CASE REPORT

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Background: Perosomus Elumbis (PE) is a rare congenital abnormality due to agenesis of the caudal spine and has been predominantly described in cattle and sheep. Associated lesions in these species include pelvic and hind limb hypoplasia, arthrogryposis, and various urogenital and gastrointestinal malformations. We describe the gross, histopathologic, and radiographic findings in the first documented case of PE in a non-human primate and relate our findings with other species.

Methods: A 128-day, stillborn, female, rhesus macaque with musculoskeletal malformations was presented for necropsy. Gross and histopathologic examinations were performed in a routine manner. Radiographs of the caudal spine and pelvis were obtained post fixation.

Results: Radiographs demonstrated the complete absence of lumbar, sacral, and coccygeal vertebrae and was confirmed by gross examination. The pelvis and hind limbs were disproportionately small and contracted. Both hind feet were inwardly rotated (talipes varus) there was a web of skin on the legs extending across the caudal aspect

of each knee which limited full extension of the joint (popliteal webs). The thoracic and cervical vertebrae were grossly normal. Additional lesions included horseshoe kidney, left adrenal gland agenesis, and mandibular-maxillary hypoplasia. Histologic findings included dysgenesis of corneal stroma and fusion of renal cortices.

Conclusions: This is the first reported case of PE in a non-human primate. The lesions are consistent with the features of PE in other animals.

D-14: PERIOCULAR T-CELL LYMPHOMA IN A HORSE

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Background: The left eye of a 26-year-old warmblood mare was enucleated due to a large, rapidly progressing mass that originated from the nictitans and had spread to the conjunctiva. Sections of the mass were submitted in formalin for histopathology.

Objective: Our objective was to identify the neoplasm and aid in determining the mare's prognosis. Primary differential diagnoses for neoplasia of periocular tissue included squamous cell carcinoma and sarcoid.

Methods: Sections of the nictitans and conjunctiva were stained with hematoxylin and eosin, and with immunohistochemistry markers for CD3, CD20, and CD79a.

Results: On hematoxylin and eosin, both the conjunctiva and nictitans were infiltrated by densely cellular sheets of round cells with marked anisocytosis and anisokaryosis. An average of five mitoses were seen per high power field. The mass was unencapsulated and poorly demarcated. Neoplastic cells were found infiltrating vascular channels and nerves. The round cell population overwhelmingly expressed a CD3 antigen signal. Low level expression of CD20 and CD79a antigens was found widely scattered in the mass and appeared to be colocalized.

Conclusions: The neoplasm was consistent with a T cell lymphoma. Lymphoma is one of the most common malignant neoplasms in horses, with peripheral T cell lymphoma being a frequent subtype. Ocular and orbital lymphoma in horses is rare. Due to the high degree of anaplasia in this mass, immunohistochemistry was essential in obtaining an accurate diagnosis.

D-15: ALGAL LYMPHADENITIS IN A DOG CAUSED BY SCENEDESMUS SPECIES GREEN ALGAE

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Background: A 6-year-old, spayed female Labrador/Weimaraner cross dog presented in Montana for an annual wellness examination, at which the general practitioner

discovered an enlarged popliteal lymph node. The node was sampled with an incisional biopsy and later excised in full. Histologic examination yielded granulomatous lymphadenitis with green algae.

Objective: Our objective was to characterize the green algal infection and identify the infecting algal agent.

Methods: Routine histology was performed on the initial incisional biopsy and on representative sections from the full lymph node, which was submitted later. DNA from the paraffin-embedded tissue was amplified with PCR using primers encompassing the internal transcribed spacer (ITS) and D1/D2 regions of the 28S ribosomal RNA gene, and the product was sequenced. Cytology, transmission electron microscopy, and bacterial culture on chocolate blood agar were also performed.

Results: Eosinophil-rich granulomas with both extracellular and intrahistiocytic green algae multifocally expanded the lymph node on histology with similar organisms were seen cytologically. When cultured, the algae quickly formed 203 mm bright green colonies on blood agar. Ultrastructurally, the algae had a multilayered cell wall and contained chloroplasts with numerous starch granules. The sequenced amplicon had high sequence identity with *Scenedesmus* sp. isolate KY655009. A year after initial submission, the retropharyngeal lymph node became enlarged and again contained green algae on cytology, but the dog remained healthy.

Conclusion: To the authors' knowledge, this is the first case of *Scenedesmus* species infection in a dog and raises the possibility of subclinical algal infection in dogs.

D-16: TWO CASES OF UNILATERAL MANDIBULAR HYPEROSTOSIS AND OSTEOMYELITIS IN JUVENILE CATS

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Over the course of 6 months, the University of California, Davis Anatomic Pathology Service received surgically-resected hemimandibles from two unrelated, approximately 1.5-year-old, domestic short-haired cats with diffuse, unilateral hyperostosis of the left mandible. Marked mandibular expansion had first been noted when the cats were less than one-year-old with no known history of trauma. On extensive diagnostic imaging, the left mandibles in both animals were expanded from the level of the symphysis to the ramus; the right mandibles and temporomandibular joints were unaffected. Microscopically, the mandibles had similar histologic features with effacement and replacement of normal mandibular architecture by concentric layers of trabecular bone that were oriented perpendicular to the mandibular surface and centered around the pre-existing alveolar bone. Trabeculae were separated by fibrous stroma that was multifocally infiltrated by dense aggregates of neutrophils with fewer lymphocytes and plasma cells. In both cases, residual premolar and molar deciduous teeth were partially to completely entrapped (impacted) within the proliferative jawbone. Grossly normal permanent canine and incisor teeth were present in only one cat, who was concurrently

diagnosed with periodontitis and aerobic culture yielded Escherichia coli bacteria. In both cases, the resected jaws demonstrated some features of proliferative fibro-osseous lesions (PFOLs). However, PFOLs are uncommon in young cats and rarely cause extensive distortion of an entire hemimandible. The patients' young age and marked architectural distortion of the jawbone suggests the possibility that these lesions represent an as of yet uncharacterized genetic or developmental abnormality.

D-17: SUDDEN DEATH CAUSED BY FETLOCK FAILURE IN A THOROUGHBRED RACEHORSE

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The most prevalent causes of death in racehorses are musculoskeletal injuries, causing app. 80% of deaths within the racing industry in California and elsewhere. The vast majority of these injuries (about 90%) have pre-existing lesions that predispose to fatal injury.

A 4-year-old Thoroughbred colt suffered from an acute suspensory apparatus failure, including biaxial proximal sesamoid bone fracture of the right front fetlock, causing loss of support of the fetlock joint and consequent fall with cervical and sacral vertebral fractures.

Evidence of pre-existing pathology was observed in association with the complete transverse fracture of the medial proximal sesamoid bone. A focal discoloration within a region of sclerotic bone was found to be osteopenic on microcomputed tomography examination. Most likely, focal osteopenia acted as a stress riser that caused initiation of complete bone fracture. A similar osteopenic lesion was present in the intact medial proximal sesamoid bone of the left forelimb.

The morphological features of the vertebral fractures are compatible with acute injury. Probably, these acute fractures occurred subsequent to the fall during the horse’s collision with the racetrack. The presence of bilateral osteopenic lesions is consistent with repetitive overuse injury, with the most severe lesion leading to bone fracture and fetlock failure.

Approximately 30% to 50% percent of California Thoroughbred racehorse deaths have been associated with proximal sesamoid bones injuries. Therefore, the understanding of the chronic pathogenesis and character of these lesions is paramount to try to reduce the number of catastrophic injuries in the racehorse population.

D-18: BILATERAL MENINGIOMA WITH FIBRINOID NECROTISING VASCULITIS IN A WESTERN LOWLAND GORILLA

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Bilateral meningiomas were diagnosed within the cranial vault of a 43-year-old female western lowland gorilla, after progressive seizure activity. Histologically, the masses were associated with a necrotising vasculitis, with features similar to those described in canine idiopathic polyarteritis (polyarteritis nodosa). To the authors' knowledge, this is the first report of meningioma in a western lowland gorilla, of paraneoplastic vasculitis in a nonhuman primate, and of multiple meningiomas in a nonhuman primate.

D-19: USE OF COMPUTED TOMOGRAPHY IN THE FORENSIC POSTMORTEM EXAM OF A CANINE GUNSHOT CASE

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In the last 15 years, the United States' simultaneous increase of the urban canine population and gun violence has increased the likelihood of canine gunshot victims, which has bolstered the need for comprehensive veterinary forensics. In Phoenix, AZ a 9-m-old male American pit bull terrier was found deceased from a suspected gunshot to the head. The scene was processed, and the animal submitted to Midwestern University's Diagnostic Pathology Center. Traditional computed tomography (CT) and three-dimensional multi-slice computed tomography (3D MSCT) complemented the routine postmortem exam. The imaging procedure confirmed gunshot; assessed the in-situ location and number of projectiles to be collected; and determined manner and cause of death. The technique enhanced the accuracy of the postmortem exam by providing a 3D model of the victim with in-dwelling projectiles, which guided dissections and limited iatrogenic artifacts. Combining the 3D model with the ballistic rods allowed a more accurate determination of the presumed projectile trajectories and facilitated their recovery as evidence. Consistent with the 12-gauge casing found at the scene, the patient had suffered a fatal buckshot to the right side of the face angled caudally across the neck towards the back. Five projectiles were retrieved, one of which fractured the C1-C2 vertebrae and severed the cervical spinal cord killing the patient. The 3D MSCT provided a degree of accuracy not achievable with routine postmortem exam alone. If possible, 3D MSCT should be routinely performed in canine gunshot cases.

D-20: BALAMUTHIA MANDRILLARIS MENINGOENCEPHALITIS IN A CAPTIVE BORNEAN ORANGUTAN (PONGO PYGMAEUS)

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Background: Pathogenic free-living amebae cause disease in humans and animals. The incidence is low, but infections are a diagnostic challenge, are difficult to treat, and are often fatal.Environmental exposure is not improbable; these amebas are ubiquitous, amphizoic, opportunistic, target immunocompetent and immunosuppressed hosts, and occupy soil, air, and water.

Objective: An 11-y-old female Bornean orangutan was examined by veterinarians when caretakers noticed minor episodes of proprioceptive ataxia. Over 72 hours, these deficits progressed to left-sided hemiparesis. An MRI revealed a focal right-sided cerebral mass. Forty-eight hours later, the animal was deceased.

Results: On postmortem exam, the medial right parietal lobe was replaced by locally extensive encephalomalacia and hemorrhage that displaced the lateral ventricle and abutted the corpus callosum. The right lung had a 0.8 cm diameter granuloma. Histopathology of the cerebral lesion revealed pyogranulomatous meningoencephalitis with myriad intralesional ameba trophozoites and rare cysts. The lung granuloma had intralesional degenerate yeast spherules (*Coccidioides* sp.). Fresh brain was submitted to the CDC's FLIA lab for multiplex free-living ameba real-time PCR and detected *Balamuthia mandrillaris* DNA at a high burden. DNA was amplified at a novel mitochondrial-based genotyping locus and sequenced. The sequence was compared to human isolates of *B. mandrillaris* and shown to be identical to four human cases of amebic encephalitis: 1998 in Australia, 1999 in California, 2000 in New York, and 2010 in Arizona.

Conclusion: *Balamuthia mandrillaris*, first reported in a mandrill in 1986, has since occurred in humans and animals, most commonly in Old World nonhuman primates.

D-21: INFLAMMATORY BOWEL-DISEASE-LIKE IN EMYS ORBICULARIS (EUROPEAN POND TURTLE).

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Introduction: Inflammatory Bowel Disease (IBD) is an idiopathic, multifactorial entity of mammals characterized by relapses of chronic enteritis. Histological features of IBD include mucosal changes, lamina propria leukocytosis, fibrosis, and lymphangiectasia.

Objective: Describe a new histological pattern of enteritis in Emys orbicularis (EO).

Methods: A wild caught EO, housed with two conspecifics in a rescue center tank, developed anorexia, dysphagia and floating problems (EO-A). Similar problems were simultaneously observed in the cohabitants (EO-B and EO-C). All the animals died within few weeks, despite intensive veterinary care. Full necropsy and intestinal and environmental microbiology were performed.

Results: Grossly, 3/3 animals had enteritis. Additionally, gelatinous celomic ascites and anasarca were observed in one individual (EO-A), stomatitis and pneumonia in the second (EO-B), and septicemic cutaneous ulcerative disease in the third (EO-C). Gastroenteritis/enteritis was histologically confirmed in 3/3 EOs. EO-A gastroenteritis was characterized by lamina propria infiltration of lymphocytes and plasma cells, fewer heterophils and macrophages, lymphangiectasia, lymphocytes exocytosis, and mucosal remodeling, with no detectable bacteria. No fibrosis was observed. Necrotizing and ulcerative enteritis, with minimal heterophilic inflammation, and intralesional yeast or bacteria were observed in EO-B and EO-C, respectively. Microbiology yielded Candida albicans in EO-B and Aeromonas hydrophila (Ah) in the others. Environment was negative for Ah.

Conclusion: Although gastroenteritis/enteritis was observed in all EOs, different pathological patterns were present. While EO-B and EO-C findings support immune-compromise, histology in EO-A suggest an immune system hyperactivation. EO-A pathological findings parallel those of other mammals' IBD. Additional studies are needed to study its pathogenesis.

D-22: GANGLIONEUROBLASTOMA IN THE ADRENAL GLAND OF A DOG

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Background: A 9-year-old female, spayed, Beagle presented for vomiting and a right cranial abdominal mass. Abdominal ultrasound revealed a heterechoic mass enlarging and replacing the right adrenal gland. Computed tomography revealed invasion into the vena cava, extension to the cardiac silhouette, and dorsal extension to, but not into, the body wall. The right adrenal mass was removed and submitted for histopathology.

Objective: To evaluate the histologic origin of an adrenal neoplasm.

Methods: The submitted mass was examined histologically. Immunohistochemistry for PGP9.5, chromogranin A, synaptophysin, and MAP2 was employed.

Results: Histologic examination revealed a large, unencapsulated, invasive mass composed of neoplastic ganglion cells, within an abundant, wispy eosinophilic stroma containing a moderate population of indistinct spindle cells arranged in streams. Ganglion cells were angular, with peripheral basophilia, and eccentric, oval nuclei, with open chromatin, and a single prominent magenta nucleolus. In one examined section was a densely cellular aggregate of small, primitive polygonal cells with indistinct cell borders, a moderate amount of eosinophilic cytoplasm, and irregularly round to oval, central nuclei. Immunohistochemistry revealed immunolabelling of both cell populations with MAP2 and synaptophysin, moderate immunolabelling of ganglion cells to PGP9.5, and strong immunolabelling of primitive cells to chromogranin A. Scattered remnant adrenal medulla and cortex was noted in and around the mass.

Conclusions: The histologic features were consistent with a ganglioneuroblastoma. These are rare neuroblastic tumors of the peripheral nervous system, originating from

embryonal cells of the sympathetic nervous system. There have been no reports of an adrenal ganglioneuroblastoma in a dog.

D-23: PRIMUM ATRIAL SEPTAL DEFECT WITH ANTERIOR MITRAL VALVE CLEFT IN A YORKSHIRE-LANDRACE CROSS PIG

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A 59 kg., male, castrated Yorkshire-Landrace cross pig was sedated for a cardiovascular study at our institution. During the study, we conducted echocardiography for research purposes and noticed the following unexpected morphological and functional abnormalities: Echocardiography showed primum atrial septal defect (ASD), and anterior mitral cleft with eccentric mitral regurgitation with color Doppler imaging. The left-to-right atrial shunt was apparent. On autopsy, the primum ASD and anterior mitral valve cleft were identified. The lateral half of the anterior mitral leaflet was continuous to the tricuspid leaflet. There was no connection between the lateral half and medial half of the anterior mitral valve. These defects are hereditary congenital cardiac anomalies frequently seen in pigs. The findings were communicated with the vendor and they were advised to cull this breeding line to prevent interference with future cardiovascular studies utilizing pigs in our institution.

Diagnostic Pathology Focused Scientific Poster Session C

D-01: RETROSPECTIVE PATHOLOGY SURVEY OF AMPHIBIANS RECEIVED AT THE TEXAS A&M SYSTEM

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Background: Knowledge of amphibian disease is limited compared to other vertebrates. Many extant amphibians are globally threatened and face extinction due to various anthropogenic and natural threats. Amphibian *ex situ* conservation programs are essential.

Objective and Methods: To understand and delineate the main health issues of amphibians submitted to the Texas A&M System, we queried the databases based upon defined selection criteria. Epidemiologic and biologic data, necropsy reports, photographic material, and ancillary diagnostics results were retrieved and further analyzed, with special emphasis on type and causes of death and/or major pathologic findings.

Results: A total of 470 amphibian cases were retrieved, including 444 Anura (138 frogs [38 species], 306 toads [8 species]) and 26 Caudata (21 salamanders [5 species], 5

newts [4 species]). The Houston toad (*Bufo houstonensis*; n=280), the African clawed frog (*Xenopus laevis*; n=16) and the Barton Springs salamander (*Eurycea sosorum*; n=15) were overrepresented. Nearly all animals came from captive settings and were adults. There was no sex bias in the sample set. The main causes of spontaneous death or major pathologic findings (CD-MPFs) leading to euthanasia were infectious (192/252; 76%), involving mycobacteriosis (52/192; 27%) and chlamydiosis (43/192; 22%), mainly in Houston toads, and mycosis (19/192; 10%); neoplastic (29/252; 11%); and skeletal deformities (12/252%; 5%). In 218 (46%) cases, no CD-MPFs were identified.

Conclusions: Our findings suggest infectious diseases are the main threat to captive amphibians in Texas. These results may be of value to diagnosticians and the personnel involved in captive amphibian rearing programs.

D-02: FIVE TOUCANS FOUND DEAD WITH GASTROINTESTINAL PARASITISM.

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Wildlife animals living freely and in captivity play an important role in the epidemiology of several diseases. There are few studies related to gastrointestinal parasites of non-domestic birds and all of them are based on fecal examination. To our knowledge this is the first pathological and parasitological report of gastrointestinal lesions caused by nematodes in toucans. Cases: The five captive toucans were from a wildlife rescue center. Four were brought together with a history of 11/54 dying suddenly. In three a complete necropsy was performed. For four case parenchymal tissues (2.0 to 2.50 cms) including intestine were submitted. Three months later, another necropsy was performed. Signalment: There were 4 Ramphastos sulfuratus and one R. swansoni. All reported as adults, with 2 females, 2 males and one gender not reported. Macroscopic: In general, the intestinal tract was dilated with watery content and yellow to red mucosa. Two mucosal scrapings were submitted for parasitological analysis, and reported as Capillaria sp. In addition, in one case the proventriculus and ventriculus had free parasites classified as Cheilospirura hamulosa. Histopathology: Mucosal necrosis of proventriculus and gizzard with nematodes mixed inflammatory response were seen. Regarding the intestine, there were free transversal and longitudinal sections of nematodes; present also in the mucosa with mixed inflammatory response. Despite the fact, that parasitic species found in this paper have already been reported in the literature, no one had described C. hamulosa in gastric tissue of toucans. Moreover, the intestinal histopathological changes associated had not been reported previously.

D-03: SYSTEMIC FERLAVIRAL INFECTION IN A BLACK-TAILED RATTLESNAKE (CROTALUS MOLOSSUS OAXACUS)

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Ophidian Paramyxoviruses (genus *Ferlavirus*) may cause epizootics or individual deaths in captive reptile collections worldwide, mainly in viperids and crotalids. Clinical signs may include respiratory noises and exudate, abnormal posture, head tremors,

anorexia, and regurgitation. A five-year-old male castrated black-tailed rattlesnake (*Crotalus molossus oaxacus*) with a history of sudden death and a three days prior regurgitation event was submitted for necropsy. At necropsy, the main gross findings were mucoid foamy fluid in the lungs, oral petechiae and intracoleomic fluid. Microscopically, the main findings were: proliferative pneumonia with faveolar exudate with mixed Gram-negative bacteria and hemorrhage; heterophilic and lymphocytic stomatitis; hyperplastic enteritis; lymphoplasmacytic pancreatitis with necrosis and fibrosis; lymphocytic conjunctivitis; and periocular adenitis. A systemic viral infection was suspected. Polymerase chain reaction and direct sequencing analysis identified genogroup C *Ferlavirus* on lung tissue. Paramyxoviral infection has been reported in different *Crotalus* species, however, it is not reported in black-tailed rattlesnakes. With the increasing captive snake population, the prevalence of ferlaviral infection in Texas is uncertain. The epidemiology of ferlaviruses in free-ranging snakes in Texas is largely unknown. Here we describe the pathologic findings in a confirmed case of ferlaviral infection among snake cases that were recently submitted to the Texas A&M Veterinary Medical Diagnostic Laboratory. These findings may be of interest to diagnosticians and personnel dealing with snake rearing.

D-04: DISSEMINATED SARCOCYSTOSIS DUE TO SARCOCYSTIS MEISCHERIANA IN A SLAUGHTERED PIG

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Background: Sarcocystis infections are common in pigs worldwide. However, to the best of our knowledge this has not been reported in Grenada. The species affecting pigs as intermediate hosts include *Sarcocystis meischeriana*, *Sarcocystis suihominis* and *Sarcocystis porcifelis*; whose definitive hosts are dogs, humans and cats, respectively.

Case description: An approximately 8-month old pig was presented to a local abattoir for slaughter. Due to multiple white lesions affecting the meat, the butcher contacted the Veterinary Department in the Ministry of Agriculture for a second opinion and further action.Consequently, the meat, representing half of the carcass was submitted to the necropsy diagnostic laboratory in the School of Veterinary Medicine at St. George's University for further evaluation.

Results: Grossly, all superficial and deep muscle groups contained hundreds to thousands of small white, spindle-shaped, rice-grain-like, soft, macrocysts (3mm x 2mm x 1mm) in parallel streaks on the surface and extending deep through the musculature. Histopathology revealed moderate multifocal granulomatous and eosinophilic myositis with intralesional degenerated parasites. Sarcocysts with bradyzoites were observed in all the muscle samples. Nucleotide sequence analysis of the 18S RNA gene showed 100% identity to *S. meischeriana* in the GenBank.

Conclusion: This case confirms the presence of *S. meischeriana* in a slaughtered pig. This report also underscores the need for a standardized meat inspection protocol on the island, for the protection of public health. Further studies on *Sarcocystis* spp in Grenada will be valuable to provide insight into the epidemiology and possible genetic predisposition of Grenadian pigs to this protozoan parasite.

D-05: MUCINOUS ADENOCARCINOMA OF THE ILEOCECAL JUNCTION WITH INTRAPERITONEAL METASTASES IN A RHESUS MACAQUE (MACACCA MULATTA)

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A 21-year-old female rhesus macaque (*Macacca mulatta*) presented with a clinical history of decreased activity and weight loss. Abnormal clinicopathologic findings included severe regenerative anemia, mild leukocytosis, and elevated alkaline phosphatase. Radiographs demonstrated an abnormal cardiac silhouette +/- pleural effusion, while abdominal ultrasonography demonstrated peritoneal effusion with abnormal serosal surfaces found on the liver capsule and small intestine. Due to the advanced age of the animal and poor prognosis, the animal was euthanized and submitted to necropsy. Gross findings at necropsy included: serosanguinous unilateral pleural and peritoneal effusion; a 7cm diameter, poorly-defined, gelatinous, tan-red mass obscuring the ileocecal junction; diffusely gelatinous and thickened omentum; multiple coalescing white plaques randomly distributed on the capsular surface of all liver lobes; and a focal mass similar to the ileocecal mass attached to the lower right abdominal wall. Histologically, the ileocecal junction contained a poorly circumscribed, unencapsulated mass of polygonal cells forming tubules and acini that were frequently dilated with abundant amorphous to fibrillar amphophilic material and collagen extending from the mucosa to the serosa. Alcian-blue stain confirmed the presence of mucin lakes within the primary neoplasm (ileocecal junction) and sites of intraperitoneal metastasis (liver capsule, omentum, abdominal wall). In humans, mucinous adenocarcinomas are a very rare subtype of adenocarcinoma originating from goblet cells and carry a poorer prognosis, due to their higher rate of local recurrence. To the authors' knowledge, this is the first description of intraperitoneal metastasis from an ileocecal adenocarcinoma in rhesus macaques.

D-06: THE USE OF PCR TO IDENTIFY PYTHIUM INDISIOSUM IN FORMALIN FIXED TISSUE SECTIONS

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Pythium insidiosum is an oomycete that is most commonly pathogenic to dogs and horses and is less commonly pathogenic to humans and other species. Infected

animals typically develop either the cutaneous or gastrointestinal form of pythiosis, and the prognosis is usually guarded to poor. This organism is not a true fungus since it lacks chitin within the cell wall and lacks ergosterol in the cell membrane. Due to these characteristics, antifungal treatment is usually unsuccessful. Identification of the organism can be difficult as the hyphae of *P. insidiosum* do not stain well with H&E, and even when visualized, cannot always be definitively distinguished from some other fungal or fungal-like organisms (such as *Lagenidium sp.*) based on morphology alone. In this study, FFPE sections of various tissues from 26 dogs with lesions consistent with *P. insidiosum* were examined histologically for the presence of hyphae. A specific *Pythium* sp. PCR was developed and used to identify the pathogen from FFPE tissue scrolls. Currently, 13 out of 25 cases have identification of hyphae on H&E and 13 of 24 on GMS. Of the 19 cases tested using PCR, 17 have been positive for *P. insidiosum* (13 of these 19 had hyphae identified histologically). Therefore, PCR was able to identify *Pythium* in 89.5% of cases, whereas histopathology was only able to identify hyphae most consistent with *Pythium sp.* in 54.2% of the cases. In conclusion, the specific *Pythium* PCR is more sensitive than histopathology alone and can be used to more accurately direct therapy.

D-07: RETROPERITONEAL EXTRAOSSEOUS PERIPHERAL PRIMITIVE NEUROECTODERMAL TUMOR IN A FORMOSAN SEROW (CAPRICORNIS SWINHOEI)

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Background: Peripheral primitive neuroectodermal tumors (PNETs) are a group of highly malignant tumors composed of small round cells, which typically arise from multipotent progenitor cells derived from neuroectoderm. They usually reveal varying degrees of neuronal differentiation and almost invariable EWSR 1 gene rearrangement, which is a consequence of a reciprocal t(11;22)(q24;q12) chromosomal translocation. Extraosseous PNETs (pPNETs) are more aggressive with a worse prognosis comparing to osseous PNETs, and local recurrence and metastasis to regional lymph nodes, lung, liver, and bone frequently occur.

Case description: A 10-y-old female captive Formosan serow in Taipei Zoo was inactive, and blood examination revealed increased serum creatinine, urea, and potassium. The animal did not improve with supportive treatment and was found dead the next day. Necropsy, histopathologic examination, and a series of immunohistochemistric (IHC) stainings were performed to diagnose the case.

Results: At necropsy, multiple irregularly-shaped masses were noted on both ureters and extended to peripheral soft tissues and organs in retroperitoneal space, including the left adrenal gland, urinary bladder, and uterus. Based on the cellular morphology/arrangement, results of IHC stainings and anatomic location, the diagnosis

of PNET with metastasis to liver and involvements of left adrenal gland, urinary bladder and uterus was made.

Conclusions: The purpose of this case report is to present gross, histological and immunohistochemical characteristics of an extraosseous pPNET in a Formosan serow.

D-08: NATURALLY OCCURRING DESMOPLASTIC MELANOMA IN A DOG

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An 11-year-old castrated male miniature Schnauzer weighing 8 kg was presented with multiple mass on humerus of both forelimbs. Grossly the masses were multiple, had clear margin and separated from soft tissue measuring in the range of 15 mm x 19 mm to 40 mm x 53 mm. Desmoplastic melanoma is an uncommon tumor in a dog with only one case reported till date and very rarely reported even in human medicine. The complete pathobiology of the lesion is opaque in veterinary medicine leading to challenging diagnosis. Our objective was to identify the neoplasm as a desmoplastic melanoma with histopathology and immunohistochemistry. Fine-needle aspiration cytology smears showed large spindled shaped cells with the high nuclear-cytoplasmic ratio. Pathology of the mass showed interlacing bundles of fibroblast-like cells, highly pleomorphic multinuclear cells, occasional atypical spindle cells separated by fibro-collagenous stroma and lymphocyte aggregates admixed with abundant collagen. Tumor cells were immunohistochemically diffusely positive for S100a, a strong marker for DM. However, desmin was found negative differentiating the lesion with other sarcomas. Thus, here we report a rare, uncommon variant of melanoma in a dog.

D-09: RNA AND PROTEIN EXPRESSION OF P-GLYCOPROTEIN AND APOPTOSIS-RELATED FACTROS IN CANINE HEPATOCELLULAR CARCINOMA

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Dysregulation of apoptosis is one of the most common feature in various cancers. P-glycoprotein, which functions as efflux pump, carries various foreign materials out of the cell, and also, can induce apoptosis. TRAIL (TNF-related apoptosis-inducing ligand) is an inducer of apoptosis and expressed in diverse normal tissues. FADD (Fas-associated protein with death domain) is an adaptor protein that deliver apoptotic signals of TRAIL, to downstream molecules. Overexpression of P-glycoprotein and impairment of apoptosis in hepatocellular carcinoma is reported in many studies, however, involving molecules to apoptosis in view of tumor progression are not fully identified. The aim of the present study is to investigate RNA and protein expression of P-glycoprotein and apoptosis-related factors in canine hepatocellular carcinoma. Five of hepatocellular carcinoma and six of normal liver tissues were included in the study. In situ hybridization was conducted using RNAscope, for P-glycoprotein and TNFRSF10B. Immunohistochemistry was applied for P-glycoprotein, TRAIL (TNF-related apoptosis-inducing ligand), and FADD (Fas-associated protein with death domain). Mann-Whitney U test and bivariate Pearson corelation test was performed for statistical analysis. Protein expression level of P-glycoprotein was higher in hepatocellular carcinoma than

in normal liver (P=0.017), and TRAIL was downregulated in hepatocellular carcinoma (P=0.017). Expression of protein or RNA was not significantly different between normal liver and hepatocellular cacinoma for other markers. RNA expression levels of P-glycoprotein and TNFRSF10B had positive correlation (P=0.001). These results implies that loss of TRAIL expression may suppress apoptosis and influence tumor progression independently from P-glycoprotein. However, larger scale study is necessary.

D-10: THYMIC LYMPHOSARCOMA AND AMOEBIC TYPHLITIS AND HEPATITIS IN A BABOON (PAPIO SPP.)

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Background: Baboons (Papio spp.) are natural hosts for protozoal species that are often associated with significant morbidity and mortality. Entamoeba spp. are occasional findings in fecal and histopathological examinations of the digestive system in baboons, however, the extra-intestinal dissemination and pathogenic behavior is a rare finding. We describe a case of thymic lymphoma with protozoal (presumptive Entamoeba spp) typhlitis and hepatitis in a baboon.

Methods: A 3-year-old male baboon presented with a history of gingivitis, right side lung consolidation, and swollen lymph nodes. Necropsy revealed a multinodular mass that filled 75% of the thorax. There were three pale, 1mm diameter, foci in the liver.

Results: The thymus was effaced and replaced by sheets of small lymphocytes consistent with lymphoma. Similar neoplastic cells effaced the hilar and mesenteric lymph nodes, expanded the hepatic and splenic sinusoids, and filled blood vessels in various tissues. Multifocally, the cecal mucosa was ulcerated and the submucosa expanded by lymphocytes, plasma cells, macrophages and neutrophils, admixed with many 12-15 micron diameter protozoal organisms (presumptive Entamoeba spp). There were multifocal hepatic granulomas with intralesional protozoa (presumptive Entamoeba spp.).

Conclusion: With the concurrence of multiple pathologies, this baboon was an ideal host for the extra-intestinal dissemination of protozoal organism (presumptive Entamoeba spp), possibly due to a compromised immune system from a pre-existing thymic lymphoma.

D-11: HISTOPATHOLOGICAL CHARACTERIZATION OF TOOTH CYCLE IN A BABOON (PAPIO SPP.)

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Background: Oral health of non-human primates has received increased focus in animal care, resulting in more biopsy submissions to our pathology service. There is currently limited knowledge regarding the normal tooth cycle of baboons (*Papio spp.),* which impacts the histopathological evaluation and diagnoses of dental diseases.

Methods: A 2-year-old female baboon presented in the clinic with loose upper incisors (I1, left and right), significant gingival recession and reddening, and rotation of the upper left (I1) incisor. The incisors were removed and submitted for histopathological evaluation. The teeth were processed by decalcification and H&E staining and reviewed by board certified pathologists.

Results: Histopathological evaluation revealed mild pulpitis with external and internal tooth resorption. The resorption was predominantly physiologic due to the eruption of the permanent teeth, yet there was minor pathologic resorption present. The case provided valuable histopathologic information regarding the pathophysiology of tooth development and associated conditions in baboons.

Conclusion: Understanding the morphological features of normal tooth cycle histology is critical in distinguishing physiologic versus pathologic processes when evaluating teeth. This case report provides valuable insights into normal tooth histology as well as associated pathology in baboons which can be a useful resource to clinical veterinarians and diagnostic pathologists.

D-12: BALL PYTHON NIDOVIRUS IN TWO PYTHON SPECIES IN A CAPTIVE COLLECTION

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Nidoviruses have recently been discovered to cause respiratory disease in various species of reptiles and chelonians. One Burmese python (*Python bivittatus*) and one reticulated python (*Python reticulatus*) in a private collection became ill within three weeks of each other, with the first python developing regurgitation and stomatitis and the second with respiratory signs. Upon necropsy, the lungs of both snakes were dark red, thickened, and heavy with copious amounts of tan mucus within the lungs and trachea. Histology revealed moderate to marked proliferative pneumonia with large amounts of edema and mucus within faveoli. The Burmese python had colonies of bacteria within air spaces, but minimal inflammation. The reticulated python had more severe heterophilic inflammation within the interstitium, but no visible bacteria. Stomatitis, glossitis, tracheitis, enteritis, and colitis were additional findings. PCR for nidovirus and direct sequencing returned ball python nidovirus in both pythons. Whether ball python nidovirus is capable of crossing species warrants further investigation. To the author’s knowledge, this is the first report of a nidovirus in a reticulated python.

D-13: INTRAMEDULLARY VASCULAR ECTASIA OF THE SPINAL CORD IN A DOG

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A 6-year-old male castrated Pekingese dog was referred to the Auburn University Veterinary Teaching Hospital for a 1-week history of painful back. Ataxic and non-ambulatory paraparesis of pelvic limbs were observed during the neurologic examination. Neurologic disease was localized to the T3-L3 spinal cord segments. Magnetic resonance imaging showed large intramedullary and intradural areas of hyperintensity and hypointensity in the lower cervical region and upper thoracic spinal cord. Differential diagnosis based on MRI findings was a neoplastic process. On gross examination, the thoracic spinal cord was enlarged by a focally extensive area of hemorrhage that compressed the surrounding white matter. Histology of the thoracic spinal cord consisted of a markedly dilated thin-walled blood-filled cavity lined by a single layer of CD31-positive flattened endothelial cells. The adjacent, compressed spinal cord contained scattered spheroids and multifocal dilated myelin sheaths with digestion chamber formation. The central canal was filled with blood and fibrin. The morphologic diagnosis was intramedullary vascular ectasia of the spinal cord.

Spinal vascular anomalies are rare in dogs with only a few case reports available. Reported vascular anomalies in dogs include cavernous malformations (hamartoma and cavernous angioma), arteriovenous malformations, and undetermined intramedullary vascular hemorrhagic anomalies. The classification of these vascular malformations is based on the histologic description of the involved vessels, the involvement of surrounding parenchyma, and the origin of the malformation. This case report describes a spinal cord vascular lesion that caused progressive spinal cord injury and myelopathy.

D-14: MULTIPLE MYELOMA WITH ABERRANT CD3 EXPRESSION IN AN AMAZON PARROT

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Background: Aberrant CD3 expression in myeloma and B-cell neoplasms is a rare event reported in humans, dogs, and cats. Myeloproliferative disorders are uncommon in avian species. To the best of the authors' knowledge, multiple myeloma has not been reported in psittacines.

Objective: Describe the clinicopathological findings in a red-lored Amazon parrot (*Amazona autumnalis*) with multiple myeloma.

Case Report: A 20-year-old, male intact, red-lored Amazon parrot (*Amazona autumnalis*) was presented for a two-week history of weakness. Radiographs and ultrasound revealed coelomic distention, increased pulmonary parenchymal opacity, renomegaly, dilated intestines, and a thickened ventricular wall. Complete blood count revealed moderate anemia (28%) and intermediate to large lymphocytes with immature

chromatin. Immunocytochemistry on peripheral blood revealed that the suspected circulating neoplastic cells were CD3+ and occasionally expressed MUM1. Biochemistry showed hyperphosphatemia (6.8mg/dL), hyperproteinemia (13.6g/L), analbuminemia (0g/dL), and severe hyperglobulinemia (13.6g/dL). Agarose gel plasma protein electrophoresis documented the lack of an albumin band and a monoclonal band which, on reduced LDS-PAGE electrophoresis, resolved as 60KD and ~25KD bands consistent with IgY heavy chain and light chain. Presumptive diagnosis was multiple myeloma; owners elected euthanasia. No gross lesions were observed on postmortem examination. Bone marrow smears revealed 17.4% plasma cells and an additional 24% large immature cells with occasional plasmacytoid features. Histopathologic findings included aggregates of neoplastic plasma cells in bone marrow, spleen, kidney, liver, gastrointestinal tract, and brain. Neoplastic cells were MUM1+, CD3+, CD79a-, PAX5-, CD20-.

Conclusions: This case was a productive CD3+ multiple myeloma.

D-15: SEGMENTAL NECROTIZING MYELITIS IN A CAT WITH TOXOPLASMOSIS

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A 14-year-old male castrated domestic shorthair cat was referred to the Ontario Veterinary College Health Sciences Centre for evaluation of acute non-ambulatory paraparesis of the hindlimbs. MRI of the thoracolumbar spine identified a focal intramedullary lesion at T8 with extensive T2-weighted hyperintensity from T4 to L1 suggestive of meningomyelitis. Cerebrospinal fluid (CSF) analysis showed marked mixed, predominantly mononuclear, inflammation with a marked elevation in total protein (>16.70 g/L). Pooled whole blood and CSF qPCR were negative for Feline Coronavirus, *Toxoplasma gondii* and Feline Leukemia virus (PCR Laboratory, University of California, Davis). Serology was negative for Cryptococcus antigen (AHDC, Cornell University), while low levels of antibodies (IgG 1:64, IgM Negative) against *Toxoplasma gondii* were detected on ELISA (VDL, Colorado State University). While awaiting these results, the patient was treated empirically with clindamycin and an anti-inflammatory dose of corticosteroids. Due to a lack of response to therapy, the patient was euthanized and submitted for necropsy. Histopathological assessment of the spinal cord identified regionally extensive necrotizing myelitis of gray and white matter with rare protozoal cysts, and mild lymphoplasmacytic meningitis. On immunohistochemistry, there were rare foci of immunoreactivity for Toxoplasma antigen. The serology results and absence of other systemic signs in this patient suggest reactivated *T. gondii* infection. The ante-mortem diagnostic work-up and post-mortem evaluation did not reveal an underlying cause for immunosuppression. The focal spinal cord lesion, poor sensitivity of PCR, and equivocal serologic results in this case highlight the difficulty associated with obtaining an antemortem diagnosis of toxoplasmosis.

D-16: CUTANEOUS ROUND CELL TUMORS IN GOATS: RETROSPECTIVE STUDY OF FIVE MAST CELL TUMORS AND TWO HISTIOCYTOMAS

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Over a 29-year period, 127 distinct tumors from 124 goats were diagnosed at the Veterinary Diagnostic Laboratory at Oregon State University (1987 to 2015). Detailed records were available for 89 cases. Of these cases, five were diagnosed as cutaneous mast cell tumors (MCT) and two were diagnosed as cutaneous histiocytomas. Breeds with cutaneous MCT included Nubian (n=2), Pygmy (n=1), and Saanen (n=1); breed was not reported in one case. Two were intact males, two were females, and sex was not reported in one case. Ages ranged from 1 month to 3 years. Cutaneous histiocytoma was diagnosed in a 14-month-old Boer goat and a 7-month-old mixed-breed goat. Metachromatic granules were visualized in only one of the five putative caprine MCT using Giemsa stain, whereas immunohistochemistry for CD117/KIT stained cytoplasmic granules in * DELET? mast cells* all mast cell tumors. KIT staining patterns were similar to those observed in canine MCT. Putative histiocytomas occurred in young goats and had not recurred 5 or 3 years after biopsy, respectively. Follow-up information was not available for those diagnosed with cutaneous MCT. CD117/KIT IHC was required to reliably differentiate MCT from histiocytoma in goats.

D-17: HIGH-THROUGHPUT SEQUENCING (HTS) AS TOOL FOR MOLECULAR DETECTION OF A NOVEL CHAPPARVOVIRUS IN WILD ANIMALS' FECES IN BRAZIL

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Background: Chapparvovirus is a new virus genus proposed that belongs to the *Parvoviridae* family, group involved in many clinical and subclinical animal infections. Chapparvoviruses exhibit a wide host range, infecting birds and mammals.

Objective: HTS constitutes a robust tool for the identification of new virus species in Cerrado fauna and was applied in the present study.

Methods: Feces samples of *Psittacara leucophtalmus*, *Didelphis aurita*, *Sapajus libidinosus* and *Galictis cuja* were collected from the Veterinary Hospital, University of Brasilia. The sequencing was performed using Illumina HiSeq. The reads were trimmed and the contigs assembled.

Results: The contigs analysis enabled the identification of a novel parvovirus. This new virus showed closer sequence identity to *Desmodus rotundus* parvovirus, a Chapparvovirus genus member, with 35,2% NS1 amino acid sequence identity. This protein is used as demarcation criteria in *Parvoviridae*. The genomic sequence found was 4434bp. The 5′- and 3′-ends showed palindromic sequences that are responsible

for the folding of the parvoviruses' terminal hairpins. Two main ORFs were identified (NS1 and VP1), occurring a large overlap between them of 62pb. The parvoviruses' conserved motifs (GPXNTGKS) and (HVH) were found coded in the genome. This new virus lacks the phospholipase A2 motif. The phylogenetic trees of NS1 protein and virus genome sustain with high bootstrap values that this genus is monophyletic.

Conclusions: Although chapparvoviruses are a highly divergent group of parvoviruses, the according to the genetic distance-based criteria, the new virus identified here belongs to a novel species of Chapparvovirus.

D-18: NEURAL INVASION OF BASOSQUAMOUS CARCINOMA IN A DOMESTIC DOG AS EVIDENCE OF NEURAL METASTASIS

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Studies in humans have reported the presence of nerve fibers in various types of tumors, in addition to visualizing tumor cells invading nerve fascicles in pancreatic, prostate, breast, colonic and skin tumors. This process is defined as perineural invasion and is believed to be one more pathway of invasion and metastasis; besides, it has been associated with poor prognosis and high recurrence rates. Histological sections of a skin biopsy of a 10 year pitbull bitch were examined, which showed neoplastic lobules of undifferentiated basaloid cells with central abrupt formation of keratinocytes, corresponding to a basosquamous carcinoma; tumor cells were found invading nervous fascicles. The origin of tumor cells and neural tissue was confirmed by immunohistochemistry. After the surgery, the patient did not receive any treatment, but until now she is under medical supervision due to the human-published background, in which recurrence and poor prognosis are reported in patients with neural tumor invasion. To our knowledge, this characteristic of malignancy has not been described in dogs. This finding opens another window to the investigation and knowledge of the neurobiology of cancer and consolidates the relationship of the physiopathogenesis of spontaneous tumors in both species (humans and dogs). Furthermore, it raises questions about the participation of the nervous system in the progression and malignization of tumors, which allows to deepen in the unknown biology of cancer, perhaps allowing in the future to establish possible novel therapeutic targets.

D-19: A METAGENOMIC APPROACH FOR VIRAL DIAGNOSIS IN WILD ANIMALS FROM BRAZIL: KNOWN AND UNKNOWN VIRUSES DETECTED

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Background: The Brazilian fauna shows great diversity and can be a potential viral reservoir. Epidemiological surveillance with focus in native fauna is a way to identify possible threats to humans and non-humans animals that are hidden in reservoirs. Moreover, the Brazilian wild animal virome is unknown. Based on this scenario, high-throughput sequencing constitutes a robust tool for the identification and

characterization of known and unknown virus species in this environment as well as for molecular diagnostics.

Objective: Feces samples from birds (*Psittacara leucophthalmus*, *Amazona aestiva* and *Sicalis flaveola*) and mammals (*Didelphis aurita*, *Sapajus libidinosus* and *Galictis cuja*) from Cerrado biome were tested at the Veterinary Hospital, University of Brasilia for viruses species.

Methods: Viral nucleic acid was extracted, and random PCR amplification were performed. Products were sequenced by Illumina HiSeq 2500 platform and reads, *de novo* assembled using Megahit v1.1.3 and SPAdes 3.13.0. Blastn and tblastx searches against the RefSeq Virus database were conducted.

Results: Most viral contigs analyzed were closely related to bacteriophages sequences. Novel archaeal viruses of the *Smacoviridae* family were detected. Moreover, sequences of *Adenoviridae*, *Anelloviridae*, *Circoviridae*, *Caliciviridae* and *Parvoviridae* families were identified. Complete and nearly complete genomes of known adenoviruses, anelloviruses, circoviruses and parvoviruses were obtained as well as putative novel species with potential pathogenic activity.

Conclusions: Taken together, results shown that metagenomics led to the identification of previously unknown viruses in Brazilian fauna.

D-20: PROGNOSTIC VALUE OF LYSYL-OXIDASE (LOX) EXPRESSION IN CANINE CUTANEOUS MAST CELL TUMORS

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Background: Mast cell tumors (MCTs) comprise almost one fourth of all malignant neoplasms in dogs. Due to their unpredictable biological behavior, these tumors have caused several therapeutic frustrations, leading to investigation regarding prognostic markers. Lysyl-oxidase (LOX) is an enzyme that promotes extracellular matrix stability through collagen cross-linking and contributes to cell migration, angiogenesis and epithelial-mesenchymal transition. LOX expression positively correlates with poor prognoses in human cancers.

Objective: To characterize the immunohistochemical expression of LOX in MCT samples and compare it with histopathological grading and post-surgical survival.

Methods: Twenty-eight canine cutaneous MCTs cases (9 high-grade and 19 low-grade) were submitted to immunohistochemistry for LOX detection. The percentages of positive nuclei and cytoplasms of neoplastic mast cells were quantified in 5 high-power

fields per case and the results compared with the two-tier histopathological grading and survival.

Results: All samples were positive for LOX, with variable percentages of cytoplasmic and nuclear positivity. The average percentages for nuclear and cytoplasmic positivity were: 56.96% ± 4.78% and 56.70% ± 6.16%, for low-grade tumors; and 72.62% ± 7.47% and 78.48% ± 4.55% for high-grade tumors, respectively. Cytoplasmic positivity was significantly higher in high grade MCTs ($p = 0.031$). A cutoff value of 78.21% for cytoplasmic positivity was established using a ROC Curve and survival analysis revealed that dogs with higher percentage of positive cells had significantly shorter survival ($p = 0.0219$, median survival = 1119 days). **Conclusion:** High expression of cytoplasmic LOX in neoplastic mast cells is an indicator of poor prognosis for canine cutaneous MCTs.

D-21: BOVINE AMNIOTIC DYSTROPHIC OXALOSIS ASSOCIATED WITH PLACENTAL INSUFFICIENCY AND STILLBIRTH

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This is a case of a stillborn heifer associated with placental insufficiency and unusual changes in the amnion. Placental insufficiency was evidenced by adventitial placentation, patchy intercotyledonary chorioallantoic fibrosis and history of multiple vaginal prolapses in the cow. The most striking finding was the development of marked diffuse amniotic hyperemia and large foci of necrosis infiltrated by fine crystalline material. With the use of X-ray diffraction and energy dispersive X-ray spectroscopy (EDS) in SEM, this material was confirmed to be whewellite (calcium oxalate monohydrate). To our knowledge, this is the first report of whewellite formation (oxalosis) within the placenta of any animal. While renal oxalosis is a well-known, obscure finding in aborted, stillborn, and neonatal calves, the calf in this case did not have crystals in any organ. No evidence for primary hyperoxaluria or fungal infection (Aspergillus sp.) was found to account for the infiltrate.

D-22: SMOOTH MUSCLE HAMARTOMA FORMING A NASAL MASS IN A DOG

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Background: A 3-year-old, female spayed pug-mixed breed dog was presented for a 2-year history of progessive nasal congestion and facial deformity. Nasopharyngoscopy, endoscopic biopsy, computed tomography (CT) scan and complete surgical resection with histopathology were performed.

Objective: Description of the clinical, radiographic, and histopathologic features of a morphologically benign lesion not previously reported in the upper respiratory tract of dogs.

Methods: Biopsy specimens were collected endoscopically and via surgical resection, fixed in 10% neutral buffered formalin and processed routinely for histopathology and

immunohistochemicstry for alpha smooth muscle actin. All sections were examined using light microscopy. Clinical records including CT scans and interpretation, surgical reports and physical examinations were reviewed.

Results: revealed a friable, polypoid mass of the right nasal conchae that occluded the right nasal passage. CT revealed a space occupying mass of the right nasal cavity extending to the cribriform plate and into the right choana with minimal lysis of the adjacent calvarium and scattered mineralization of the mass. In endoscopic and excisional biopsies the mass was comprised of bundles of well differentiated smooth muscle cells that were strongly immunoreactive for alpha smooth muscle actin. There was moderate mixed submucosal inflammation.

Conclusion: The mass was diagnosed as a smooth muscle hamartoma with no infiltration of the associated bone and with a secondary, moderate rhinitis. There is no reported recurrence or complications 1-year post excision. Nasal hamartomas are rare causes of nasal mass effects in dogs and they have a good prognosis.

D-23: CASE REPORT: SYSTEMIC COCCIDIOIDOMYCOSIS IN A LLAMA CRIA BORN IN MISSOURI

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Background: Coccidioidomycosis is a rare, highly infectious fungal disease that can affect humans and other mammals. The disease is caused by two soil-dwelling species, *Coccidioides immitis* and *Coccidioides posadasii*, which are endemic to the southwestern United States. In domestic mammals, common routes of infection include inhalation and cutaneous inoculation.

Objective: To describe a case of a llama cria born in Missouri with systemic coccidioidomycosis.

Methods: A 3-month-old llama cria presented with a history of illthrift and abnormal mentation. Fixed and fresh tissues were submitted for testing after the necropsy exam. Serum from the cria was submitted for coccidioidomycosis and blastomycosis AGID. Serum from seven herdmates was submitted for coccidioidomycosis AGID. Computed tomography was performed on the dam.

Results: Generalized lymphadenomegaly, a brainstem mass at the fourth ventricle, and dilation of the lateral ventricles was observed grossly. Diffuse pyogranulomatous lymphadenitis and pyogranulomatous encephalitis, both with intralesional fungal spherules were identified histologically. The cria was positive for coccidioidomycosis AGID. Only the dam was positive for coccidioidomycosis AGID within the herd. Computed tomography of the dam revealed multiple nodules within the lung and liver.

Conclusion: Coccidioidomycosis has been characterized in llamas and alpacas with the most common route of infection being inhalation. Transplacental infections are rare but have been reported in domestic mammals. The dam was moved from Arizona 2.5

years ago. With no travel history to endemic areas, the examined cria probably represents a transplacental infection of coccidioidomycosis. Diagnostic screening suggests the dam is the likely source of infection for the cria.

D-24: WHITE CHICK SYNDROME IN THE UNITED STATES: PATHOLOGY AND DIAGNOSTIC FINDINGS

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Background: White chick syndrome is a vertically-transmitted disease of broiler chickens caused by Chicken Astrovirus. This disease is associated with a decrease in hatchability, increased mid-to-late embryonic deaths, high mortality in chicks as well as a high number of weak chicks that have whitish plumage.

Objective: The objective of this study was to characterize the gross and microscopic lesions of White Chick Syndrome and to report the diagnostic laboratory findings in chicks with this disease.

Methods: Day-old chicks and pipped chicken embryos with White Chick Syndrome from two broiler complexes in two different states were submitted to the Poultry Diagnostic and Research Center at the University of Georgia. Necropsy was performed and tissues were collected for histopathology, virus isolation and Polymerase Chain Reaction (PCR) for Chicken Astrovirus.

Results: Necropsy revealed greenish discoloration of the liver and mild urolithiasis in most affected chicks. Histopathology in liver samples revealed heterophilic and necrotizing cholangiohepatitis with bile duct hyperplasia, abundant extramedullary granulopoiesis as well as multifocal hepatic degeneration and necrosis with fibrinoheterophilic inflammation. Multifocal renal tubular nephrosis and atrophy of pancreatic acinar cells were observed in a few samples. Liver, intestine and bile samples were positive for Chicken Astrovirus by virus isolation and PCR.

Conclusions: White chick syndrome appears to be a sporadic disease of broiler chicks in the United States. Pathologists and diagnosticians should consider white chick syndrome as a differential diagnosis when high mortality associated with heterophilic and necrotizing cholangiohepatitis is encountered in pipped embryos and broiler chicks presenting whitish plumage.

Young Investigator Poster Abstracts: Diagnostic Pathology

DY-01: MENINGEAL GRANULAR CELL TUMOR IN THE CEREBRUM OF A GREEN TREE PYTHON (MORELIA VIRIDIS)

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Background: Central nervous system (CNS) neoplasia is rarely reported in reptiles, with snake gliomas representing most cases. Granular cell tumors (GCT) are thought to arise from the neural crest and are composed of sheets of round to polygonal cells with eosinophilic granules that likely represent autophagosomes or autophagolysosomes. Most GCTs manifest as oral neoplasia in people, dogs, and cats, cerebral meningeal neoplasia in dogs, pituitary gland neoplasia in people, and pulmonary neoplasia in people and horses.

Methods: A full set of tissues, including decalcified serial sections of the head, were examined histologically with HE stain. Immunohistochemistry (S100) and special stains (periodic acid-Schiff [PAS] with diastase) were performed on sections of the head.

Results: A 10-year-old female green tree python (*Morelia viridis*) presented for severe constipation and hyporexia. Despite treatment, she was found dead the following morning. Histologically, she had ulcerative colitis with transmural hemorrhage (trauma from constipation). Additionally, a large neoplasm was arising from the ventral meninges and markedly compressing the cerebrum and midbrain. The neoplasm was composed of sheets of round to polygonal cells with cytoplasmic immunoreactivity to S100 and eosinophilic cytoplasmic granules that were PAS positive and diastase resistant. These findings are diagnostic for a meningeal granular cell tumor.

Conclusions: CNS neoplasia is uncommonly reported in snakes. Routine examination of the brain *in situ* via decalcified sections of the head is recommended in reptiles. In this case, neurological deficits secondary to the meningeal GCT may have caused inappetence and led to dehydration and constipation.

DY-02: CHARACTERIZATION OF NEUROPATHOLOGIC LESIONS ASSOCIATED WITH AVIAN REOVIRUS INFECTION IN COMMERCIAL BROILER CHICKENS

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Avian reovirus is an important pathogen within the commercial poultry industry, and is often associated with significant economic impact, especially in broilers. Typical lesions caused by the virus include tenosynovitis/arthritis, myocarditis, multiorgan lymphoid depletion, and enteritis. Neurologic disease linked to reovirus is rarely reported with natural infection in chickens. This report describes histologic brain lesions associated with reovirus infection in commercial broiler chickens in Mississippi. Affected birds ranged from 27-40 days old, and exhibited severe neurologic signs including torticollis and head tremors. Necropsy confirmed severe inflammation in the brain in 26 birds submitted from affected houses. The histologic lesions were most severe in the brainstem and cerebellar white matter, characterized by robust mononuclear perivascular cuffs, neuronal degeneration and necrosis, sometimes accompanied by microglial nodules, and neuropil vacuolation with axonal degeneration. Similar lesions were found occasionally in the cerebral cortex and spinal cord. Affected birds often had concurrent lesions characteristic for avian reovirus, such as lymphonodular

tenosynovitis, pericarditis or myocarditis, and widespread lymphoid aggregates in multiple organs. Fresh brain tissues were PCR positive for avian reovirus and reovirus was isolated from pooled brain samples. Other major causes for neurologic disease in chickens were ruled out based on further diagnostic testing. While the histologic findings from these cases can mimic other common viral encephalitides in chickens, the lesion distribution and inflammatory pattern appeared to be consistent within our cases. These findings suggest that avian reovirus should be considered as a possible cause for chickens with similar neurologic presentations and histologic features.

DY-03: GRANULOMATOUS BRANCHITIS AND NEPHRITIS IN A JUVENILE BROWN TROUT (SALMO TRUTTA) WITH RENIBACTERIUM SALMONINARUM INFECTION

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Sixty hatchery raised brown trout were submitted to Michigan State University - Aquatic Animal Health Laboratory (MSU-AAHL) as part of a pre-stocking evaluation. In one examined fish, there was a white to tan raised nodule focally encompassing the gill filaments and gill arch. Concurrently, there was a white to tan multinodular mass affecting the majority of the cranial third of the kidney. Histologically, the gill nodule consisted of a densely cellular infiltrate comprised of epithelioid macrophages centered around regions of necrosis and peripherally lined by variable amounts of fibrous connective tissue. Similar microscopic changes were noted within the renal mass. *Renibacterium salmoninarum* was detected at high loads in kidney tissues from the affected fish using a semi-quantitative enzyme linked immunosorbent assay (ELISA). Gram staining of serial sections of the gill and kidney specimens revealed numerous Gram-positive coccobacilli. *Renibacterium salmoninarum* is a facultative intracellular pathogen, the causative agent of bacterial kidney disease, and is associated with significant mortality in salmonids. To the best of our knowledge, this is the first documented case of granulomatous branchitis associated with *Renibacterium salmoninarum*infection.

DY-04: CHRONIC PROLIFERATIVE RHINITIS IN A SHEEP ASSOCIATED WITH SALMONELLA ARIZONAE

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A 3-year-old, female sheep (*Ovis aries*) of unknown breed with a history of persistent labored breathing and nasal discharge was euthanized and submitted for postmortem examination. There was a moderate amount of mucoid discharge in the nares and on coronal section the nasal turbinates (conchae) were diffusely, markedly thickened, with a roughened appearance and the meatuses were narrowed. Histopathology of the nasal turbinates showed a markedly thickened mucosa, with polypoid to finger-like projections of hyperplastic epithelial cells, often containing intracytoplasmic aggregates of coccobacilli which were visible with Gram or Giemsa stains. The submucosa and lamina

propria contained dense infiltrates of plasma cells and lymphocytes as well as scattered pyogranulomas. Aerobic culture of a nasal swab isolated *Salmonella enterica* subspecies *arizonae* (serotype III 61:k:1,5,7).

This case represents an example of chronic proliferative rhinitis of sheep, an apparently rare and striking disease, which has previously been reported in Europe and the United States in association with *Salmonella enterica* serotype IIIb 61:-:1,5,7. Curiously, this serotype is common in sheep flocks in the US and is considered host-adapted and factors that lead to the development of this disease remain unknown. This report expands the number of reported cases of this rare disease and reinforces this as a differential diagnosis for upper respiratory disease in sheep.

DY-05: PROVENTRICULAR PENTASTOMIASIS IN A BROWN PELICAN

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A 3-year-old female captive Brown pelican (*Pelecanus occidentalis)* was observed isolating itself from the rest of the flock. On physical examination the bird had flaccid neck paralysis and paraplegia. The bird was treated once with antibiotics and fluids and found dead later in the afternoon and submitted for necropsy. Gross examination revealed too numerous to count firm, dark green to black granulomas measuring 0.1 to 0.5cm in diameter throughout the coelomic fat and serosa of the gastrointestinal tract and mesentery. On cut surface the nodules were gritty and contained dry, black material. The ventricular lumen contained abundant, thin, tan to cream-colored nematodes measuring approximately 1 to 3 cm in length. The nematodes were identified as *Contracaecum* sp., which is typically transmitted from fish serving as the intermediate host. On histologic examination, the granulomas were observed in the proventricular wall, mesentery, and spleen, composed of central necrotic debris and occasional mineralization (presumptive degenerate parasites) surrounded by multinucleated giant cells, macrophages, heterophils and eosinophils with peripheral lymphoplasmacytic infiltration and fibrosis. In addition to the granulomas, the proventriculus revealed parasites histomorphologically compatible with pentastomes (hooks, acidophilic glands, striated muscles, intestines, and sclerotized openings) in the hemorrhagic deep submucosa. Previous reports of pentastomiasis in avian species were in the respiratory tract, whereas this pelican had proventricular pentastomiasis, which also suspected to be the cause of the numerous granulomas seen in the proventriculus, mesentery, and spleen.

DY-06: SYSTEMIC MASTOCYTOSIS PRESENTING AS SUBCUTANEOUS HEMORRHAGE AND EDEMA IN A GREYHOUND

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A 5-year-old, spayed female greyhound with a one-month history of progressive ventral cutaneous edema, hemorrhage, and pain was submitted for autopsy. Grossly and

histologically, the subcutaneous tissues of the entire ventrum and all limbs were severely expanded by hemorrhage and edema. Superficial to the panniculus carnosus was a dense sheet of neoplastic mast cells. The neoplastic cells contained toluidine blue positive granules and formed aggregates and nodules within several visceral organs including the liver, spleen, heart, kidney, and bone marrow. Systemic mastocytosis, characterized by infiltration of multiple organs by neoplastic mast cells, is a well described entity in human medicine with specific criteria for diagnosis, but is ill defined in veterinary literature. Diffuse edema and hemorrhage is an unusual presentation of mast cell tumors in dogs. Antemortem diagnostics including complete blood count, coagulation profile, and viscoelastic coagulation testing were suggestive of a primary hemostatic defect. Hemostatic disorders are reported in humans affected by systemic mastocytosis, but have not been well described in veterinary literature.

DY-07: BACILLUS PUMILUS-ASSOCIATED PLACENTITIS AND ABORTION IN A MARE

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Bacillus pumilus is a Gram-positive, aerobic, spore-forming bacillus rarely associated with clinical disease in man and veterinary species. *Bacillus pumilus* is part of the subtilis subgroup of bacilli, which are widely spread in the environment and used in agriculture for their antifungal and antibacterial properties. *Bacillus pumilus* has been implicated in veterinary literature as a cause of mastitis in dairy cows, while other subtilis subgroup bacilli have been linked to abortions in cattle, sheep, and pigs. A 7-month-old equine fetus and placenta aborted by a 16-year-old Westphalian mare presented for post-mortem examination. Grossly, the chorioallantois was diffusely thickened and mottled dark red with a 30x12 cm, sharply demarcated pale area covered with pinpoint coalescing fibrinous plaques and surrounded by a bright red area of congestion and hemorrhage located near the junction of the gravid horn and the body of the placenta. On histological examination, a locally extensive area of the chorionic epithelium and chorionic villi was overlaid by an exudate of degenerative neutrophils admixed with necrotic debris and fibrin. Small colonies of 1 µm, Gram-positive bacilli were scattered throughout the exudate and within macrophages. Aerobic culture of a uterine swab from the mare and fetal lung tissue resulted in the growth of *Bacillus pumilus*. To the best of our knowledge, this is the first report of *B. pumilus* causing infectious abortion and placentitis in any species.

DY-08: ISOLATED CNS PROTOTHECOSIS IN A DOG

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Background: A 1.5-year old-female spayed Miniature Schnauzer presented for a 2-week progressive history of lethargy, inappetence, and unlocalizable pain, and for a recently diagnosed fever and mild thrombocytopenia. Clinical examination revealed marked multifocal spinal pain. CBC, serum biochemistry, fundic exam, rectal scraping, and spinal radiographs were unremarkable. MRI and CSF analysis raised suspicion of protothecosis and despite treatment with fluconazole and steroids, the dog continued to decline and was euthanized days later.

Objective: To describe clinical and diagnostic findings in a case of isolated CNS protothecosis in a dog.

Methods: MRI, CSF analysis and cytology, CSF mycologic culture, histology of all major tissues, and PCR of CNS tissues were employed to diagnose protothecosis.

Results: MRI revealed severe syringohydromyelia and diffuse myelitis. CSF analysis and cytology revealed a mixed cell pleocytosis with suspected Prototheca organisms. Histology with GMS and PAS stains revealed protothecal meningoencephalomyelitis with brain, spinal cord, and optic nerve involvement. Despite the significant CNS Prototheca burden, definitive diagnosis and Prototheca subspeciation were not obtained due to negative results of CSF culture and PCR of CNS tissues.

Conclusions: To the authors' knowledge, this is the first documented case of isolated CNS protothecosis in which organisms were detected by antemortem CSF cytology. However, definitive diagnosis of protothecosis can be challenging given the diagnostic delay and complicated by the lack of validated PCR for dogs. In cases with neurologic involvement, prognosis is grave given the short window for therapeutic intervention after clinical presentation.

DY-09: UNUSUAL HISTOLOGICAL APPEARANCE OF BILATERALLY SYMMETRICAL SUBCLINICAL OSTEOCHONDROSIS IN A FOAL

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A 4-month-old, intact male Standardbred foal was euthanized and presented for necropsy due to mild lameness of the right hind limb with lateral rotation and asymmetry of the tuber coxae. At necropsy, there was a right coxofemoral subluxation with fragmentation of the ligamentum teres and a right acetabular fracture. In addition, on transection of the distal third metatarsal bilaterally, there was a focal, locally extensive region of pallor immediately subjacent to the articular cartilage at the central sagittal ridge (with approximate size of 2 mm x 4 mm x 5 mm). Histologically, the lesions were composed of small caliber blood vessels admixed with loose fibrous connective tissue (granulation tissue) that abutted the deep articular cartilage and replaced a locally extensive area of subchondral bone. On close examination, a thin zone of necrotic cartilage was identified between the deep zone of the articular cartilage and the

subjacent granulation tissue. The histologic appearance, location, and bilateral nature of the lesion was consistent with a diagnosis of chronic, subclinical osteochondrosis manifesta. Comparison with a documented osteochondrosis dissecans lesion in the distal humerus of a 5-month-old domestic pig revealed an essentially identical area adjacent to the articular cartilage cleft. Although subclinical osteochondrosis manifesta lesions are uncommonly identified, it is important to recognize and differentiate them from other developmental and traumatic osseous lesions in young animals. The chronicity of these lesions suggested that they had been present for at least several weeks. The bilateral symmetry of the lesions is consistent with the pathogenesis of osteochondrosis.

DY-10: PATHOLOGICAL AND FLOW CYTOMETRIC FEATURES OF A CASE OF CANINE LYMPHANGIOSARCOMA

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Background: An 11-year-old female spayed German Shepherd was presented for a second opinion of ventral cervical swelling for three months duration. Upon examination, the dog had significant fluctuant swelling of the ventral neck. Enlarged cystic lymph nodes and severe facial edema were noted on computed tomography. Yellow-tinged fluid was obtained upon aspiration of the swelling. Analysis of the fluid revealed predominance of lymphoid cells. A cervical exploratory surgery was performed and the left mandibular lymph node was extirpated.

Objective: To describe the clinicopathologic, cytologic, and histopathologic findings of a case of naturally-occurring lymphangiosarcoma in a dog.

Methods: Impression smear cytology, flow cytometry, polymerase chain reaction for antigen receptor rearrangements (PARR), and histology of the left mandibular lymph node were utilized for diagnosis. Histopathologic sections were assessed for vimentin, cytokeratin AE1/AE3, von Willebrand's factor and prospero-related homeobox gene-1 (PROX-1) immunoreactivity.

Results: Impression smear cytology revealed reactive lymphoid hyperplasia and a population of discrete, large, atypical cells. Flow cytometry demonstrated normally distributed small lymphocytes, and a separate large cell population (30% of which were CD34+ and CD45-). Histopathologic examination and immunohistochemistry revealed a PROX-1 positive neoplastic endothelial population, leading to the diagnosis of lymphangiosarcoma.

Conclusions: To the authors' knowledge, this is the first case report to describe the use of flow cytometry to aid in the diagnosis of lymphangiosarcoma. In the future, flow cytometry may be a useful minimally invasive diagnostic technique for cases that present for poorly-defined subcutaneous masses, subcutaneous edema and regional lymphadenomegaly to further evaluate for possible lymphangiosarcoma.

DY-11: CONGENITAL LIPOBLASTOMA IN A NEONATAL BRANGUS BULL CALF

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Background: A 2-day-old Brangus bull calf presented to the Auburn University JT Vaughan Large Animal Teaching Hospital for evaluation of several congenital abnormalities. Due to a guarded long-term prognosis, the calf was euthanized shortly after presentation and submitted for postmortem examination. Significant findings included a 25 cm x 30 cm x 20 cm pale tan, multilobulated, retroperitoneal mass that encompassed the left kidney, regional cervical dermal fibrosis, goiter, and kyphosis.

Objective: Our objective was to characterize the retroperitoneal and perirenal mass observed grossly during postmortem examination.

Methods: Samples of the perirenal mass were fixed in neutral buffered formalin, routinely processed for histopathology, stained with hematoxylin and eosin, and examined histologically.

Results: On histopathologic evaluation, the perirenal mass consisted of two populations of polygonal cells arranged in moderately-sized lobules surrounded by a fine fibrovascular stroma. The first population have well-defined cell borders, eosinophilic cytoplasm with multiple discrete, clear vacuoles, and round to ovoid nuclei with finely stippled chromatin and a small basophilic nucleolus. The second population is composed of cells resembling mature white adipocytes with well-defined cell borders, scant eosinophilic cytoplasm, and a single, large, clear cytoplasmic vacuole that displaces and deforms a hyperchromatic nucleus with indistinct nucleoli. No mitotic figures are observed within 10 standardized 400x fields.

Conclusions: The perirenal neoplasm most resembles a lipoblastoma, a rare and benign mesenchymal embryonal tumor of which only few cases have been reported in domestic animals and in humans. This case report expands the current literature of lipoblastomas in domestic animals.

DY-12: OSTIUM SECUNDUM TYPE ATRIAL SEPTAL DEFECT IN A NEW ZEALAND WHITE RABBIT

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Background: An adult female New Zealand White rabbit presented for necropsy after being found dead in its cage 5 days after being shipped to Johns Hopkins University for use as a research animal. There was no history of therapy or experimental manipulation.

Objective: Our objective was to identify the cause of death in this rabbit and to determine if there was any threat to herd health in this research colony.

Methods: A necropsy was performed, and representative sections of major tissues were submitted for histologic examination.

Results: On gross examination, a 5 mm-diameter defect in the atrial septum was identified and interpreted as an ostium secundum type atrial septal defect (ASD). Associated with the defect were marked right atrial dilatation and thickened tricuspid valve leaflets. On histologic examination, there was myxomatous degeneration of the tricuspid valve leaflets and increased collagen in the right atrium. Other histologic findings included mild hepatic lipidosis and moderate renal tubular degeneration and necrosis.

Conclusions: ASD is an uncommon congenital heart disease in domestic animals. To our knowledge, this is the second report of ostium secundum type ASD in a rabbit, and the first report in the New Zealand White breed.

DY-13: EPIZOOTIC OF CUTANEOUS MESENCHYMAL NEOPLASIA IN A CAPTIVE COLLECTION OF CICHLIDS (ORDER: CICHLIFORMES)

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Introduction: Neoplasia is caused by genetic mutations that may be inherited or acquired by exposure to environmental agents, including chemical carcinogens, radiation, and infectious diseases, particularly viruses. In fish, chemical carcinogens and oncogenic viruses are well-documented causes of epizootic neoplasia; inherited causes are less commonly documented.

Objective: Describe epizootiology and pathologic findings in cichlids with epizootic neoplasia.

History and Methods: From 2008-2019, multiple Cichliformes species along with 3 Synodontis catfish were kept in a closed system tank without any changes to their diet, enclosure, or water quality parameters. Animals reproduced within the exhibit. Only a small group of Frontosa Cichlids (*Cyphotilapia frontosa*) were added to the system throughout the exhibit's lifetime. From 2015-2019, approximately 70% of 300 cichlids developed cutaneous neoplasms. Neoplasms occurred only when animals reached adulthood. Ten affected cichlids were euthanized for histologic examination. Masses from two cichlids were examined using transmission electron microscopy (TEM).

Results: Ten cichlids had mesenchymal neoplasia of the body wall, including sarcoma in six *Labidochromis caeruleus* (one of which also had a fibroma), two *Melanochromis auratus*, and one *Pseudotropheus johannii*, and chondroma in one *Labidochromis caeruleus*. Viral particles were not identified using TEM.

Conclusion: Inherited genetic mutations are an unlikely cause for neoplasia in these cases as multiple genera were affected. Chemical carcinogens are also unlikely as there were no changes to the environment or diet for approximately 11 years prior to the onset of neoplasia. Although TEM was negative, viral induction is still a possible cause. Further research could include viral culture.

DY-14: NEUN AND DOUBLECORTIN IMMUNOLABELING IN FELINE GLIOMA

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Background: Gliomas are thought to arise from neural stem cells or from dedifferentiated astrocytes that regain immature glial properties, but their cell of origin is still a matter of debate. Canine gliomas exhibit variable immunolabeling for doublecortin (DCX) and no immunolabeling for NeuN, immunomarkers for neuronal progenitor cells and mature neurons, respectively. DCX expression has been associated with tumor invasion in human and canine gliomas, as well as in canine meningiomas; human gliomas are often positive for NeuN. These immunomarkers have not been evaluated in feline gliomas.

Objectives: To characterize the immunohistochemistry (IHC) features of DCX and NeuN in feline glioma.

Methods: A retrospective database search of the Athens Veterinary Diagnostic Laboratory was performed for cases of feline glioma. All cases were reviewed histologically and subjected to immunohistochemistry (IHC) for DCX and NeuN. Immunostaining was recorded as absent, weak, moderate, or strong, and further as patchy, multifocal, or diffuse.

Results: Eleven feline gliomas were retrieved. All tumors had been diagnosed as oligodendroglioma (7 cases) or astrocytoma (4 cases) based on morphology and IHC (Olig2 and GFAP). Tumors exhibited variable immunolabeling for DCX, with no staining variations related to tumor invasion and tumor type. NeuN immunolabeling was absent in all cases.

Conclusions: DCX and NeuN immunolabeling in feline gliomas are similar to those reported for canine gliomas. DCX immunolabeling suggests that neuronal progenitor cell antigens may be present within the tumors, but staining did not vary when comparing invasive versus non-invasive tumors, or oligodendrogliomas versus astrocytomas.

DY-15: CARDIOMYOPATHY AND AGE-RELATED FINDINGS IN A CAPTIVE CALIFORNIA SEA LION (ZALOPHUS CALIFORNIANUS)

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Background: A 31-year-old spayed female California sea lion (*Zalophus californianus*) presented to the Louisiana Animal Disease Diagnostic Laboratory at Louisiana State University in December 2018 for necropsy. For two years prior, the sea lion had been undergoing treatment for right-sided congestive heart failure, which had worsened over the last 10 months. Additional clinical history included bilateral ocular enucleation, a laparoscopic spay three years prior, a two-year history of multifocal subcutaneous cysts on the ventral abdomen, and a three-month history of a presumed abdominal mass.

Observation: Necropsy findings included bilateral atrial dilation, endocardial fibrosis, marked dilation of major heart vessels, thickened cardiac valves, multifocal to coalescing subcutaneous cystic expansion of the mammary glands over the caudal abdomen, collapsed and mottled lungs with a focal abscess, and pyloric obstruction due to an osteobezoar.

Results: The most significant histopathologic lesions were observed within the heart, including severe endocardial and interstitial fibrosis and vascular dilation and valvular endocardiosis. Subintimal proliferation within cardiac and renal vessels were indicative of systemic hypertension. In addition, there was a severe pyogranulomatous pneumonia, mild hepatitis with biliary hyperplasia and cholestasis, a pheochromocytoma within the adrenal gland, C-cell adenoma within the thyroid, membranoproliferative glomerulonephritis, exocrine pancreatic atrophy, and active lactation of the mammary glands despite undergoing a laparoscopic ovariohysterectomy three years prior.

Conclusions: Gross and histopathological examination findings were consistent with the clinical history of congestive heart failure, with additional secondary and age-related disease processes.

DY-16: ABERRANT MICRORNA EXPRESSION IN CANINE DIFFUSE LARGE B-CELL LYMPHOMA: POTENTIAL DIAGNOSTIC TOOL

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Background: Lymphomas are among the most common cancers in dogs and are likely the leading cause of cancer-related deaths. The diffuse large B-cell lymphoma (DLBCL) is the most common type, accounting for up to half of all cases. MicroRNAs are post-transcriptional regulators of gene expression that play a major role in several cellular processes including cell differentiation, cell cycle progression, and apoptosis. They also play a role in carcinogenesis through their influence on oncogene and tumor suppressor genes. The high stability of microRNAs make them promising biomarkers for diagnostic testing, prognostic classification, and disease monitoring.

Objectives: This study aimed to identify microRNA signatures within DLBCL to develop diagnostic tools and to serve as the core for future studies of microRNA regulation in DLBCL.

Methods: Total RNA was extracted from archived formalin-fixed paraffin-embedded lymph nodes from dogs with DLBCL collected at the time of diagnosis (n=20) and from control dogs (n=15). Total RNA was extracted (miRNeasy FFPE Kit, QIAGEN) and cDNA was synthesized using 500 ug RNA (miScript II, QIAGEN) according to the manufacturer’s specifications. Comparative expression of mir-34, mir155 and let-7 family of microRNAs was performed by RT-qPCR (miScript SYBR Green, QIAGEN) using RNUB6 as a normalizer.

Results: The comparative expression results showed upregulation of mir-34 ($p<0.0001$), while microRNAs mir-155 ($p=0.0154$), let-7a ($p=0.0029$), let-7b ($p=0.0002$), let-7c ($p=0.0021$), let-7e ($p<0.0001$), let-7f ($p<0.0001$), and let-7g ($p<0.0001$) were downregulated in DLBCL compared to the control samples.

Conclusion: This study emphasizes the potential of mir-34, mir-155 and let-7 microRNAs as biomarkers for canine DLBCL.

DY-17: YOLK PERITONITIS IN A BEARDED DRAGON (POGONA VITTICEPS)

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Background: A 6-year-old, intact female bearded dragon was submitted for postmortem examination when she was found deceased overnight after recently laying eggs. She had no history of any prior medical illness.

Objective: Our objective was to identify the cause of the sudden onset of death in this client-owned bearded dragon.

Methods: A full postmortem examination was performed. Samples of lung, liver, intestines, spleen, kidneys, and the abdominal mass were fixed in 10% neutral buffered formalin, routinely processed, embedded in paraffin, sectioned at 4 to 6 um, and stained with hematoxylin and eosin (H & E).

Results: Postmortem examination identified a fibrinous exudate within the coelomic cavity, a yolk granuloma, pneumonia, and hepatic lipidosis. Histopathology revealed a fibrinous, histiocytic and lymphocytic coelomitis. The yolk granuloma was composed of monocytic inflammatory infiltrates, hemorrhage, calcification, and necrosis with a large amount of yolk material.

Conclusions: Yolk peritonitis is a common condition in backyard hens of all ages, however, it is uncommon in reptile species with only a few published case reports in the literature. Peritonitis associated with the presence of egg yolk material can range in severity from nonclinical to life threatening. This condition can present prior to, during, and after ovulation. Additionally, egg yolk peritonitis may occur from trauma, salpingitis, rupture of the oviduct, neoplasia, ovarian cystic hyperplasia, and ectopic ovulation due to reverse peristalsis of the oviduct.

DY-18: PUTATIVE EPIDERMOLYSIS BULLOSA IN A LITTER OF NEONATAL BASSET HOUNDS

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Background: Epidermolysis bullosa (EB) is a group of blistering diseases affecting skin and mucus membranes. Three inherited types of EB have been described in dogs, distinguished by the level of splitting at the basement membrane zone. Epidermolysis bullosa simplex (EBS) affects basal keratinocytes, junctional epidermolysis bullosa

(JEB) affects the lamina lucida, and dystrophic epidermolysis bullosa (DEB) affects the anchoring filaments between the lamina densa and the superficial dermis. Herein, we describe suspected inherited EB in a single litter of neonatal Basset hounds, in which this condition has not been previously reported.

Case description: A clinically normal bitch was bred to a relative (grandfather) by artificial insemination. Out of seven puppies, one was stillborn, and another died twelve hours later. Within two days following birth, three male puppies were noted to have blisters variably involving the bridge of the nose, nasal planum, and pawpads. Multiple nails were sloughed and crusts were noted around the ear canals. Two such affected puppies died naturally and one was humanely euthanized. The two remaining puppies remain clinically normal.

Results: Three puppies were submitted for routine necropsy evaluation. All had severe, subacute to chronic, multifocal, cutaneous bullous dermatopathy with ulceration and superficial infection predominated by gram positive cocci. In addition to bullae/ulcers involving the pawpads and nasal planum, ulcers were also noted in the oral cavity and esophagus. PAS positive material was noted at the roof of the blisters.

Conclusions: An autosomal recessive genodermatosis is suspected. Genetic analysis and ultrastructural evaluation are underway.

DY-19: THYMIC TERATOMA IN A CHICKEN (GALLUS GALLUS DOMESTICUS)

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Background: A 3-year-old rooster was submitted for postmortem evaluation for a progressive necrotic dermatitis on the right hock. Upon gross examination several discrete thymic masses were observed, which histologically were consistent with a thymic teratoma. Teratomas are germ cell tumors with at least two germinal layers present. Teratomas have been reported in chickens, but only derived from gonadal tissue. Mature thymic teratomas have been described in humans, and are considered a rare entity.

Objectives: To describe gross and histopathologic features of thymic teratomas in a chicken.

Methods: A complete gross post mortem examination and routine histopathology was performed. Additional special and immunohistochemical stains were pursued to confirm the presence of multiple germinal layers and include multi-cytokeratin (MCK), glial fibrillary acidic protein (GFAP), and desmin.

Results: Gross examination revealed 6 bilateral globoid and discrete masses, which run along the caudal ventral cervical musculature and extending into the proximal coelomic cavity. Histology of masses demonstrates three germinal layers including muscle (mesoderm), nervous tissue (ectoderm), and glandular tissue (endoderm)

associated with thymic epithelium and lymphocytes. Immunohistochemical stains confirmed the presence of nervous tissue (GFAP), muscle (desmin), and epithelium (MCK).

Conclusions: HIstopathology and immunohistochemistry identified three distinct germinal layers, consistent with the diagnosis of a teratoma. To the knowledge of the authors, this is the first described report of a thymic teratoma in chickens. A teratoma represents a novel differential for a thymic mass in chickens.

DY-20: TOPHACEOUS PSEUDOGOUT IN A POODLE

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Introduction: Pseudogout, also known as calcium pyrophosphate dihydrate (CPPD) deposition disease or chondrocalcinosis, is caused by crystalline deposits composed of calcium pyrophosphate dihydrate within the extracellular matrix of articular hyaline and fibrocartilages, and within articular and periarticular connective tissue. Though CPPD commonly occurs in older people, few cases are reported in the veterinary literature.

Objective: The aim of this work is to assist veterinary pathologists in diagnosing this uncommon condition, and familiarize readers with these diagnostic techniques.

Materials and Methods: Standard HE histology with polarizing filters and a first order red compensator, Scanning Electron Microscopy with Energy Dispersive X-ray Analysis (SEM/EDXA), and Fourier Transform Infrared microspectroscopy (FT-IR) were performed on formalin-fixed paraffin-embedded sections of the mass. Standard HE histology with decalcification was performed on sections of the mass with bone and joint.

Results: A 12-year-old, standard poodle had amputation of digit V (right hind limb) due to a digital mass. Histologically, the joint, bone, tendon, and dermis were expanded and effaced by lobules of mineralized crystals surrounded by macrophages, multinucleated giant cells, fibrous connective tissue, chondroblasts, and chondroid and osseous matrix. Using a first order red compensator filter and polarized microscopy, rhomboid crystals exhibited weak positive birefringence. SEM/EDXA confirmed rhomboid crystals composed of calcium, phosphorous and oxygen. FT-IR confirmed the presence of calcium pyrophosphate.

Conclusion: Combined results are diagnostic for pseudogout. In dogs, tophaceous pseudogout often occurs as a single, tumor-like periarticular mass. In this case, the mass was invasive, mimicking a malignant neoplasm. The pathogenesis of CPPD is unknown.

DY-21: ORAL MALIGNANT MELANOMA WITH MULTIORGAN METASTASES IN A EURASIAN EAGLE-OWL (BUBO BUBO)

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A 29-year-old, intact female Eurasian eagle-owl (*Bubo bubo*) was euthanized and submitted for autopsy at the University of Tennessee Veterinary Medical Center after an acute period of depression and lethargy. Two months prior, the owl was diagnosed with and began chemotherapeutic treatment (carboplatin) for oral malignant melanoma. Postmortem findings included a dark-brown, infiltrative, 5.5 x 3.5 x 0.6 cm mass originating from the soft palate and numerous, dark-brown, variably-sized nodules throughout the subcutis, myocardium, oviduct, cranial air sacs, lung lobes, intestinal serosa, right kidney, peritoneum, and bone marrow. Additionally, the liver was diffusely firm, dark-brown, and enlarged. Microscopically, select tissues had neoplastic polygonal cells with variably distinct cell borders, moderate amounts of pale, eosinophilic cytoplasm containing few to abundant, dark-brown granules (melanin), and irregularly round to oval nuclei, with stippled chromatin and one variably-prominent nucleolus. Anisocytosis and anisokaryosis were marked (up to 5x). Four mitotic figures were in ten high-power fields (400x). The final diagnosis was oral malignant melanoma with multiorgan and coelomic metastases. Malignant melanoma is a rarely reported neoplasm in birds of prey, though it has been observed in a merlin and a red-tailed hawk. This is the first report of malignant melanoma with metastasis in an owl.

DY-22: MYCOBACTERIAL INFECTION IN A SCALLOPED HAMMERHEAD SHARK (SPHYRNA LEWINI)

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Background: A male scalloped hammerhead shark (Sphyrna lewini) of unknown age which presented anorexia was found dead at the Shimane Aquarium in Shimane Pref., Japan. Necropsy was performed on site, and some of the gross findings included marked erythema and inflammation of testicles and melting epigonal organ.

Objective: To describe a case of mycobacteriosis in Elasmobranchii, which is rarely reported.

Methods: Select formalin- and ethanol-fixed tissues were submitted to the Laboratory of Aquatic Medicine at Nippon Veterinary and Life Science University for histopathological and molecular biological evaluations, respectively.

Results: Histopathologic examination revealed severe inflammatory infiltrate in the testicular interstitial stroma consisting mostly of large numbers of lymphocytes associated with macrophages having afoamy cytoplasm. A portion of seminiferous tubules was degenerated and necrotic, and infiltrated by macrophages and lymphocytes. Giemsa and gram stains did not reveal significant microbes. However, numerous acid-fast bacilli were observed within macrophages accumulating at the edge

of testicle adjacent to epigonal organ by Ziehl-Neelsen stain. DNA was extracted from the ethanol-fixed testicle and universal Mycobacterium primers were used to amplify a portion of the 65-kDa heat shock protein gene. Sequencing of the PCR amplicon matched Mycobacterium marinum with 100% sequence identity in GenBank.

Conclusions: In teleostei, mycobacteriosis has been frequently diagnosed and M. marinum is the most common causative agent. In elasmobranchii, mycobacteriosis has been rarely reported with no cases involving M. marinum. This report describes the first case of mycobacteriosis in scalloped hammerhead shark associated with M. marinum as the causative agent.

DY-23: METASTATIC CERVICAL PARAGANGLIOMAS WITH BONE INVASIONS IN TWO DOGS

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Background: A malignant cervical paraganglioma is rarely reported in dogs. Case presentation: Case #1) The computed tomography (CT) of a dog with neck pain demonstrated (1) a right-sided cervical mass with intracalvarial and vertebral canal invasion, and (2) a mass at the heart base. Case #2) A magnetic resonance imaging (MRI) of a dog with tetraparesis demonstrated a left-sided cervical mass with vertebral canal invasion, and resultant extradural spinal cord compression. Post-mortem examination: Case #1) Two multinodular masses were associated with the right common carotid artery. Smaller nodules extending to the atlanto-occipital joint that were seen protruding into the C1 spinal canal and invading into the petrous temporal bone. Of note, there was a nodular mass at the heart base. Case #2) A mass at *THE* left retropharyngeal space near *DELETE the near the* C1 was found with bony invasion into the calvarium involving tympanic bulla and petrous temporal bone, and a mass within the C2 spinal canal was observed compressing the spinal cord. The masses were composed of neuroendocrine cells forming packets and nests. The majority of cells within masses stained positive with Grimelius stain and were immunoreactive with synaptophysin immunohistochemistry (IHC). About 20-25% of cells were variably immunoreactive with neuron specific enolase (NSE) IHC. **Conclusions:** Both neoplasms were diagnosed as malignant paragangliomas. In dogs, there are rare reports of these tumors with bone metastasis. In both of the presented cases, the neoplasms exhibited atypical aggressive bon*Y* invasion into the axial skeleton resulting in neurologic clinical signs.

DY-24: SYSTEMIC ANAPLASTIC LARGE-CELL LYMPHOMA AND ITS IMMUNOHISTOCHEMICAL CHARACTERIZATION IN A POMERANIAN DOG

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Background: Anaplastic large-cell lymphomas (ALCL) comprise a group of T/null-cell non-Hodgkinlymphomas in humans characterized by enormous cell and nuclear

pleomorphism and CD30 expression. The prognosis is poor. Here we report systemic canine ALCL and characterize its immunohistochemical features with a panel of markers. Case Description: A 13-year-old, intact male Pomeranian dog presented to the National Taiwan University Veterinary Hospital (NTUVH) for hypoalbuminemia of 3-week duration. Laboratory tests revealed leukocytosis, hypoalbuminemia and elevated ALKP. Urinalysis detected ketonuria, proteinuria and bilirubin crystals. Ultrasound showed multiple hypo- or hyper-echoic nodules in the liver and diffuse thickening of the intestinal wall. The dog died three days after presentation and was submitted for necropsy.

Results: At necropsy, numerous distinct white to gray nodules were found in the liver, kidneys, lungs, heart and GI tract. Except for the pancreatic lymph nodes which were swollen and lost the corticomedullary arrangement, the remaining lymph nodes and spleen remained intact. Microscopically, each nodule consisted of solid sheets of pleomorphic neoplastic round cells with scattered areas of necrosis and hemorrhage. Bizarre cells with bi- to multi-nucleation or multilobulated nuclei were occasionally encountered. Immunohistochemically, the neoplastic cells were positive for CD30, CD3, MUM-1, MHC-II, Granzyme B and negative for CD79a, PAX-5 and CD15. Surprisingly, a portion of cells were also positive for CD20. PARR revealed a monoclonal T-cell receptor gamma gene rearrangement.

Conclusion: This case provided the first comprehensive immunophenotypic profile of canine ALCL. The unusual CD20 and CD3 expression observed herein highlights the importance of PARR in diagnosing canine ALCL.

DY-25: CO-INFECTION WITH TESTUDINID HERPESVIRUS 3 (TEHV-3) AND INTRANUCLEAR COCCIDIOSIS OF TORTOISE (TINC) IN TWO RED-FOOTED TORTOISES (CHELONOIDIS CARBONARIA)

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Background: Herpesvirus infection and intranuclear coccidiosis of tortoise (TINC) are important diseases in tortoises with increasing clinical importance and conservation significance. Their causative pathogens exhibit a strong epithelial tropism and could lead to high mortality in affected collections. Pathologically, herpesvirus causes diphtheritic lesions in the upper respiratory tract, oral cavity and esophagus whereas intranuclear coccidium often results in systemic diseases with unspecific clinical signs. Here we report co-infection with Testudinid herpesvirus 3 (TeHV-3) and TINC in two captive red-footed tortoises (*Chelonoidis Carbonaria*).

Case Description: Twelve adult red-footed tortoises were transported to a new enclosure and then presented with lethargy, anorexia, and oronasal discharge. Two of them died and were submitted for necropsy.

Results: Both tortoises were thin, and extensive ulceration covered by fibrinous substance was noted on the oral, esophageal and tracheal mucosal surfaces.

Microscopically, the sloughed and remnant epithelial cells nearby the ulceration occasionally contained eosinophilic to amphophilic intranuclear viral inclusions. Myriad and different stages of intranuclear coccidia were observed in the epithelial cells of lung, pancreas and thyroid gland accompanied with mixed inflammatory infiltrates. PCR determined co-infection with TeHV-3 and TINC.

Conclusion: The current report highlights the tremendous impact of stress on host immunity and the risk of disease outbreak due to transportation and environmental change. The course of disease development and the pattern of lesion distribution in this co-infection case also raise the concern on the interaction among TeHV-3, TINC, and host immunity. To the authors' knowledge, this is the first case report of TINC in Asia.

DY-26: CANINE EXTRA-GASTROINTESTINAL STROMAL TUMORS

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Background: Gastrointestinal stromal tumors (GISTs) are mesenchymal neoplasms of the gastrointestinal tract that arise from the interstitial cells of Cajal in the submucosa or muscularis of the stomach, small intestine, cecum, and colon. GISTs have been reported in humans, dogs, and horses. In humans, GISTs are rarely found as primary neoplasms of extragastrointestinal tissues such as omentum and mesentery, and are termed extra-gastrointestinal stromal tumors (eGISTs). There are no published reports of canine eGISTs.

Objective: To determine if eGISTs occur in canine submissions received at the Diagnostic Medicine Center (DMC) - Colorado State University.

Methods: Biopsy cases submitted to the DMC from 2013-2018 were considered. Criteria for inclusion were canine cases with a morphologic diagnosis of primary omental/mesenteric mesenchymal neoplasm/sarcoma (without intestinal wall involvement). An immunohistochemical panel was used to identify eGISTs and included desmin, DOG1, and CD117.

Results: 25 cases of primary omental neoplasms were identified. 7 cases were sarcomas. 3 of 7 cases were positive for DOG1 and diagnosed as eGISTs. The excluded cases included true GISTs, fibrosarcomas, liposarcomas, and myxosarcomas. The selected cases are omental masses from an 11 year old neutered Coonhound, 10 year old neutered Curly Coated Retriever, and a 10 year old neutered Standard Poodle.

Conclusions: From our small sample size, we conclude that eGISTs occur in older dogs and have a low incidence. In humans, the incidence of eGISTs is reportedly low and omental eGISTs have aggressive behavior. eGIST should be considered as differential diagnosis for omental masses in dogs.

DY-27: ATYPICAL GRANULOMATOUS STROMAL KERATITIS IN A DOMESTIC SHORTHAIRED CAT

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Background: The left globe from an 8.5-year-old male neutered domestic shorthaired cat was submitted for investigation of a rapidly developing vascular mass on the left cornea. The clients were unable to medicate the cat, so opted for enucleation. The enucleated globe was fixed in 10% formalin and submitted to the Comparative Ocular Pathology Laboratory of Wisconsin (COPLOW).

Objective: To report an atypical case of granulomatous stromal bacterial keratitis in a cat exploring its etiology and pathogenesis.

Methods: The formalin-fixed globe was bisected dorsoventrally and processed for histopathology. Histopathologic sections were stained with H&E, Gram's stain, modified Steiner's, Gomori's methenamine silver (GMS), and Fite's stainfor acid-fast bacteria. Scrolls from the formalin-fixed, paraffin-embedded tissue were submitted to the Michigan State University Diagnostic Laboratory for 16S rRNA gene sequencing for bacterial identification.

Results: Grossly, the eye had a temporal paraxial tan exophytic corneal mass. Histopathologic examination revealed a well-delineated mass infiltrating and expanding the axial corneal stroma, composed of multifocal to coalescing pyogranulomas containing large numbers of epithelioid and multinucleated macrophages surrounding a core of degenerate neutrophils and myriad 2um, beaded, filamentous gram-positive bacilli forming dense colonies. Modified Steiner's stain and GMS highlighted the filamentous nature of the bacteria. The bacteria were negative for Fite's stain.

Conclusions: Themorphologic and staining characteristics of the organisms suggest *Actinomyces*spp. Confirmatory 16S rRNA gene sequencing is pending. The localized nature of the lesion, intense granulomatous response and large numbers of opportunistic bacteria suggest a contaminated penetrating trauma was involved in the pathogenesis.

DY-28: A PERITONEAL INFLAMMATORY MYOFIBROBLASTIC TUMOR IN AN ARABIAN MARE

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This report describes an inflammatory myofibroblastic tumor in a five year old Arabian mare who presented for colic signs. At postmortem examination there were multifocal to coalescing variably sized tan nodules on the abdominal serosa, including the abdominal wall, spleen, diaphragm, and large intestine, with the number of nodules on the serosal surface of the colon and cecum. Histologically, the masses are composed of a densely

cellular, poorly demarcated and infiltrative mesenchymal neoplasm composed of spindle cells arranged in interlacing bundles or occasional storiform whorls within a collagenous stroma. Neoplastic spindle cells have little atypia arranged in interlacing bundles or storiform whorls with prominent nuclei and no mitotic figures. with Infiltrating the masses, often surrounding vessels, are large numbers of plasma cells, lymphocytes and large foamy macrophages. Neoplastic cells are diffusely immunopositive for smooth muscle actin, variably for vimentin and cytokeratin, rarely for desmin and negative for Factor VIII, CD117 and S100. Based on the histological pattern, staining characteristics and inflammatory cell infiltration, the tumor was classified as an inflammatory myofibroblastic tumor. Inflammatory myofibroblastic tumors are uncommon in the human literature and rarely described in the veterinary literature. Neoplastic cells exhibit a myofibroblastic phenotype which are typically reactive to vimentin, smooth muscle actin and desmin. It is proposed that cytokines produced by the neoplastic cells signal remarkable inflammatory cell infiltration. Inflammatory myofibroblastic tumor is a diagnosis of exclusion, and the variable appearance has resulted in inconsistent nomenclature.

DY-29: LACK OF CONSISTENCY IN URINARY AND RENAL LESIONS OF DISTEMPER IN RACCOONS (PROCYON LOTOR)

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Background: Distemper is a highly contagious and potentially lethal disease caused by Canine morbillivirus (CM) that affects dogs and several species of wild carnivores. Raccoons can act as reservoirs of the disease and spread it to susceptible species including sensitive populations. The urinary bladder is commonly collected for CM histopathological and immunohistochemical (IHC) diagnosis in many wild carnivores. Some of our submissions have even consisted solely of urinary bladder.

Objective: To evaluate if urinary bladder is a desirable testing organ for distemper in raccoons, as it can be in other species. Methods. We analyzed the collection of raccoons diagnosed with distemper from the Southeastern Cooperative Wildlife Disease Studies archives from 1999 to 2012.

Results: The were 87 raccoons from this 13-year-period in which the kidney or urothelium were evaluated. The percentage of cases in which a diagnosis of distemper could be achieved by either histopathology or IHC was low in urinary bladder (46.43%) and even lower in kidneys (32.88%). In these cases, characteristic eosinophilic inclusion bodies were observed in the urothelium from 73.08% of the bladders and in 54.17% of the epithelium from renal tubules. In some cases, renal tubules but not urothelium were positive.

Conclusion: These results indicate that sampling for diagnosing CM in raccoons should be done using alternative organs, such as brain, in which 93.48% of 92 cases

had evident lesions or tested positive for CM for the same time period. This work highlights the importance of choosing adequate sample collection for testing depending on the host species.

DY-30: A NOVEL BURSAL VIRUS, VISCERAL GOUT, AND CEREBRAL VASCULAR URATE DEPOSITION IN A DOUBLE-CRESTED CORMORANT, PHALACROCORAX AURITUS

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A juvenile, wild caught, great black cormorant, Phalacrocorax carbo, was submitted for necropsy at the University of Tennessee Veterinary Medical Center after a history of neurologic signs and seizures. Gross necropsy findings included marked inanition and severe multifocal disseminated urate deposition (visceral gout). Microscopically, the myocardium, lungs, kidneys, meningeal and cerebral vessels had urate deposition surrounded by epithelioid macrophages and fewer multinucleated giant cells. The gross and microscopic findings of visceral gout, specifically with deposition in the meningeal and cerebral vasculature likely resulted in the neurologic signs and seizures. Additional microscopic findings included lymphocytolysis within the bursa of Fabricius and the spleen. The bursa of Fabricius had large basophilic intranuclear viral inclusions and next generation sequencing was performed. A novel aviadenovirus was sequenced with only 76% homology to the closest related adenovirus, fowl aviadenovirus D. This is the first report of a novel bursal adenovirus and urate deposition in the cerebral vasculature of a cormorant.

DY-31: BILATERAL RENAL CYSTS RESEMBLING POLYCYSTIC KIDNEY DISEASE IN AN ADULT GUINEA PIG

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A 4-year-old intact female guinea pig was submitted for necropsy with a reported history of sudden death. Postmortem examination revealed marked bilateral enlargement of the kidneys. Both kidneys were expanded by myriad, 2-40mm diameter, fluid filled cysts. The bladder and urethra were grossly unremarkable with no evidence of urolithiasis or obstruction. Histologic examination of both kidneys revealed severe disruption of the cortical and medullary architecture by mildly to markedly dilated renal tubules separated by multifocal to coalescing areas of interstitial fibrosis with few remaining glomeruli. Tubules were lined by a variably attenuated intact squamous to cuboidal epithelium and contained large amounts of homogenous eosinophilic fluid mixed with small amounts of necrotic cellular and karyorrhectic debris. PKD is a genetic disease caused by mutation of the polycystic kidney disease genes, PKD-1 or PKD-2, and involves alteration in polycystin proteins. In veterinary species, PKD occurs in two forms: autosomal-dominant and autosomal-recessive. The autosomal-dominant form is similar to the adult-onset form described in humans and involves the insidious growth of renal cysts

over time leading to chronic renal failure in adult life. Gross and histologic findings in this guinea pig are most consistent with an autosomal-dominant (adult-onset) form of polycystic kidney disease (PKD) and death is attributed to chronic renal failure. PKD has been reported across a wide variety of species, however there are no previous reports in guinea pigs.

*Molecular detection of an autosomal-dominant mutation in the PKD-1 and PKD-2 genes by polymerase chain reaction amplification and sequencing is pending.

DY-32: NEURODEGENERATIVE DISEASE WITH DISTINCTIVE CEREBELLAR PATHOLOGY IN A GOLDEN RETRIEVER DOG

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An intact male Golden Retriever presented at the age of 4 months with a reportedly acute onset of cerebellar/hypermetric ataxia, marked intention tremors, and intermittent bouts of aggressive behavior. Between the initial presentation and euthanasia at 15 months of age, the patient had progressive, waxing and waning signs including seizures, vestibular signs, and severe bouts of aggression. The CBC, chemistry panel, and CSF analysis were within normal limits. At necropsy, cerebral cortices were moderately, symmetrically collapsed, with appropriate gyrification; the dorsal aspect of the cerebellar vermis was narrow and had decreased volume; there was moderate to marked hydrocephalus, with rupture of the septum pellucidum and atrophy of the periventricular white matter. Histologically, in the vermis, cerebellar granule cells and stellate cells were markedly swollen with an abundant, granular eosinophilic cytoplasm and an enlarged eccentric nucleus; there was marked Purkinje cell loss in these areas. This intracytoplasmic material was negative for autofluorescence under UV illumination, and failed to stain with PAS, luxol fast blue, sudan black, oil red O, Giemsa, and toluidine blue, and did not label with GFAP or Iba-1. Ultrastructurally, an increased numbers of mitochondria were present within affected cells. Further diagnostic tests such as IHC for mitochondria and genetic testing is in progress in an attempt to confirm the diagnosis. The histological appearance of the affected cells in this case is unique and does not match any reported canine diseases.

DY-33: EXTRAMEDULLARY PLASMACYTOMA IN A HAMSTER

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Background: An extramedullary plasmacytoma is a neoplastic proliferation of plasma cells arising from outside of the bone marrow. It is a relatively common tumor in old dogs and occurs most frequently on the skin and mucous membranes. Salivary gland plasmacytomas are extremely rare within all species.

Case Description: A 1.5-year-old, intact female Syrian hamster (*Mesocricetus auratus*) showed respiratory distress and a ventral cervical mass was palpated. Due to the overall poor prognosis, the animal was humanely euthanized and submitted for necropsy. On gross examination a subcutaneous, round, loosely attached, semi-firm

mass measuring approximately 1 cm in diameter was identified at the ventral neck region. Histopathologic examination of the cervical mass revealed neoplastic proliferation of mononuclear cells with plasmacytoid morphology adjacent to and infiltrating the submaxillary salivary gland. The neoplastic cells were positive for CD79a and negative for CD3. Based on the diagnostic test results and histopathologic evaluation of the mass tissue sections, a diagnosis of an extramedullary plasmacytoma was determined. In addition, the neoplastic cells were occasionally surrounded by moderate amounts of amorphous eosinophilic material, which was negative for Congo red stain and Periodic acid–Schiff.

Summary: This is a case report of an extramedullary plasmacytoma in a hamster arising from the submaxillary salivary gland. Prior reports of extramedullary plasmacytomas originating in Syrian hamster salivary glands suggests a predisposition to development of plasmacytic tumors in this location.

DY-34: PULMONARY TOXOPLASMOSIS IN A DOG

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Background: Toxoplasmosis is a worldwide disease caused by *Toxoplasma gondii,* which is an obligate intracellular parasite. *T. gondii* infects virtually all species of warm-blooded animals, including humans, as intermediate hosts. Domestic cats and other Felidae are the definitive hosts that excrete oocysts. Toxoplasmosis is seen most frequently in young animals, often facilitated by immunosuppression. Clinical signs of toxoplasmosis may be localized to the respiratory, neuromuscular, or gastrointestinal systems, or may be related to generalized infection. Canine toxoplasmosis is similar to infection with *Neospora caninum*; toxoplasmosis is more prevalent in cats, while neosporosis is seen more frequently in dogs.

Case Description: A 9-year-old, spayed female, Pit-bull dog presented for behavior changes, anorexia, and respiratory distress. She had been treated with radiation therapy for a pituitary macroadenoma three months prior to presentation. Thoracic radiographs revealed severe nodular pattern in the lungs. Metastatic neoplasia or fungal pneumonia was suspected. Due to poor prognosis, the dog was humanely euthanized and submitted for necropsy. Grossly, all lung lobes contained multifocal, white, firm, nodules ranging in size from 0.2 to 2.0 cm diameter. Microscopic examination revealed the nodules to consist of densely cellular pyogranulomatous inflammation associated with multifocal areas of necrosis. Intralesional macrophages frequently contained basophilic, 2-4 µm, round to oval protozoal zoites within cytoplasmic vacuoles. Immunohistochemical stains for *T. gondii* antibody were moderately positive. PCR performed on the formalin-fixed, paraffin-embedded lung tissue was positive for *T. gondii.*

Summary: Pulmonary toxoplasmosis is generally a diffuse, interstitial disease, whereas this dog had an unusual, multi-nodular, pyogranulomatous presentation.

DY-35: BRONCHOPULMONARY DYSPLASIA WITH SECONDARY BULLA RUPTURE AND BACTERIAL BRONCHOPNEUMONIA IN A YOUNG SPHYNX CAT

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Background: Bronchopulmonary dysplasia occurs in human infants and has been reported in one cat previously. A 2.5-year-old, spayed female Sphynx cat was managed for two years for chronic cough and exercise-induced tachypnea that was initially responsive to corticosteroids. Serial imaging studies revealed progression from a marked diffuse bronchial and focal alveolar pattern to saccular bronchiectasis and bullous emphysema. Bronchoalveolar lavage revealed mixed inflammation and chronic hemorrhage. The cat ultimately presented for acute pneumothorax with suspected bulla rupture and was subsequently euthanized.

Objective: Our aim was to confirm bulla rupture and further characterize the features of bronchopulmonary dysplasia in this cat.

Methods: Postmortem lung samples were collected, submitted for bacterial culture, and processed routinely for histologic analysis.

Results: Gross evaluation revealed marked multifocal to coalescing bullous emphysema with pleural fibrosis, endogenous lipid pneumonia, and congestion in non-emphysematous parenchyma. A focal ruptured bulla was confirmed. Histopathology revealed marked distortion of pulmonary architecture by thickened, disrupted, and blunted alveolar septae that were lined by hypertrophied type II pneumocytes. Alveolar septae formed large bullae or markedly narrowed air spaces, with frequent consolidation of thickened septae into areas of fibrosis. These findings are consistent with bronchopulmonary dysplasia. A mild neutrophilic bronchopneumonia was also present and aerobic culture grew *Bordetella bronchiseptica*.

Conclusions: Bronchopulmonary dysplasia should be considered as a differential in young feline patients with radiographic evidence of bronchiectasis and bullous emphysema. The pathogenesis of this disease in the cat is unknown, but can be further complicated by secondary bacterial infection and bulla rupture.

DY-36: CHOLEDOCHAL CYST WITH SECONDARY CHOLANGITIS, CHOLEDOCHITIS, DUODENAL PAPILLITIS, AND PANCREATITIS IN A YOUNG DOMESTIC SHORTHAIR CAT

Megan Schreeg[1], Sybille Miller[2], John Cullen[1]
[1]North Carolina State University College of Veterinary Medicine, Raleigh, NC, USA, [2]Veterinary Centers of America, Leesburg Veterinary Internal Medicine, Leesburg, VA, USA

Background: Choledochal cysts, congenital segmental dilations of the common bile duct, have been reported in cats but histologic characterization is lacking. A 1.75 year-old spayed female domestic shorthair cat was presented for vomiting and weight loss. There was progressive elevation of liver enzyme activity (ALT>ALP, GGT) and hyperbilirubinemia. Diagnostic imaging identified a focal cystic dilation of the common bile duct, dilation and tortuosity of adjacent hepatic ducts, and a prominent duodenal papilla. A choledochal cyst was suspected and the animal was euthanized.

Objective: Our aim was to characterize the gross and microscopic pathology of the choledochal cyst and associated tissues.

Methods: The liver, gallbladder, duodenum, and pancreas were fixed whole for further gross evaluation and were processed for histologic evaluation.

Results: Grossly there was a 2 cm cystic dilation of the common bile duct that was moderately firm and thickened but patent with adjacent ducts. Histologically the cyst wall was expanded by myofibroblasts, collagen, and lymphoplasmacytic inflammation. Adjacent bile ducts were markedly dilated and tortuous, with similar inflammation and papillary mucosal infolding that extended to the major duodenal papilla. There was moderate to marked chronic neutrophilic cholangitis with fibrosis, suggesting chronic bacterial infection and obstruction. There was mild lymphoplasmacytic pancreatitis with similar duct changes, suggesting a link with biliary disease. Duodenal lymphoid follicles were prominent, suggesting chronic immune stimulation.

Conclusions: Choledochal cysts can be associated with chronic obstructive cholangitis, choledochitis, duodenal papillitis, and pancreatitis, and should be a differential for increased liver enzymes and hyperbilirubinemia in young cats.

DY-37: MYCOBACTERIUM ABSCESSUS PNEUMONIA IN A CAT

Cheng-Hsin Shih[1], Ying-Chen Wu[2], Wei-Hsiang Huang[1]
[1]Graduate Institute of Molecular and Comparative Pathobiology, National Taiwan University, Taipei, Taiwan, [2]Animal Disease Diagnostic Center, National Chung Hsing University, Taichung, Taiwan

Background: Mycobacterium abscessus, classified among the rapidly-growing and nontuberculous mycobacteria, is one of the causative agents of atypical mycobacterioses. These bacteria are ubiquitous in the environment. They can cause opportunistic infection in both healthy and immunocompromised animals or people. Here we report the first case of feline Mycobacterium abscessus pneumonia. Case description: A ten-year-old spayed female domestic shorthair cat presented with chronic cough for three years. Although treatments, including antibiotics and steroids, were administered, the respiratory signs still worsened. CT scan showed extensive lung consolidation except for the right caudal lobe. Pneumonia was suspected, but the etiology was not identified. The cat died at home and the body was submitted for pathological examination.

Results: On necropsy, the cat was obese without any trauma. The lungs were diffusely consolidated, failed to collapse and contained multifocal to coalescent beige, bulging, plaque-like lesions. Microscopically, diffuse granulomatous pneumonia characterized by numerous lipid-laden macrophages, some lymphocytes, and rare neutrophils effaced the pulmonary parenchyma. The alveolar inflammatory infiltrates often surrounded a central lipid vacuole which contained small numbers of acid-fast bacilli. Mycobacterium abscessus was cultured from frozen lung samples.

Conclusions: The current case, which presented with granulomatous pneumonia with intralesional acid-fast bacilli and lipid vacuoles, is consistent with atypical mycobacterial pneumonia caused by ubiquitous nontuberculous Mycobacterium abscessus. To the author's knowledge, this is the first case report of feline Mycobacterium abscessus pneumonia.

DY-38: CD45 EXPRESSION IN CANINE NODAL T-ZONE LYMPHOMA

Leah Stein[1], Cynthia Bacmeister[2], Matti Kiupel[1]
[1]Michigan State University Veterinary Diagnostic Laboratory, East Lansing, MI, USA,
[2]Antech Diagnostics, Irvine, CA, USA

Background: T-zone lymphoma (TZL) is a subgroup of nodal T-cell lymphoma that has been well characterized both histologically and immunophenotypically in dogs. Impairment of CD45 gene expression has recently been identified in canine TZL, and as such, loss of CD45 expression has become a key feature in diagnosing TZL via flow cytometry only without morphologic confirmation.

Objective: The goal of this study was to further characterize the immunophenotype of canine nodal TZL.

Methods: Twenty-six TZLs were selected based on their morphologic features and tissue micro arrays were generated to evaluate expression of CD3, CD5, CD21, CD25, CD45, Bcl-6, and Ki67.

Results: TZLs were characterized by proliferating small to intermediate sized T cells with abundant, water clear cytoplasm and densely staining nuclei that expanded the paracortex and compressed lymphoid follicles. Neoplastic T cells in all cases were positive for CD3, CD5, and CD25, and were negative for CD21 and Bcl-6. One of the 26 cases was positive for CD45. The average Ki67 proliferation index was 19.45.

Conclusion: The observed immunophenotype and low proliferation index for canine TZL is similar to previous publications. Interestingly, one case expressed CD45. Loss of CD45 expression in CD3+ lymphomas has been utilized as the sole criterion to diagnose TZL using flow cytometry. Our results indicate that loss of antigenicity of a single CD molecule may be insufficient to accurately diagnose each TZL and a final diagnosis should include morphologic assessment to confirm the characteristic architecture of this neoplastic entity.

DY-40: PROXIMAL URETERAL FIBROEPITHELIAL POLYP WITH SUBSEQUENT RENAL ATROPHY IN A DOG

Charles Talbot, Laura Lowe, Maninder Sandey
Auburn University, Auburn, AL, USA

The current report describes the gross and histologic features of a ureteral fibroepithelial polyp resulting in renal atrophy in a 12-year-old entire male Papillon dog. The dog was presented to the Auburn University Veterinary Teaching Hospital Emergency and Critical Care Service after being hit by a car with an incidental history of chronic hematuria. During necropsy examination, the proximal right ureter was markedly dilated and contained a 1.5 x 2.2 cm multi-lobulated, tan-white to dark red soft mass adhered to the ureteral mucosa by a fibrous stalk. The right kidney was markedly smaller compared to the left. Histologically, the mass was exophytic, moderately cellular and composed of numerous papillary projections with thick fibrovascular cores lined by transitional epithelium. The right kidney had diffuse interstitial fibrosis and atrophic changes. This case is a typical presentation of this neoplasm within the ureter resulting in chronic renal injury associated with luminal obstruction. Ureteral neoplasms are rare in both veterinary and human patients with fibroepithelial polyps, leiomyoma, leiomyosarcoma and transitional cell carcinomas the most common. Only eight documented cases of fibroepithelial polyps have been described, with the most recent in 2006. The etiology is not completely understood, and believed to represent either a benign neoplasm or chronic inflammatory reaction associated with urinary incontinence, urinary tract infections, hydronephrosis or hydroureter. Despite the rarity of this condition, it is important to be aware of this neoplasm and should be considered as a possible differential in animals presenting with hematuria, recurrent urinary tract infections or urinary incontinence.

DY-41: MORPHOLOGIC CHARACTERIZATION OF NASOPHARYNGEAL CICATRIX SYNDROME IN TEN HORSES

Brianne Taylor, Keith Chaffin, Raquel Rech
Texas A&M University, College Station, TX, USA

Nasopharyngeal cicatrix syndrome (NCS) is a poorly understood respiratory condition frequently diagnosed in Texas but not reported outside of the southeastern U.S. NCS involves the development of a regionally extensive cicatrix or scar in the upper respiratory tract, and horses typically present with increased respiratory noise and exercise intolerance. Several risk factors have been associated with NCS, but the pathogenesis remains unclear. From 2016 to 2018, ten clinically diagnosed horses were submitted to Texas A&M University for necropsy. Horses ranged from 9 to 28 years of age. Eight of the ten horses were diagnosed endoscopically, and one horse had previously undergone a palliative permanent tracheostomy. Grossly, the laryngeal structures were affected in all ten cases, and lesions varied from granulation tissue to severe, circumferential fibrosis. The arytenoids were involved in all ten cases, and the epiglottis was involved in eight cases. The proximal trachea was affected in seven cases and in one case extended to the thoracic inlet. Moderate to marked airway occlusion was observed in seven cases. The glottic cleft was partially occluded in three cases, and the proximal trachea rostral to the fourth tracheal ring was partially occluded

in four cases. Histologically, all cases revealed varying degrees of mucosal and submucosal granulation tissue and fibrosis. Lymphoplasmacytic inflammation with frequent erosion and ulceration was seen in eight cases. In this report, cicatrix formation predominantly involves the larynx and proximal trachea.

DY-42: EPICARDIAL ECTOPIC LIVER IN A CAT WITH FELINE INFECTIOUS PERITONITIS

Xiaobo Wang, Charles Talbot, Katharine Horzmann, Lydia Pena
Auburn University, Auburn, AL, USA

Background: A 1-year-old neutered male American Domestic Shorthair cat presented to the Auburn University Small Animal Emergency and Critical Care service after a 4-day-history of anorexia, lethargy, and anemia. The clinical signs continued to decline and the cat was euthanized.

Objective: The current report describes the gross and histologic features of a case of feline infectious peritonitis (FIP) with ectopic liver in a 1-year-old neutered male American Domestic Shorthair cat.

Methods: After a standard necropsy and gross evaluation, samples of major organs and an epicardial mass were fixed in neutral buffered formalin, routinely processed for histopathology, stained with hematoxylin and eosin, and examined histologically.

Results: The kidney, lungs, mesentery, and mesenteric lymph nodes had gross and histological evidence of pyogranulomatous vasculitis and inflammation characteristic of FIP. Incidentally on gross examination, a 1.8 cm × 1.2 cm × 0.1 cm, brown, soft, mass was firmly adhered to the pericardium and underlying right ventricular epicardium. Histologically, the mass was attached to the epicardium and had the appearance of a normal liver, with cords of hepatocytes, portal triads, and central veins. The ectopic liver had no connection with the main liver. The Feline Coronavirus indirect fluorescent antibody titer was 1:102400.

Conclusions: The gross, histopathological, and virological testing in this case were consistent with feline infectious peritonitis with a co-incidental finding of epicardial ectopic liver. Ectopic liver has been previously described in two cats. It is presumed, based on similar human cases, that these abnormalities are congenital in origin.

Education Focused Scientific Session

Tuesday, November 12, 2019 | 8:00 a.m. – 12:00 p.m.
Session Chair: Mee-Ja Sula, DVM, DACVP, University of Tennessee, Knoxville, TN

Tuesday, November 12, 2019
11:50 a.m. – 12:00 p.m.

USE AND BENEFITS OF DISTANCE MENTORING IN VETERINARY PATHOLOGY

Nicola Parry
Midwest Veterinary Pathology, LLC, Lafayette, IN, USA

Background: In veterinary pathology, positive mentorship is of critical importance in exposing students to a career in pathology, providing scholarly guidance and career counseling for residents, and promoting the success of colleagues or offering them support.

Objective: However, the gap between supply (mentors available) and demand (mentors wanted) may be disproportionately large. Distance mentoring has great potential to bridge that gap and improve the effectiveness of mentorship, by facilitating a customized solution that better fits the individual person and their situation. It also avoids either overburdening local mentors or forcing some pathologists into mentoring positions if they prefer not to participate. Additionally, for students or residents looking to subspecialize in a specific area of pathology, on-site mentors who work in this area may not be available.

Methods: For 10 years, I have served as a distance mentor for many students and residents in the US and the UK, making use of different methods of communication, such as email, telephone, and video conferencing.

Results: This has been successful in various ways, including for offering informal ad hoc career guidance, providing ongoing advice and support for students and veterinarians pursuing a path to residency, and even for temporarily providing semi-structured training support for residents studying for the ACVP certifying examination.

Conclusion: Although more challenging than in-person mentoring, this distance format can be highly effective and mutually beneficial. It also holds additional potential as a tool to tailor the mentorship experience for underrepresented minorities, and to support students and pathologists in resource-limited countries.

Education Focused Scientific Poster Session A

E-01: IN THE PALM OF YOUR HAND: AN EDUCATIONAL TOOL FOR UNDERSTANDING AND EXPERIENCING RODENT ANATOMY

Erin Cox, Alyssa Ivy, Symantha Berzynski, Nourah Abusada, Wesley Estrada, David Wadkins, Hannah Brown, Georgina Ofori-Amanfo, Mariah Leidinger, Katherine Gibson-Corley
University of Iowa, Iowa City, IA, USA

Background: Toxic tissue fixatives present a frustrating roadblock for pathologists preserving specimens for educational use. This is especially true when working with children, who are vulnerable to chemical exposure and unfamiliar with the use of personal protective equipment.

Objective: To create a tool we could use for educational outreach in our communities that would allow students of all ages to experience real anatomy safely in a self-directed or group environment.

Methods: Select rodent organs were fixed in 10% neutral buffered formalin, embedded in a clear casting resin, and set in molds. Using these tissue blocks we created an interactive, table-top activity mat which encourages students to identify organ function and location in the body.

Results: We curated four full sets of tissue blocks, each with a play mat, and presented them to an elementary science fair and to a summer program for elementary-aged students. The blocks generated excitement and interest from students, parents, and educators, and were durable during normal handling and dropping. The activity mat and blocks provided students a hands-on, visual tool to learn basic anatomy and organ function through cooperative effort.

Conclusions: This tool benefits both educators and students. Educators appreciate the value of real tissues and a student-driven group learning experience. Students engage in collaborative learning and enjoy the novelty this intuitive educational tool brings to the classroom.

E-02: PAPERLESS PATHOLOGY SERVICE IN A UNIVERSITY SETTING: ARE WE READY TO CONVERT?

Pompei Bolfa[1], Antoine Laws[1], Imani Phipps[1], Candita Chapman[1], David Hilchie[1], Maurice Matthew[1], Michelle Dennis[1], Oscar Illanes[1,2], Elpida Artemiou[1]
[1]Ross University School of Veterinary Medicine, Basseterre, Saint Kitts and Nevis, [2]College of Veterinary Medicine, Long Island University, Brookville, NY, USA

Background: Most veterinary pathology diagnostic laboratories use, predominately, paper submission forms to request a wide range of diagnostic procedures. This requires large archival storage space and makes information retrieval difficult and time consuming. Nowadays modern technology allows for the implementation of a fully digital, environmentally-conscious and time efficient workplace.

Objective: To promote an environmentally friendly workplace by providing a paperless working environment.

Methods: Paper forms utilized in the RUSVM pathology services were digitalized, using Adobe Acrobat Pro DC®, and archived in a friendly fillable interactive format. They included the Autopsy request, Biopsy-Histopathology request, Cremation request, Incineration request, Student autopsy sign-up and Guideline acknowledgement forms. Technical considerations included dropdown options for sex, date, as well as yes or no option responses, signature, reset form button, and submit button configured to email the form to the end user. Customized email addresses were created to which the forms were automatically sent. Adobe Acrobat Reader® was installed on all electronic devices (iPads®) that were placed in key areas within the lab.

Results: The digitalized forms created work on computers, tablets or smartphones, using both Apple® and Microsoft® technology. This paperless system connects in a

timely manner the clients, university clinic, pathology technicians office, trimming room, autopsy floor and histology lab.

Conclusions: This digitalized format model was implemented successfully; it has improved the pathology service communication, efficiency, tracking and filing procedures, as well as reduced costs surrounding use of paper and labor. This environmentally-conscious system can easily and cost-effectively be implemented in similar laboratory settings.

Education Focused Scientific Poster Session C

E-01: CYTOLOGY ATLAS IN WILD ANIMALS

Marina Guadarrama Olhovich[1], Mariela Díaz Negrete[2], Carlos Reyes Cetina[1], Javier Ojeda Chávez[1,2]
[1]Veterinary Medicine and Zootechnic Faculty, National Mexican Authonomous University, Mexico, Mexico, [2]General Direction of Zoos and Conservation of Wild Animals, México, Mexico

Wild and exotic animal medical care demands have increased during the last few years as they require minimal invasive and highly specific diagnostic techniques to improve and accelerate treatment implementation to cure different diseases. The present cytology (OR Cytopathology) atlas illustrates different lesions in wild and exotic animals, emphasizing the distinction between inflammatory and non-inflammatory lesions and between benign and malignant neoplastic diseases. Information such as effective treatment, prognosis and frequency of each clinical case are recommended to develop special diagnostic tests. Fifty cytology slides were selected from mammals (34), birds (8), reptiles (4) and amphibian (4). Twenty seven of 50 cases were neoplastic lesions and the remaining cases were inflammatory diseases. Malignant mesenchymal neoplasia was the most common finding, followed by benign mesenchymal neoplasia, malignant epithelial tumors and finally benign epithelial tumors. No round cell tumors were diagnosed. Skin was the most frequent site of the origin of lesions followed by lesions in the kidney, bowels, muscle, liver, mammary gland, ovary and uterus. Septic suppurative inflammatory diseases were the most common findings in the skin followed by thoracic and/or abdominal effusions, joints, uterus, bowel and tracheal. The main objectives of this atlas were to generate a digital cytology document depicting the most common lesions in wild animals, to teach undergraduate students about cellular changes characteristics of different diseases/tumors, and to correlate the cytologic diagnosis (es) with the clinical course of different diseases in these animals.

E-02: CLINICAL PATHOLOGY COURSE REVIEW FOR DAY 1 COMPETENCIES

Maxey Wellman[1,2], Melinda Rhodes-DiSalvo[1,2], Jay Hsiao[1,2], Linda Lord[1,2]
[1]College of Veterinary Medicine, Columbus, OH, USA, [2]The Ohio State University, Columbus, OH, USA

Some courses in veterinary curricula are taught by faculty members who have had limited or no experience in a private practice setting, making it difficult for these faculty

members to know whether the material being taught in their didactic courses is relevant to general practice. In this study, general practitioners reviewed lecture notes for the Clinical Pathology core curriculum course presented to first-year veterinary students during the first semester of their four-year program at The Ohio State University. Practitioners used a Likert scale to rank relevance of the material to general practice, how often they encounter the topic in practice, and their perception of whether the material should be kept in the curriculum. Practitioners also responded to three questions about material taught in the laboratories for this course: How often do you look at a blood smear? How often do you look at a cytology smear? and How important is it for new graduates to know how to use a microscope? Lastly, practitioners ranked how often they see various hematologic abnormalities in practice from a list of topics presented in the course. Results of the surveys were summarized so that course instructors could incorporate key findings into course design for the upcoming academic year. This information will be an important contribution to the relevance of the veterinary curriculum and the American Veterinary Medical Association's Standard 11 Outcomes Assessment requirements for Day 1 competencies.

Experimental Disease Focused Scientific Session

Sunday, November 10, 2019 | 1:30 p.m. – 5:00 p.m.
Session Chair: Oded Foreman, DVM, DACVP, Genentech Inc., South San Francisco, CA

Sunday, November 10, 2019
2:30 p.m. – 2:45 p.m.

PATHOLOGY OF THE BLOOD-TUMOR BARRIER IN THE DEVELOPMENT OF NON-SMALL CELL LUNG CANCER BRAIN METASTASES

L. Tiffany Lyle, Alexandra Dieterly, Chinyere Kemet, Hsin-Yi Weng, Arvin Soepriatna, Craig Goergen, Aparna Shinde, Michael Wendt, Gozde Uzunalli
Purdue University, West Lafayette, IN, USA

Objective: Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related deaths. Approximately 50% of NSCLC patients are diagnosed with brain metastasis during the course of their disease with a 2-month survival time. Brain metastases confer a dismal prognosis due to the shift of the blood-brain barrier (BBB) to the insufficiently characterized blood-tumor barrier (BTB). Effective drug delivery through the BTB is one of the greatest therapeutic obstacles in brain metastases. We hypothesize that the growth of NSCLC brain metastases leads to time-dependent pathologic alterations in the BTB.

Methods: An experimental model of NSCLC brain metastasis was developed using ultrasound-guided intracardiac injection of NSCLC cells. Components of the BBB and the BTB were evaluated over a six-week period using immunofluorescence microscopy. Statistical analysis was performed with a mixed-regression model.

Results: Our results demonstrated a dynamic time-dependent phenotype of the BTB. Capillaries of the BTB were dilated with increased CD31 expression compared to the healthy BBB. There was a 4.0-fold ($p<0.001$) increase in desmin^{+}- contractile pericytes

in the BTB compared to the BBB. The most striking changes were identified in astrocyte water channels with a 12.2-fold ($p<0.001$) decrease in aquaporin-4 in the BTB. The BTB of human NSCLC brain metastases consisted of dilated capillaries and loss of aquaporin-4 expression; corroborating experimental findings.

Conclusion: This research has provided the first comprehensive time course analysis of the BTB formation in NSCLC brain metastasis. Our results demonstrated that astrocytic endfeet, and pericytes can be modulated to enhance targeted therapeutics and improve patient survival.

Sunday, November 10, 2019
2:45 p.m. – 3:00 p.m.

REQUIREMENT OF STEROL REGULATORY ELEMENT-BINDING PROTEIN PATHWAY IN PANCREATIC DUCTAL ADENOCARCINOMA

Stephanie Myers, Meredith McGuire, Wei Shao, Chune Liu, Theodore Ewachiw, Zeshaan Rasheed, William Matsui, Peter Espenshade
Johns Hopkins University, School of Medicine, Baltimore, MD, USA

Background: Pancreatic ductal adenocarcinoma (PDAC) is an aggressive tumor with limited diagnostic and therapeutic options. PDAC tumor cells are extremely proliferative with a high requisite demand for lipids. However, the tumor microenvironment is poorly vascularized and hypoxic. As lipid synthesis is oxygen-consumptive, neoplastic cells are challenged with meeting the demand for lipids. Cancer cells respond to lipid conditions through the sterol regulatory element-binding protein (SREBP) pathway, which requires SREBP cleavage activating protein (SCAP) during signaling.

Objective: To test the requirement of SCAP in PDAC using both *in vitro* and *in vivo* model systems.

Methods: Utilizing four patient-derived PDAC cell lines, *SCAP* was knocked out and followed by gene rescue. Cell growth assays were performed in lipid-poor and lipid-rich media conditions. Subcutaneous and pancreatic orthotopic xenografts were performed in nude mice using wild type and *SCAP* knockout cells. Using an established PDAC mouse model, *LSL-Kras^{G12D}; LSL-Trp53^{R172H}*; *Pdx-1 Cre* (KPC), KPC and other mice lacking *Scap* in one or both alleles were generated.

Results: In lipid-poor conditions, *SCAP* knockout cells showed significantly reduced growth. In subcutaneous xenografts, *SCAP* knockout cells exhibited reduced tumor volume in 3 out of 4 cell lines. Similarly, *SCAP* knockout cells grew poorly in pancreatic orthotopic xenografts with reduced splenic metastases. Mice lacking *Scap* are clinically and phenotypically normal.

Conclusions: Loss of *SCAP* in PDAC tumor cells alters growth both *in vitro* and *in vivo*. Additionally, *Scap* is not required for pancreas function in mice. These findings suggest SCAP may be useful as a therapeutic target in PDAC.

Sunday, November 10, 2019
4:30 p.m. – 4:45 p.m.

EARLY INFLAMMATORY LESIONS IN THE PROXIMAL OVIDUCT OF THE LAYING HEN: PRECURSORS TO OVARIAN CARCINOMA

K Denise Apperson[1,2], Karyn Bird[2], Gita Cherian[2], Christiane Löhr[2]
[1]Arkansas Veterinary Diagnostic Lab, Little Rock, AR, USA, [2]Oregon State University, Corvallis, OR, USA

Background: There is strong genetic and histopathological evidence that high-grade serous ovarian carcinomas in women originate in proximal oviductal epithelium and metastasize to the ovary. Laying hens spontaneously develop ovarian carcinomas and are a preferred animal model for the disease.

Objectives: It is not known if laying hens develop precursor lesions in proximal oviductal tissue similar to those described in women.

Methods: Reproductive tissues were collected from two groups of laying hens at 78 weeks and 90 weeks of age and examined for microscopic histological characteristics.

Results: In 86% of 90-week hens, we observed transitional intra-epithelial carcinomas (TICs). These lesions consisted of localized, multilayered, proliferative regions of hyperplastic epithelium. Cells facing the lumen retained apical cilia. Affected hens had multiple TICs throughout the proximal oviduct. Poorly differentiated, invasive carcinomas in the proximal oviducts of the same hens were also observed. The lesions did not protrude into the oviductal lumen and were not grossly visible. The carcinomas were comprised of nests of epithelial cells; apical cilia were present in visible lumens. The nests were either tightly packed with no intervening stromal cells, or they were intermixed with normal glandular acini. Similar oviductal lesions were not observed in 78-week hens.

Conclusions: Neoplastic transformations in proximal oviductal epithelium appear to be an early process, occurring well before detectable loss of function or formation of grossly detectable ovarian tumors in laying hens. The tissue of origin of ovarian carcinomas has significant implications for disease screening, chemotherapy drug targets, and appropriate surgical interventions in women.

Sunday, November 10, 2019
4:45 p.m. – 5:00 p.m.

AGE-RELATED DIFFERENCES IN THE PATHOGENESIS OF CLADE 2.3.4.4A H5N2 HPAIV IN COMMERCIAL BROAD BREASTED WHITE TURKEYS

Silvia Carnaccini[1], Daniel Perez[1], Daniela Rajao[1], Jefferson Santos[1], Adebimpe Obadan[1], Mary Pantin-Jackwood[2], David Suarez[2]
[1]Poultry Diagnostic and Research Center, University of Georgia, College of Veterinary Medicine, Athens, GA, USA, [2]Southeast Poultry Research Laboratory, U.S. National Poultry Research Center, U.S. Dept. of Agriculture, Agricultural Research Service, Athens, GA, USA

Background: The 2.3.4.4A newly emerged highly pathogenic avian influenza (HPAI) H5 clade quickly spread through migratory birds and caused economic losses worldwide. In 2014-15, this reassortant H5 virus led to high mortality in more than 200 commercial poultry operations across the United States costing millions of dollars in economic losses. Although AIV is one of the most studied poultry diseases, only few studies have characterized the pathogenesis of HPAIV in turkeys. Furthermore, there are no studies reporting pathologic findings of HPAIV in commercial turkeys at “market age”.

Objective: Our aim was to characterize the pathology and antigen tissue distribution in naïve commercial turkeys intranasally inoculated with H5N2 clade 2.3.4.4A HPAIV at different ages.

Methods: Turkeys were challenged with 10^6.5 EID50/bird of H5N2 HPAIV A/turkey/Minnesota/12582/2015 respectively at 6 and 16-weeks of age. Clinical signs, mortality, pathology and histopathology were paired with AIV antigen detection in multiple organs by immunohistochemistry. RT-qPCR was used to quantify viral shedding in tracheal swabs and viral loads in the tissues.

Results: Older birds survived longer and shed higher titers of virus. Both age-groups were able to get infected and reached 100% mortality by day 5 post-challenge. Lesions ranged from none to severe hemorrhagic and fibrinoheterophilic pneumonia, necrotizing pancreatitis and splenitis, heterophilic meningoencephalitis and myocarditis. Influenza A virus immunohistochemistry confirmed systemic virus antigen dissemination.

Conclusions: Longer age-related survival time may be critical for disease transmission as prolonged subclinical virus shedding increases the chances of viral spread between individuals and populations.

Combined Experimental Disease and Industrial & Toxicologic Pathology Focused Scientific Session

Tuesday, November 12, 2019 | 1:30 p.m. – 5:00 p.m.
Session Chairs: Oded Foreman, DVM, DACVP, Genentech Inc., South San Francisco, CA and Katherine A.B. Knostman, DVM, PhD, DACVP, StageBio, Mount Jackson, VA

Tuesday, November 12, 2019
2:30 p.m. – 2:45 p.m.

FLOW CYTOMETRY PROVIDES QUANTITATIVE DATA FOR BONE MARROW SAFETY EVALUATION IN INVESTIGATIVE ONCOLOGY MOUSE MODELS

Clare Hoover, Denise Hughes, Laura Prickett, Courtney Andersen, Jay Mettetal, Mark Anderton, Sean Redmond, Kim Maratea
AstraZeneca, Boston, MA, USA

Oncology therapeutics frequently have dose-limiting toxicities due to on-target or off-target bone marrow effects. Safety evaluation has traditionally relied on bone marrow histopathology evaluation to identify these toxicities, which can miss subtle shifts in cell

populations due to the high cell density and tissue complexity. Hematology evaluation is restricted to mature populations in the bloodstream and may not accurately reflect acute bone marrow alterations. NSG strain (NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ, Jackson Laboratories) immunocompromised mice are used for investigative oncology studies due to their ability to support patient derived xenografts. Despite being immunocompromised, these mice provide opportunities to acquire preclinical mechanistic and toxicity information early in the drug discovery process. To establish methods of bone marrow evaluation in this mouse strain, we first validated a bone marrow flow cytometry protocol that quantifies the three bone marrow cell lineages: erythroid, myeloid, and megakaryocyte. We then applied flow cytometry and histopathology analysis to compare bone marrow toxicity between monotherapy and combination oncology therapeutic studies in NSG mice. In these studies, histopathology identified minimal to marked treatment-related cytotoxicity in bone marrow populations, with greater severity following combination versus monotherapy. Flow cytometry cell lineage differential counts correlated with these histopathology changes and identified cytotoxicity in both myeloid and erythroid lineages, which was not able to be determined microscopically. In comparison, hematology data displayed minimal changes when compared with vehicle-treated cohorts. These data demonstrate the usefulness of flow cytometry quantitative data in bone marrow safety evaluation from investigative studies early in the drug discovery process.

Tuesday, November 12, 2019
2:45 p.m. – 3:00 p.m.

I SPY WITH MY (VIRTUAL) EYE: ASSESSMENT OF A MURINE MODEL OF CLOSTRIDIUM DIFFICILE TYPHLITIS BY TRADITIONAL MICROSCOPY AND BY A DEEP LEARNING ARTIFICIAL NEURAL NETWORK

Ingrid Bergin, Evan Czyzycki, Benjamin Li, Michael Dieterle, Kathryn Eaton, Mark Hoenerhoff, Jenna Wiens, Vincent Young University of Michigan, Ann Arbor, MI, USA

Background: Histology severity scoring is frequently used to assess interventions, genetic perturbation, and other factors in experimental animal models. Traditional scoring can be tedious and subject to bias. Further, few universal scoring standards exist and formal validation is infrequently performed.

Objective: We validated traditional microscopy-based scoring of our established murine model of *Clostridium difficile* typhlitis. Additionally, we constructed a convolutional neural network (CNN)-based deep learning model to improve efficiency.

Methods: We compared inter-observer and intra-observer reproducibility for edema, inflammation, and epithelial damage and validated scoring against weight loss as an independent disease marker. We trained a CNN model using pathologist-annotated training slides and tested performance on a held-out set of images.

Results: Traditional histology scoring had good inter-observer reproducibility (Kendall's W for concordance 0.818-0.868) and correlation with weight loss (Spearman correlation coefficients 0.652 -0.864) across parameters. The trained CNN model performed

comparably or superiorly to pathologists in predicting weight loss, with 95% confidence intervals for the area under the receiver operating characteristic curve (AROC) of 0.694-0.846 for the CNN, 0.565-0.731 for an individual pathologist, and 0.741-0.873 for the mean of three pathologists. Further, the trained model improved efficiency over traditional histologic scoring.

Conclusions: This project validated histology scoring for a murine model of *Clostridium difficile* and illustrated use of a deep learning model that met or exceeded traditional microscopy scoring in terms of reproducibility and accuracy (predicting weight loss). Similar pipelines may be useful for validating other preclinical histology scoring systems and generating artificial intelligence models for improved pathologist efficiency.

Tuesday, November 12, 2019
4:30 p.m. – 4:45 p.m.

LONGITUDINAL PHENOTYPIC DEVELOPMENT IN A PIG MODEL OF NEUROFIBROMATOSIS 1

David Meyerholz[1], Johanna Uthoff[1], Jared Larson[1], Shawn Sato[1], Emily Hammond[1], Frank Rohret[2], Christopher Rogers[2], Dawn Quelle[1], Benjamin Darbro[1], Rajesh Khanna[3], Jill Weimer[4], Jessica Sieren[11]University of Iowa Carver College of Medicine, Iowa, IA, USA, [2]Exemplar Genetics, Sioux Center, IA, USA, [3]University of Arizona, Tucson, AZ, USA, [4]Sanford Research, Sioux Falls, SD, USA

Neurofibromatosis type 1 (NF1) is an autosomal dominant RASopathy that predisposes patients to disfiguring tumors along with other phenotypes including skeletal abnormalities, learning and memory dysfunction, and enhanced pain. Imaging and pathology approaches in longitudinal investigations of large animal models are powerful techniques to study the model and directly translate to NF1 patients. We further characterized a recently developed NF1 pig model by longitudinally studying NF1 (heterozygous) and WT (wildtype) littermates from 4 to 20 months of age by clinical evaluation, non-invasive medical imaging and pathology. Café au-lait macules were present on all NF1 pigs with minor size/shape changes during the study. Decreased length of long bones (femur, tibia, humerus, metacarpal) was detected as early as 4 months of age indicating shorter stature, similar to humans. A neurofibroma was detected in one NF1 boar by 12 months, but retrospective CT and MRI scans showed indicators as early as 4 months. Comparison of quantitative image-based characteristics demonstrated the neurofibroma closely followed intensity and texture trends found in published human plexiform neurofibroma image data. At 20 months, the neurofibroma was collected at necropsy and showed scattered infiltration of the dermis/subcutis by myxoid tumors composed of S100+ Schwann cells and other cell types. Comparison with other cutaneous neurofibromas from the pig model showed infiltrative myxoid tumor tissue that dispersed and effaced the normal dermis/subcutis architecture with plasticity toward collagenous matrix with maturation. Taken together, NF1 pigs display a phenotype that mimics human NF1 and allows for translational imaging/pathology studies to improved clinical diagnostics.

Tuesday, November 12, 2019
4:45 p.m. – 5:00 p.m.

3-DIMENSIONAL HISTOLOGIC MODELS OF CANINE HEPATIC FIBROSIS FOR QUANTIFICATION AND TEACHING MODEL DEVELOPMENT

T. William O'Neill, Christiane Löhr
Oregon State University, Corvallis, OR, USA

The use of 3-dimensional (3D) reconstructions from advanced imaging (CT, MRI) is widespread in medicine as a way to obtain volumetric measurements, for surgical planning, and to create teaching models. Histologic slides provide excellent cellular detail and serial sections have been used previously to create 3D models, which have great utility in describing natural disease as well as in toxicological research. Patterns of hepatic fibrosis can be difficult to discern in 2-dimensional slides and present a challenge for veterinary students in understanding the clinical implications. Tissue blocks were curated from diagnostic cases received through the Oregon Veterinary Diagnostic Laboratory. Serial sections of Trichrome and Reticulin-stained slides from tissues with various patterns of fibrosis were digitized, including centrilobular, periportal, portal bridging, and end-stage cirrhosis. Acquired photomicrographs were aligned using ImageJ, converted to an 8-bit grayscale TIFF stack, and imported into 3D Slicer. Using a combination of computer-calculated thresholding and human input on each section, we generated 3D models that can be manipulated in real-time, digitally cross-sectioned, and provide quantifiable data regarding the amount of fibrosis present. Qualitative aspects of fibrosis, such as the areas affected and the patterns across the volume examined, were also evaluated by manipulating the models. The next step is to assess the utility of the generated 3-D models in teaching veterinary students.

Experimental Disease Focused Scientific Poster Session A

E-01: SPECTRUM AND GRADING OF MELANOCYTIC LESIONS IN A MOUSE MODEL CARRYING THE TYR-NRAS*Q61K TRANSGENE

Charles-Antoine Assenmacher[1], Sara Santagostino[2], Jean-Christophe Marine[3], Elise Bonvin[4], Enrico Radaelli[1]
[1]Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA, [2]Department of Safety Assessment, Genentech, South San Fransisco, CA, USA, [3]Laboratory for Molecular Cancer Biology, VIB Center for Cancer Biology, Leuven, Belgium, [4]Laboratory of Cancer Epigenetics, Cancer Research Center, Université Libre de Bruxelles, Brussels, Belgium

Background: The use of mouse models to study melanomagenesis allowed for great advances and discoveries both in the pathobiology and treatment of this neoplasm in humans. The mouse line carrying the Tyr-NRAS*Q61K transgene is widely used to model *in vivo* NRAS-driven melanomagenesis. Histopathological features of the model are well-described; however, the lack of molecular and biological characterization hampers the ability to further classify and interpret the different proliferative lesions observed in this mouse line in regards to their origin, evolution, grading, and pathobiological significance.

Material and Methods: Immunohistochemistry and qPCR for selected biomarkers (including Ki67, MITF and nestin) were used to further characterize the classification and grading of cutaneous and lymph nodal melanocytic lesions in this mouse model.

Results: The analysis of cutaneous and lymph nodal proliferations demonstrated that Ki67, MITF, and nestin expression linearly correlated with significantly increased expression in high-grade lesions (i.e. melanomas and atypical nevi) compared to lower grade lesions. Although high- and low-grade lymph nodal lesions could be clearly distinguished based on histopathological criteria, their metastatic or primary nature remained largely undetermined. No correlation between cellular morphology or pigmentation and Ki67 or MITF could be established in cutaneous lesions.

Conclusion: This study suggests that incorporation of MITF, Ki67, and nestin analysis into the histopathological classification/grading scheme of the melanocytic proliferations described for this model is warranted to assess with accuracy the nature and evolution of the phenotype, monitor disease progression, and predict response to experimental treatment or other preclinical manipulations.

E-02: EXPERIMENTAL WEST NILE VIRUS TRANSMISSION CYCLES USING WILD BIRDS AND MOSQUITOES

Alex Byas[1], Angela Bosco-Lauth[1], Claudia Rückert[1], Alexis Robison[1], Michael Young[1], Dalit Talmi-Frank[1], Todd Felix[2], Aaron Brault[3], Richard Bowen[1], Gregory Ebel[1]
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West Nile virus (WNV) is the leading cause of mosquito-borne disease in the United States. The rapid adaptation of WNV to local transmission ecologies and its continued diversification highlight the ability of RNA viruses to evolve in changing conditions. Different WNV hosts impose unique evolutionary pressures on virus populations, with genetic diversification in mosquitoes and restriction in vertebrates. Additionally, species-specific impacts of birds (American robins, house sparrows and American crows) and mosquitoes (Culex [Cx.] pipiens, Cx. quinquefasciatus and Cx. tarsalis) have been documented. However, the impact of distinct vector-vertebrate pairs on virus transmission and evolution remain poorly understood. Accordingly, we developed laboratory transmission systems involving pairings of ecologically-relevant mosquitoes and birds to evaluate whether the effects of evolutionary pressures including avian purifying selection and mosquito bottlenecks are maintained when WNV is forced into a combined vector-vertebrate system. We predict that mutations that arise during infection of highly competent Cx. tarsalis will be removed by purifying selection following transmission to robins, resulting in fitness gains. Conversely, we expect that strong population bottlenecks imposed by the moderately competent vector Cx. pipiens, coupled with relatively weak purifying selection in crows will result in diminished virus fitness and possible extinction. A field isolate of WNV was used to infect wild birds and three consecutive cycles of bird-to-mosquito transmission were performed. Bird serum and mosquito saliva were sequenced to assess viral populations. Ultimately this work

will provide knowledge on how viral genotypes emerge and help understand the evolutionary forces that shape their populations in nature.

E-03: CHARACTERIZING THE TISSUE TROPISM OF EQUINE PARVOVIRUS-HEPATITIS (EQPV-H)

Mason Jager[1], Joy Tomlinson[1], Gerlinde Van de Walle[1], Thomas Divers[2], Grace Klass[1], Emily Locolano[1]
[1]Baker Institute for Animal Health, Cornell University College of Veterinary Medicine, Ithaca, NY, USA, [2]Department of Clinical Sciences, Cornell University College of Veterinary Medicine, Ithaca, NY, USA

Background: Theiler's disease, a.k.a serum hepatitis or idiopathic acute hepatic necrosis, is a devastating, highly fatal disease of horses. Recently, our research team identified a new parvovirus, Equine Parvovirus-Hepatitis (EqPV-H), as the likely cause of Theiler's disease. The virus was present in samples from 27 of 28 consecutive Theiler's disease cases (2014-2017) and hepatitis occurred in 7 experimental horses inoculated with EqPV-H-positive serum, providing a strong link between this virus and Theiler's disease. The pathogenesis of EqPV-H is currently poorly understood.

Objective: Identify the cellular tropism of this novel equine parvovirus.

Methods: Tissue samples were collected from horses during the acute (n=3) and chronic (n=3) stages of infection and analyzed for the presence of viral genetic material using qPCR. Tested tissues included serum, cerebrospinal fluid, liver, spleen, bone marrow, lymph node, lung, heart, salivary gland, small intestine, colon, kidney, synovium, and spinal cord. RNAscope *in situ* hybridization was used to confirm the presence of the viral genome within hepatocytes of formalin-fixed, paraffin-embedded liver tissue.

Results: Our qPCR data demonstrated a high viral load in the liver consistent with hepatotropism. *In situ* hybridization revealed large amounts of viral genomes within small numbers of scattered hepatocytes. Infected hepatocytes displayed variable degrees of degeneration and necrosis.

Conclusions: Our data indicate that EqPV-H is hepatotropic, providing further evidence for its etiological role in Theiler's disease in horses.

E-04: THE POTENTIAL ANTI-FIBROTIC ROLE OF MESALAZINE, AN OSTEOPONTIN INHIBITOR IN INDUCED LIVER FIBROSIS IN RATS.

A. Ramadan[1], Nehal Afifi[1], Nemat Yassin[2], Rehab Abdel-Rahman[2], Sahar Abd El-Rahman[1], Hany Fayed[2]
[1]Faculty of Veterinary Medicine, Cairo University, Egypt., Cairo, Egypt, [2]National Research Centre, Giza, Egypt, Giza, Egypt

Objectives: Liver fibrosis is considered as a reversible healing process of a wound, the end result of chronic liver injury arising from various aetiologies. The current study

aimed to evaluate the potential anti-fibrotic activity of mesalazine through inhibition of osteopontin (OPN), an extra cellular matrix (ECM) component.

Methods: Liver fibrosis was induced by i.p. injection of TAA (200 mg/kg) for 6 weeks twice weekly. In the hepato-protective study, animals were administered mesalazine orally (50 and 100 mg/kg) for 4weeks before fibrosis induction then concomitantly with TAA injection. In the hepato-therapeutic study, mesalazine was administrated for 6weeks after TAA discontinuation with the same doses.

Results: In both studies, mesalazine administration improved liver biomarkers through decreasing serum levels of AST, ALT and total bilirubin and increasing total protein and albumin levels compared to fibrotic group. Mesalazine significantly decreased hepatic MDA level and counteracted the depleted hepatic GSH content and SOD activity. Additionally, it significantly lowered hepatic levels of OPN, TGF-β1, TNF-α and α-SMA. Histopathologically, mesalazine as a treatment showed a good restoration of the hepatic parenchymal cells with decreased intensity and retraction of fibrous proliferation, while as a prophylaxis it didn't achieve enough protection against TAA effect, although it decreased fibrosis intensity and pseudolobulation. Furthermore, mesalazine could suppress the expression of both α-SMA and caspase-3 in immunohistochemical sections.

Conclusions: Mesalazine could have a potential new indication as anti-fibrotic agent through limiting the oxidative damage and altering TNF-α pathway as an anti-inflammatory drug with down-regulating TGF-β1, OPN, α-SMA and caspase-3 signaling pathways.

E-05: SPLENIC MICROSTRUCTURE AND IMMUNE ACTIVATION IN U.S. DOGS WITH CANINE LEISHMANIOSIS

Erin Cox, Breanna Scorza, Kurayi Mahachi, Diogo Valadares, Angela Toepp, Katherine Gibson-Corley, Christine Petersen
University of Iowa, Iowa City, IA, USA

Background: Dogs are the primary reservoir for zoonotic visceral leishmaniasis and canine leishmaniosis (CanL) closely models human disease. Dogs may remain subclinical for many years after infection before converting to clinical disease. Clinical disease is associated with hypergammaglobulinemia and antibody associated pathologies. As disease progresses, secondary lymphoid organs undergo alterations in immune function and microstructural organization that are not fully understood or described.

Objective: To examine microstructural elements of splenic white pulp as a method of identifying immune activation in symptomatic and asymptomatic dogs with CanL.

Methods: Spleen was collected from an outbred cohort of dogs with naturally occurring *L. infantum* infection. Dogs were considered subclinical or clinical based on the results of ante-mortem physical examination. H&E stained tissue sections were evaluated with light microscopy, and elements were further analyzed using digital imaging software.

Results: Clinically ill dogs trended towards having larger follicles and more follicles per mm^2 of splenic tissue evaluated compared to subclinical adult dogs. Spleens from ill dogs had a lower ratio of primary to secondary follicles than subclinical dogs, approaching statistical significance, indicating more follicles containing germinal centers in ill dogs. Based on digital measurements, clinically ill dogs had a trend toward greater total white pulp area in their spleens.

Conclusions: Taken together, these preliminary results suggest that adult clinical dogs had more white pulp structural elements present within the spleen, a possible indicator of higher B cell activation. Going forward, we aim to identify splenic B and T cell populations associated with these changes.

E-06: SPONTANEOUS KRT5 GENE MUTATION IN RHESUS MACAQUES (MACACA MULATTA): A NOVEL NONHUMAN PRIMATE MODEL OF EPIDERMOLYSIS BULLOSA SIMPLEX

Amanda Johnson, Samuel Peterson, Margaret Terry, Betsy Ferguson, Lois Colgin, Anne Lewis
Oregon National Primate Research Center, Beaverton, OR, USA

Background: Epidermolysis bullosa simplex is an inherited skin disorder characterized by increased skin and mucous membrane fragility. The majority of cases are caused by mutations in keratin 5 *(KRT5)* and keratin 14 *(KRT14)*, genes that encode cytokeratins found in the basal layer of the epidermis. Mutations of these genes result in cytoskeletal disruption of the basal keratinocytes.

Objective: This report describes the gross and histopathologic findings of two clinically affected homozygous rhesus macaques and six phenotypically normal heterozygous animals with a mutation in *KRT5*.

Methods: Two *KRT5* homozygous and six *KRT5* heterozygous male and female rhesus macaques were identified through retrospective DNA sequence analysis. Archival materials, including gross images and paraffin embedded formalin fixed tissues, as well as periodic acid-Schiff and immunohistochemistry for anti-cytokeratin 5 were evaluated by board certified veterinary pathologists.

Results: Homozygous animals were stillborn and had severe generalized ulceration and sloughing of the epidermis. Microscopic examination revealed severe ulceration and acute dermal hemorrhage. Histologic features of intact epidermis included basal cell vacuolation, and basilar vesicle formation. DNA analysis confirmed a 34 base pair insertion variant in exon 5 of the *KRT5* gene.

Conclusion: The phenotypic and genotypic changes described in the rhesus macaque make it a viable alternative to the currently available animal models of *KRT5* EBS. In addition to the advantage of being a spontaneous model of a naturally occurring disease in a species more closely related to humans.

E-07: USE OF FELINE TRACHEAL TISSUE EXPLANTS TO EVALUATE REPLICATION AND SPREAD OF FELINE HERPESVIRUS-1 DELETION MUTANTS

Yao Lee, Roger Maes, Matti Kiupel, Gisela Soboll Hussey
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Feline herpesvirus-1 (FHV-1) is the primary cause of viral respiratory and ocular disease in cats. Current vaccines labeled for systemic use do not protect from infection or prevent latency. Moreover, they are not safe for intranasal administration.

The objective is to develop mucosal vaccine against FHV-1 disease.

We previously performed bacterial artificial chromosome mutagenesis with deletion of glycoprotein C (gC-), gE (gE-), protein kinase (PK-), and both gE and thymidine kinase (gE-TK-). In this study, tracheal tissue explants from four cats were sham-inoculated or exposed to wild-type virus (WT), gE-, gE-TK-, or PK- mutants at a multiplicity of infection (MOI) of 1 or 0.1, and stained by immunohistochemistry (IHC) for FHV-1 at 24, 48, or 72 hours post-inoculation (hpi). Sections were scored for staining intensity and distribution.

WT-inoculated explants reached highest scores of IHC staining scales at 72 hpi at an MOI of 1 or 0.1. At an MOI of 1, inoculation with the gE- mutant reached highest scores at 48 hpi, whereas inoculation with the gE-TK- mutant were scored lower than 1 at all timepoints. Only one sample at each timepoint inoculated with the PK- mutant at an MOI of 0.1 showed mild infection. Overall titers of PK- did not allow for experiments at an MOI of 1.

We concluded that the reduced replication and spread in the tracheal explant system of both the gE-TK- and PK- mutants compared to gE- and WT is indicative of their reduced virulence and makes them attractive candidates for mucosal immunization.

E-08: INFLAMMATORY RESPONSE IN EQUINE HERPESVIRUS TYPE 1 INFECTION IN MICE

Leonardo Mesquita[1,2], Rafael Costa[1], Laís Rodrigues[1], Eliana Villalobos[3], Maria do Carmo Lara[3], Enio Mori[4], Cláudia Mori[1], Paulo Maiorka[1]
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Background: Equine herpesvirus type 1 (EHV-1) is an emerging pathogen that causes neurological disease in horses. We have previously identified two EHV-1 strains that induced a severe and necrotizing meningoencephalitis in mice, which is a potential model to study the pathogenesis of viral encephalitis.

Objective: The aim of this study was to characterize the inflammatory response to infection by neurovirulent EHV-1 strains in mice.

Methods: C57BL/6 male mice (n = 4/group), 8-12 weeks-old, were intranasally infected with 25 µL of A4/72 and A9/92 ($10^5 TCID_{50}$/ml) EHV-1 strains. Mice were euthanized on

1, 2 and 3 days post-inoculation (dpi), and brains were collected for virus titration and flow cytometry. Control mice were inoculated with Minimum Essential Medium.

Results: EHV-1 strains reached the brain of infected mice on 2 dpi, with an increase in viral titers on 3 dpi, when EHV-1-infected mice exhibited severe neurological signs. At 3 dpi, EHV-1-infected mice had a large infiltration of leukocytes ($CD45^{hi}$) in the brain. These cells were further characterized as macrophages/monocytes ($CD45^{hi}F4/80^{+}CD11b^{+}$), which also exhibited a MHC-II phenotype, and cytotoxic T lymphocytes ($CD45^{+}CD3^{+}CD8^{+}$). Overall, the number of infiltrating leukocytes in A4/72-infected brains was significantly higher than in A9/92-infected brains.

Conclusions: A4/72 and A9/92 EHV-1 strains are extremely neurovirulent and induce a significant influx of monocytes and T cytotoxic lymphocytes into the brain of mice, which might contribute to the extensive neuroparenchymal lesions seen in histology. This model could also be useful to study the inflammatory response in the brain caused by neurotropic viruses.

E-09: LOSS OF SKELETAL MUSCLE SPECIFIC P53 FUNCTION PRODUCES OSTEOSARCOMAS IN AGING B6 MICE

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Age-related skeletal muscle atrophy is a very common, but remains poorly understood. Contradictory evidences suggest that the tumor suppressor p53 may or may not play a central role in atrophy. To help address this question, we studied aging mice with p53 deficient skeletal muscle (p53 mKO) causing lifelong absence of p53 expression in skeletal muscle fibers. We found that the absence of p53 expression in skeletal muscle fibers had no apparent deleterious or beneficial effects on skeletal muscle mass or function under basal conditions up to 6 months of age, when mice exhibit peak muscle mass and function. Furthermore, at 22 months of age, when age-related muscle atrophy is evident, p53 mKO mice demonstrated no differences relative to controls. At advanced ages, p53 mKO mice began to die prematurely and an unexpected contributing factor was an increased incidence of osteosarcoma, precluding rigorous analyses of muscle in very old p53 mKO mice. Based on these results, we conclude that p53 expression in skeletal muscle fibers has minimal if any direct effects on skeletal muscle mass up to at least 22 months of age. We also identified the development of osteosarcoma as an unexpected consequence of p53 loss in skeletal muscle.

E-10: EXCLUSION OF PATHOLOGISTS FROM AUTHORSHIP DIMINISHES THE RIGOR AND REPRODUCIBILITY OF PATHOLOGY DATA IN PUBLICATIONS

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Pathologists are key contributors to multidisciplinary biomedical research teams. However, they are often omitted from authorship in publications. To better understand this pattern, we identified publications (n=24) where pathologists had contributed significantly to the project but were excluded from publication authorship. Here, pathologists either generated data for the paper (79%) or provided critical support (21%). Pathologists were supported either through grants/collaborations (62%) or fee-for-service (38%). They were acknowledged for their contributions in 67% of the papers but were rarely asked to review manuscripts to ensure their data was correctly presented (4%). We identified a subset of these papers (n=16) that had pathologist-generated data in the results section to compare the expected scope of contributions for the average co-author vs. the pathologist's data in each paper; significant differences were not detected. We then identified a different group of 16 papers in which the same pathologists were listed as co-authors, to assess the impact of inclusion of pathologists as co-authors on pathology data quality. The reproducibility of the methods section and the rigor of pathology figures were both significantly higher when pathologists were listed as authors ($P<0.05$). Lastly, significantly more errors were detected in the pathology figures when pathologists were excluded from authorship ($P<0.05$). Our data suggests the pathologist's participation is necessary from data generation to publication for accuracy and reproducibility of results. Further, this study also raises several questions as to why pathologists are so commonly omitted from authorship, even when their data are deemed essential for publication of the paper.

E-11: A COMPARISON OF TFR-1 EXPRESSION IN HUMANS AND ANIMALS: A POSSIBLE NEW MARKER FOR CANCER THERAPY

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Background: TFR-1 is one of the two transmembrane glycoproteins crucial for the internalization of the iron within cells and therefore expressed on the membrane of any cell type. TFR-1 has been shown in humans to be more expressed in tumors than in healthy tissue. Moreover, TFR-1 shows an increased expression in tumoral metastatic progression. In veterinary medicine TFR-1 has not been largely investigated.

Objective: Both dog and cat mammary tumor shares some characteristics with human breast cancer. Level of TFR's protein and gene expression in primary (p) and metastatic (m) cancer cell lines from adenocarcinoma of dog (CIPp - primary, CIPm - metastatic, CF-33 - primary), cat (FMCp - primary, FMCm - metastatic) and human (ER+ MCF-7, triple-negative MDA-MB231) was examined.

Methods: To achieve the aim, immunofluorescence (IF), flow cytometry (FC) and RT-qPCR were carried out.

Results: Protein and gene expression of TFR-1 was confirmed in all cell lines. qPCR showed a higher expression of TFR-1 in FMCm than FMCp, in CIPm than CIPp and CF-33, in MDA-MB231 than MCF-7. Similarly, also FC showed an increased expression of TFR-1 in FMCm and CIPm when compared to FMCp and CF-33, respectively ($p<0,05$).

Conclusions: These results show higher level of TFR-1 in metastatic than in primary cell lines and with qPCR in triple negative HBC than in ER+ HBC. The highest increase was found in FMCm and in MDA-MB231. TFR-1 expression could therefore be related with increased tumor aggressiveness as typical for feline mammary carcinoma and triple negative HBC.

Experimental Disease Focused Scientific Poster Session C

E-01: DETECTION AND QUANTIFICATION OF FOAMY MACROPHAGES USING REAL AND ARTIFICAL INTELLIGENCE

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Background: Deep learning and artificial intelligence are used by veterinary pathologists in many settings. The technologies provide advantages, one of which is producing highly granular quantified data that can be analyzed and integrated with other data to gain biological insight.

Objectives: We had three objectives: 1) To create algorithms that recognize and quantify foamy macrophage areas, and count within the areas; 2) To validate algorithm performance against human pathologists; and 3) To use algorithm-quantified results in context of differential susceptibility to tuberculosis.

Methods: We scanned ~400 hematoxylin & eosin lungs from *Mycobacterium tuberculosis*-infected Diversity Outbred mice. We then used the aiforia *Create* platform iteratively to train, test, and validate 20 algorithms using ~25 slides.

Results: We selected the best performing algorithm based on high agreement with the pathologist and acceptable error rates/types when validated on new tissues, i.e. those that had not been used for training. We achieved lung tissue error rate of 0.01%; foamy macrophage area error rate of 0.03%, and counter error rate of 4.99%. The counter error reflected false negatives, not false positives, and this was by design. We applied the algorithm to ~400 lungs and it took <15 seconds per slide. The humans are still analyzing the quantified foamy macrophage data.

Conclusions: Both real and artificial intelligence detect foamy macrophages in *M. tuberculosis*-infected lungs with high agreement. The algorithm was much faster than the pathologist. The pathologist now has more time analyze, generate new hypotheses, and think about results in context of this globally important disease.

E-02: DETECTION OF MARKERS FOR THE ERADICATION OF INFECTION IN AN EXPERIMENTAL MODEL OF EQUINE SEPTIC ARTHRITIS USING DISCOVERY PROTEOMICS APPROACH

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Septic arthritis (SA) is debilitating in horses of any age. There is a lack of reliable biomarkers for the detection of eradication of infection, complicating treatment monitoring. The objective of this study is to identify differentially expressed proteins in equine plasma and synovial fluid which can be used as potential markers for the eradication of infection in an experimental equine SA.

Horses were injected with either *E. coli* (Septic group), LPS (LPS group) or saline (Control group) in midcarpal joint (day 0). After 24 hours (day 1) the horses underwent a standard therapeutic protocol (arthroscopic lavage, regional limb perfusion and systemic antimicrobials). Samples of plasma and SF were collected at regular intervals during the experiment. The point of eradication of infection occurred at day 4 based on culture, cytology and PCR. A subset of samples from days 0, 1, 4 and 7 (2 horses per group) were used for the tandem mass spectrometry analysis.

We have identified 7,915 proteins in plasma and 8,537 proteins in SF. Mass-spectrometric peptide intensities in plasma and SF were compared by two-way ANOVA. Proteins with a 2-fold intensity change in Septic group on day 1 compared to all other groups were identified as potential biomarker candidates. A list of candidates for potential biomarkers for the eradication of infection consists of 9 proteins in plasma and 75 proteins in synovial fluid.

We are currently conducting further analyses to narrow down this list and develop a targeted mass-spectrometric assay for validation of the identified putative biomarkers.

E-03: INCREASED INTERLEUKIN-6 LEVELS ASSOCIATED WITH EARLY, CHRONIC INFLAMMATORY CHANGES IN LAYING HEN OVARIES

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Background/Objectives: Laying hens are a robust animal model for ovarian carcinomas in women. The link between chronic inflammation and cancer is well established, although little is known about early inflammatory changes in the ovaries of hens or women.

Methods: Reproductive tissues were collected from two groups of laying hens at 78 weeks and 90 weeks of age. Birds were grouped by ovarian lesion type, and levels of IL-6 in serum and ovarian tissue were measured.

Results: Extensive ovarian heterophil infiltrates were observed in 44% of the 78-week hens. These hens had a higher mean serum IL-6 value (348 pg/mL) than hens without heterophil infiltrates (215 pg/mL). Older hens had a wider array of lesions including

heterophil infiltrates, and cortical cysts that have been linked to inflammatory processes in hens and women. Ovarian cysts were observed in 57% of 90-week hens. These hens had a higher mean serum IL-6 concentration (2669 pg/mL) than hens without cysts (1882 pg/mL). Hens with cysts also had a higher mean ovarian tissue IL-6 concentration (44,136 pg/mL) than hens without cysts (17,183 pg/mL).

Conclusions: Inflammatory lesions in laying hen ovaries form early and increase in intensity as hens age. All 90-week hens had severely elevated serum IL-6 values similar to those measured in women diagnosed with advanced stage ovarian carcinoma. Increasing levels of IL-6 are linked to chronic inflammation and early, pervasive changes to ovarian tissue that set the stage for eventual development of ovarian carcinomas in laying hens and women.

E-05: MACROPHAGE ACTIVATION SYNDROME IN HUMANIZED NOD.CG-PRKDCSCIDIL2RGTM1WJL /SZJ (NSG) EXPRESSING HUMAN CYTOKINES IL-3, GM-CSF, AND SCF (NSG-SGM3) AFTER ENGRAFTMENT WITH HUMAN CD34+ HEMATOPOIETIC STEM CELLS

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Eight, female, commercially available, NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1wjl}(NSG) mice humanized with transgenes for human interleukin-3 (IL-3), granulocyte/macrophage-colony stimulating factor (GM-CSF), and stem cell factor (SCF) (NSG-SGM3), were engrafted at 3 weeks of age with human CD34+ hematopoietic stem cells from a single donor. Mice were considered to be successfully engrafted. Within 2-3 weeks of arrival, and before any experimental manipulation, all eight, 19-week-old mice exhibited hunched posture, rough coat, weight loss, and died or required euthanasia. Two mice were submitted for pathologic evaluation. Severe pancytopenia was present. Postmortem examination revealed emaciation, roughened hair coat, fecal staining, mucous membrane pallor, splenomegaly, and hepatomegaly. Histologically, large numbers of epithelioid macrophages and/or multinucleated giant cells effaced up to 90% of the liver. Hemosiderin-laden, epithelioid macrophages and multinucleated giant cells also expanded splenic red pulp and alveolar septa in the lungs. Hemophagocytosis was evident on blood and bone marrow smears. The severe pancytopenia and proliferation of activated macrophages with hemophagocytosis in these mice are similar to features observed with Macrophage Activation Syndrome (MAS), a subclassification of hemophagocytic lymphohistiocytosis in humans. MAS is defined by overactive immune response and hemophagocytosis, as well as high levels of cytokines including IFNγ, TNFα, and GM-CSF which activate macrophages and result in systemic inflammation and release of other proinflammatory cytokines. The lesions in NSG-SGM3/hu-CD34+ mice are consistent with sustained supraphysiologic human cytokine expression causing dysregulation of engrafted human myeloid cells. The use of this model for immuno-oncology studies may be limited by this syndrome of uncontrolled activation and proliferation of macrophages.

E-06: NEUROPATHOLOGIC EVALUATION OF A NOVEL NEUROSURGICAL TECHNIQUE IN SHEEP FOR HUMAN TRANSLATION

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Background: Tay-Sachs and Sandhoff are fatal inherited neurologic diseases of children. Adeno-associated viral (AAV) gene therapy has shown success in Tay-Sachs sheep and Sandhoff cats, however, human translation required surgical adaptation. Preclinical studies were done using rigid catheters, however a larger volume is required in humans, increasing anesthetic risk and brain shift. Use of flexible catheters would enable simultaneous thalamic infusions, negating these risks.

Objective: In this study we wanted to determine 1) if a flexible catheter is an option for thalamic delivery, (2) fastest rate of infusion that minimizes neuroinflammation (3) the correlation of lesion size between MRI, gross, and histopathology.

Methods: Lambs (n=9) underwent thalamic injections using Flexible Catheters (Brainlab). Each thalamus was injected with a volume varying between 0-800ml of phosphate buffered saline at a rate between 0.5-5ml/min. MRI (T1 & T2) and necropsy were performed 30 days post-surgery. Brains were sectioned into 6mm blocks, H&E stained, quantified (ImageJ, AMIRA Thermo-Fisher) and compared to MRI.

Results: In the thalamus, several animals had focally extensive areas of necrosis with associated infiltrates of macrophages and reactive gliosis, however these were not correlated with volume, or infusion rate. Damage to the brain parenchyma was caused by fixation failure of the flexible catheter.

Conclusion: The lesions observed using the flexible catheters were not observed using rigid catheters, suggesting that catheter movement led to hemorrhage and inflammatory response. Based on these results, a 6.5-month-old Tay-Sachs child successfully and safely received a thalamic infusion of AAV using the rigid catheter system.

E-07: SYNONYMOUS CHANGES IN SPECIFIC LEUCINE CODONS IMPACT PROTEIN PRODUCTION FROM HUMAN AND CANINE CODON OPTIMIZED AND SUBOPTIMIZED MORBILLIVIRAL N GENE CONSTRUCTS

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Background: Global and localized optimization of human measles virus (HMV) or canine distemper virus (CDV) genes to human or canine codon usage bias (CUB) changes protein expression. For HMV and CDV nucleoprotein (N) genes, protein production was increased by optimization to human or canine codon usage bias (CUB) and decreased by suboptimization. Six different leucine codons were replaced by CTG with optimization to human CUB and by CTG & CTC in canine CUB optimized constructs. For CUB suboptimized N gene constructs CTT coded for leucine.

Objective & Methods: Our objective was to characterize the impact of leucine codons CTG, CTC and CTT on protein production from the human and canine CUB optimized and suboptimized HMV and CDV N gene constructs. The various viral N gene constructs were cloned into expression plasmids (JM109/DH5a) and transfected into human HEK 293 cells. Protein production from the constructs was compared using flow cytometry.

Results: Changing CTG to CTT resulted in decreased protein production from the human/canine CUB optimized HMV N and the human CUB optimized CDV N gene constructs. When CTT was used for leucine in canine CUB optimized CDV N gene protein production from the construct was increased. Changing CTT to CTG or CTC in the suboptimized CUB constructs increased protein production. All differences were significant at $p<0.05$.

Conclusions: These results indicate that changes in specific leucine codons can significantly impact morbilliviral protein production and indicate that synonymous codon changes could have significant effects on viral pathogenesis.

E-08: LAPATINIB EFFECTS ON HER-2 POSITIVE CANINE MAMMARY CARCINOMA PRIMARY CELL LINES

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Background: Mammary gland tumors (CMT) are spontaneous and frequent in intact female dogs. The treatment of choice is mastectomy, and for metastatic tumors, chemotherapy protocols can be stablished. As in women breast cancer (WBC), CMT can be classified molecularly. In WBC, HER-2 expression it is a pivotal protein for molecular classification, directing the treatment. Lapatinib is a HER-2 receptor tyrosine kinase inhibitor and a therapeutic alternative for metastatic HER2+ BC, resistant to trastuzumab.

Objective: This study aimed to evaluate the antitumoral effect of lapatinib in HER2-positive canine mammary carcinoma cells in vitro.

Methods: From our tumor cell bank, we selected six CMT HER-2 positive cell lines. HER-2 expression was evaluated by qPCR and immunohistochemistry using Herceptest ™ kit (Dako, Carpinteria, CA, USA). The cells were treated for 24 hours with different concentrations of Lapatinib, and cellular viability test was performed using MTT assay.

Results: Lapatinib showed a good anti-tumoral effect and the response was directly related to *HER*-2 levels (RQ).

Conclusions: This study demonstrated a dose dependent effect of Lapatinib in canine mammary gland cells with higher HER-2 protein/gene expression.

Financial support: FAPES and CNPq

E-09: PARTHENOLIDE: A PROMISING PHYTOMEDICINE FOR CANINE AND HUMAN HEMATOPOIETIC NEOPLASMS

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Background: Many canine and human hematopoietic neoplasms have constitutively active NFκB signaling, which promotes tumor cell proliferation, suppression of apoptosis, angiogenesis, and metastasis. Parthenolide (PTL) perturbs cellular redox balance, inhibits NFκB signaling, and selectively induces apoptosis, sparing normal hematopoietic cells from off-target effects.

Objective: The purpose of this research is to explore the therapeutic potential of PTL to treat hematopoietic neoplasms in dogs. Additionally, some dog breeds predisposed to developing mast cell neoplasia and histiocytic sarcoma (HS) provide translational study populations for rare and deadly human diseases.

Methods: Using immortalized canine cells, growth inhibition assays were performed with PTL alone or in combination with redox-perturbing standard-of-care therapeutics. Cell death was assessed using flow cytometry. NFκB-DNA binding was assessed with an electrophoretic mobility shift assay (EMSA). Immunofluorescence and immunoblotting were used to assess NFκB localization and phosphorylation, respectively. An H2DCFA assay was performed to assess reactive oxygen species (ROS) generation.

Results: All canine cell lines evaluated are sensitive to PTL and undergo dose-dependent apoptosis, and some synergistic interactions are observed with combination therapeutics. Some cell lines have constitutively active NFκB activity. PTL exposure generates ROS and leads to inhibition of NFκB, as evidenced by immunofluorescent nuclear exclusion and decreased p65 phosphorylation.

Conclusions: These initial studies show that PTL is a promising therapeutic for hematopoietic neoplasms in dogs. Murine modeling, RNA sequencing, and investigation of basal NFκB activity in a large number of spontaneous canine neoplasms are in progress to further assess PTL's potential in the clinical setting.

Young Investigator Poster Abstracts: Experimental Disease

EY-44: REQUIREMENT OF STEROL REGULATORY ELEMENT-BINDING PROTEIN PATHWAY IN PANCREATIC DUCTAL ADENOCARCINOMA

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Background: Pancreatic ductal adenocarcinoma (PDAC) is an aggressive tumor with limited diagnostic and therapeutic options. PDAC tumor cells are extremely proliferative with a high requisite demand for lipids. However, the tumor microenvironment is poorly vascularized and hypoxic. As lipid synthesis is oxygen-consumptive, neoplastic cells are challenged with meeting the demand for lipids. Cancer cells respond to lipid conditions through the sterol regulatory element-binding protein (SREBP) pathway, which requires SREBP cleavage activating protein (SCAP) during signaling.

Objective: To test the requirement of SCAP in PDAC using both *in vitro* and *in vivo* model systems.

Methods: Utilizing four patient-derived PDAC cell lines, *SCAP* was knocked out and followed by gene rescue. Cell growth assays were performed in lipid-poor and lipid-rich media conditions. Subcutaneous and pancreatic orthotopic xenografts were performed in nude mice using wild type and *SCAP* knockout cells. Using an established PDAC mouse model, *LSL-Kras^{G12D}; LSL-Trp53^{R172H}*; *Pdx-1 Cre* (KPC), KPC and other mice lacking *Scap* in one or both alleles were generated.

Results: In lipid-poor conditions, *SCAP* knockout cells showed significantly reduced growth. In subcutaneous xenografts, *SCAP* knockout cells exhibited reduced tumor volume in 3 out of 4 cell lines. Similarly, *SCAP* knockout cells grew poorly in pancreatic orthotopic xenografts with reduced splenic metastases. Mice lacking *Scap* are clinically and phenotypically normal.

Conclusions: Loss of *SCAP* in PDAC tumor cells alters growth both *in vitro* and *in vivo*. Additionally, *Scap* is not required for pancreas function in mice. These findings suggest SCAP may be useful as a therapeutic target in PDAC.

EY-45: THERAPEUTIC DELIVERY OF ALX-0171 NANOBODY REDUCES DISEASE SEVERITY OF HUMAN RESPIRATORY SYNCYTIAL VIRUS STRAIN M37 AFTER ESTABLISHED RSV INFECTION

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Respiratory syncytial virus (RSV) is the most common reason for hospitalization of infants with viral pneumonia, but lacks a therapy that is fully satisfactory and effective. ALX-0171 is a Nanobody directed against RSV F protein that reduces RSV infection and disease severity. In order to determine the optimal dose of therapeutic delivery of ALX-0171 after RSV infection, lambs were nebulized with RSV (Memphis 37 strain), then three doses of ALX-0171 (1.4, 2.8 and 11 mg) were delivered to the lambs at 3, 4, and 5 days after RSV. Efficacy assessed at 3, 4 and 6 days post infection. There was a dose-effect relationship yielding *in vivo* IC50 and lambs treated with ALX-0171 had reduced lesions and viral parameters at 4 and 6 days post RSV infection compared to lesions in lambs not receiving treatment. Lambs not receiving ALX-0171 had limited or lacked RSV gross and microscopic lesions, RSV viral antigen in lung and RSV titers at 3 days post infection, but these lesions and viral parameters increased progressively at days 4 and 6. These studies determined that ALX-0171 reduces RSV disease severity when administered after RSV inoculation in lamb model for human infants. Clinically, infants for commonly do not receive treatment RSV until after clinical signs develop and work in this study suggests that ALX-0171 has potential to reduce RSV disease severity in the face of established RSV infection at optimized doses.

EY-46: PEDV INFECTION IMPAIRS MUCOSAL IMMUNITY BY REDUCING NUMBERS OF MICROFOLD CELLS IN THE SMALL INTESTINE

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Background: In the small intestine, microfold (M) cells are essential for maintaining mucosal immunity. M cells capture and deliver antigens to lamina propria and stimulate lymphocyte activities. Based on the location, M cells are divided into two subtypes: villus M cells scattered on villi and Peyer's patch M cells located on dome epithelium in ileum. Porcine epidemic diarrhea virus (PEDV) infection in weaned pigs induces villus injury in the small intestine and impairs digestive function.

Objective: The aim of this study is to evaluate changes in M cells during PEDV infection.

Methods: Sixty-four 4-week-old pigs were randomly arranged in two treatments [PEDV-inoculated (n = 40) and mock (n = 24)] and orally inoculated with PEDV IN19338 strain

or mock. On day after inoculation (dpi) 2, 4, and 6, pigs were euthanized, and jejunum and ileum were collected for histological examination and immunohistochemistry.

Results: Results show that, compared with the mock, PEDV-inoculated pigs significantly decreased villus height to crypt depth (VH:CD) ratio in the jejunum on dpi 2, 4, and 6 and in the ileum on dpi 4 ($P < 0.05$). Also, PEDV-inoculated pigs had significantly higher numbers of Peyer's patch M cells on dpi 2 but lower numbers of Peyer's patch M cells on dpi 6 ($P < 0.05$).

Conclusions: Reduced numbers of Peyer's patch M cells impair induction of antigen-specific IgA and M cell-dependent lymphocyte activation. As a result, the immune response to other pathogens in the small intestine and effectiveness of oral vaccine might be affected.

EY-47: CANINE THYROID CANCER: MOLECULAR CHARACTERIZATION AND CELL LINE GROWTH IN NUDE MICE

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Background: Thyroid cancer is the third most common solid malignancy in dogs. Dogs and humans are similar in the spontaneous development of thyroid cancer and metastasis to lungs; however, thyroid cancer has a higher incidence of metastasis in dogs.

Objective: Our objective was to develop a preclinical nude mouse model of canine thyroid cancer using a canine thyroid follicular adenocarcinoma cell line (CTAC) and to measure the expression of important invasion and metastasis genes in spontaneous canine thyroid carcinomas and CTAC.

Methods: CTAC cells were examined by electron microscopy. Short tandem repeat (STR) analysis was performed for both the original neoplasm and CTAC cells. CTAC cells were transduced with luciferase and injected subcutaneously and into the tail vein. Tumors and metastases were monitored using bioluminescent imaging and confirmed with gross necropsy and histopathology. Molecular characterization of invasion and metastasis genes was conducted in 8 follicular thyroid carcinomas, 4 C-cell thyroid carcinomas, CTAC and 3 normal thyroids.

Results: The CTAC cells grew well as xenografts in the subcutis, and they resembled the primary neoplasm. Metastasis to the kidney and lung occurred infrequently following subcutaneous and tail vein injection of CTAC cells. STR analysis confirmed that CTAC cells were derived from the original neoplasm and were of canine origin. Finally, 24 genes were differentially expressed in spontaneous canine thyroid carcinomas, CTAC and normal thyroids.

Conclusions: This study demonstrated the usefulness of a nude mouse model of experimental canine thyroid carcinoma and identified potential molecular targets of canine follicular and C-cell thyroid carcinoma.

EY-48: RNA-SEQ TRANSCRIPTOME ANALYSIS REVEALS DISTINCT GENE EXPRESSION PATTERNS BETWEEN INVASIVE AND NON-INVASIVE CANCER CELLS

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Background: The majority of pancreatic adenocarcinoma (PDAC) patients die due to metastasis. Local invasion, the initial step of the metastatic cascade, is hard to be accessed nor studied in *in-vivo* settings without invasive diagnostic modalities. However, the organoid model recapitulates the invasive phenotype of the primary tumor, and retains the inter-patient heterogeneity of such invasive potential. Here we present a striking disparity in gene expression patterns in the “invasive” and “non-invasive” PDAC organoids.

Objective: The aim of this study was to identify the key mechanism driving local invasion and metastasis of PDAC using organoid models.

Methods: 6 PDAC organoid lines were created from surgically resected PDACs. Individual organoids were plated in each well in 96 well plates and cultured for 7 days. Organoids were sorted into the “invasive” or “non-invasive” populations based on presence of the invasive protrusions and their relative length to the core of the organoid. RNA was sequenced to find differentially expressed genes and pathways associated with local invasion.

Results: Total of 2,306 organoids (1,164 invasive and 1,142 non-invasive organoids) from 6 PDAC organoid lines were analyzed with RNA-seq. The list of differentially expressed genes between invasive and non-invasive organoids includes 425 genes with FDR 5%. Pathway analysis revealed many up & down-regulated pathways which include metabolic processes and wnt signaling pathways.

Conclusions: RNA-seq revealed a distinct gene expression program between invasive and non-invasive cells in patient-derived organoids. More in-depth analysis is underway to find gene expression signatures driving local invasion and metastasis.

EY-49: ALTERATION OF COLONIC MUCIN AND CYTOKINE EXPRESSION IN SWINE DYSENTERY

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Background: Swine dysentery (SD) is a re-emerging disease in grower-finisher pigs associated with strongly beta-hemolytic *Brachyspira* infection that manifests as mucohemorrhagic diarrhea and typhlocolitis. The spirochetes colonize the mucus layer and within crypts and infected pigs frequently show marked mucus production.

Objectives: To evaluate the histochemical and immunohistochemical characteristics of colonic mucin and investigate cytokine expression in colon specimens of pigs with and without SD by RNA in situ hybridization (RNA ISH).

Materials and Methods: Formalin fixed spiral colon samples were obtained from 9 controls and 19 pigs with SD from an experimental infection study. Histochemical staining for sialomucin and sulfomucin, immunohistochemical staining of mucin 2 (MUC2) and mucin 5 AC (MUC5AC), and RNA ISH for interleukin 1-beta (IL-1 beta) and transforming growth factor beta 1 (TGF-beta1) was performed. Standardized images were captured and quantification of staining specific for the above targets was analyzed using a commercial software program.

Results: Pigs with SD showed significantly increased expression of MUC5AC and sialomucin, and significantly decreased expression of sulfomucin within colonic crypts. RNA ISH revealed significantly lower expression of TGF-beta1 in SD pigs while IL-1 beta expression was generally higher but without significance. MUC2 expression was not significantly different between groups.

Conclusions: There is markedly altered expression of mucins and cytokines in pigs that developed SD. Specifically, there is a reduction in sulfomucin concurrent with de novo expression of MUC5AC and increased expression of sialomucin. Alterations in cytokines detected may suggest potential pathways involved in the pathogenesis of SD.

EY-50: RENAL MACROPHAGES AS POTENTIAL VIRAL RESERVOIRS IN SIV-INFECTED PIGTAIL MACAQUES

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HIV-1-infected individuals are at risk for developing kidney disease. Recently, the first HIV-to-HIV living donor kidney transplant was completed successfully in the United States to circumvent the limited supply of kidney donors. Anti-retroviral therapy (ART) makes transplantation possible by controlling viral replication. However, if antiretroviral therapy is discontinued, viral replication may resume in latent cellular reservoirs including both CD4+ T cells and macrophages. In this SIV/macaque study, to determine whether SIV could emerge from renal reservoirs, we measured SIV RNA levels in the kidneys of SIV-infected macaques released from antiretroviral therapy using qRT-PCR. 5 of 16 animals (31%) had SIV RNA detectable in kidney within one month after stopping ART. We used immunostaining with the macrophage markers CD68 and CD163 followed by digital image analysis to compare macrophage populations in the kidney, and found a significant increase in macrophage immune activation markers in the kidneys of SIV-infected animals ($P = 0.02$ and $P = 0.04$ respectively) versus uninfected animals. Macrophage levels did not correlate with clinical signs of renal disease, H&E histopathology, nor clinical pathology parameters including SDMA, BUN, and creatinine, which were all similar between infected and uninfected animals. This study demonstrates that in the absence of clinical renal disease, SIV-infected pigtail macaques nonetheless had increased numbers of macrophages and detectable viral

RNA in kidneys. After stopping ART, SIV RNA was detectable in a subset of animals suggesting that donor kidneys could serve as a source of latent HIV that might reactivate if ART was interrupted.

EY-51: PATHOGENICITY OF DIFFERENT U.S. PEDV STRAINS IN PIGS OF DIFFERENT AGES

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Background: Currently, two main strains of porcine epidemic diarrhea virus (PEDV) i.e. U.S. non S-INDEL and S-INDEL PEDVs, circulate in U.S. swine but their pathogenicity has not been characterized in older pigs.

Objective: This study aimed to compare pathogenicity of these two PEDVs in three ages of pigs.

Methods: Thirty 3-week-old ('weaned'), thirty 8-week-old ('grower'), and thirty 23-week-old ('finisher') pigs were included with each age divided into 3 groups (10 pigs/group) and orogastrically inoculated with PEDV isolate USA/IN19338/2013 (non S-INDEL), USA/IL20697/2014 (S-INDEL), or virus-negative medium. Half the pigs in each group were randomly selected for necropsy at 4 DPI and remaining pigs were necropsied at 28 DPI.

Results: In 'weaned' pigs, non S-INDEL had longer duration of fecal shedding than S-INDEL, and significantly higher fecal virus load at 2 and 10 DPI. In 'grower' pigs, S-INDEL PEDV had longer fecal shedding than non S-INDEL; S-INDEL fecal virus load was significantly higher than non S-INDEL at 7 and 14 DPI but was opposite at 10 DPI. In 'finisher' pigs, non S-INDEL had slightly longer fecal shedding than S-INDEL but fecal virus load was similar. For non S-INDEL PEDV, onset of fecal virus shedding and shedding level was highest in 'weaned', followed by 'grower' and 'finisher'. S-INDEL PEDV trended similarly when compared across age groups.

Conclusions: Pathogenicity of PEDV is pig age-dependent and more severe in younger pigs. Non S-INDEL appeared to be more pathogenic than S-INDEL PEDV in 'weaned' and 'finisher' pigs, but pathogenicity difference was less distinct in 'grower' pigs.

EY-52: CHARACTERIZATION OF BRUCELLA CANIS INFECTION IN MICE

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Background: Canine brucellosis, caused by *Brucella canis*, is a disease of dogs and zoonotic pathogen. Infection in dogs in United States is increasing and there is currently no available vaccine. Mice have been extensively utilized to investigate vaccine candidates for other *Brucella* species and could serve a similar role for studying *B*. *canis*.

Objective: The objective of this study was to characterize the kinetics of colonization and pathogenicity of *B*. *canis* in mice in order to evaluate the mouse as a model for studying vaccine candidates.

Methods: C57BL/6 mice were inoculated intraperitoneally with varying doses of *Brucella canis* RM6/66 and euthanized at regular intervals to monitor organ colonization and gross and histopathologic lesions.

Results: *B*. *canis* induced splenomegaly in mice infected with 10^9 colony forming units (CFU) while no gross lesions were observed in lower dose groups. Infection resulted in dose-dependent granulomatous hepatitis and histiocytic infiltration of the spleen and mesenteric lymph nodes by 1–2 weeks. *B*. *canis* was cultured from the liver, spleen, uterus, bone marrow, lung, and kidney in all groups with colonization declining at a slow but steady rate. Clearance in all groups was achieved by 12 weeks, apart from persistence in the spleen through 12 weeks in the 10^9 dose group.

Conclusions: *B*. *canis* has the ability to establish an infection, induce splenomegaly, and persist for several weeks in multiple organs in mice. Moreover, 10^7 CFU appears to be a suitable challenge dose for investigating canine brucellosis vaccine candidates.

EY-53: KINETICS OF NERVOUS SYSTEM CYTOKINE EXPRESSION IN A CANINE MODEL OF MUCOPOLYSACCHARIDOSIS IIIB

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Background: Mucopolysaccharidosis (MPS) IIIB is a neuropathic lysosomal storage disease characterized by a deficiency in a lysosomal enzyme important for degradation of the glycosaminoglycan heparan sulfate. The pathogenesis of neuroinflammation and neurodegeneration in MPS type IIIB is incompletely understood. Elevated proinflammatory cytokines are thought to be the result of lysosomal dysfunction and defects in the autophagy pathway; however, glycosaminoglycan storage may also induce the production of proinflammatory cytokines. Microgliosis and astrocytosis in canine MPS IIIB occur early and progress over time. Corresponding changes in cytokine levels are likely to occur in conjunction with gliosis, however, these changes still remain to be elucidated.

Objective: Characterize the temporospatial expression of pro- and anti-inflammatory cytokines in the brain, spinal cord and dorsal root ganglion in a canine model of MPS IIIB.

Methods: We utilized a naturally occurring canine model of MPS IIIB to determine the expression of TNF-a, IL-1b, IL-10, and TGF-b by ELISA in five brain regions (cerebral cortex, centrum semiovale, dorsal thalamic nucleus, cerebellar peduncle, and vermis), cervical spinal cord, and dorsal root ganglion at 2, 18, and 26 months of age. We then

correlated pro- and anti-inflammatory cytokine protein expression to the rate of gliosis, as determined by quantitative immunohistochemistry, in the corresponding regions.

Results: TNF-a was elevated in brain, spinal cord and dorsal root ganglion at 18 and 26 months of age, corresponding with progressive gliosis.

Conclusions: Characterization of cytokine expression in this model suggests a role for pro-inflammatory cytokines in the progression of neurological disease in MPS IIIB.

EY-54: AGE-RELATED DIFFERENCES IN THE PATHOGENESIS OF CLADE 2.3.4.4A H5N2 HPAIV IN COMMERCIAL BROAD BREASTED WHITE TURKEYS

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Background: The 2.3.4.4A newly emerged highly pathogenic avian influenza (HPAI) H5 clade quickly spread through migratory birds and caused economic losses worldwide. In 2014-15, this reassortant H5 virus led to high mortality in more than 200 commercial poultry operations across the United States costing millions of dollars in economic losses. Although AIV is one of the most studied poultry diseases, only few studies have characterized the pathogenesis of HPAIV in turkeys. Furthermore, there are no studies reporting pathologic findings of HPAIV in commercial turkeys at “market age”.

Objective: Our aim was to characterize the pathology and antigen tissue distribution in naïve commercial turkeys intranasally inoculated with H5N2 clade 2.3.4.4A HPAIV at different ages.

Methods: Turkeys were challenged with 10^6.5 EID50/bird of H5N2 HPAIV A/turkey/Minnesota/12582/2015 respectively at 6 and 16-weeks of age. Clinical signs, mortality, pathology and histopathology were paired with AIV antigen detection in multiple organs by immunohistochemistry. RT-qPCR was used to quantify viral shedding in tracheal swabs and viral loads in the tissues.

Results: Older birds survived longer and shed higher titers of virus. Both age-groups were able to get infected and reached 100% mortality by day 5 post-challenge. Lesions ranged from none to severe hemorrhagic and fibrinoheterophilic pneumonia, necrotizing pancreatitis and splenitis, heterophilic meningoencephalitis and myocarditis. Influenza A virus immunohistochemistry confirmed systemic virus antigen dissemination.

Conclusions: Longer age-related survival time may be critical for disease transmission as prolonged subclinical virus shedding increases the chances of viral spread between individuals and populations.

EY-56: THE CHRONIC WASTING DISEASE AGENT FROM MULE DEER HAS WIDESPREAD LYMPHOID DISTRIBUTION IN SHEEP AFTER SECOND PASSAGE INTRACRANIAL INOCULATION

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United States Department of Agriculture, Agricultural Research Service, National Animal Disease Center, Ames, IA, USA

Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy characterized by fatal neurodegeneration and wasting in cervids. The disease is associated with the accumulation of misfolded prion protein (PrP^{Sc}). We previously demonstrated that sheep were susceptible to the agent of CWD from mule deer (CWD^{md}) after intracranial inoculation. A sheep with the $V_{136}R_{154}Q_{171}$/ARQ genotype developed clinical disease and had detectable PrP^{Sc}in the brain, tonsils, lymph nodes, and limited amounts in the spleen after a 35-month incubation period (IP). The objective of the present experiment was to further explore the permissiveness of PrP^{Sc}lymphoid replication and tissue distribution in sheep with different genotypes after passage of VRQ/ARQ brain inoculum. Sheep with the VRQ/ARQ (n=6), ARQ/ARQ (n=4), and ARQ/ARR (n=2) *PRNP* genotypes were intracranially inoculated with 100 mg tissue equivalent of brain homogenate from a VRQ/ARQ sheep that had received the agent of CWD^{md}. Sheep of all genotypes had PrP^{Sc}accumulation with IPs of 14.6 months (VRQ/ARQ), 11.3 months (ARQ/ARQ), and 60 months (ARQ/ARR). $PrP^{Sc}$was detected in their brains by immunohistochemistry, enzyme immunoassay, and western blot analysis. In sheep with the VRQ/ARQ and ARQ/ARQ genotypes, there was widespread dissemination of PrP^{Sc}in the lymphoid tissues including the tonsils, lymph nodes, spleen, Peyer's patches, and rectal mucosa. These results demonstrate that sheep are permissive to PrP^{Sc}replication in the brain and lymphoid tissue after interspecies transmission of the agent of CWD^{md}; unexpectedly, sheep with the ARQ/ARQ genotype had a shorter IP than VRQ/ARQ genotype sheep.

EY-57: IMMUNOGENICITY OF RSV ATTACHMENT PROTEIN USING AAV VECTOR SYSTEM

Margaret Martinez, Stefan Niewiesk
The Ohio State University, Columbus, OH, USA

Background: Human respiratory syncytial virus (RSV) is a leading cause of respiratory tract infections in infants and young children. The receptor-binding attachment (G) protein has two forms, a membrane bound (mG) and secreted form (sG), with a distinct CX3C motif that binds to host receptor CX3CR1.

Objective: Our goal was to determine the effect of the various forms of the G protein on the immune system.

Methods: We tested the expression of the G protein (both forms produced), sG, or mG using an adenovirus associated virus (AAV) vector system in the cotton rat. We also expressed a G protein with a mutated receptor binding site (C186S), interferon alpha (INFa), as well as GFP as a negative control.

Results: There was a significant increase in total IgG antibody production against RSV 3 weeks post-inoculation with AAV-G. When both forms of the G protein were expressed, AAV-G, the greatest level of total antibody production was achieved. However, none of the forms resulted in neutralizing antibody production. There was partial protection from RSV challenge when rats were inoculated with AAV-G, which was mostly attributed to a CD8 T cell response. Partial protection was dependent on an intact CX3C motif, and complete protection was accomplished with the combination therapy of AAV-INFa and AAV-G.

Conclusions: The RSV attachment protein induces partial protection, which was enhanced with the addition of type I interferon. Thus it would be beneficial to incorporate the complete G protein in a vaccine candidate along with an adjuvant that stimulates innate immunity.

EY-58: HTLV-1 CTCF-BINDING SITE IS DISPENSABLE FOR IN VITRO IMMORTALIZATION AND PERSISTENT INFECTION IN VIVO

Michael Martinez[1,2], Xiaogang Cheng[3], Ancy Joseph[3], Jacob Al-Saleem[1,2], Amanda Panfil[1,2], Wessel Dirksen[1,2], Lee Ratner[3], Patrick Green[1,2,4]
[1]The Ohio State Univeristy Center for Retrovirus Research, Columbus, OH, USA, [2]The Ohio State University Department of Veterinary Biosciences, Columbus, OH, USA, [3]Washington University, St. Louis, MO, USA, [4]The Ohio State University Comprehensive Cancer Center, Columbus, OH, USA

Background: Human T-cell leukemia virus type 1 (HTLV-1) is the retroviral etiologic agent of adult T-cell leukemia/lymphoma and the neurological disorder HTLV-1-associated myelopathy/tropical spastic paraparesis. The exact mechanisms through which latency and disease progression are regulated are not fully understood. CCCTC-binding factor (CTCF) has been shown to play a major role in organization of metazoan higher-order chromatin structure, gene expression, chromatin insulation, and genomic imprinting. A CTCF-binding site was identified within the HTLV-1 genome (vCTCF-BS). Therefore, HTLV-1 integration randomly inserts a vCTCF-BS into the host genome. CTCF-mediated interactions between proviral and host CTCF-binding sites has been shown to alter host chromatin structure and flanking host gene expression.

Objective: This study examines the effects of the vCTCF-BS on HTLV-1-induced *in vitro* immortalization and *in vivo* persistence.

Methods: The vCTCF was mutated to abolish CTCF binding (HTLV-1ΔCTCF). *In vitro*PBMC immortalization capacity of HTLV-1ΔCTCF was evaluated via short-term co-cultivation assay. New Zealand White rabbits were then inoculated with irradiated viral producer cell clones followed by serial blood collection over a twelve-week period. Collected samples were used to assess HTLV-1-specific antibody response, proviral load, gene expression, and total lymphocyte count.

Results: HTLV-1 and HTLV-1ΔCTCF *in vitro* immortalization capacity was comparable. HTLV-1-specific antibody response was significantly decreased in HTLV-1ΔCTCF-

inoculated rabbits. Proviral load, gene expression, and total lymphocyte count were not significantly different.

Conclusions: CTCF binding to the vCTCF is dispensable for early *in vivo* persistence, but plays a role in the host immune response. Future studies will investigate the effects of CTCF binding on tumorigenesis.

EY-59: THE POTENTIAL USE OF BIOLOGICAL PRODUCTS FOR THE ALLEVIATION OF NEUROINFLAMMATION IN THE FELINE MODEL OF GANGLIOSIDOSES

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Background: Gene therapy approaches using adenovirus-associated vectors (AAV) in animal models of gangliosidoses, including GM1 and GM2 in mice and cats, have significantly improved clinical disease outcomes. However, a strong inflammatory response is present in later stages of gangliosidoses, which is characterized by marked activation of microglia cell populations and significant upregulation of MHC-II and MIP-1. Platelet and stem cell-derived products have been proposed as therapeutic tools for modulation of inflammatory responses and might hold therapeutic potential for the alleviation of neuroinflammation in lysosomal storage diseases.

Objectives: The objectives of this study were to characterize cultures of feline microglia cells and assess whether the addition of platelet lysate (PL) or mesenchymal stem cell-derived conditioned media (MSC-CM) would alleviate the inflammatory responses induced by lipopolysaccharide (LPS).

Methods: Feline microglia were isolated and immunolabeled for Iba1, GFAP and MAP-2 by immunofluorescence. PL and MSC-CM were obtained according to established protocols and were added to LPS-stimulated microglia. Following incubation, the cells were immunolabeled for microglia activation markers such as CD68 and MHC-II, and expression levels of inflammatory cytokines TNF-α, IL-1β and MIP-1 were measured.

Results: Feline microglia were positive for Iba1 and negative for GFAP and MAP-2. Variable expression of inflammatory cytokines and chemoattractant factors were detected following the addition of PL or MSC-CM to LPS-stimulated microglia cells.

Conclusions: Biological therapeutics could serve as promising tools to modulate neuroinflammation in the feline model of gangliosidoses.

EY-60: INFECTION POTENTIAL OF RICKETTSIA FELIS VIA INGESTION

Matthew Schexnayder, Hanna Laukaitis, Mariano Carossino, Ingeborg Langohr, Kevin Macaluso
Louisiana State University School of Veterinary Medicine, Baton Rouge, LA, USA

Background: *Rickettsia felis* is the agent of flea-borne spotted fever (FBSF), an emerging disease in humans and a cause of fever in animals. It is primarily transmitted by the cat flea *Ctenocephalides felis*. Known routes of *Rickettsia felis* transmission to vertebrate hosts include cutaneous bites and contamination of cutaneous wounds with infective flea feces. The bulk of FBSF infections occur in young African children, though infections of people of all ages worldwide have been confirmed. As mammals and toddlers are exposed to flea feces through routine grooming and indiscriminate oral hygiene, we speculate that ingestion of infective flea feces may account for a portion of natural infections.

Objective: We designed experiments using a BALB/c mouse model to determine if *Rickettsia felis* could establish an infection via an oral route from culture and infective cat flea feces.

Methods: *Rickettsia felis* was cultured in ISE6 cells, purified, and administered orally. Infective flea feces were generated and similarly administered. In both experiments necropsy was performed at 1, 7, and 14 days post-ingestion and heart, liver, spleen, stomach, and intestine were collected for extraction of DNA and RNA with qPCR analysis. Select tissues were submitted for histopathology and anti-*Rickettsia* immunohistochemistry. Serum was tested via an indirect immunofluorescence assay.

Results: Ingestion from culture resulted in a 17% infection rate with detection of *Rickettsia felis* in the heart, liver, and spleen. Inflammation was not induced and the gastrointestinal tract was not infected. An humoral response developed.

Conclusion: Ingestion is a potential route for *Rickettsia felis* infection.

EY-61: LACTOBACILLUS ACIDOPHILUS AS AN ORAL VACCINE AGAINST ROTAVIRUS

Allison Vilander[1], Kimberly Shelton[1], Akinobu Kajikawa[2], Gregg Dean[1]
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Background: Despite the availability of two attenuated live oral rotavirus vaccines, rotavirus continues to cause 215,000 deaths in children yearly. Rotavirus most commonly affects children living in developing countries where efficacy of the current vaccines is lower (50-60%) than in developed nations (>85%). We have developed the Gram positive lactic acid probiotic bacterium *Lactobacillus acidophilus* (LA) as an oral vaccine against rotavirus by expressing a 10 amino acid neutralizing rotavirus capsid peptide (RV-VP8) within a LA surface layer protein in addition to three immune stimulating adjuvants: the inflammasome cytokine-IL-1beta, Toll-Like Receptor 5 ligand–FliC, and the microfold cell targeting protein–FimH.

Objective: Confirm LA expression of RV-VP8 and adjuvants and evaluate the immune response following oral vaccination in Balb/C mice.

Methods: RV-VP8 was inserted into a LA surface layer protein by homologous genetic recombination and adjuvants were expressed via transfected plasmids. Surface

expression of adjuvants was confirmed by flow cytometry and soluble IL-1beta by ELISA. Immune responses of orally vaccinated mice were assessed by VP8-specific IgG serum and fecal IgA ELISA as well as IgA ELISPOT of mucosal B cells.

Results: The VP8 peptide was robustly expressed on the surface of LA along with surface expression of FimH and FliC and secretion of IL-1beta. Results from serum IgG, fecal IgA, and mucosal IgA B cells will be reported as will proinflammatory cytokine expression in the mesenteric lymph nodes.

Conclusions: rLA surface-expressing VP8 with and without adjuvants has the potential to be a powerful next generation oral vaccine against human rotavirus.

Industrial and Toxicologic Pathology Focused Scientific Session I

Sunday, November 10, 2019 | 8:00 a.m. – 12:00 p.m.
Session Chair: Katherine A.B. Knostman, DVM, PhD, DACVP, StageBio, Mount Jackson, VA

Sunday, November 10, 2019
9:00 a.m. – 9:30 a.m.

VALIDATING RASH2 CARCINOGENICITY STUDIES USING ARTIFICIAL INTELLIGENCE

Beth Dray[1], Colin Doolan[2], Mark Gregson[2], Daniel Rudmann[1]
[1]Charles River Laboratories, Ashland, OH, USA, [2]Deciphex, Dublin, Ireland

Preclinical carcinogenicity studies require considerable pathology effort, in part due to the sheer number of slides reviewed. A typical carcinogenicity study has approximately 10000 tissues (200 animals x 50 tissues), with a large proportion of these tissues being normal or only having background changes common for the species (mouse or rat). In transgenic carcinogenicity models using the rasH2 mouse, a positive control group is included that is treated with a carcinogen such as urethane or N-Nitroso-N-methyurea (NMU). The purpose of the positive control group is to verify that the study was valid based on the presence of the expected neoplams in the transgenic mice (lymphomas and papillomas/squamous cell carcinomas for NMU and lung adenoma/adenocarcinomas for urethane). We hypothesized that artificial intelligence (AI) could be used to verify that these neoplasms were present and reduce pathologist time examining positive control animals. Whole slide scanned (WSS) lungs and stomachs from positive control groups (1 urethane and 1 NMU-treated) were used for supervised training of a convolutional neural network (CNN) by Deciphex. A subset of tissues were used for testing and two pathologists verified the accuracy of tumor identification by the program. The developed AI method was able to identify tumor positive and negative animals from the positive control group and eliminate the need of the pathologists to evaluate these animals individually. Time savings using AI was substantial and estimated at 3 working days per study.

Sunday, November 10, 2019
9:30 a.m. – 9:45 a.m.

IQ DRUSAFE PHARMA SURVEY ON NONCLINICAL AND CLINICAL USE OF EMERGING SAFETY BIOMARKERS

John Burkhardt[1], Deidre Dalma[2], Dominique Brees[3], Jean-Charles Gautier[4], Warren Glaab[5], Magali Guffroy[6], JoAnn Harding[7], Eric McDuffie[8], Lila Ramaiah[9], William Reagan[1], Albert Schultze[10], James Smith[11], Tanja Zabka[12]
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IQ DruSafe Biomarker Working Group conducted a survey of 20 pharmaceutical companies to assess the current/future state of emerging safety biomarkers (ESBs) found in biofluids (i.e. protein-, activity-, metabolites-, miRNA/mRNA-biomarkers). Because translatable biomarker utilization by pharmaceutical/biotech companies is rarely disclosed, the true impact of their use on speed of drug development (DD) remains unclear. Sixty-seven questions were designed, to determine the following: current use in the nonclinical and clinical space; identification of opportunities, gaps or barriers to greater implementation; to understand impact on advancement of DD; and to benchmark perspective on regulatory acceptance. re was general agreement among the following areas: percent of companies indicating current use of ESBs in the nonclinical and clinical space; use in non-GLP and GLP studies; and use for forward and reverse translation. Frequency of use, however, varied among companies. Inclusion of ESBs in investigational new drug applications was limited and was similar across companies. A desired future state would be characterized by broader use in both nonclinical and clinical DD phases and by better balance between robust science and practicality of approaches that evaluate strengths and weaknesses of ESBs. For increased biomarker acceptance, value of clear written criteria by Health Authorities was identified. Additionally, there is a need for increased cross-industry collaborations to develop well-validated assays and decrease the time/resources required for ESB development.

Sunday, November 10, 2019
9:45 a.m. – 10:00 a.m.

GOING GREEN – REDUCING WORK-RELATED TOXICITY IN ANATOMIC PATHOLOGY

Polina Aukon, Wendy O'Rourke, Andrew Ernest, Jennifer Nelson, Justin Griffin, Alexandra Brower
Midwestern University College of Veterinary Medicine, Glendale, AZ, USA

Background: Techniques that allow the chemical alteration of tissues form the basis of anatomic pathology, and two of the most commonly used chemicals are formalin for fixation and xylene for processing. Each comes with potential hazards for people and the environment.

Objective: To identify, implement, and evaluate laboratory initiatives that reduce exposure to formalin and xylene.

Methods: Areas of potential use and exposure to formalin and xylene were identified in a diagnostic pathology laboratory. For formalin, engineering controls that combined ventilated equipment and increased air exchanges, and two glyoxal-based formalin substitutes (Prefer and ExCell Plus) were investigated. Formalin substitutes were trialed for tissue fixation for histology slides and for gross teaching specimens. For xylene, complete elimination and replacement with alcohol-based processing was implemented. Engineering controls resulted in undetectable levels of formalin and low enough exposure levels to allow the option of terminating monitoring based on OSHA standards. There were no differences in the color or texture of gross teaching specimens between those preserved in modified Klotz solution using formalin and those using an equivalent volume of ExCell Plus. Both formalin substitutes created an unacceptable level of artifact when compared to formalin fixation. Implementation of alcohol-based tissue processing resulted in high quality diagnostic slides that were equivalent to those produced with xylene.

Conclusions: Pathology laboratories should be able to discontinue xylene use and reduce formalin exposure to negligible levels. Formalin alternatives failed to produce diagnostic quality slides but did produce good results for long-term preservation of teaching specimens.

Sunday, November 10, 2019
11:30 a.m. – 12:00 p.m.

IMPACT OF FOOD RESTRICTION, WATER DEPRIVATION, RESTRAINT, OR ISOPROTERENOL ON PLASMA CARDIAC TROPONIN I AND N-TERMINAL PRO-ATRIAL NATRIURETIC PEPTIDE IN MALE SPRAGUE-DAWLEY RATS

James Turk[1], George Hu[2], Sally Lei[2], Jean Fu[2], Kathy Chai[2], Nancy Poy[3], Ron Tyler[1]
[1]Amgen (ATO), Thousand Oaks, CA, USA, [2]Amgen Shanghai, Shanghai, China, [3]Amgen (ASF), San Francisco, CA, USA

Background: Qualification of circulating biomarkers may facilitate assessment of cardiotoxicity without histopathology thereby reducing the number of animals required for future terminal studies.

Objective: Our objective was to examine cardiac troponin I (cTnI) and N-terminal pro-atrial natriuretic peptide (NT-proANP) for detection of (A) physiologic stressors and (B) Isoproterenol-induced (ISO) cardiotoxicity in male Sprague-Dawley rats.

Methods: Histopathologic examination and assay of cTnI and NT-proANP from: (A) Rats randomized into 4 groups of 15 each exposed for 72 hours to: (1) Control, (2) food-restriction, (3) water-deprivation, or (4) 3-hours daily restraint. Five rats per group were sacrificed at 72, 96, or 264 hours; (B) Seventy rats randomized into groups of 6-10 each receiving subcutaneous ISO at 0,1,3,10,30,100, or 500 µg/kg with sacrifice at 0.5,1,2,3,4,6,24,48, and 72 hours post-dose.

Results: (A) Minimal sporadic increase in hscTnI in the absence of histopathologic changes was detected in individual rats in all groups. NT-proANP was detected in all groups and fell below the limit of detection at 72 hours in water deprivation. (B) Increased plasma cTnI correlated with histopathological lesions in the heart. Peak plasma occurred at 2-3 hours and returned to baseline within 24 hours at < 500 µg/kg ISO. NT-proANP increased in 4 sudden deaths that occurred within 1 hour of ISO, but was minimal and sporadic at scheduled timepoints

Conclusions: (A) physiologic stressors may alter cTnI and NT-proANP. (B) cTnI correlates with histopathologic evidence of ISO-induced cardiotoxicity. The relative sensitivity of histopathology and cardiac biomarker changes were not differentiated in these studies.

Combined Experimental Disease and Industrial & Toxicologic Pathology Focused Scientific Session

Refer to page ____ for the Combined Experimental Disease and Industrial and Toxicologic Pathology Focused Scientific Session II abstracts.

Industrial and Toxicologic Pathology Focused Scientific Poster Session A

T-01: SARCOMA OF HISTIOCYTIC ORIGIN IN THE HOCK OF TWO ATHYMIC NUDE RATS

Kendall Langsten[1], Lauren Neidig[2], M. O'Sullivan[1], Piper Treuting[2], Cathy Carlson[11]
University of Minnesota, St. Paul, MN, USA, [2]University of Washington, Sealttle, WA, USA

Two athymic nude rats (Hsd:RH Foxn1^{rnu}) developed unilateral soft tissue swelling around the hock; the cases presented approximately 2 years apart. Both animals were euthanized and complete necropsies were performed, including histologic evaluation of all major organs and affected joints. Histologically, the affected tibiotarsal joints were expanded by a population of highly pleomorphic and occasionally multinucleated round to polygonal neoplastic cells supported by abundant myxomatous and edematous matrix. Neoplastic cells had well-defined cell borders, a moderate amount of homogeneous eosinophilic cytoplasm, a round nucleus with finely stippled chromatin, and one to two prominent nucleoli. Increased numbers of neoplastic cells were present surrounding tendon sheaths and were often associated with invasion into tendon sheaths, skeletal muscle, and occasionally bone and joint cavities. Neoplastic cells exhibited strong CD68 (ED-1) and vimentin immunoreactivity, weak S-100 positivity, and no immunoreactivity to cytokeratin. It is likely that these lesions represent a sarcoma of histiocytic origin. Differential diagnoses include myxofibrosarcoma and giant cell tumor of the tendon sheath. Differentiating between sarcoma types can be challenging and is not always possible. Although the pathoetiology of these lesions is unclear, the presence of similar lesions in the hock of two athymic nude rats may

represent a predisposition to sarcoma in the hock that has not previously been reported in this rat strain.

T-02: HISTOLOGICAL DIFFERENCES BETWEEN HUMAN AND MINIPIG SKIN, AND REGIONAL VARIANCE IN MINIPIGS SKIN

Bunichiro Ogawa, Yutaka Nakanishi, Satoshi Wakabayashi, Minoru Sasaki
Taisho Pharmaceutical Co., Ltd., Saitama, Japan

Background: Minipig skin is more similar to human skin histologically than other experimental animals, but apparent differences in pharmacokinetics of percutaneous application drugs are sometimes observed between human and minipigs. Although histological differences are considered as a factor in addition to physic-chemical properties, few reports that analyzed histopathological parameter of skin are available.

Objective: In this study, detailed histologic parameters of human skin and each site of minipig skin were compared to collect background data for nonclinical pharmacokinetics studies of percutaneous application drugs.

Methods: The HE-stained sections of the skin (n=4) in human (dorsal) and minipig (dorso-cervical, dorso-lumbar, ventro-lateral, and abdominal) were prepared to measure the thickness of epidermis, stratum corneum and dermis, the dermal adipose tissue, and the number of hair follicles. Immunohistochemistry for von-Willebrand-factor (vWF) was also conducted to compare quantity of blood vessels.

Results: Minipig skin showed thicker epidermis and thinner dermis compared to human skin. The number of hair follicles, dermal adipose tissue, and the number of vWF-positive cells were greater in human than in minipigs. Regarding the regional differences in minipig skin, the number of hair follicles was high in the dorso-lumber site, and the number of vWF-positive cells was higher in the abdominal site than in the other sites.

Conclusions: In this study, some remarkable histological differences in skin were observed between human and minipigs, and the differences among the sites in minipig skin were also be confirmed. This histological information could promote better understanding for results of nonclinical pharmacokinetics in percutaneous application drugs.

Industrial and Toxicologic Pathology Focused Scientific Poster Session C

T-01: PRECLINICAL PHARMACOLOGICAL SAFETY TEST OF COMBINATION OF TEMOZOLAMIDE AND RU-486 FOR TREATMENT GLIOBLASTOMA

Ramón Sebastián Leon-Zetina[1,2], Patrica Garcia-Lopez[2], Laura Romero-Romero[1], Marina Guadarrama-Olhovich[1], Isabel Muñoz-Duarte[1]
[1]National Autonomous University of Mexico, Mexico city, Mexico, [2]National Institute of Cancerology, Mexico City, Mexico

Glioblastoma is the most aggressive malignant central nervous system neoplasm included in the World Health Organization schemes. Treatment of choice is composed by combination of radiotherapy and chemotherapy (Temozolomide, Tz), however, even with treatment, glioblastoma has chemoresistance to Tz which gives lower survival times. Recent research has showed that RU-486 (Mifepristone) increases cytotoxicity of several antineoplastic agents, including Tz during Glioblastoma treatment, however, there are currently no preclinical safety studies comprising the combination of both drugs which are necessary for the development and application in clinical practice. The purpose of this study was to determine if there are toxic effects during and after administration of temozolamide and RU-486 combination, to generate preclinic information about its pharmacological safety. The studies carried out were acute toxicity, local tissue damage and sub chronic toxicity in Wistar rats; genotoxicity evaluation was carried out using micronucleus essay using ICR mice. In all essays, groups were stablished in which administration of temozolamide and RU-486 combination was compared with temozolamide alone, following the guidelines established by the Organization for Economic Co-operation and development (OECD): chemical test guides 423, 452 and 474. Within acute and sub-chronic toxicity tests, hepatic and renal blood function test were made as well as post mortem evaluation to all individuals. Genotoxicity evaluation in mice was performed by micronucleates polychromatophilic erythrocytes count in peripheral blood. Results showed that RU-486 addition to temozolamide treatment had the same pharmacological safety as temozolamide administration alone in a preclinic model.

T-02: IN VITRO EVALUATION OF SMALL MOLECULE INHIBITORS OF FELINE ISLET AMYLOID POLYPEPTIDE FIBRILLOGENESIS

Noah Kos, Thomas Thompson, Nurhanis Isa, Anisa Rashid, Malikah O'Dell, Jessica Fortin
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Background: In a majority of feline diabetic patients, amyloid deposits have been detected in the islets of Langerhans. These deposits originate from islet amyloid polypeptide (IAPP), a satiety hormone produced and co-secreted with insulin by beta-cells. Islet amyloid deposits have been associated with beta-cell death during progression of diabetes. Several molecular entities have been shown to inhibit human IAPP aggregation, including silibinin and resveratrol. However, these agents have poor bioavailability and cause multiple pharmacological effects. An effective means to stop or prevent pancreatic amyloidosis in feline diabetes mellitus with small drug-like molecules is still not available.

Objective: To demonstrate that the aggregation of feline IAPP can be modulated by IAPP-interactive compounds *in vitro*. The overall objective of this project is to establish the structure-activity relationship and underlying molecular mechanism of our in-house designed small molecules to impede feline pancreatic amyloid deposits and to prepare new analogs for further drug development.

Methods: The potency of our molecules to reduce the formation of feline IAPP fibrils and toxic oligomers was assessed *in vitro* using biophysical methods such as Thioflavin T (ThT) fluorescence and photo-induced cross-linking of unmodified protein (PICUP) assays.

Results: Based on the screening of one hundred newly synthesized molecules, we discovered seven inhibitors (TT-19-004, TT-19-005, AR-19-006, NBMI-19-009, JF-19-043, JF-19-047 and JF-19-069) of feline IAPP fibrillization and oligomerization.

Conclusion: This project represents a novel strategy for modulating feline IAPP aggregation by small molecules. It has the potential to point towards new therapeutic strategies for cats with diabetes mellitus.

Young Investigator Poster Abstracts – Industrial & Toxicologic Pathology

TY-63: FOLLICLE PROTECTIVE ACTION OF TAMOXIFEN IN CANINE OVARIAN EXPLANT TISSUE CULTURES IN PRESENCE OF CYCLOPHOSPHAMIDE

Puja Basu[1], Rebecca Egbert[2], Agnew Dalen[2], Margaret Petroff[2], Brian Petroff[2]
[1]Comparative Medicine and Integrative Biology, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA, [2]Department of Pathobiology & Diagnostic Investigation, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA

Background: Recent studies in rodents have demonstrated that tamoxifen treatment prevented follicular loss and apoptosis from chemotherapeutic drugs and radiation-mediated injury.

Objective: Evaluate the ovarian-sparing effect of tamoxifen on canine ovarian follicle reserve (primordial and primary follicles), ovarian cortical explant tissue from pre-pubertal canines (N = 10) at elective ovariohysterectomy were randomly assigned and treated for 72 hours with active metabolites of alkylating agent cyclophosphamide (4-hydroxycyclophosphamide; CTX) (0, 1, and 10 mM; C, CTXL, and CTXH) and tamoxifen (4-hydroxytamoxifen; TAM) (0 and 10 mM; C and TAM). Results presented as total normal follicle reserve based on histological morphology. Primordial follicles were morphologically characterized as ‘normal’ if the nucleus of the oocyte and the single flattened layer of surrounding granulosa cells were structurally intact. Similarly, ‘normal’ primary follicles were oocytes surrounded by a single layer of cuboidal granulosa cells. The follicles were ‘abnormal’ if the oocyte and/or granulosa cells contained pyknotic, fragmented or shrunken nucleus or the follicle was not structurally sound with one or more of the following changes: the oocyte was vacuolated; loss or absence of more than 2 granulosa cells surrounding the follicle; piling up of granulosa cells towards one pole. High dose CTXH caused marked follicular loss ($P < 0.05$) whereas treatment with TAM decreased follicular loss ($P < 0.05$) from CTX in-vitro. TAM alone did not have an effect on morphologically normal reserve follicle counts ($P > 0.05$).

Conclusion: The results suggest that TAM has a protective action on follicles when exposed to CTX mediated loss.

TY-64: THE EFFECT OF GLUTATHIONE ON ITRACONAZOLE-ASSOCIATED HEPATOCYTE TOXICITY IN AN IN VITRO CANINE MODEL

Natalie Kirk, Miranda Vieson, Jennifer Reinhart
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Background: Itraconazole (ITZ) is used to treat many fungal infections in dogs. However, therapy is associated with dose-dependent increases in liver enzymes in up to 42% of dogs. Glutathione (GSH) precursors are commonly used to treat ITZ-associated hepatotoxicity, although no studies have investigated the efficacy of this practice.

Objective: To identify the effect of GSH on cell survival in an *in vitro* model of ITZ-associated hepatotoxicity using canine primary hepatocytes.

Methods: Using a sandwich culture technique, pooled canine primary hepatocytes were incubated for 24 hours with 10-fold dilutions of GSH (0 to 500 µM). Following GSH pre-incubation, the cells were exposed to 5-fold dilutions of ITZ (0 to 50 µM). Cell viability was determined using the neutral red assay after 4 (n=2) and 24 (n=3) hours of ITZ exposure. The effect of ITZ and GSH concentration on cytotoxicity was assessed using a two-way ANOVA for each time point.

Results: Canine hepatocyte cytotoxicity significantly increased with ITZ concentration ($p<0.001$) and there was a trend toward increased cytotoxicity with longer incubation times ($p=0.092$). At 4 hours, ITZ-induced cytotoxicity significantly decreased with GSH concentration ($p=0.001$) and a similar trend was noted at 24 hours ($p=0.097$).

Conclusions: The *in vitro* model demonstrates dose- and time-dependent ITZ cytotoxicity. Pre-treating with GSH appears to provide a protective effect. These results suggest that GSH precursors may have a role in the management or prevention ITZ-associated hepatotoxicity in dogs. Clinical trials are needed to evaluate their utility for this adverse drug reaction.

TY-65: 3-DIMENSIONAL HISTOLOGIC MODELS OF CANINE HEPATIC FIBROSIS FOR QUANTIFICATION AND TEACHING MODEL DEVELOPMENT

T. William O'Neill, Christiane Löhr
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The use of 3-dimensional (3D) reconstructions from advanced imaging (CT, MRI) is widespread in medicine as a way to obtain volumetric measurements, for surgical planning, and to create teaching models. Histologic slides provide excellent cellular detail and serial sections have been used previously to create 3D models, which have great utility in describing natural disease as well as in toxicological research. Patterns of hepatic fibrosis can be difficult to discern in 2-dimensional slides and present a challenge for veterinary students in understanding the clinical implications. Tissue blocks were curated from diagnostic cases received through the Oregon Veterinary

Diagnostic Laboratory. Serial sections of Trichrome and Reticulin-stained slides from tissues with various patterns of fibrosis were digitized, including centrilobular, periportal, portal bridging, and end-stage cirrhosis. Acquired photomicrographs were aligned using ImageJ, converted to an 8-bit grayscale TIFF stack, and imported into 3D Slicer. Using a combination of computer-calculated thresholding and human input on each section, we generated 3D models that can be manipulated in real-time, digitally cross-sectioned, and provide quantifiable data regarding the amount of fibrosis present. Qualitative aspects of fibrosis, such as the areas affected and the patterns across the volume examined, were also evaluated by manipulating the models. The next step is to assess the utility of the generated 3-D models in teaching veterinary students.

TY-66: THE HISTOPATHOLOGICAL EFFECTS OF COPPER EXPOSURE ON THE OLFACTORY ORGAN OF DELTA SMELTS: LINKING PATHOLOGY WITH BEHAVIOR IN AN ENDANGERED FISH.

Pedro Alejandro Triana Garcia, Swee Teh
University of California, Davis, CA, USA

Background: Delta Smelt (*Hypomesus transpacificus*) is native to the San Francisco Bay-Delta and currently is critically endangered. Copper is a contaminant of concern in the Bay-Delta used in agricultural, industrial and boating practices. The effects of copper exposure on the sensory systems of Osmerid species have not been well documented.

Objective: We aimed to characterize the histopathological effects of copper (Cu^{+2}) exposure on the olfactory rosette of Delta Smelts and to correlate these changes with functional impairment using behavioral endpoints.

Methods: We did histological evaluation on olfactory rosettes of 6 fish from a control group, 5 fish exposed to 5 µg CU^{+2}/L and 3 fish exposed to 80 µg CU^{+2} /L for 96 hours. We evaluated the behavioral response of 10 fish to alarm cues after exposure to 0 and 5 µg CU^{+2}/L. We analyzed the number of apoptotic cells by TUNEL.

Results: Copper exposure affected the olfactory epithelium in a dose-dependent manner. The control fish had a well-conserved sensory epithelium and few apoptotic cells. The groups exposed to 5 and 80 µg CU^{+2} /L had increased numbers of apoptotic cells, lack of surface structures, and a thinner sensory epithelium. The fish not exposed to CU^{+2} responded to the alarm cues by sudden changes in speed, dashing and freezing behaviors. The fish exposed to CU^{+2} did not show any of these behaviors when exposed to alarm cues.

Conclusion: A 96 hours copper exposure can impair the detection of odorants in Delta Smelts causing alterations in pivotal behaviors for survival.

Natural Disease Focused Scientific Session I

Sunday, November 10, 2019 | 8:00 a.m. – 12:00 p.m.
Session Chair: Angela Pillatzki, MS, DVM, DACVP, South Dakota State University, Brookings, SD

Sunday, November 10, 2019
9:00 a.m. – 9:15 a.m.

THE EFFECT OF DEXAMETHASONE ON HEMATOLOGICAL PROFILES, HAEMOSPORIDIAN INFECTION, AND SPLENIC HISTOLOGY IN HOUSE FINCHES (HAEMORHOUS MEXICANUS)

Esther Crouch[1], Maria Teresa Reinoso-Perez[2], Keila Dhondt[3], André Dhondt[2], José Cruz Otero[1], María Forzán[4]
[1]Department of Biomedical Sciences, Cornell University, Ithaca, NY, USA, [2]Cornell Laboratory of Ornithology, Cornell University, Ithaca, NY, USA, [3]Department of Microbiology & Immunology, Cornell University, Ithaca, NY, USA, [4]Cornell Wildlife Health Lab, Department of Population Medicine, Cornell University, Ithaca, NY, USA

Background: The emergence and rapid spread of *Mycoplasma gallisepticum* (MG) in house finches (*Haemorhous mexicanus*) has prompted both experimental and field studies investigating the effects of mycoplasmosis on house finch survival. In other bird species, the administration of glucocorticoids has induced immunosuppression with increased susceptibility to infectious agents, involution of immune organs, and lymphopenia and heterophilia.

Objective: Dexamethasone was used to induce stress-like immunosuppression in house finches following recovery of an experimental MG infection, allowing characterization of alterations in hematological profiles, haemosporidian infection, and splenic histology.

Methods: Eighteen birds were injected subcutaneously with either dexamethasone (experimental group, n=7) or saline (control group, n=8) daily for 8 days. Blood smears were prepared at day 0, 4, 8 and 9 of treatment, and birds were then euthanized. Necropsies and histopathologic examination were performed. Sections of spleen were graded on the histologic presence of follicles and CD3 immunoreactivity. White blood cell differential counts and analysis for hemoparasites were performed on blood smears.

Results: A significant decrease in lymphoid follicles in dexamethasone-treated birds was noted ($p < 0.001$). Dexamethasone induced a relative lymphopenia and heterophilia, severe on day 4 and almost absent by day 9. Increased Leucocytozoon spp. and Plasmodium spp. parasitism in the dexamethasone-treated birds was detected. No difference in intestinal coccidiosis, splenic CD3 immunoreactivity, and ocular lesions were seen between the control and treatment group.

Conclusions: Dexamethasone is capable of inducing changes consistent with immunosuppression in house finches, likely comparable to that of stress-induced immunosuppression. The role of MG infection is unclear.

Sunday, November 10, 2019
9:15 a.m. – 9:30 a.m.

CURRENT STATE OF THE GOLDEN RETRIEVER LIFETIME STUDY: SEVEN YEARS OF DATA, DIAGNOSES, AND POTENTIAL INSIGHTS INTO CANINE CANCER EPIDEMIOLOGY

Missy Simpson[1], Rod Page[2], Kelly Diehl[1], Michael Cinkosky[1], Janet Patterson-Kane[1]
[1]Morris Animal Foundation, Denver, CO, USA, [2]Flint Animal Cancer Center, Colorado State University Veterinary, Fort Collins, CO, USA

Background: Prospective studies are a robust design for establishing temporality of exposures and studying multiple diseases. The Golden Retriever Lifetime Study is the most extensive veterinary cohort study to date. Our goal is to identify risk factors for lymphoma, hemangiosarcoma, high-grade mast cell tumor, and osteosarcoma. We chose golden retrievers because cancer is overrepresented in this breed. We focused on these cancers because of their high prevalence and poor prognosis.

Objective: Describe the health and vital status of enrolled dogs and compare with original estimates.

Methods: We enrolled 3,044 golden retrievers throughout the contiguous United States at 2-24 months of age. We collect owner- and veterinarian-reported data, clinical pathology results, and biological samples annually. In the event of malignancy, we collect additional veterinarian-reported data, clinical pathology, and tissue samples. At death, full medical records are curated and, if an owner is willing, necropsy tissues are collected.

Results: Currently, 2808 dogs are actively enrolled, with accrual of 15,200 dog-years of data. The mean age is 6.3 ±1.1 years, with 85% compliance. One hundred forty-two dogs have died and 68 (48%) of those are cancer related. We have 64 total diagnoses of the primary cancers and 44 (69%) have died. Compared to estimates, we have 57% of the projected primary cancer diagnoses, 119% of deaths due to all cancers, and 111% of all-cause mortality.

Conclusion: This study offers a groundbreaking opportunity to gain insights into canine cancer. Banked samples are available via application. Curated data can be retrieved via open access portal.

Sunday, November 10, 2019
9:30 a.m. – 9:45 a.m.

CANINE GASTRIC CARCINOMAS - A HISTOPATHOLOGICAL STUDY

Alexandros Chardas[1], William Becker[1], Alejandro Suarez-Bonnet[1], Sam Beck[2], Simon Priestnall[1]
[1]The Royal Veterinary College, Hatfield, United Kingdom, [2]VPG Histology, Bristol, United Kingdom

Background: Carcinoma is the commonest canine gastric tumor, accounting for around 90% of gastric malignancies, with reported predisposing factors of diet and breed.

Histological classification, based on WHO and Lauren classifications, is based on predominant growth pattern, architectural and cytological features. *Helicobacter* are reported frequently in the canine stomach but their role in disease, including neoplasia, is uncertain.

Objective and Methods: To characterize and histologically classify a large series of canine gastric carcinomas and to evaluate possible risk factors for tumor development. To assess the presence of *Helicobacter* in relation to gastric inflammation, as scored by WSAVA grading, and histological tumor classification.

Results: 137 cases of canine gastric carcinoma (surgical and endoscopically-retrieved samples) from the archives of the Royal Veterinary College and VPG Histology were included. Carcinomas were most frequently present in Staffordshire bull terriers, Collies and Labrador retrievers. Mean age was 9 years but no sex predilection was identified. Signet-ring cell type carcinoma was the most frequent histological type (38%, 52/137), followed by undifferentiated (26%), tubular (24%), mucinous (9.5%) and papillary (1.5%) types. Signet-ring cell types were more frequently diagnosed in endoscopic samples. Mean gastric inflammatory score (MGIS) was 3.3 for Helicobacter-positive cases and 2.6 for negative cases. MGIS was greater (3.7) in cases with high bacterial numbers compared to those with few (3.0) and mucinous carcinomas were most often positive (53.8%, 7/13).

Conclusions: This study contributes further to our understanding of the potential role of *Helicobacter* spp. inducing inflammation and possible tumor formation in canine gastric carcinomas.

Sunday, November 10, 2019
9:45 a.m. – 10:00 a.m.

ASSOCIATION OF DOMESTIC CAT HEPADNAVIRUS WITH HEPATITIS AND HEPATOCELLULAR CARCINOMA

Patricia Pesavento[1], Tiffany Tse[1], Bronte Hampson[2], Kenneth Jackson[1], John Munday[3], Julia Beatty
[1]School of Veterinary Medicine, University of California, Davis, Davis, CA, USA, [2]Sydney School of Veterinary Science, New South Wales, Sydney, Australia, [3]School of Veterinary Science, Massey University, Aukland, Massey, New Zealand

Background: In 2018, the first carnivore hepatitis B-like virus was discovered in a sick feline patient by metagenomics. Domestic cat hepadnavirus (DCH) is phylogenetically similar to Hepatitis B virus (HBV),which in humans causes hepatitis and cancer, and is responsible for 1-2 million deaths annually. Establishing whether there is an association of DCH with liver disease is of interest for feline health.

Objective: The goal of the work is to identify whether any DCH-associated subset of hepatitis or hepatic cancer exists in cats, and if so, to determine the cellular location of DCH in infected livers.

Methods: DNA extracted from liver of cats with biliary or hepatocellular carcinomas, cats with chronic hepatitis, and cats that were histologically normal were screened for the presence of DCH by PCR. All PCR-positive livers, and subsets of normal and PCR-negative cases were examined by in situ hybridization using probes that distinguish predicted nuclear and cytoplasmic forms of DCH.

Results: In livers collected from 3 countries (US, AU, NZ), DCH was detected by PCR in 6/39 cats with hepatitis or carcinoma. DCH was not detected in normal livers. By ISH, a subset of PCR positive cases had cccDNA present in hepatocyte nuclei, and in patchy regions viral nucleic acid was present within hepatocellular cytoplasm: the distribution is consistent with the predicted viral lifecycle, and is similar to human HepB.

Conclusions: DCH is associated with a subset of chronic hepatitis and carcinomas in a distribution that supports a causal role for DCH in feline liver cancer.

Sunday, November 10, 2019
10:30 a.m. – 10:45 a.m.

CULTURE AND METAGENOMIC MINION SEQUENCING TO DETECT AND CHARACTERIZE RNA VIRUSES

James Stanton[1], Kevin Lahmers[2], Kelsey Young[1], Salman Butt[1], Holly Sellers[1], David Stallknecht[1], Jerry Saliki[1], S Tompkins[1], Ian Padykula[1], S Todd[2], Chris Siepker[1], Elizabeth Howerth[1]
[1]University of Georgia, Athens, GA, USA, [2]Virginia Tech University, Blacksburg, VA, USA

Background: Due to the rapid mutability of RNA viruses, they frequently jump species and can drift from vaccine protection or from detection by targeted techniques. Additionally, most diagnostic assays provide only limited genomic characterization. Thus, in specific situations there is a need to efficiently detect and characterize RNA viruses without having prior knowledge of the viral sequence.

Objective: The objective of this study was to determine if viral culture coupled with random MinION sequencing can efficiently identify and characterize RNA viruses from different classes, including positive- and negative-stranded viruses, and segmented viruses.

Methods: Egg-cultured infectious bronchitis virus (IBV) and canine-origin influenza A virus, along with cell-cultured porcine reproductive and respiratory syndrome virus (PRRSV), canine distemper virus (CDV), epizootic hemorrhagic disease virus (EHDV), bovine viral diarrhea virus (BVDV), and porcine-origin influenza A virus were used to determine if random, strand switching, MinION-based sequencing could detect and genetically categorize these viruses. Raw reads were processed using a stock MacBook Pro.

Results: This method accurately identified the targeted viruses, including determining the lineages for the IBV, PRRSV, CDV and influenza samples. With a minimum of 20× depth, five viruses were completely sequenced with an average of 86% genome

coverage across all viruses. Also, two viruses were detected from a single sample (EHDV and BVDV), highlighting the ability of this approach to detect co-infections.

Conclusions: The results demonstrate the utility of using standard viral culture followed by random, MinION-based strand switching for the identification and characterization of viruses.

Sunday, November 10, 2019
10:45 a.m. – 11:00 a.m.

HEPATIC DISTOMIASIS IN UPPER MIDWEST FETAL AND NEONATAL BEEF CALVES

Heidi Pecoraro, Teresa Newell, Brett Webb
North Dakota State University Veterinary Diagnostic Laboratory, Fargo, ND, USA

Background: Hepatic trematodes, such as *Fasciola hepatica* (common or sheep liver fluke) and *Fascioloides magna* (giant or deer liver fluke), are present in the upper Midwest and have been associated with clostridial infections such as redwater disease and black disease in cattle. Liver flukes may also result in monetary loss due to carcass condemnation and the increased time taken for affected animals to reach slaughter weight. Since 2016, over 50 cases of liver fluke have been identified in weaned calves and adult beef cattle at the North Dakota State University (NDSU) Veterinary Diagnostic Laboratory (VDL). Rarely, signs of fluke infestation have been present in much younger calves, including aborted fetuses.

Objective: To describe the gross and histopathologic changes in fetal and neonatal beef calves with signs of liver fluke infection.

Methods: Gross examinations were performed and histopathology was described for four fetal and neonatal beef calves submitted to the NDSU VDL. Ancillary testing, including bacteriology, virology, and toxicology, was completed.

Results: On gross examination, black linear tracts were noted in all four livers examined. Microscopically, the linear tracts corresponded to black pigment (hematin) variably admixed with trematode eggs, fibrosis, and inflammation. In one case, liver fluke was associated with peritoneal hematoma. Bacterial and fungal infections were identified in two of the calves and one calf had cardiomyocyte degeneration and necrosis, possibly associated with monensin toxicity.

Conclusion: Liver fluke infestation can occur *in utero* and may lead to increased susceptibility to other common causes of death in fetal and neonatal calves.

Sunday, November 10, 2019
11:00 a.m. – 11:15 a.m.

THE CONTRIBUTION OF KERATINOCYTE STRESS TO EQUINE LAMINITIS PATHOGENESIS: MACRO- AND MICRO-ANATOMICAL LESIONS AND UP-REGULATION OF A STRESS MARKER, GRP78/BIP.

Hannah Galantino-Homer[1], Julie Engiles[1], Lynne Cassimeris[2], Caitlin Armstrong[1], Kimberly Hildreth[1]

[1]University of Pennsylvania, School of Veterinary Medicine, Kennett Square, PA, USA, [2]Lehigh University, Bethlehem, PA, USA

Background: Equine laminitis is divided into categories based on the initiating risk factor, including endocrinopathic laminitis (EL), associated with hyperinsulinemia, supporting limb laminitis (SLL), associated with decreased limb unloading due to a non-weight-bearing injury in the contralateral limb, and sepsis-associated laminitis. Although a common feature is damage to the epithelial and connective tissue integrity of the hoof lamellae resulting in failure of the suspensory apparatus of the distal phalanx (SADP), relatively little is known about the molecular pathogenesis or histologic lesions between different initiating risk factors.

Objective: To present the macro- and micro-anatomical hoof lamellar lesions of spontaneous EL (n=12) and SLL (n=18) cases, and the associated immunolocalization of Grp78/BiP protein chaperone, a molecular marker of cell stress, in comparison to age-matched controls.

Results: The macroanatomical stratum internum-corium measurement, representing the “lamellar wedge,” was significantly increased in both SLL and EL feet. Microanatomically, this corresponded to epidermal hyperplasia and dysplasia characterized by acanthosis and keratinocyte metaplasia with loss of cell-cell and cell-matrix adhesion, and keratinocyte apoptosis/necrosis. Lesions consistent with failure of lamellar epithelial integrity more often occurred at the junction between the basal cells and suprabasal cells of the secondary epidermal lamellae than at the basal cell-basement membrane junction, sometimes resulting in complete separation from the basal layer. In laminitic feet, Grp78/BiP-positive keratinocytes often co-localized within metaplastic keratinocytes located adjacent to the keratinized axes of abaxial and middle lamellar regions.

Conclusion: Lamellar epidermal cell stress, epidermal metaplasia, and apoptotic cell death may contribute to EL- and SLL-associated SADP failure.

Sunday, November 10, 2019
11:15 a.m. – 11:30 a.m.

IMMUNOHISTOCHEMICAL EVALUATION OF REGULATORY T LYMPHOCYTE INFILTRATION OF CANINE GLIOMA

Gregory Krane[1,2], David Malarkey[1], Andrew Miller[3], C. Miller[4], Debra Tokarz[5], Christopher Mariani[2]
[1]National Institute of Environmental Health Sciences: National Toxicology Program, Research Triangle Park, NC, USA, [2]North Carolina State University: College of Veterinary Medicine, Raleigh, NC, USA, [3]Cornell University: College of Veterinary Medicine, Ithaca, NY, USA, [4]University of North Carolina: Lineberger Comprehensive Cancer Center, Chapel Hill, NC, USA, [5]Experimental Pathology Laboratories, Research Triangle Park, NC, USA

Background: Glioma evasion of the immune system is an integral part of its pathogenesis. In humans, an important mechanism of evasion is recruitment of

regulatory T lymphocytes (Tregs). Tregs suppress effector immune cells and contribute to their inability to eliminate cancer cells. Canine glioma has a robust immune cell microenvironment; however, the distribution of Tregs has not been determined.

Objective: To characterize Treg infiltration in canine glioma.

Methods: 85 cases from the NCSU archives (2006-2018) originally diagnosed as glioma were independently reviewed via consensus diagnosis by a group of five pathologists (4 DVM, 1 MD), resulting in 73 glioma cases for analysis. Treg populations in these gliomas were manually quantified via immunohistochemistry for FoxP3, a marker of Tregs.

Results: 44/73 gliomas had infiltrating Tregs. Mean Tregs per high-powered field (ocular FN 22 mm, 400X magnification, 0.237 mm^2area) were 2.7 (range 0.1-21.6, standard deviation 5.2). This subset of gliomas was predominantly high grade oligodendrogliomas. Tregs were generally evenly distributed throughout the tumor tissue and extended slightly beyond the brain-tumor interface, though they were not otherwise detected in normal brain.

Conclusions: Treg infiltration occurred in the majority of gliomas although the numbers of Tregs were relatively low in most tumors. However, a subset of gliomas predominantly composed of high grade oligodendrogliomas showed more robust Treg infiltration. Treg blockade or depletion may be potential therapeutic targets in this group of tumors.

Sunday, November 10, 2019
11:30 a.m. – 11:45 a.m.

MITOTIC COUNT IN CANINE CUTANEOUS MAST CELL TUMORS: MANUAL COUNTS CONSISTENT WITH HIGHEST MITOTIC DENSITY?

Christof Bertram[1], Marc Aubreville[2], Corinne Gurtner[3], Alexander Bartel[4], Sarah Corner[5], Martina Dettwiler[3], Olivia Kershaw[1], Erica Noland[5,6], Anja Schmidt[7], Dodd Sledge[5], Rebecca Smedley[5], Tuddow Thaiwong[5], Matti Kiupel[5,6], Andreas Maier[2], Robert Klopfleisch[1]
[1]Institute of Veterinary Pathology, Freie Universität Berlin, Berlin, Germany, [2]Pattern Recognition Lab, Computer Science, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany, [3]Institute of Animal Pathology, Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland, [4]Institute for Veterinary Epidemiology and Biostatistics, Freie Universität Berlin, Berlin, Germany, [5]Michigan State University Veterinary Diagnostic Laboratory, Lansing, MI, USA, [6]Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University, Lansing, MI, USA, [7]Vet Med Labor GmbH - Division of IDEXX Laboratories, Ludwigsburg, Germany

Background: Current prognostication of canine cutaneous mast cell tumors (ccMCT) requires determining the mitotic count (MC) in tumor areas with the highest mitotic density, which is assumed to correlate best with biological behavior. Due to inconsistent

area selection, we speculated that identifying highest density might be hampered by inter-observer variation.

Objective: To compare the manual MC with the true mitotic distribution in ccMCTs.

Methods: All mitotic figures in digital slides of 28 ccMCTs were labeled by two pathologists. Image analysis was used to determine ground truth MC distribution throughout the tumor section. Thereafter, manual MCs were performed on scanned sections by eleven pathologists.

Results: The ground truth MC varied between different tumor areas in most examined cases. Manual MCs were within the highest ground truth quartile in only 51.9% of the cases (random chance: 25%). However, there was substantial agreement between participants ($\kappa = 0.865$) in consistently identifying either a MC≥7 or MC<7. High accuracy was due to the majority of MCTs having either overall high or low MCs. In contrast, in there were 95.5% falsely low counts in four borderline cases.

Conclusion: Manual MCs often do not represent the most mitotically active tumor regions and area selection may have a significant impact on the MC, in particular in borderline cases. Further standardization of area selection, such as by computerized analysis, is required to achieve higher accuracy of MCs. Regardless of the high variability of mitotic density, prognostication based on manual MCs was very reproducible when published cut-off values were applied.

Sunday, November 10, 2019
11:45 a.m. – 12:00 p.m.

A HIGH MORTALITY OUTBREAK OF MYCOPLASMA BOVIS IN WYOMING PRONGHORN (ANTILOCAPRA AMERICANA)

Jennifer Malmberg[1], Terry Creekmore[2], Marguerite Johnson[1], Erika Peckham[2], Hally Killion[1], Madison Vance[1], Donal O'Toole[1], Kerry Sondgeroth[1]
[1]University of Wyoming, Laramie, WY, USA, [2]Wyoming Game and Fish Department, Laramie, WY, USA

Background: Mycoplasma bovis (M. bovis) is an economically important pathogen of cattle that also causes virulent pneumonia in ranched North American bison. In 2019, M. bovis was identified as the cause of a high mortality outbreak of pneumonia in pronghorn near Gillette, Wyoming. Of at least 60 animals found dead, M. bovis was detected in 10 by PCR, immunohistochemistry (IHC), or both.

Objective: Reports of M. bovis in free-ranging wildlife are uncommon, and infections have not been previously reported in pronghorn. In this study, we characterized the pathology and genetics of M. bovis in pronghorn and compared our findings to those documented in cattle and bison.

Methods: Isolates from 5 cases of *M. bovis* in pronghorn were grown in culture and characterized by whole genome sequencing. We used multilocus sequencing typing

(MLST) to compare pronghorn isolates to those from cattle and bison, and used IHC and PCR to retrospectively screen pronghorn lung tissue for *M. bovis*.

Results: We report that *M. bovis* acts as a sole or primary pathogen in pronghorn and causes fatal fibrinosuppurative pleuropneumonia, similar to infections seen in bison. MLST revealed that pronghorn isolates are most similar to those from cattle in the United States, suggesting spillover from cattle to pronghorn. Additionally, *M. bovis* was not detected in archived lung tissue (N=19) evaluated by IHC and PCR. We conclude that *M. bovis* has continued to expand in disease expression and host range in recent years, and should be considered as cause of pneumonia in free-ranging ungulates.

Natural Disease Focused Scientific Session II

Sunday, November 10, 2019 | 1:30 p.m. – 5:00 p.m.
Session Chair: Angela Pillatzki, MS, DVM, DACVP, South Dakota State University, Brookings, SD

Sunday, November 10, 2019
2:30 p.m. – 2:45 p.m.

CATASTROPHIC TIBIAL FRACTURES IN RACEHORSES

Monika Samol[1], Susan Stover[2], Francisco Uzal[1], Rick Arthur[3]
[1]California Animal Health and Food Safety Laboratory, University of California Davis, San Bernardino, CA, USA, [2]J.D. Wheat Veterinary Orthopedic Laboratory, Veterinary Medicine Teaching Hospital, University of California Davis, Davis, CA, USA, [3]Veterinary Medicine Teaching Hospital, University of California Davis, Davis, CA, USA

The most prevalent cause of death in racehorses are musculoskeletal injuries. This study characterized tibial fractures in racehorses. The database of CAHFS, including 7,068 racehorse necropsy reports was searched for horses that died because of a tibial fracture between 1990 and 2018. One hundred nineteen horses had complete tibial fracture. The majority of fractures (66%) occurred during training, whereas racing-related and non-exercise fractures occurred in 17% and 14% of horses, respectively. For the remaining 3% of cases the information regarding circumstances of the injury was unavailable. Most fractures in Thoroughbreds occurred in 2- and 3- year old horses (39% and 33%, respectively), while 100% of the Quarter Horses for which age was available were 2-year-olds. Most horses (97%) had unilateral fractures that affected the left (50%) or the right (47%) hindlimb, the remaining 3% of horses fractured both tibiae. The most commonly recognized fracture site and configuration was diaphyseal (44%), and oblique (40%); 97% of the fractures were comminuted. Out of 65 cases examined for pre-existing callus formation, 60% of horses had raised periosteal callus bridging the line of catastrophic fracture. The most prevalent location for callus was the cortical bone in the proximal third of the diaphysis/proximal metaphysis, underneath the fibula (64%). The second most common area of callus formation was the distocaudal surface of the distal third of the diaphysis/distal metaphysis (26%). Our results suggest that stress fractures on the proximolateral aspect of the tibia are challenging to diagnose clinically. These calluses may predispose to catastrophic fractures.

Sunday, November 10, 2019
2:45 p.m. – 3:00 p.m.

MOLECULAR CHARACTERIZATION OF 119 MAMMARY CARCINOMAS IN PET RABBITS

Sophie Degner[1], Heinz-Adolf Schoon[1], Mathias Baudis[2], Claudia Schandelmaier[3], Heike Aupperle-Lellbach[3], Sandra Schöniger[4]
[1]Institute of Veterinary Pathology, University of Leipzig, Leipzig, Germany, [2]Tierarztpraxis Ralf Bischoff, Melle-Markendorf, Germany, [3]Laboklin GmbH & Co. KG, Bad Kissingen, Germany, [4]Targos Molecular Pathology GmbH, Kassel, Germany

Rabbits are popular pets. Studies showed, that most mammary tumors in female pet rabbits are carcinomas. So far, no data on prognostic features are published. The only treatment option is surgical excision.

This study examined mammary carcinomas of pet rabbits for the expression of estrogen and progesterone receptors (ERα, PR) as well as the myoepithelial marker calponin. Immunohistochemical data (ERα, PR and calponin expression) were statistically correlated with histological findings of the tumors (mitotic count, histotype, histological grade).

Most carcinomas (n = 75; 63%) were negative for ERα and PR, whereas solely ERα was detected in 20% (n = 24) and 18% (n = 22) were positive for both receptors. Retained nonneoplastic myoepithelial cells (NME) were detected in all carcinomas; 93% (n = 111) contained additional calponin-positive tumor cells. These encompassed up to 22% of the neoplastic cells. The ERα and PR status was not influenced by tumor histotype or grade. Significantly higher mitotic counts were observed in ERα- and/or PR-negative carcinomas than in ERα- and/or PR-positive carcinomas. A higher percentage of calponin-positive tumor cells was statistically associated with increased tubular growth, a lower tumor grade and a lower mitotic count.

This study provides first insights into molecular features of pet rabbit mammary carcinomas. The detection of NME in all carcinomas suggest their progression from in situ carcinomas. Results reveal an association between mitotic count, hormone receptor status and calponin expression. A prognostic relevance of these features appears likely. This has, however, to be examined in long-term follow-up studies.

Sunday, November 10, 2019
3:30 p.m. – 3:45 p.m.

SEVERE ESOPHAGITIS IS A COMMON CAUSE OF MORBIDITY IN CAPTIVE NAKED MOLE RATS

Wesley Siniard[1,2,3], Megan Smith[4], Rochelle Buffenstein[4], Denise Imai-Leonard[1,3]
[1]School of Veterinary Medicine, University of California, Davis, Davis, CA, USA, [2]Zoological Society of San Diego, San Diego, CA, USA, [3]Comparative Pathology Laboratory, Davis, CA, USA, [4]Calico Life Sciences, South San Francisco, CA, USA

Objective: Characterize inflammatory esophageal lesions in captive naked mole rats (NMRs) and assess relevance of select predictive factors or consequences to developing severe esophagitis.

Design: Prospective and retrospective case series.

Animals: 53 NMRs from the San Diego Zoo (SDZ) and 20 from a research colony.

Procedures: Data including age, history of weight loss, and body condition were obtained. Also, 18 research NMRs with a history of weight loss and 2 control NMRs were used for gross morphometry and gastroesophageal junction analysis. Archival esophageal sections were evaluated by light microscopy for presence and severity of esophagitis, presence of intralesional *Candida* sp., intestinal metaplasia of the distal thoracic esophagus, and cecal dybiosis. Data were analyzed as categorical variables. Associations were tested by Fisher's exact test, with calculation of relative risk and odd's ratio.

Results: Esophagitis was identified in 14/20 research NMRs (70%) and 40/53 (75.5%) SDZ NMRs. History of weight loss ($p < 0.001$), poor to fair body condition ($p = 0.003$), and presence of *Candida* sp. ($p = 0.013$) were statistically significantly associated with severe esophagitis. Intestinal metaplasia of the distal thoracic esophagus was identified in two research NMRs and one SDZ NMR, all three of which also had severe esophagitis.

Conclusions and Clinical Relevance: Based upon these studies, esophagitis appears to be an important and common lesion within captive NMR populations, and likely contributes to significant morbidity (weight loss and poor body condition). Intestinal metaplasia of the distal thoracic esophagus suggests the pathogenesis of disease could involve gastroesophageal reflux.

Sunday, November 10, 2019
3:45 p.m. – 4:00 p.m.

PATHOLOGY OF OPHIDIAN SERPENTOVIRUS INFECTIONS IN SNAKES

Robert Ossiboff
University of Florida, Gainesville, FL, USA

Background: Ophidian serpentoviruses (nidoviruses) are increasingly being identified as important agents of clinical disease in captive snake populations. Nidovirus infections can cause severe and sometimes fatal upper respiratory and upper alimentary disease that can result in significant losses to private and zoological collections.

Objective: The objective of this study is to detail comparative gross and histopathologic findings in snake species naturally infected with ophidian serpentoviruses from both published and unpublished data.

Methods: Postmortem, microbiologic, molecular, and in situ hybridization findings were compared from multiple serpentovirus outbreaks to identify common manifestations of ophidian serpentovirus infections.

Results: Gross and histologic findings in nidovirosis cases are primarily restricted to the oral, nasal, and upper respiratory tracts. Secondary bacterial infections are common. The degree of epithelial proliferation and inflammation in certain tissues, particularly the oral cavity and esophagus, can be useful in differentiating nidoviral infections from other ophidian viral diseases. RNAscope® *in situ* hybridization identifies abundant viral nucleic acids within the inflamed and hyperplastic epithelium of the oral cavity, nasal cavity, trachea, esophagus, and lung, as well as within the lumen of the gastrointestinal tract and oronasal mucus secretions. The abundance of viral nucleic acid present within the oral, cranial esophageal, and pharyngeal regions makes oral/choanal swabs the preferred diagnostic sample for molecular diagnostics or virus isolation.

Conclusions: Serpentoviruses are an important cause of morbidity and mortality in captive snakes, particularly pythons. They should be considered an important differential diagnosis in snakes with respiratory disease.

Natural Disease Focused Scientific Poster Session A

N-01: VALIDATION OF DIAGNOSTIC TECHNIQUES FOR INFECTION BY THE TILAPIA LAKE VIRUS (TILV)

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According to the information reported by the OIE, the confirmatory methods of TiLV diagnosis include the molecular techniques described by Eyngor, Tsofack, Dong and Tattiyapong. The aim of this study was to validate and compare the sensitivity and specificity of the Eyngor and Dong techniques in relation to histopathological lesions compatible with TiLV infection. Fifty eight cases were selected, corresponding to 19 epidemiological events between June 2016 to April 2018. Eighty four percent (84%) of the positive cases to TiLV were presented in the dry season in Colombia (December-March and June-September), compared to 31% in the rainy season. The Eyngor RT-PCR compared to the histopathology had a sensitivity of 70% and a specificity of 100%, with a kappa index of 0.472. The sensitivity of the Dong technique compared to the histopathology was 68%, the specificity 63% and the kappa index was 0.229. The Dong nested RT-PCR detected a greater number of healthy carriers (without lesions but with viral molecular detection). In conclusion, it is suggested to routinely use the nested Dong's RT-PCR to improve the detection of healthy carriers and always include histopathological evaluation in order to determine this epidemiological status. Additionally, continue the studies of the viral genomic structure to optimize the diagnostic techniques given the mutability of the TiLV.

N-03: AN UNUSUAL MORTALITY EVENT OF GREY WHALES ALONG THE WEST COAST OF NORTH AMERICA

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Background: Between January 1 and July 1, 2019, approximately 171 grey whale mortalities were reported along the eastern North Pacific coast. Necropsies were performed on 15 strandings in Washington State, 13 in California primarily within San Francisco Bay area, 5 in British Columbia and 3 in Alaska.

Objective: A marked increase in grey whale mortalities along the western seaboard of North America prompted enhanced efforts to necropsy animals to determine cause of death.

Methods: Marine mammal mortalities observed by the public are reported to regional response networks and teams of trained personnel mobilized to examine animals. Signalment, morphometric, gross pathologic and environmental data were compiled and reviewed.

Results: Of 32 necropsied whales, 24 were females, 10 males and 2 of undetermined sex, with 23 adults, 8 subadults, 3 yearlings and 2 weanlings. A specific cause of death was not resolved in all cases; however, suboptimal body condition was noted in 27 animals, confirmed or suspect ship strike in 6 and possible live stranding in 4.

Conclusions: As with a prior unusual mortality event of grey whales in 1999 and 2000, many mortalities examined to July 1, 2019 were often associated with emaciation, likely related to metabolic demands associated with migration, although individual cases had further pathology. Animals foraging in high vessel traffic areas were vulnerable to ship strike, and loss of condition may have contributed to reduced buoyancy and live stranding on mud flats in some cases.

N-04: HISTOLOGIC, ULTRASTRUCTURAL, AND DEEP SEQUENCING BASED MOLECULAR CHARACTERIZATION OF A NOVEL HERPESVIRUS ASSOCIATED WITH BRANCHITIS IN A TIGER SHARK (GALEOCERDO CUVIER)

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Viral diseases in elasmobranch fishes, essential predators in marine ecosystems and popular in aquarium displays, are largely unknown. Herpesvirus-like and adenovirus-like particles have been observed in gill, oral, and skin lesions in dogfish using electron microscopy, although none have been characterized molecularly. The only complete disease-associated viral genome assembled from an elasmobranch causes papillomatous skin disease in the giant guitarfish. The agent represents a novel viral family, suggesting great opportunity for viral discovery in these understudied species. Herein, we describe a case of necrotizing branchitis in a juvenile tiger shark (*Galeocerdo cuvier*) and partial description of an associated herpesvirus. The wild-caught, 5.8 kg, 120 cm male declined following capture and was euthanized several days later. Microscopically, gills contained multiple foci of epithelial loss and necrosis, often associated with lamellar thromboses. Scattered groups of markedly enlarged lamellar epithelial cells contained basophilic, intranuclear and intracytoplasmic inclusion bodies. Transmission electron microscopy revealed arrays of intracytoplasmic and fewer intranuclear viral particles within epithelial cells. Complete virions possessed an electron-dense core, 90-100 nm icosahedral capsid, tegument, and outer envelope. Next-generation sequencing (Illumina Hi-Seq) performed on formalin-fixed gill tissue produced a partial 200 kb genome, including complete terminase, major capsid protein, and polymerase gene sequences that share <65% protein identity to corresponding core genes of known Herpesviridae and facilitated production of a TaqMan-based quantitative PCR (qPCR). Ongoing work includes development of an in situ hybridization assay that along with qPCR will be used to elucidate virus-associated pathology and screen additional animals for infection.

N-05: DISSEMINATED XANTHOGRANULOMATOUS INFLAMMATION IN EIGHT ECLECTUS PARROTS (ECLECTUS RORATUS) AND ONE PARAKEET (MELOPSITTACUS UNDULATES); A CASE SERIES

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Background: Lipid related lesions in psittaciform species are commonly associated with lipid metabolic disorders, and include xanthomas, atherosclerosis, lipomas, steatitis, pancreatic necrosis, hepatic, intestinal and splenic lipidosis, yolk coelomitis and storage disorders.

Objective: We describe a trend of disseminated or less frequently focal xantho granulomatous inflammation in Psittaciform species with a possible species predisposition (Eclectus parrots).

Methods: Case material from 9 psittacine birds was reviewed including history, dietary information, imaging findings, gross, histologic lesions and ancillary tests.

Results: Some birds had a history of poor diet (4/9). Eclectus parrots predominated (8/9). Six of 9 birds were male. Ages ranged from 4 years to 24 years, with 2 birds of unknown age. Two birds were Bornavirus positive by PCR. Imaging findings were available in 7 of 9 birds and included poorly defined, irregularly marginated coelomic nodules. Gastrointestinal dilation was commonly observed. On gross examination, disseminated coelomic inflammation was observed in 6 of 9 cases, characterized by irregular, soft, tan to yellow amorphous plaques distributed over the viscera and body wall. Focal nodules (2/9) cases were grossly similar. Concurrent atherosclerosis of great vessels was found in 4/9 birds. Histologic evaluation revealed xanthogranulomatous inflammation with intralesional and intracytoplasmic lipid, cholesterol clefts and mineralization. No infectious organisms were found with special stains.

Conclusions: Disseminated xanthogranulomatous inflammation was observed in a group of psittacine species from multiple institutions. These findings suggest a possible lipid metabolism disorder as a cause of nodular inflammation or coelomitis in psittacines. Eclectus parrots may be predisposed to lipid related lesions.

N-06: PATHOLOGY ASSOCIATED WITH STREPTOCOCCUS SPP. INFECTION IN BABOONS (PAPIO SPP.)

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Background: *Streptococcus* spp. are a source of morbidity and mortality in captive, non-human primate (NHP) populations. However, little is known about the multiple pathologies associated with naturally occurring *Streptococcus* spp. infection in baboons (*Papio* spp.).

Methods: A computer search of the pathology database was performed for all baboon necropsies at the Southwest National Primate Research Center from 1988 to 2018 in which a *Streptococcus* spp. was cultured. Baboons on experimental protocol were excluded. The gross necropsy and histopathology reports were individually reviewed. Archived specimens were retrieved and reviewed as needed for confirmation or clarification.

Results: Fifty-four cases of *Streptococcus spp*. infection were identified. Gross pathology associated with bacterial lesions included suppurative exudate, fibrinous and fibrous adhesions, hemorrhage, mucosal thickening, organ enlargement, and abscessation. Microscopic diagnoses/findings include: suppurative inflammation,

abscessation, necrosis, hemorrhage, fibrin accumulation, and thrombosis. Lungs were the most commonly infected organ followed by central nervous system, spleen, soft tissues (skin, subcutaneous tissue, and muscle), air sacs, liver, peritoneum, and adrenal glands. Infections by non-Beta-hemolytic *Streptococcus* appeared to predominate in the lung and air sacs; the most common isolate was *Streptococcus pneumoniae*. Infections by Beta-hemolytic *Streptococcus* appeared to predominate in the reproductive tract and soft tissues.

Conclusion: Naturally occurring Beta-hemolytic *Streptococcus spp*. and non-Beta-hemolytic *Streptococcus spp*. infections are causes of morbidity and mortality in captive baboon populations. In addition, pathology associated with streptococcus infection in baboons is similar to reports of human infection and may represent an underutilized model for studying *Streptococcus spp*. as a pathogen.

N-07: THE FIRST REPTILIAN CIRCOVIRUS IDENTIFIED INFECTS GUT AND LIVER TISSUES OF BLACK-HEADED PYTHONS

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Background: Viral metagenomic analysis was performed on the liver of a black headed python (*Aspidites melanocephalus*) euthanized for a proliferative spinal lesion of unknown etiology.

Objective: Characterize the first reptile-infecting circovirus genome (black- headed python circovirus; BhPyCV). Screen additional samples for initial prevalence and possible pathogenicity of this virus in snake population.

Methods: BhPyCV-specific PCR assay and in situ hybridization (ISH) probes were developed. Seventeen snakes in the python family with spinal disease were screened with ISH. PCR was used to screen available frozen tissues from 13 of these pythons, four additional deceased pythons with and without spinal disease, and fecal samples from 37 live snakes of multiple species with unknown disease status.

Results: In the index case, BhPyCV-specific ISH staining was intense in the intestine and multifocal in the liver. Intestine of a second BhP and liver of a Boelen's python (*Morelia boeleni*) were also positive. PCR detected multiple positive tissues in both of the ISH positive BhP, the feces of two live BhP, and feces of two live annulated tree boas (*Corallus annulatus*).

Conclusions: Preliminary analysis indicates this circovirus can infect BhPs where it was found in 4/5 BhPs tested (2/2 with spinal disease, 2/3 live with unknown status), Boelen's python (1/2 with spinal disease), and annulated tree boa (2/6 live with unknown status) but was not detected in other python species with the same spinal lesions. This circovirus' causal or contributory role in spinal disease remains speculative and not well supported by these initial data.

N-08: FOLLOW-UP STUDY OF TMEM154 MUTATED SHEEP EXPERIMENTALLY AND NATURALLY EXPOSED TO SMALL RUMINANT LENTIVIRUS (SRLVS)

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Background: Small Ruminat Lentivirus (SRLVs) cause chronic inflammatory changes in the central nervous system, mammary gland and lung of sheep. It has been proved that susceptibility to SRLVs infection is modulated by an amino acid substitution (E/K) at position 35 of the transmembrane protein 154 (TMEM154) in sheep belonging to different breeds.

Objective: Herein, we studied whether these variants of TMEM154 gene are associated to SRLVs susceptibility in Sarda breed sheep.

Methods: SRLV genotype B3 was inoculated in lambs (n.13) with distinct TMEM154 genotypes (E35E and E35K) and in supposed resistant lambs (n. 4) carrying the TMEM154 variant (K35K). Furthermore, 40 rams representing the three different genotypes of TMEM154 at position 35 were naturally exposed within a flock with high SRLV prevalence. A clinical, serological and virological long-term follow-up was performed on all animals. Experimental infected lambs were serially culled and submitted to histopathological examinations.

Results: In the experimentally infected E35K and E35E lambs, SRLV DNA was detected by PCR since 14 days p.i., whilst antibodies were evident by ELISA since 21 days p.i.. Moreover, a number of susceptible lambs showed lymphoproliferative lesions in the mammary glands and in the lungs. Interestingly, SRLV were never determined in the 4 K35K lambs by serologically and virological means. During the follow-up of the naturally exposed rams, exclusively those E35E and E35K revealed SRLV antibodies and/or SRLV DNA positivity.

Conclusions: Our preliminary results suggested that the mutation K35K is associated to SRLV resistance in Sarda breed sheep.

N-09: PROLIFERATIVE TUMOR-LIKE LESIONS IN LAMBS AND KIDS AFFECTED BY ORF VIRUS

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Background: In sheep and goats, ORF virus (ORFV) causes contagious ecthyma (ORF), a disease characterized by crusted pustular lesions and multifocal coalescing verrucous nodules on the skin and mucosa. We studied potential oncogenic factors

implicated in the pathogenesis of the proliferative tumor-like lesions in lambs and kids affected by ORFV.

Methods: We employed virological, histopathological and immunohistochemical means to investigate oral and cutaneous proliferative lesions in neonatal lambs and kids from sheep flocks and goat herds with ongoing ORFV infections.

Results: Macroscopically, we observed crusted pustules on the lips and coalescing hyperemic, proliferative, verrucous outgrowths with papilloma-like appearance along the lips and the gingival and lingual mucosa. Microscopically, epithelial hyperplasia and hyperkeratosis with ballooning degeneration and eosinophilic inclusion bodies, and marked neovascular proliferation in the lamina propria were found. Hyperexpression of vascular endothelial growth factor (VEGF) and its receptor (VEGFR2), as well as of epidermal growth factor receptor (EGFR), were detected by immunohistochemistry in epithelial cells and endothelial cells of newly-formed vessels. PCR identified ORFV DNA, and immunohistochemistry detected ORFV in epithelial and endothelial cells.

Conclusions: Our study reveals that, in lambs and kids, ORFV causes neovascular proliferation in the papillary and reticular layers of the lamina propria and papilloma-like changes in the epithelium by a mechanism utilizing VEGFR-2 and EGFR signaling for endothelial and epithelial cell mitogenesis. These data give insight into possible oncogenic properties of ORFV.

N-10: EPIZOOTIC HEMORRHAGIC DISEASE VIRUS IN ELK WITH ENCEPHALOMYELITIS

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Background: Epizootic hemorrhagic disease (EHD) is an arthropod-borne disease caused by an orbivirus. White-tailed deer are most susceptible and typically develop systemic hemorrhages and infarcts following endothelial damage. Surviving deer can develop hoof deformities and oral and forestomach ulcers. Other cervids and domestic ruminants rarely exhibit clinical disease or lesions. Viremia and mild fever are documented in infected elk.

Objective: To report central nervous system lesions in elk naturally infected with EHD virus (EHDV).

Methods: Three captive and one wild adult elk *(Cervus canadensis nelsoni)* necropsied in Utah between 2013 and 2018 were investigated. One elk was euthanized due to aimless circling; three were found dead.

Results: All elk had central nervous system lesions suggestive of ischemia consisting of random multifocal foci of necrosis in the brain and/or foci of axonal swelling with spheroids in the spinal cord. In three elk, perivascular lymphocytic cuffs were in the

cerebrum, brainstem, spinal cord, and rarely in the cerebellum. In all elk, EHDV was detected by qPCR in spleen or liver and identified as EHDV-2 in two elk. Immunoreactivity for EHDV-2 in endothelial cells was detected in the brain or spinal cord in all elk. Bluetongue virus was not detected by qPCR in any elk. Two elk were tested for *Listeria* spp., equine herpesvirus-1, bovine herpesvirus-1, West Nile virus, and/or malignant catarrhal fever, either by culture or qPCR; none of these agents were detected.

Conclusions: These findings suggest that EHDV-2 may cause encephalomyelitis with ischemic necrosis in elk.

N-11: LESIONS OF OPHIDIOMYCES OPHIODIICOLA IN NATURALLY INFECTED SNAKES IN ONTARIO, CANADA

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Background: Ophidiomycosis (i.e., snake fungal disease) is caused by the fungus *Ophidiomyces ophiodiicola* and is the most common cause of skin disease in free-ranging snakes in the northeastern U.S. It was first reported as a distinct entity in 2011 and common lesions include facial swelling, granulomatous dermatitis, emaciation, and death.

Objectives: To describe lesions and outcomes of ophidiomycosis cases in Ontario, Canada, at the northern limit of its known range.

Methods: All free-ranging snakes diagnosed with ophidiomycosis at the Canadian Wildlife Health Cooperative (CWHC) Ontario/Nunavut node from 2012-2018 were reviewed, including carcasses (9) and skin biopsies (12). Criteria for inclusion included: available formalin-fixed, paraffin-embedded tissues, a positive quantitative PCR result for *O. ophiodiicola*, and lesions consistent with the disease. A novel grading scheme was used to describe microscopic skin lesions.

Results: Of 66 snake cases submitted to the CWHC, 21 (32 %) met the inclusion criteria. Affected species included eastern foxsnakes (*Pantherophis vulpinus*; 15), gray ratsnakes (*Pantherophis spiloides*; 3), eastern massasaugas (*Sistrurus catenatus*; 2) and a queensnake (*Regina septemvittata*; 1). Lesion severity varied from mild microscopic skin lesions to fatal, necroulcerative facial lesions. Ophidiomycosis was the primary cause of death in three cases and secondary in two cases. Most carcasses (6/9) had gross lesions, which were also reported in all 12 biopsy samples. All cases had microscopically visible fungal elements.

Conclusion: Ophidiomycosis affects numerous, native, free-ranging snake species in Ontario, sometimes with fatal outcomes. Lesions in snakes in Canada are similar to those reported in snakes in the U.S.

N-12: MORTALITY EVENT INVOLVING A NOVEL BACTERIA (FAMILY PASTEURELLACEAE) IN TERNS, MARCO ISLAND, FLORIDA, USA

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Background: Terns are globally distributed shorebirds that frequently nest in large colonies. Mass mortalities have been associated with adverse weather and biotoxin exposure; however, bacterial infections are rarely implicated. A mortality event involving neurologic disease and death in hundreds of sandwich terns (*Thalasseus sandvicensis*) and common terns (*Sterna hirundo*) occurred in Florida, USA in November-December 2018.

Objective: Twelve tern carcasses from this mortality event underwent diagnostic evaluation to determine cause of death.

Methods: Terns originating from a wildlife rehabilitation center underwent full postmortem examination. Diagnostic workup included histopathology (H&E, Gram), toxicology (GC/MS screen, botulism, brevetoxins, domoic acid), bacteriology (blood agar culture, MALDI-TOF, sequencing), and virology (PCR for herpesviruses, paramyxoviruses, influenza viruses).

Results: Nine of twelve terns had heterophilic and lymphoplasmacytic inflammation associated with necrosis and short, gram-negative bacilli in one or more organs, including brain, heart, lungs, spleen, skeletal muscle, and adrenal gland. Bacterial culture of liver and heart samples and subsequent molecular testing identified a presumed novel bacterium most similar to sequences for Bisgaard Taxon 40 and those in the Pasteurellaceae family. Brevetoxin values in gastrointestinal tract, liver and kidney were moderately elevated. All other ancillary tests yielded insignificant results.

Conclusions: Tern morbidity and mortality was likely precipitated by sepsis caused by a novel bacterium. Terns and other shorebirds are vulnerable to mounting environmental stressors, such as suboptimal resource availability and changing climatic conditions. The contributing factors to mortality events in terns and other seabirds may reflect larger oceanic and global health concerns and warrant continued vigilance and study.

N-13: A RETROSPECTIVE SUMMARY OF CERVID MORBIDITY AND MORTALITY IN ONTARIO/NUNAVUT REGIONS OF CANADA (1991-2017)

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Background: Wildlife in northern latitudes such as Ontario, Canada are challenged by the effects of global climate change, human population expansion and northward spread of disease vectors, parasites and pathogens.

Objective: We sought to retrospectively identify and characterize causes of morbidity and mortality in free-ranging cervids submitted to the Canadian Wildlife Health Cooperative (CWHC)-Ontario/Nunavut for diagnostic evaluation.

Methods: We evaluated diagnostic data from wild cervid carcasses and samples submitted to CWHC-Ontario/Nunavut from 1991-2017. Most submissions involved full postmortem examination (gross/histopathology), but some consisted of select samples collected at field necropsy. Ancillary laboratory tests were performed as needed for a diagnosis.

Results: Trauma was the most common cause of mortality (21%; 121/582), with vehicular collisions comprising 33% (40/121) and most often diagnosed in wapiti (*Cervus canadensis*; 64/121; 53%). Eight percent (48/582) died of emaciation, most commonly wapiti (52%; 25/48), juveniles (52%; 25/48), and nearly all in winter/spring (90%; 43/48). Fatal infections were diagnosed in 34% (199/582), most commonly in moose (*Alces alces*; 42%; 85/199). Forty-six percent (91/199) of infections were bacterial and 43% (87/199) parasitic. Most infections in moose were parasitic (78%; 67/85), often *Parelaphostrongylus tenuis* (64%; 43/67). Those in caribou (*Rangifer tarandus* spp.) were bacterial (93%; 25/27), including foot rot (32%; 8/25) and *Brucella suis* biovar 4 (28%; 7/25). No cases of chronic wasting disease were identified, consistent with current provincial surveillance results.

Conclusions: These results reveal species and pathogen-associated patterns as well as anthropogenic and environmentally-associated deaths. Some of these may represent population-level threats that warrant continued monitoring.

N-14: PREVALENCE OF FELIS CATUS GAMMAHERPESVIRUS-1 IN FELINE ORONASAL SWABS AND TISSUES

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Background: The first gammaherpesvirus to affect cats, *Felis catus* gammaherpesvirus-1 (FcaGHV1), was isolated in 2014. Sequential epidemiologic studies suggest that more than 30% of wild and domestic cats worldwide are infected with FcaGHV1. To determine the significance of this virus, its prevalence and mechanisms of infection must be defined. Given recent detection of FcaGHV1 in oronasal secretions, we hypothesized that oronasal tissues are sites of viral persistence.

Objective: We aimed to estimate the prevalence of oronasal FcaGHV1 in cats and identify cellular target(s) of infection.

Methods: Formalin-fixed tissues (turbinates, oropharyngeal mucosa, tongue, salivary gland, tonsils, submandibular lymph node) and fresh swabs of oropharyngeal and nasal secretions were collected from 24 cats submitted for routine necropsy. DNA was extracted from the swabs and tested for FcaGHV1 using PCR that targets the virus'

glycoprotein B gene. For PCR-positive cases, tissues were examined for FcaGHV1 through in situ hybridization (ISH).

Results: A total of 4/24 (16.6%) cats, all of which were adults, were PCR-positive for FcaGHV1. Virus was detected in the oropharyngeal swabs of 3/4 cats and in the nasal swab of 1/4 cats. One was a client-owned patient and 3 were shelter-housed animals. No formalin-fixed tissues exhibited reactivity for FcaGHV1 through ISH.

Conclusions: Our data suggests that FcaGHV1 may persist in oronasal tissues of adult, shelter-housed and client-owned domestic cats. The microanatomical site of infection was not identified and will be further investigated in future studies so as to better elucidate FcaGHV1's impact on feline health and morbidities

N-15: OPHIDIOMYCES OPHIODIICOLA EPIZOOTIC IN A CAPTIVE COLLECTION OF SNAKES IN ARIZONA

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Background: In May 2018, a sanctuary received snakes including 42 *Lachesis* spp. adults and 24 *Lachesis melanocephala* eggs from North Carolina, and 12 additional eggs were laid. Between October and February 2019, 25 hatchlings developed lethargy, anorexia, crusting dermatitis, pit organ swelling, and death (100%). Beginning in January 2019 and over the next six months, crusting dermatitis and subcutaneous nodules developed in 18 snakes from 12 other species.

Results: Skin lesions from 20 live and dead snakes were PCR positive for *Ophidiomyces ophiodiicola*. Postmortem exams (10 dead; 3 euthanized) documented heterophilic granulomatous dermatitis and myositis with intralesional mixed morphology of bacteria and fungus. Additionally, 3 bushmasters had granulomatous hepatitis PCR negative for *O. ophiodiicola* in the two tested; 2 snakes had *O. ophiodiicola* pneumonia PCR confirmed in one; 4 snakes had gouty nephrosis; at least 5 snakes had bacteremia; and 4 snakes had fungemia, 3 with *O. ophiodiicola* morphology and PCR confirmed in one. Bacteriology and mycology of skin and liver from 6 deceased snakes with dermatitis yielded 33 bacteria and > 6 other fungi. Given postmortem evidence of co-infections, O. *ophiodiicola* dermatitis may compromise the epithelial barrier predisposing to septicemia, while uncommonly causing pneumonia and systemic mycosis.

Conclusion: This epizootic is ongoing and surviving snakes are receiving terbinafine nebulization therapy. This is the first report of *O. ophiodiicola* in Arizona and the first time diagnosed in 12 novel species. The fungus may have entered the collection with the shipment from NC, reaffirming the risk of anthropogenic spread of pathogens.

N-16: ENZOOTIC CHLAMYDIOSIS IN A HERD OF JERSEY CATTLE

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Background: Over a 10-month period, a 2000 lactating cow Jersey dairy farm submitted deceased and euthanized cows, calves, and late term aborted fetuses for postmortem examination. Cows and calves suffered from a variety of pathology, most commonly pneumonia due to *Mycoplasma bovis* and *Pasteurella multocida*. Additionally, at least 6 cows and 4 calves had thrombotic meningoencephalitis with vasculitis that was consistently negative for *Histophilus somni*, and negative for virus isolation in two calves. Eleven late term aborted fetuses had similar meningoencephalitis with vasculitis, and variably splenitis, fibrinous polyserositis, hepatitis, and enteritis. In multiple fetuses, cerebral endothelium had intracytoplasmic basophilic organisms, and one fetus had similar cytoplasmic organisms in reactive pericardial mesothelium.

Results: Abortion diagnostics included: bacteriology of at least two fetal samples (abomasal fluid, lung, liver, pleural fibrin, brain, kidney, and heart epicardium) from 5 fetuses yielded no significant growth; PCR performed on brain or pooled tissues from two fetuses was negative for BVD, IBR, Neospora, Ureaplasma, Listeria, and Leptospira; and Chlamydia PCR was positive on brain or pooled tissues from 1 calf and 4 fetuses. 16S rRNA sequencing was 100% homologous to *Chlamydia pecorum*, with one base mismatch from *C. abortus* and *C. psittacii.* Immunohistochemistry for *Chlamydia* sp. on the brain from 1 calf, 1 cow, and 3 fetuses was in all cases immunopositive within lesions. Additional molecular (i.e. ompA gene) and epidemiological studies are actively being pursued.

Conclusion: Enzootic chlamydiosis was found to be responsible for late term abortions and multiage vasculitis and meningoencephalitis.

Natural Disease Focused Scientific Poster Session C

N-01: WHAT THE FOXA2?! PROTEIN DOWNREGULATION CAUSES MUCUS HYPERSECRETION IN CANINE AND FELINE FUNGAL PNEUMONIA

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Background: Pulmonary mycotic infections are uncommon and more difficult to treat when compared to bacterial pneumonia. Microbial infections of the lung modulate pulmonary immune responses and induce goblet cell hyperplasia and metaplasia as well as mucus hypersecretion into airways culminating in decreased oxygen exchange

and respiratory distress. The Forkhead Box protein A2 (FOXA2) is a transcription factor that regulates mucus homeostasis in the airways. Bacterial infections in human and canine lungs reveal FOXA2 depletion causing mucus hypersecretion. However, similar investigations of FOXA2 in mycotic pneumonia are lacking.

Objective: Study the relationships between fungal pulmonary infections and the expression of FOXA2 and mucin proteins in airway epithelium.

Methods: Immortalized canine bronchoalveolar cancer cells (BACA) were infected with *Blastomyces dermatiditis* and *Histoplasma capsulatum* or exposed to heat-killed fungi or fungal cell wall components. Nuclear and cytoplasmic protein levels of FOXA2, MUC5AC and MUC5B mucins were then evaluated by Western Blot. Clinical cases of feline and canine pulmonary blastomycosis were also analyzed by immunohistochemistry to evaluate the correlation between presence of fungal infection, FOXA2 depletion, and hypersecretion of mucins.

Results: Fungal-infected canine and feline lung downregulates expression of FOXA2, resulting in overexpression of MUC5AC and MUC5B mucins. Molecular studies indicate inhibition of FOXA2 is mediated through the activation of the antagonistic Dectin-ROS-EGFR-AKT/ERK 1/2 pathway.

Conclusions: Further understanding the role of FOXA2 in mucus hypersecretion may lead to novel therapeutics to aid in the treatment of fungal pulmonary infections in human and veterinary patients.

N-02: GENE EXPRESSION PROFILING IDENTIFIES ONCOGENIC PATHWAYS UPREGULATED IN CANINE PERIPHERAL T-CELL LYMPHOMA AND MOLECULAR HOMOLOGY TO AN AGGRESSIVE SUBSET OF HUMAN PERIPHERAL T-CELL LYMPHOMA-NOT OTHERWISE SPECIFIED

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Introduction: Peripheral T-cell lymphoma-not otherwise specified (PTCL-NOS) encompasses a heterogeneous group of aggressive and poorly-understood neoplasms in both human and canine patients. Because PTCL-NOS is relatively uncommon in human patients, an appropriate model is needed to investigate the underlying pathobiology, identify therapeutic targets, and perform clinical trials. In this study, we aimed to characterize the gene expression profile of canine PTCL and evaluate the molecular similarity to PTCL-NOS in human patients.

Methods: RNA-sequencing was performed on lymph node aspirates from dogs diagnosed with PTCL and sorted T-cells from lymph nodes of control dogs. Differential gene expression and pathway enrichment analysis were performed, and results were compared to similar studies on human PTCL-NOS. In vitro studies were subsequently performed on canine cell lines and primary lymphoma cells to interrogate driving molecular pathways.

Results: Canine PTCL was significantly enriched for the gene set representative of human PTCL-NOS. Canine cases overexpressed GATA3 and CCR4; two markers which characterize a subset of human PTCL-NOS associated with worse outcomes. Canine cases also exhibited significant enrichment for the PI3K and mTOR induced gene signatures. In vitro inhibition of PI3K resulted in increased cell death and impaired proliferation. Overexpression of platelet derived growth factor receptors (PDGFRs) were also conserved across both human and canine cases.

Conclusions: We conclude that canine PTCL is molecularly similar to an aggressive subset of human PTCL-NOS and has potential to serve as a useful, naturally occuring model. We also identify the PI3K-AKT-mTOR pathway as a potential driving molecular pathway of this disease.

N-03: DETECTION AND QUANTIFICATION OF ERBB2 MRNA USING RNA IN SITU HYBRIDIZATION IN CANINE MAMMARY TUMORS: CORRELATION BETWEEN RNA AND PROTEIN EXPRESSION

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Background: Canine mammary tumor (CMT) have many similarities with human breast cancer. In human breast cancer, human epidermal growth factor (HER2) is an important prognostic factor in the selection of targeted chemotherapy. Since there is controversy over the cross-reactivity of HER2 antibodies in dogs, the value of HER2 as a prognostic factor has not been fully understood.

Objective: We aimed to detect and quantify single-cell levels of ERBB2 (encoding HER2) mRNA expression in CMTs by RNA-in situ hybridization (ISH). We also sought to compare the levels of RNA and protein levels by immunohistochemistry (IHC).

Method: Forty CMT samples were used in the study. RNA-ISH analysis was performed by RNAscope® using a canine-specific probe, and quantitative measurement was performed using the housekeeping gene together. IHC analysis was performed using the anti-human HER2 antibody with serial sections of the sample. Quantification of ERBB2 mRNA was performed using image analysis software, and evaluation of HER2 protein was performed manually according to the ASCO/CAP guidelines.

Results: Expression of ERBB2 mRNA molecule was confirmed in tissues from CMT at the single-cell level. RNA-ISH showed high concordance with IHC in the same regions of serial sections with the lower background. Correlation between HER2 score using IHC and the quantified ERBB2 mRNA using ISH was confirmed.

Conclusion: We identified ERBB2 mRNA expression in CMT using ISH and quantitatively assessed its expression, suggesting that RNA-ISH is suitable for diagnosing the HER2 status of CMTs. These findings suggest the possibility that the dog may benefit from HER2-target chemotherapy.

N-04: CORNEAL ENDOTHELIAL DYSTROPHY IN BOSTON TERRIERS: CLINICAL PRESENTATION, HISTOPATHOLOGICAL CHANGES AND PRELIMINARY GENETIC STUDY

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Background: Corneal endothelial dystrophy (CED) is a bilateral disease in dogs whereby the corneal endothelial cells (CECs) prematurely degenerate resulting in progressive corneal edema, vision loss, and ocular discomfort due to corneal ulceration. Advanced cases can require enucleation. Boston Terriers have a higher prevalence of CED when compared with other breeds, suggestive of an underlying genetic component. A similar disease, termed Fuchs´endothelial corneal dystrophy, is described in humans.

Objective and methods: The purpose of this study was to describe the clinical characteristics, histologic features and a preliminary genetic analysis of CED in Boston Terriers by using *in vivo* confocal microscopy (IVCM), histological examination, and a genome-wide association study (GWAS), respectively.

Results: Clinically, affected dogs presented with corneal edema of variable intensity in one or both eyes. A significant decrease (Student *t*-test, $p<0.001$) in CEC density was observed with IVCM in 15 CED cases (1042 ± 212 cells/mm^2) versus 40 breed-matched controls (2784 ± 544 cells/mm^2). On histological examination of enucleated eyes, flattening and loss of CECs with variable thickening and lamination of Descemet´s membrane was observed in 10 CED-affected Boston Terriers. Using 45 cases and 38 controls, our preliminary GWAS did not identify any genome-wide statistically significant single nucleotide polymorphisms (SNPs), indicating that the genetic component of CED is likely complex.

Conclusion: Boston Terriers with CED are a useful animal model for the study of human Fuchs´ endothelial corneal dystrophy. Further studies are required to fully understand the underlying genetic basis of CED in the Boston Terrier.

N-05: HISTOLOGICAL CHARACTERISTICS AND PHYSICAL PARAMETERS OF BROILER BREAST MYOPATHIES IN ONTARIO, CANADA

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Background: Spaghetti meat (SM), wooden breast (WB), and white striation (WS) are emerging myopathies of the breast muscle which are associated with rapid growth rates

and large pectoral muscles in broilers. These myopathies cause severe economic losses because of poor meat processability and reduced consumer acceptance. In-line detection of breast myopathies is challenging due to the high processing speed and subjective detection by operators.

Objectives: We aimed to identify specific morphologic characteristics of breast myopathies that could be applied to an automated in-line detection system, such as image analysis. For this purpose, we sought to 1) identify how specific physical parameters of fillets (weight, height, compression resistance) correlate with the severity of myopathies, and 2) establish a histological grading scheme that could be used as a gold standard to inform macroscopic detection methods.

Methods: A total of 180 breast fillets were collected, grossly evaluated for the presence of myopathies, and tested to obtain physical parameters (i.e., weight, height, surface area, length, width, and compression force). Histological changes were graded from three (cranial, medial, caudal) regions of fillets.

Results: Affected breast fillets were significantly thicker, heavier, and harder than grossly unaffected fillets. The odds of WB and WS were higher in thicker breast fillets. Histologic detection of myodegeneration showed a strong positive correlation with lipidosis and fibrosis ($r = 0.79$, $p < 0.05$).

Conclusions: Height, weight, force are good predictors for determining the severity of breast myopathies. Our histologic grading scheme could be implemented for in-line detection systems in Canadian processing plants.

N-06: IMMUNE CHECKPOINT MOLECULE EXPRESSION IN CANINE LYMPHOMA AND CANINE REACTIVE LYMPHOID HYPERPLASIA

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Background: Lymphoma is the most common hematopoietic tumor in dogs, but remission rates and survival times remain stagnant. Targeted immunotherapy using checkpoint molecule blockade of PD-1 and PD-L1 shows promise for various types of lymphoma in people; however, less is known regarding the roles and expression of checkpoint molecules in canine lymphoma.

Objectives: Determine the patterns of expression of mRNAs encoding PD-1 and its ligands PD-L1 and PD-L2 in lymphoma and in reactive lymphoid hyperplasia controls.

Methods: Retrospective: formalin-fixed paraffin-embedded (FFPE) tissue from dogs with untreated lymphoma (n=10) and reactive lymphoid hyperplasia (n=10). Prospective: fine-needle aspirates (FNAs) from dogs with untreated lymphoma (n=10) and reactive lymphoid hyperplasia (n=10). Total RNA was extracted, and expression of PD-1, PD-L1, and PD-L2 was measured using qRT-PCR analysis of random-primed cDNA.

Results: Overall, expression of PD-1, PD-L1, and PD-L2 (normalized internally to 18S rRNA) trended lower in lymphoma compared to reactive lymphoid hyperplasia; however, a statistically significant decrease was only identified for PD-1 in FFPE samples. The PD-L1:PD-1 and PD-L2:PD-1 ratios were >2-fold higher in lymphoma than reactive lymphoid hyperplasia.

Conclusions: In this study, checkpoint molecule expression was not upregulated in canine lymphoma relative to canine reactive lymphoid hyperplasia, suggesting a limited application of PD-1 and PD-L1 blockade in canine lymphoma. The ligand:receptor ratio imbalances reflect the lower PD-1 expression relative to PD-L1 and PD-L2 in lymphoma. Although these results do not suggest that checkpoint inhibitors would be useful for treatment, they give insight into the mechanisms of unchecked lymphocyte proliferation in canine lymphoma.

N-07: IMMUNOHISTOCHEMICAL CHARACTERIZATION OF FELINE DIFFUSE IRIS MELANOMA

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Objective: The goal of this study was to investigate potential prognostic indicators in FDIM using immunophenotyping and markers that have prognostic significance in melanocytic neoplasms from extraocular sites.

Methods: Globes from 32 cats with FDIM were evaluated for degree of pigmentation, mitotic count, nuclear atypia, number of multinucleated cells, and degree of infiltration. A tissue microarray with 5 repetitive 0.6 mm punches of each FDIM was created, and immunohistochemistry for Melan-A, PNL2, CD18, CD172a, CD204, HMB45, and Ki-67 was performed. The percentage of positive cells was determined for each series of microarray punches.

Results: Neoplastic melanocytes in 31/32 cases were positive for PNL2, and 30/32 cases were positive for Melan-A and HMB45. Two PNL2 positive cases were negative for Melan-A, and one Melan-A positive case was negative for PNL2. Thirteen cases had neoplastic melanocytes that also labelled with CD18 or CD172a. Eighteen cases had a high Ki-67 index while only 15 of these cases had a high mitotic count.

Conclusions: FDIM label positive for PNL2, Melan-A, or HMB45. This study adds HMB54 to previously described diagnostic markers. Routinely, both PNL2 and Melan-A should be used to diagnose amelanotic melanomas. While expression of melanoma markers confirmed melanocytic origin, significance of dual labeling with histiocytic and melanoma markers is unknown. Ki-67 provides the most accurate assessment of proliferation activity.

N-08: FOLLICLE STIMULATING HORMONE RECEPTOR IS EXPRESSED IN HISTOLOGICALLY NORMAL AND NEOPLASTIC CANINE MAMMARY GLAND TISSUES

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The roles of Follicle Stimulating Hormone (FSH) in reproductive physiology have been recognized for a long time. The hormone achieves its functions by binding to the Follicle Stimulating Hormone Receptor (FSHRc) that is strongly expressed in both male and female gonads. Over the last 30 years FSH and its receptor have been discovered also in normal and neoplastic extragonadal tissues including: placenta, uterus, and most recently mammary glands. In neoplasias they contribute to cancer development, cancer cell migration, invasion and neovascularization. Canine mammary gland tumors (MGTs) are the most frequent tumors in intact bitches. Though frequently studied, their pathogenesis is still not completely understood and the presence of FSHRc has not been investigated. The purpose of this study was to evaluate histologically normal and neoplastic mammary glands for the presence of FSHRc using immunohistochemistry and in situ hybridization. Positive immunohistochemical cytoplasmic staining is noticed in epithelial and the underlying myoepithelial cells of normal and neoplastic mammary glands and also in the cytoplasm of chondrocytes in mixed MGTs. In situ hybridization detected the expression of FSHRc in the nucleus and perinuclear cytoplasm of epithelial and myoepithelial cells of normal and neoplastic mammary glands. The signal was observed in the cytoplasm and, to a lesser degree, in the nucleus of chondrocytes in mixed MGTs. Further studies are necessary to elucidate the physiological and pathophysiological roles of FSH and FSHRc in histologically normal and neoplastic mammary glands and to investigate their potential use as targets for highly specific chemo- or immunotherapies of MGTs.

N-09: CHARACTERIZATION OF OCT3/4, NESTIN, NANOG, CD44 AND CD24 AS STEM CELL MARKERS IN CANINE PROSTATE CANCER

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Background: The cancer cell population is heterogeneous, and cancer stem cells (CSCs) are important for tumor growth and maintenance. The CSC population is associated with different neoplastic characteristics, such as cell migration, metastasis, resistance to apoptosis, radiation therapy and chemotherapy.

Objective: Our aim was to characterized CSC markers in canine prostate cancer (PC) samples and in tumorspheres.

Methods: We performed immunohistochemistry of OCT3/4, Nestin, NANOG, CD44 and CD24 in 10 normal canine prostatic tissue samples, 10 prostatic hyperplastic (PH) tissue samples and 28 PC tissue samples. Then, we established two canine prostate cancer cell cultures and characterized the CSC profile of tumorspheres grown from these cultures.

Results: Normal and PH tissues were positive for Nestin, NANOG, CD44 and CD24 only in the basal cell layer. OCT3/4 was expressed in the luminal cells of normal and PH tissues. There was no significant difference in Nestin expression among the prostatic tissues. However, we found higher expression of NANOG and CD44 in canine PC tissues than that in normal and PH tissues. Tumorspheres from canine prostate cancer cells express OCT3/4, Nestin, NANOG and CD44, indicating that these markers may be potential cancer stem cell markers in canine PC.

Conclusions: The results obtained can be useful to better characterize the stem cell population in canine prostatic cancer and to guide future studies in comparative oncology.

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N-10: IS THERE PREFERENTIAL USE OF LAMBDA LIGHT CHAIN IN THE DOG?

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Background: Canine κ:λ ratio is reported as 1:9 based on immunohistochemistry using non-validated anti-human kappa or lambda light chain (LC) antisera. Human κ:λ ratio is approximately 0.5; ratio perturbations are used for detection and prognosis of plasma cell-proliferative disorders.

Objective: Evaluate labeling of anti-human kappa or lambda antisera to mass-spectrometry characterized neoplastic clonal canine κ-LCs and λ-LCs.

Methods: LC bands from nine archived canine samples with IgG-LC, IgA-LC or IgM-LC monoclonal/biclonal gammopathies previously characterized by canine-specific immunofixation were captured and characterized using reduced LDS-PAGE and liquid chromatography tandem mass-spectrometry (LC-MS/MS). Immunofixation using anti-human kappa and anti-human lambda antisera (Sebia IF antisera kit, 4815) was performed. As needed, antisera was modified by addition of polyethylene glycol 6000 and serum concentrations were increased.

Results: LC-MS/MS identified six κ-LC and three λ-LC canine cases. Anti-human kappa antisera labeling was only possible with the modified protocol; two κ-LC cases labeled strongly and two κ-LC cases labeled faintly. One κ-LC case labeled faintly with anti-human kappa and strongly with anti-human lambda antisera. Three cases labeled strongly with anti-human lambda antisera (2 λ-LC and 1 κ-LC) but one λ-LC case did not label with either anti-human antisera, independent of the method.

Discussion: Non-specific anti-human lambda labeling and low-avidity anti-human kappa labeling of canine LCs by immunofixation may translate to immunohistochemical labeling. Although low-n, the observed number of κ-LC gammopathies by LC MS/MS suggests higher use of κ-LC. Evaluation of canine κ:λ ratio may be diagnostically useful.

Conclusions: Further work should confirm canine κ-LC and λ-LC usage.

N-11: CANINE GLIOMATOSIS CEREBRI: AN INVASIVE GROWTH PATTERN OF GLIOMA RATHER THAN A SEPARATE ENTITY?

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Background: Gliomatosis cerebri (GC) is a rare glioma subtype characterized by diffuse infiltration of the neuroparenchyma without disruption of its architecture. The term was first employed in human medicine and remained as a controversial entity for many years, until its recent elimination from the diagnostic lexicon. GC is now defined as a glioma growth pattern rather than a distinct entity.

Objectives: To classify canine GC as oligodendroglioma, astrocytoma, or undefined glioma.

Methods: A retrospective database search for cases of canine GC was performed. Selected cases were reviewed and immunostained with GFAP, Olig2, and CNPase.

Results: Eleven cases were studied. The mean age of affected dogs was 7.5 years; 5 dogs were brachycephalic. Histologically, widespread neoplastic glial cell infiltration was present predominantly within the white and gray matter of the telencephalon (8 cases) and spinal cord (3 cases). Neoplastic cells with oligodendroglial morphology (4 cases) had scant or lost cytoplasm (clear halo) and round nuclei with coarse chromatin on a basophilic matrix. Neoplastic cells with astrocytic morphology (6 cases) had abundant, fusiform, eosinophilic cytoplasm and elongate nuclei with finely stippled chromatin; one case was consistent with an undefined glioma. The overall immunohistochemistry (IHC) results were consistent with neoplastic cell morphology and were supportive of the diagnosis.

Conclusions: The morphologic and IHC features of GC allow for a diagnosis of oligodendroglioma, astrocytoma, or undefined glioma, suggesting that GC represents an invasive growth pattern of canine glioma rather than a distinct entity.

N-12: CARDIAC FINDINGS IN DOGS WITH DIETARY EXPOSURE TO DOG FOOD LABELED AS “GRAIN FREE”

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Background: FDA Center for Veterinary Medicine, Veterinary Laboratory Investigation and Response Network, university and private partners initiated an investigation in July 2018 of cardiac conditions including dilated cardiomyopathy (DCM) in dogs eating pet foods containing high proportions of peas, lentils, other legumes, and potatoes ("grain-free").

Objective: To determine histopathologic findings in hearts from nine dogs exposed to food labeled as "grain-free" with antemortem heart disease, including ultrasound-confirmed DCM (6), sudden death (1), endocardiosis with a lung mass (1), and cardiomegaly with congestive heart failure (1).

Methods: Medical records and tissues were collected for dogs which had consumed grain-free diets and due to suspected heart disease. Complete necropsies including cardiac weights and measurements were conducted on three female and six male dogs

Results: Six dogs had antemortem, gross, and histopathologic diagnosis of primary DCM. One dog's DCM was secondary to myocarditis and myocardial fibrosis. The five dogs with DCM had mild to moderate cardiomyocyte atrophy (5/5), fatty infiltration (4/5), interstitial edema (3/5), mild lymphoplasmacytic interstitial myocarditis (3/5), and endocardiosis (5/5). The three remaining dogs without antemortem-confirmed DCM had non-DCM primary cardiac disease (pericardial mesothelioma, restrictive cardiomyopathy) or cardiopulmonary disease (moderate endocardiosis, bronchoalveolar carcinoma).

Conclusions: Histopathology showed no pathognomonic lesions common to dogs consuming diets labeled "grain-free" regardless of confirmed DCM or non-DCM heart disease. A causal link has not been established between consuming diets labeled "grain-free" and developing DCM, continued evaluation of hearts correlating clinical, diagnostic, gross histopathologic findings, and product testing will provide a dataset to evaluated potential associations.

N-13: BRONCHIAL ABNORMALITIES UNDERLIE DEVELOPMENT OF PULMONARY BULLAE, SPONTANEOUS PNEUMOTHORAX, AND INTRAPULMONARY SEQUESTRATION IN SEVEN DOGS

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Background: Ruptured pulmonary bulla is associated with spontaneous pneumothorax in dogs. The pathogenesis of bulla development is unknown.

Objective: Bronchial abnormalities were investigated in dogs with bulla and spontaneous pneumothorax and/or intrapulmonary sequestration (IPS). IPS was

diagnosed based on presence of aberrant systemic arteries associated with cystic lung changes.

Methods and Results: Lung lobes from 7 dogs were submitted to Michigan State University. Six of 7 dogs developed pulmonary bulla; 3/7 had gross evidence of IPS. Three dogs were Siberian Huskies; 4 represented other breeds. The average age was 6.3 years; 5 were male-castrated and 2 female-spayed. Computed tomography (CT) scans showed bulla in 5/7 of dogs. One IPS case was misidentified as lung lobe torsion on CT. Grossly, the diameter of affected lobar bronchi was markedly reduced in 7/7 cases compared to bronchi from 4 control dogs. In IPS cases, aberrant blood vessels were noted along the pleura (3/3) associated with cystic lung parenchyma (2/3). Histologically bronchial abnormalities included irregularly organized cartilage and curled and small cartilage plates. Bronchial mucus cell hyperplasia was present in the Siberian Huskies and in 1 dog, the bronchial mucosa was replaced by fibrosis. Dilated alveoli distal to abnormal bronchi were noted in all cases. In 1/3 IPS cases, severe pulmonary inflammation was present.

Conclusions: We conclude that IPS and development of bulla and spontaneous pneumothorax in dogs reflects abnormal bronchial development. Further, diagnosis of these entities requires lung lobe removal for gross and histologic evaluation and not just distal resection of the bulla.

N-14: PATHOLOGIC CHARACTERIZATION OF A CANINE MULTIPLE SYSTEM DEGENERATION DISORDER IN THE IBIZAN HOUND

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Canine multiple system degeneration (CMSD) is a progressive hereditary neurodegenerative movement disorder characterized by neuronal degeneration and loss in the cerebellum, olivary nuclei, substantia nigra, and caudate nuclei. This disorder has been reported in Kerry blue terriers and, more recently, in Chinese crested dogs. Here we describe three cases of CMSD in three related Ibizan hounds. Clinically, all patients had marked cerebellar ataxia and cerebellar atrophy on magnetic resonance imaging. At necropsy, all cases showed varying degrees of cerebellar atrophy and, in one case, gross cavitation of the caudate nuclei. Histologic findings included severe degeneration and loss of all layers of the cerebellum and neuronal loss and gliosis within the olivary nuclei, substantia nigra, and the caudate nuclei. Intraneuronal intracytoplasmic and intranuclear eosinophilic inclusions were in the caudate nuclei, thalamus, and substantia nigra. Minimal axonal degeneration within the thoracic spinal cord was present in all cases. The SERAC1 gene has been implicated in both the Kerry blue terrier and Chinese crested type CMSDs. Pedigree analysis of these Ibizan hounds showed an autosomal recessive mode of inheritance, but the causative gene has not been identified. CMSD resembles human multiple system atrophy.

N-15: LYMPHONODULAR SYNDROME IN FARMED AUSTRALIAN SALTWATER CROCODILES (CROCODYLUS POROSUS)

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Background: Crocodile farming is a growing industry in Australia. In recent years, several disease syndromes have emerged with preliminary characterization revealing possible associations with crocodyline herpesviruses. One of these conditions is a lymphonodular syndrome, characterized by multifocal lymphohistiocytic infiltrates in skin and variably oral cavity, conjunctiva and internal organs.

Objective: To further characterize the cellular infiltrate in lymphonodular syndrome of crocodiles.

Results: Large raised ulcerative lesions were identified on the tongue of two subadult (2-4 year old) male farmed saltwater crocodiles. Grossly similar tongue, pharyngeal, and/or skin lesions were also detected in 20 other animals within a group of 100. Microscopic evaluation of both tongues revealed dense nodular to confluent aggregates of variably sized lymphocytes within the submucosa. On immunohistochemical evaluation, these aggregates comprised predominately CD3-positive T cells with fewer PAX5-positive B cells and Iba-1-positive macrophages, the latter often in small clusters and with abundant foamy vacuolated cytoplasm. Variable numbers of T lymphocytes also infiltrated the mucosal epithelium, which in one case was focally ulcerated. Crocodyline herpesvirus was detected by PCR within the tongue lesion, conjunctiva, and tonsil of the single animal for which fresh tissue was available.

Conclusions: This report identifies the mixed T and B lymphocyte and macrophage components of the infiltrate in lymphonodular syndrome of crocodiles. The pathogenesis of this condition and the role of crocodyline herpesviral infection, if any, remain unknown. Comparison with infection-associated lymphoproliferative conditions of other animals will be discussed.

N-16: COMPARATIVE ANATOMY AND PATHOLOGY OF UTERINE DISORDERS IN PET RABBITS (ORYCTOLAGUS CUNICULUS) AND GUINEA PIGS (CAVIA PORCELLUS)

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Background: Rabbits and guinea pigs are popular small mammal pets. Although uterine disorders seem to be common in both species, comparison has not been previously made.

Objective: To describe particularities of uterine anatomy and pathology in pet rabbits and guinea pigs.

Methods: Uterine gross and microscopic anatomy was investigated in animals without uterine disorders. Frequency and age distribution of uterine disorders were analyzed retrospectively of cases examined between 1995 and 2018.

Results: Rabbits and guinea pigs have a uterus duplex bicollis. The cervix of guinea pigs is lined by characteristic mucinous glands. Uterine disorders were found in 27.1% and 17.4% of post-mortem examinations in rabbits and guinea pigs, respectively. For both species, incidence of neoplasia and hyperplasia increased with age. Whereas epithelial tumors were very frequent and almost exclusively malignant in rabbits, they were less common and mostly benign in guinea pigs. Mesenchymal tumors occurred occasionally and were mostly benign in both species. Uterine inflammation was most frequent in young to middle-aged rabbits and guinea pigs with similar disease incidences. Uterine disorder types that were exclusively found in rabbits were hydro/mucometra, endometrial venous aneurysms and malformations. Specific disorders of guinea pigs were decidual proliferations (deciduoma, decidualization, deciduosarcoma) and endocervical proliferations (hyperplasia and neoplasia).

Conclusion: Although age distributions of uterine neoplasia, hyperplasia and inflammation was quite similar between rabbits and guinea pigs, there were substantial differences in the frequency of the different uterine disorder. Anatomical particularities of guinea pigs are the endocervical glands that occasionally had proliferative or neoplastic disease.

Young Investigator Poster Abstracts: Natural Disease

NY-68: AN OUTBREAK OF DISTEMPER PNEUMONIA IN TIGERS

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Background: Despite its name, canine distemper virus (CDV), a single stranded RNA virus in the family *Paramyxoviridae* and genus *Morbillivirus*, has been implicated in disease causation in a variety of carnivores.

Objective: To describe spontaneous CDV infection in a group of captive tigers in northern Colorado.

Methods: Postmortem and histopathologic examinations were performed on three of four tigers submitted to the Diagnostic Medicine Center – Colorado State University. Ancillary tests performed on lung samples include real-time PCR and whole genome sequencing is in progress.

Results: The lungs on two of three male tigers were consolidated. One tiger was affected in a diffuse pattern and the second was affected in a cranioventral pattern with mucopurulent bronchiolar exudate. Microscopic findings included severe interstitial

pneumonia in one tiger and bronchointerstitial pneumonia in the second. A diagnosis of CDV-induced pneumonia was confirmed via real-time PCR. The fourth tiger was female, negative for distemper by PCR, and euthanized due to metastatic mammary carcinoma.

Conclusions: We herein present an outbreak of canine distemper in captive tigers. It is unclear why only males were infected and whether infection was passed from tiger to tiger or purely from interactions with CDV-infected wildlife. Selective pattern of infection in the cohort suggests tiger to tiger transmission is less likely. Previously, two distinct clades of CDV have been sequenced from raccoons inhabiting northern Colorado. Sequencing results from these tigers is in progress to determine if both tigers were affected with the same strain and if raccoons are the source of infection.

NY-69: A SUBSET OF EQUINE GASTRIC SQUAMOUS CELL CARCINOMAS ARE ASSOCIATED WITH EQUUS CABALLUS PAPILLOMAVIRUS 2

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Background: Squamous cell carcinoma (SCC) is the most common neoplasm of the equine stomach. However, the mechanisms underlying malignant transformation are unknown.

Objective: As Equine caballus papillomavirus-2 (EcPV-2) has been identified as a likely etiologic agent for a subset of genital SCCs, we hypothesized that EcPV-2 was associated with a subset of equine gastric SCCs.

Methods: To this aim, we performed PCR and in situ hybridization (ISH) for EcPV-2 E6/E7 oncogenes on 12 cases of gastric SCC. As controls, we included eight non-SCC gastric lesions including hyperplastic gastritis (5 cases) and leiomyomas (3 cases), as well as normal stomach (2 cases).

Results: Horses with gastric squamous cell carcinoma ranged from 3 to 24 years old and were exclusively male (12/12; 100%). Metastatic disease was detected in 7/12 horses (58%) and ranged from carcinomatosis to single or multiple nodules in the spleen, liver, lung, kidney, and lymph nodes. PCR for EcPV-2 was positive in 7 cases (58%) of equine gastric SCC and ISH demonstrated EcPV-2 E6/E7 nucleic acid within tumor cells in 5 cases (42%). EcPV-2 nucleic acid was not detected within any of the control cases.

Conclusions: This study provides evidence for a potential association between EcPV-2 and a subset of equine gastric SCC.

NY-70: COMPARATIVE HISTOPATHOLOGICAL FEATURES OF BOVINE ACUTE INTERSTITIAL PNEUMONIA AND BOVINE RESPIRATORY SYNCYTIAL VIRUS INFECTION

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Background: Acute (atypical) interstitial pneumonia (AIP) is a group of diseases causing acute-onset respiratory distress in cattle. Many etiologies of AIP have been proposed, including plant-derived toxins, bacterial, viral, and parasitic infections. Bovine respiratory syncytial virus (BRSV) has been linked to AIP based on histopathological lesions in some cases. Controversy is still present in BRSV as a cause of natural AIP in cattle.

Objective: Our aim was to compare the histopathological findings of AIP and BRSV-related pneumonia in natural cases.

Methods: We randomly selected 20 cases of bovine AIP, diagnosed by board-certified veterinary pathologists, including 10 BRSV-positive and 10 BRSV-negative cases. Characteristic findings were evaluated and scored on the scale of 0 (none) to 4 (marked to severe). The results of two groups were analyzed by Student's T-test.

Results: The scores of alveolar septal necrosis, type II pneumocyte hyperplasia, and multinucleated giant cells in AIP group were significantly greater than that for BRSV group ($p = 0.047$, 0.047, and 0.01, respectively). The score of bronchiolitis in BRSV group was significantly greater than that for AIP group ($p = 0.0007$). No significant difference was observed in overall scoring of animals, hyaline membranes, pneumocyte necrosis, interstitial cellularity, alveolar histiocytosis, syncytia, bronchiolar degeneration, pulmonary edema, and emphysema.

Conclusions: Although differences were noted in several criteria assessed, the variability among cases and the lack of difference in overall scoring would preclude using them as histologic criteria for diagnosis. We concluded that overall there was no significant difference between AIP and BRSV-related pneumonia on histopathology.

NY-71: H1N1 PANDEMIC LINEAGE INFLUENZA A IN FARMED MINK IN UTAH

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Background: A mink farm with approximately 5000 breeding females reported increased mortality in kits limited to a single barn with 640 breeding females. A total of 300-325, 1 to 2-week-old kits died over a three-week period. The producer reported dyspnea or death without clinical signs in kits. No clinical signs or deaths occurred among breeding females.

Methods: Five kits that were found dead and three euthanized breeding female mink were submitted for postmortem examination.

Results: All kits were in poor body condition and had moderate to severe, lymphohistiocytic and neutrophilic interstitial pneumonia. The adults were in good body condition with minimal to moderate, lymphohistiocytic bronchointerstitial pneumonia. Influenza A was detected in one kit's lung by qPCR targeting the matrix gene. Virus isolation and genetic analysis identified an H1N1 pandemic lineage influenza A.

Conclusions: Mink are known to be susceptible to influenza A of swine and human origin. Human-to-mink or food-borne transmission are possible virus sources for this outbreak but the source was not determined. This case emphasizes the need for close monitoring for interspecies transmission of influenza A virus and safe work practices in farms and diagnostic laboratories, including influenza vaccination.

NY-72: CANINE LEISHMANIOSIS: SYSTEMIC HISTOPATHOLOGIC FINDINGS AND OTHER ODDITIES

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Background: Canine leishmaniosis (CanL) is generally considered a tropical disease vectored by sand flies in endemic regions. However, in the United States, CanL is transmitted vertically within some populations of purebred dogs. As an outbred naturally infected cohort, they present unusual challenges and unique opportunities to observe CanL disease progression and concurrent infections outside the laboratory setting.

Objective: To describe histologic findings of canine visceral leishmaniasis through study of multiple organ systems, and identify lesions from concurrent disease processes.

Methods: Tissues were collected during necropsy from dogs donated to the University of Iowa. Dogs were all purebred and positive for *Leishmania infantum* by PCR, serology, and/or tissue culture, and represented a range of ages and clinical disease severity. H&E stained slides were examined by light microscopy, and findings were recorded and compared with clinical signs, concurrent tick-borne disease, age, and laboratory bloodwork.

Results: Lesions characteristic of CanL were identified in the liver, kidney, spleen, and bone marrow of most of the dogs, with varying degrees of severity. Other frequent findings included necrotizing arteritis and vasculitis, calcinosis of vessel endothelium and other tissues, bronchopneumonia, polymyositis, orchitis, and malignant neoplasia.

Conclusions: Although leishmaniosis poses a significant systemic burden, infected dogs can survive for many years before presenting with clinical disease. Advanced age, comorbid infections and environmental factors present confounding elements when characterizing histopathology of naturally-occurring disease, and should be factored for when working with outbred populations and uncommon pathogens.

NY-73: RETROSPECTIVE ANALYSIS OF NECROPSY FINDINGS IN 36 BEARDED DRAGONS (POGONA VITTICEPS) AT A UNIVERSITY TEACHING PATHOLOGY SERVICE AND A ZOOLOGICAL INSTITUTION (1999 – 2018)

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Background: The bearded dragon (*Pogona vitticeps*) is common in both the pet reptile trade and zoological institutions. Despite their prevalence, no extensive pathological review detailing common causes of mortality has been performed.

Objective: The goal of this study was to summarize significant and incidental findings identified during necropsy examination of captive North American bearded dragons.

Methods: Necropsies were performed on 36 bearded dragons at two institutions: the Cornell University Animal Health Diagnostic Center and the Wildlife Conservation Society's Zoological Health Program. Post-mortem findings were categorized based on body system and 104 sub-categories.

Results: The two most common findings were hepatic lipidosis (75%) and tubular epithelial pigmentation (52.8%). Pneumonia (22.2%), hepatic fibrosis (19.4%), pulmonary hyalinosis (19.4%), gout (16.7%), and granulomatous hepatitis (16.7%) were also seen but were less common. Hepatic lipidosis, tubular epithelial pigmentation, and pulmonary hyalinosis were considered non-specific pathologic changes.

Conclusions: This study is the first to summarize findings noted in a necropsy series of captive bearded dragons. This information increases our knowledge of significant diseases and non-specific and incidental histologic changes in this species. Both may be useful when investigating the relevance of various pathological conditions in the live animal.

NY-74: HEALTH ASSESSMENT OF WILD TURKEYS IN KENTUCKY

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Background: Wild turkey (*Meleagris gallopavo*) populations have reportedly declined in localized areas in Kentucky.

Objective: A survey was initiated to understand baseline health parameters of wild turkeys in Kentucky.

Methods: Thirty-six frozen, hunter-harvested, adult, tom wild turkey carcasses obtained from voluntary hunter check stations underwent postmortem examination (30 from the

populations with declines; 6 from non-affected areas). Necropsies were performed and tissues were collected for histopathology, virology, parasitology, and toxicology.

Results: No significant gross lesions were observed besides occasional macroparasites. All but two birds were in fair to good nutritional condition. Histologic lesions were rare and minimal in most cases, including mild, lymphoplasmacytic infiltrates in kidney (8/36), heart (8/36), and testicle (3/36). Four toms displayed gastrointestinal inflammation, including fungal enteritis (1/36), chronic proventriculitis (2/36), and acute bacterial proventriculitis (1/36). Testicles were weighed and examined histologically to gauge reproductive health. Light to moderate tick burdens were observed on 34/36 birds and lice were recovered from 11 /36 birds. Prevalence and burdens of nematodes and cestodes were considered insignificant. *Tetratrichomonas* sp., without lesions, was detected in 3/36 birds. Intestinal coccidia and non-intestinal helminth prevalences were low. The prevalence of lymphoproliferative disease and reticuloendotheliosis viruses were 39% and 11%, respectively. Based on PCR testing, two genetic lineages of *Haemoproteus* in 30/36 birds were detected. Gas chromatography-mass spectrometry in 32/36 turkeys revealed no toxic compounds. Elevated liver iron concentrations were occasionally detected.

Conclusions: The submitted Kentucky turkeys were in apparent good health. Continued investigation is warranted to monitor this population for morbidity and mortality.

NY-75: PROLIFERATIVE RESPIRATORY AND GASTROINTESTINAL STRONGYLOIDIASIS IN A COLONY OF MIXED COLUBRID SNAKES

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An outbreak of respiratory disease associated with increased morbidity and mortality in multiple species of colubrid snakes (*Pantherophis* and *Lampropeltis* spp.) was investigated in a large breeding colony. Signs included respiratory distress, stomatitis, facial deformation, anorexia, and waning body condition. Widespread epithelial proliferation and inflammation associated with high numbers of adult and larval nematodes was a consistent microscopic finding throughout the oral cavity, upper respiratory tract, lungs, and gastrointestinal system of affected snakes. A few snakes had involvement of the facial tissues, characterized by periodontal, periorbital, and cranial cellulitis, uveitis, and subspectacular nematodes. Histomorphology of the adult nematodes with a characteristic paired reproductive tract and rhabditiform esophagus was consistent with *Strongyloides*. Parasitologic examination of worms from the oral mucosa and lung of two affected snakes identified the parasite as a *Strongyloides* species. PCR amplification and sequencing of both the small subunit ribosomal RNA gene and the internal transcribed spacer (ITS) from affected oral mucosa and lung of two snakes identified a novel *Strongyloides* species. Parasites from the genera *Strongyloides* are capable of alternating between parasitic (homogonic) and free-living (heterogonic) life cycles and can be a significant cause of morbidity in captive reptiles and amphibians. Due to the parasite's ability to proliferate and produce infective larvae in the captive environment, there is increased potential for superinfection and

disease. The unique biology of these parasites taken together with common husbandry practices in captive reptile collections can result in significant disease manifestations as documented in this snake colony.

NY-76: MYCOLACTONE-PRODUCING MYCOBACTERIA INFECTION IN CAPTIVE REPTILES, TAIWAN

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Background: A group of mycobacteria harboring a mycolactone-producing plasmid (namely mycolactone-producing mycobacteria; MPM) is able to generate an immunosuppressive and cytotoxic toxin (mycolactone). The ability of mycolactone-producing has been considered a major determinant virulent factor in mycobacterial infection. In Taiwan, cases of MPM infection has been identified in a high mortality of captive Hong Kong warty newts.

Objective: Our objective aims to investigate the MPM infection in amphibians and reptiles in the same captive facility.

Methods: Samples were collected from the captive amphibians and reptiles with confirmation of mycobacterial infection by histopathology and acid-fast staining. PCR targeting the mycolactone-producing plasmid were performed to confirm the presence of mycolactone-producing plasmid in these mycobacteria.

Results: Samples from 14 individuals, including 9 chelonians, 3 snakes and 2 amphibians with multifocal necrogranulomatous or necrotic lesions were collected. 10 of them (7 chelonians, 1 snake and 2 amphibians) were positive for the mycolactone-producing plasmid.

Conclusions: Although the source of MPM in Taiwan is still undetermined, the current study has demonstrated that MPM are circulating and causing sporadic mortality in this captive facility. The possibility of MPM and/or mycolactone-producing plasmid circulating in Taiwan should not be overlooked, and it may be a threat for the health of endemic amphibian and reptile species.

NY-77: EVALUATION OF METALLOTHIONEIN AND KI-67 EXPRESSION IN CHRONIC CHOLANGIOHEPATITIS IN CATS

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Background: Chronic cholangiohepatitis is a common disease in cats with a guarded prognosis and unknown etiology. Enhancing liver defense mechanisms is an emerging therapeutic alternative for liver diseases with poor prognosis in human medicine. Metallothionein (MT) is a heavy metal binding protein, widely researched for its role in liver defense through antinflammatory, antifibrotic and regenerative properties.

Evaluation of the expression of MT in diseased cat livers has not been attempted before.

Objective: To evaluate the role of MT in chronic cholangiohepatitis in cats by assessing the correlation between hepatocellular MT and Ki-67 expression with histological hallmarks of the disease, namely, inflammation, bile duct proliferation and fibrosis.

Methods: We used immunohistochemistry to evaluate and quantify MT and Ki-67 expression in 34 liver tissues from cats, histologically diagnosed with chronic cholangiohepatitis. Number of hepatocytes with positive MT staining and labeling intensity were measured to quantify MT expression. Inflammation, fibrosis and bile duct proliferation were also quantified. Correlation between all the parameters were assessed using Spearman's rank correlation test.

Results: Inflammation was correlated with the number of hepatocytes with positive MT staining (r=0.36, $P<0.05$) and labeling intensity (r=0.37, $P<0.05$). Labeling intensity was markedly increased in hepatocytes near centrilobular areas in 16 of 34 (47%) samples with no obvious staining pattern in the remaining samples.

Conclusions: The results suggest that MT expression is induced in hepatic inflammation, which is speculated as a host defense mechanism to protect liver from inflammation mediated oxidative injury. Similar to humans, cats may benefit from therapeutic interventions utilising MT.

NY-78: EQUINE SALSA IS EXPRESSED ON MUCOSAL SURFACES AND IS DOWNREGULATED IN ASTHMATIC HORSES

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Background: Salivary Scavenger and Agglutinin (SALSA, also known as Deleted in Malignant Brain Tumors 1; DMBT1) is a protein with putative functions in innate immunity and tissue remodeling. Preliminary transcriptomic studies suggest relatively lower expression of SALSA in asthmatic horses compared to non-asthmatic horses.

Objectives: Characterize tissue expression and protein structure in horses, and compare gene expression between asthmatic and non-asthmatic horses.

Methods: The SALSA gene from bronchial cDNA samples of multiple horses was amplified and sequenced. A qPCR assay was designed, and an unpaired t-test and a ratio paired t-test were used to compare gene expression between six asthmatic and six non-asthmatic horses, pre- and post-asthmatic challenge. Tissue microarrays from 4 horses containing 22 tissues each were constructed. Immunohistochemical assays for SALSA were validated and applied to equine tissue microarrays.

Results: The gene in horses includes three to five scavenger receptor cysteine-rich (SRCR) domains, two CUB (C1r/C1s, uegf, bmp-1) domains and one Zona Pellucida domain. These domains mediate the binding of ligands such as those involved in innate immunity. The number of SRCR domains varied between horses, indicating isoforms.

Asthmatic horses had significantly lower gene expression for SALSA than non-asthmatic horses, but differences in expression before and after asthmatic challenge were not statistically significant. SALSA was highly expressed at mucosal sites, including the epithelium of large airways.

Conclusions: SALSA is a multifunctional protein with multiple isoforms in different individuals with a predilection for mucosal cells. Downregulation in asthmatic horses suggests dysregulation of immune balance in equine asthma.

NY-79: PAPILLARY UROTHELIAL HYPERPLASIA, DYSPLASIA, AND TRANSITIONAL CELL CARCINOMA IN CATS WITH NATURALLY OCCURRING CHRONIC KIDNEY DISEASE

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Background: Endemic nephropathy encompasses a group of chronic tubulointerstitial diseases of human beings that are endemic to certain areas of the world. The tubulointerstitial changes in Balkan endemic nephropathy (BEN) are strikingly similar to those of cats with chronic kidney disease (CKD). Although BEN has been associated with transitional cell carcinoma (TCC) of the renal pelvis and ureter, no such association has been made between feline CKD and TCC.

Objective: To describe the renal papillary changes in cats with naturally occurring CKD.

Methods: Thirteen client-owned cats with biochemical evidence of CKD presenting for euthanasia were prospectively recruited for an unrelated study. For each cat, right and left kidneys were collected postmortem and examined histologically.

Results: CKD was characterized by tubular atrophy and tubulorrhexis, with interstitial inflammation and fibrosis in all affected cats. Papillary urothelial hyperplasia was present in 11/13 cats; urothelial hyperplasia with dysplasia was present in 5 of the 11 affected cats. TCC was present in 3/11 cats and consisted of non-papillary (2 cases) or papillary (1 case) neoplasms that invaded the underlying renal papilla.

Conclusions: Most cats with CKD in this case series had hyperplastic urothelial changes in the renal pelvis. Future studies are warranted to investigate a possible association between CKD and urothelial hyperplasia in cats. Further, a subset of cats also had dysplastic urothelial changes and TCCs, suggesting that there may be an association between CKD and TCC, similar to what has been reported for BEN in human beings.

NY-80: SOX2 EXPRESSION IN CANINE NEOPLASIA

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Background: SOX2 is a major transcriptional regulator of embryonic stem cell pluripotency and self-renewability. Its expression in cancer stem cells from at least 25 different tumors in humans and rodent models has directly implicated it in tumorigenicity, metastasis, drug resistance, recurrence, and poor survival.

Objective: To investigate the expression of SOX2 in canine neoplasia.

Methods: Immunohistochemistry for SOX2 was performed in sets of ten archived formalin-fixed paraffin-embedded tissues from 45 distinct canine neoplasms embryologically originating from the three germ cell layers. Normal expression of SOX2 was evaluated in a canine tissue microarray.

Results: Strong and diffuse intranuclear SOX2 immunolabeling was consistently found in all tumors originating from the surface ectoderm (11/11) and ectodermal neural tube (1/1), in the majority of epithelial endodermal tumors (4/7), in a single type of ectodermal neural crest tumor (1/5), and in a single type of mesodermal tumor (1/16). Only variable and inconsistent intranuclear SOX2 immunolabeling was detected in ectomesodermal (3/3) and neuroendocrine endodermal tumors (2/2).

Conclusions: Although further studies are necessary to investigate the involvement of SOX2 in oncogenesis, metastasis, drug resistance, and prognosis, this wide-ranging study is the first step to demonstrate the expression of SOX2 in a wide variety of canine cancers. In the future, screening methods based on cellular plasticity and pluripotency biomarkers may provide avenues for the rational design of therapeutic strategies that target vulnerable signals upstream or downstream of SOX2 in different cancers, and possibly offer novel clinical applications for SOX2 as a prognostic indicator.

NY-81: CHARACTERIZATION OF GLOMERULAR DISEASE IN BLACK-FOOTED FERRETS

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Background: Black-footed ferrets (Mustela nigripes), on the brink of extinction in the 1980s, exist in the wild today due to successful captive breeding and reintroduction programs. All wild black-footed ferrets are now descended from a small original group of founder animals, and as a result, succumb to diseases that may have a familial basis. Renal amyloidosis is a commonly reported necropsy finding in captive black-footed ferrets.

Objective: The objective of this study is to characterize glomerular disease in black-footed ferrets histologically, histochemically, and ultrastructurally.

Methods: Formalin fixed tissues from black-footed ferrets (n=13) were submitted by the Louisville Zoo between the years of 2012 and 2018; kidneys from these cases were examined histologically. Glomerular morphology was evaluated with PASH, Jones silver, Masson's trichrome, and Congo Red histochemical stains and via transmission electron microscopy.

Results: Of the 13 ferrets examined, two (15.4%) had membranoproliferative glomerulonephritis, two (15.4%) had glomerular amyloidosis, one (7.7%) had membranous glomerulonephropathy, and one (7.7%) had focal segmental glomerulosclerosis based on light microscopy. Tubulointerstitial changes were generally mild.

Conclusions: Glomerular disease is a common necropsy finding in black-footed ferrets, occurring in 6 of the 13 examined ferrets. While 33% of the ferrets with glomerular disease had amyloidosis, other primary glomerular diseases were evident histologically or ultrastructurally.

NY-82: IMMUNE PROFILING TO DIAGNOSE CANINE MENINGOENCEPHALITIS OF UNKNOWN ETIOLOGY

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Idiopathic inflammatory neurologic diseases in dogs are also termed meningoencephalitis of unknown etiology (MUE) and encompass granulomatous meningoencephalitis (GME), necrotizing meningoencephalitis (NME) and necrotizing leukoencephalitis (NLE). The pathogenesis of MUE remains obscure but is suspected to be multifactorial, and related to both genetic and autoimmune factors. Small breeds are generally overrepresented and despite immunosuppressive treatment the prognosis for cases of MUE is guarded to poor. Ante-mortem diagnosis is by exclusion, and imaging and cerebrospinal fluid analysis remain insufficiently specific to categorize clinical MUE into subtypes of inflammatory disease, whereas post-mortem histopathology of the brain generally provides a more dependable and specific diagnosis.

The goal of this project is to investigate the utility of immune profiling as a novel diagnostic tool to improve the prognostication and treatment of MUE. Given the different histologic characteristics and acknowledged breed dispositions, subtypes of MUE may have different etiologies and which could be associated with different lymphocyte antigen receptor (LAR) repertoires. LAR repertoires are sequenced from 82 post-mortem brain samples from the archives of the University of Guelph and the University of California Davis. These sequences are assessed by principle component analysis to determine if repertoires correspond to MUE subtype, and with clinical parameters such as signalment, response to therapy, or survival time. Successful application of immune profiling to the ante-mortem diagnosis of MUE will establish proof-of-principle to apply these methods to the diagnosis of other inflammatory diseases.

NY-83: COMPARATIVE EXAMINATION OF CHEMOKINE SIGNATURES IN T-CELL MEDIATED CANINE SKIN DISORDERS

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Background: T-cell mediated skin diseases represent a significant morbidity for both companion animals and humans, including autoinflammation (allergy, immune-mediated disorders) and neoplasia (epitheliotropic lymphomas). Prior work on autoinflammation and cancer has highlighted that these conditions exist on a spectrum of immune dysregulation. Companion animals, including cats and dogs, are known to develop inflammatory or neoplastic skin conditions similar to humans, providing opportunities to study spontaneous disease from biopsies taken for diagnostic purposes. A strength of comparative immunology approaches is that immune profiles may be assessed across species to better identify pathways that might drive inflammation or development of neoplasia.

Objective: Compare the cytokine gene expression patterns of different canine T-cell mediated skin diseases using nanostring technology and compare these gene expression patterns to both human and mouse model datasets.

Methods: RNA was isolated from formalin fixed paraffin embedded canine skin biopsies submitted to the Cummings School of Veterinary Medicine at Tufts with a diagnosis that included an interface dermatitis or epitheliotropic lymphoma. Extracted RNA was processed using Nanostring cartridges to determine cytokine gene expression profiles from a custom panel of 208 different genes.

Results: The expected outcome is that a CXCR3 chemokine signature will be present in interface dermatitis, and CCR4 will be present in epitheliotropic lymphoma. Final results are currently pending.

Conclusions: We will compare these data to published human datasets to determine overlap between canine and human T-cell mediated skin disorders. We anticipate that these studies will form the basis for future mechanistic studies and clinical trials.

NY-84: HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL EVALUATION OF FELINE PITUITARY ADENOMAS

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Background: Pituitary adenoma is the cat's most common hypophyseal and third most common intracranial neoplasm, yet our knowledge is mostly based on individual cases with limited trophic hormone immunohistochemistry.

Objective: Correlation of histologic and clinical features in 18 feline pituitary adenomas.

Methods: Histochemistry included reticulin and periodic acid-Schiff (PAS) plus immunohistochemistry for adrenocorticotrophic hormone (ACTH), melanocyte stimulating hormone (MSH), growth hormone (GH), prolactin (PRL), thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH), and luteinizing hormone (LH). The proliferation index was calculated in hypophysectomy specimens as the percentage of Ki-67 immuno-reactive cells.

Results: Seven hypophysectomy and 11 postmortem pituitary adenomas were evaluated. The cats were 5.5-13.5 years old, 15/18 were castrated males, and 11 were domestic shorthairs. The reticulin framework was disrupted. Eight somatotroph adenomas were acidophilic, PAS-negative, and GH-positive; all 8 cats had hypersomatotropism with diabetes mellitus. Six gonadotroph adenomas expressed FSH and LH, and were chromophobic and PAS-negative; 2/6 cats had diabetes mellitus. Four PAS-positive adenomas expressed ACTH (one also expressed MSH); 3/4 cats had hypercortisolism and 2/4 cats had diabetes mellitus. No adenoma expressed PRL or TSH. The proliferation index was 0-0.68% for somatotroph (n=5) and 1.21-1.32% for corticotroph (n=2) adenomas.

Conclusions: Pituitary adenomas were diagnosed mainly in male cats. Most were derived from somatotrophs or gonadotrophs with fewer ACTH-expressing adenomas. Whereas somatotroph adenomas were usually associated with hypersomatotropism and diabetes mellitus, and ACTH-expressing adenomas with hypercortisolism, gonadotroph adenomas were clinically silent, at least in these neutered cats.

NY-85: OPHIDIOMYCES OPHIODIICOLA IN WILD-CAUGHT TERRESTRIAL AND AQUATIC SNAKES: A STANDARDIZED APPROACH TO GROSS AND HISTOLOGIC EVALUATION TO UNDERSTAND THE EFFECT OF LESION DISTRIBUTION, ECOSYSTEM, AND SELECT COMORBIDITIES ON DISEASE

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Background: *Ophidiomyces ophiodiicola*, the causative agent of Snake Fungal Disease (SFD), is an emerging threat to both captive and free-ranging snakes, and has been implicated in several specific population declines. While cutaneous lesions have been described, the pathogenesis of SFD and its contribution to mortality remains poorly understood. Currently, there is limited documentation regarding lesion distribution or correlation between cutaneous and visceral involvement in natural infections.

Objectives: To standardize a protocol for postmortem examination of snakes naturally infected with *Ophidiomyces ophiodiicola* in an effort to detect a wider range of lesions than have been previously documented; to determine differences in lesion distribution in snakes from different ecosystems; and correlate cutaneous and visceral involvement.

Methods: 35 naturally-infected snakes received a postmortem examination utilizing our novel systematic gross, histologic, and molecular approach. Lesion type and distribution was compared between groups.

Results: SFD lesions were most consistently identified in the head (94%), cloaca (28.6%), tail (77.1%), and viscera (37.1%). Lesions were present in all skin survey sections in 17/23 (74%) of Lake Erie Water Snakes (LEWS). In terrestrial snakes, lesion distribution varied among species and individuals.

Conclusions: This approach allowed a repeatable method of assessing natural SFD infection. Head and tail remained the most affected locations. Among our population, LEWS were most affected, with nearly diffuse cutaneous involvement; terrestrial species varied in cutaneous lesion distribution. Visceral granulomas were present in snakes with and without cutaneous lesions, demonstrating that a lack of cutaneous lesions does not rule out the possibility of SFD infection.

NY-86: HISTOLOGIC CHARACTERIZATION OF THE MAJOR DUODENAL PAPILLA OF CATS AND CORRELATION TO THE INCIDENCE OF CONCURRENT HEPATIC, PANCREATIC, AND INTESTINAL INFLAMMATION AND NEOPLASIA

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Background: Conjoining of the major pancreatic and common bile duct at the major duodenal papilla (MDP) is suspected to predispose cats to the clinical syndrome of “triaditis”. However, the pathogenesis is poorly understood and presence of pathology at the MDP has not been assessed in cats with or without triaditis.

Objective: Our aim was to identify any association between MDP anatomy or pathology and presence of biliary, pancreatic, or intestinal inflammation or neoplasia.

Methods: Histologic assessment was performed on duodenum, liver, pancreas, jejunum, and ileum from 82 cats presented for postmortem examination.

Results: The majority of cats (83%, 68/82) had a complex ductular network at the MDP, with no distinction between pancreatic and common bile ducts. Lymphoid aggregates at this site were common (46%, 38/82). Inflammation of the MDP was present in 30% of cats (25/82), and was frequently associated with cholangitis, pancreatitis, or enteritis (92%, 23/25). Cholangitis (29%, 24/82), pancreatitis (67%, 55/82), and enteritis (45%, 37/82) were common, but none was individually associated with inflammation at the MDP. Triaditis was less common (13%, 11/82), but 8/11 had concurrent inflammation at the MDP. Neoplasia was present in 24% of cats (20/82), with lymphoma (15/20) predominating. Metastatic disease and/or inflammation was present in the other two examined systems in 9/20 cats, including six with concurrent metastasis/inflammation of the MDP.

Conclusions: These findings suggest that immune activation or inflammation of the MDP may play a role in the pathogenesis of triaditis and facilitate local metastasis of neoplasia in cats.

NY-87: CANINE HEPATOCYTOTROPIC LYMPHOMA IS FREQUENTLY CD3+/CD20+

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Background: Hepatocytotropism is a distinctive feature of some canine lymphomas, with only two cases described in the literature.

Objective: To characterize hepatocytotropic lymphomas (HL) including clinicopathologic, histopathologic, and immunophenotypic data.

Methods: Retrospective analysis identified 11 cases of HL over the past 15 years. Clinicopathologic data, immunophenotyping, and PCR for antigen receptor rearrangements (PARR) were analyzed.

Results: No breed predilection was identified. Spayed females were overrepresented (8/11). The average age at presentation was 9.4 years. Dogs presented with anorexia (10/10), lethargy (5/10), nausea or vomiting (5/10), and icterus (6/10). Clinicopathologic findings included: severe cholestatic hepatopathy (9/9), hypoalbuminemia (7/7), either high or low cholesterol (5/7), an inflammatory and/or stress leukogram (7/7), anemia (4/7), thrombocytopenia (6/7), and abnormal hemostasis (7/7). Dogs were euthanized within 9 days of presentation (7/7). Post-mortem identified hepatomegaly (6/6), abdominal effusion (5/6), and lymphadenopathy (4/6). Neoplastic lymphocytes were intermediate to large, with varying patterns of infiltration, proportions of hepatocytotropism, and biliary epitheliotropism (8/11). Histopathology identified involvement of the spleen (7/7), lymph nodes (5/7), and bone marrow (5/7). The predominant immunophenotype (8/11) was dual B and T cell (CD3+/CD20+/PAX5-/CD11d-). Two cases had a T cell phenotype (CD3+/CD20-/PAX5-/CD11d-) and a single case had a B cell phenotype (CD3-/CD20+/PAX5-/CD11d-). PARR analysis identified clonal TCR gene rearrangement in 4 cases and clonal immunoglobulin gene rearrangement in 2 cases.

Conclusion: Hepatocytotropic lymphoma is uncommon in dogs, but has consistent clinicopathologic findings and a rapid clinical course. Immunophenotyping and PARR indicate that hepatocytotropic lymphomas are best classified as T cell lymphomas with aberrant CD20 expression.

NY-88: MECHANISMS OF TUMORIGENESIS AND IMMUNE TOLERANCE IN CANINE MELANOMA

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Background: Melanoma is highly resistant to conventional therapies for humans and dogs. Whereas mutations in RAF family oncogenes have been described in ~50% of human cases, little is known about driver mutations in dogs. Additionally, although

melanoma is considered to be highly immunogenic, the function of tumor-infiltrating lymphocytes (TILs) is apparently suppressed in the tumor microenvironment. Accordingly, current immunotherapies target checkpoint molecules, e.g., PD-1, expressed by cells in the tumor, inhibiting their suppressing effect over TILs. PD-L2, an alternative PD-1 ligand, is also overexpressed in malignant tumors and patients with anti-PD-L1 resistance. The role of PD-L2 remains poorly understood.

Objective: To study comparative mechanisms of tumorigenesis in humans and dogs, identifying points of commonality and divergence that may inform therapeutic options in either species.

Methods: Genotyping of BRAF was performed in genomic DNA from FFPE specimens of melanoma and melanocytoma in the Virginia Tech Animal Laboratory Services archives. Analysis of checkpoint molecule gene expression was performed by qRT-PCR using total RNA isolated from the same cases.

Results: Only one of 30 melanoma and melanocytoma cases screened showed the Val/Glu BRAF mutation common in human melanoma. Analysis of checkpoint molecule expression revealed a marked increase in PD-1 and trends upward for PD-L1 and PD-L2 in melanomas relative to melanocytomas.

Conclusions: The low observed incidence of BRAF mutation is consistent with previous reports and suggests that other driver mutations predominate in canine melanoma. As in human melanoma, overexpression of the PD-1/PD-L1/PD-L2 axis may be a common feature of canine melanoma.

NY-89: THE USE OF THREE DIMENSIONAL IMAGE RECONSTRUCTION TO CHARACTERIZE LUNG VASCULAR ABNORMALITIES IN CANINE DEVELOPMENTAL LUNG DISEASE

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Background: Neonatal respiratory distress syndrome is a significant cause of neonatal deaths in puppies; however, the underlying pathogenesis is not understood. We have identified histologic features of abnormal lung development in multiple breeds of domestic puppies that died suddenly and unexpectedly, including incomplete alveolar development, thickening of alveolar septae, and a spectrum of pulmonary vascular abnormalities. 3-dimensional reconstruction techniques have been used in human neonatal developmental lung diseases to investigate underlying vascular abnormalities, including intrapulmonary bronchopulmonary anastomoses.

Objectives: To characterize pulmonary vascular pathology utilizing standard histopathology and 3D image reconstruction. Histopathologic evaluation of 35 cases of developmental lung disease in puppies that died less than 3 weeks after birth was performed on cases that were submitted for routine necropsy to MSU Veterinary Diagnostic Laboratory. To better understand pulmonary vascular pathology we

performed 3D image reconstruction of five bronchovascular bundles (n=1 affected, n=1 control) using Free-D software.

Results: Canine neonatal puppy lungs showed pulmonary artery medial hypertrophy, increased numbers of thin-walled venous profiles, and abnormal capillary placement and numbers. 3D reconstruction identified vascular abnormalities suggestive of arterio-venous shunts, including possible intrapulmonary bronchopulmonary anastomotic vessels in all five bronchovascular bundles examined.

Conclusions: 3D reconstruction technique helped identify significant pulmonary vascular abnormalities in one puppy that died suddenly in the neonatal period. We speculate that increased hypoxemia due to abnormal vascular growth, remodeling and suspected intrapulmonary bronchopulmonary shunts may be central to the underlying developmental abnormality in affected puppy lungs and may contribute to neonatal mortality of domestic dogs.

NY-90: PREVALENCE OF CYTAUXZOON FELIS IN EASTERN KANSAS, A PRELIMINARY REPORT

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Background: *Cytauxzoon felis* is a tick-borne hemoprotozoal organism of cats that causes cytauxzoonosis. Bobcats (*Lynx rufus*) are the natural host for this pathogen in North America. Domestic cats infected with this pathogen often present with acute illness, which is frequently fatal within 1-2 weeks. Cats that survive acute disease remain persistently infected and serve as transmission reservoirs. Studies have demonstrated that multiple *C. felis* strains, which may vary in pathogenicity, are circulating in the United States. *Amblyomma americanum*, a major transmission vector for *C. felis,* is the dominant tick in eastern Kansas. Therefore, cats in this area are at risk for acquiring and serving as reservoirs for *C. felis*.

Objective: Determine the infection prevalence of *C. felis* in domestic cats in eastern Kansas using blood samples opportunistically collected from cats undergoing routine clinical procedures. Identify and compare *C. felis* strains obtained from clinically-ill and asymptomatic cats to evaluate if certain *C. felis* strains are more commonly associated with clinical disease

Methods: EDTA blood samples were collected from cats undergoing routine procedures at animal shelters and veterinary clinics in eastern Kansas. DNA was extracted for real-time PCR. Specific *C. felis* genes were cloned and sequenced from positive PCR samples to determine their *C. felis* genotype.

Results: Preliminary data indicates 5% (7/138) of domestic cats in eastern Kansas are persistently infected with *C. felis*.

Conclusions: Further investigation is warranted to determine ways to block *C. felis* transmission (e.g. vaccine development) and/or improve treatment options for acutely and chronically infected cats.

NY-91: IMMUNOHISTOCHEMICAL EVALUATION OF REGULATORY T LYMPHOCYTE INFILTRATION OF CANINE GLIOMA

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Background: Glioma evasion of the immune system is an integral part of its pathogenesis. In humans, an important mechanism of evasion is recruitment of regulatory T lymphocytes (Tregs). Tregs suppress effector immune cells and contribute to their inability to eliminate cancer cells. Canine glioma has a robust immune cell microenvironment; however, the distribution of Tregs has not been determined.

Objective: To characterize Treg infiltration in canine glioma.

Methods: 85 cases from the NCSU archives (2006-2018) originally diagnosed as glioma were independently reviewed via consensus diagnosis by a group of five pathologists (4 DVM, 1 MD), resulting in 73 glioma cases for analysis. Treg populations in these gliomas were manually quantified via immunohistochemistry for FoxP3, a marker of Tregs.

Results: 44/73 gliomas had infiltrating Tregs. Mean Tregs per high-powered field (ocular FN 22 mm, 400X magnification, 0.237 mm^2area) were 2.7 (range 0.1-21.6, standard deviation 5.2). This subset of gliomas was predominantly high grade oligodendrogliomas. Tregs were generally evenly distributed throughout the tumor tissue and extended slightly beyond the brain-tumor interface, though they were not otherwise detected in normal brain.

Conclusions: Treg infiltration occurred in the majority of gliomas although the numbers of Tregs were relatively low in most tumors. However, a subset of gliomas predominantly composed of high grade oligodendrogliomas showed more robust Treg infiltration. Treg blockade or depletion may be potential therapeutic targets in this group of tumors.

NY-92: CANINE GASTRIC CARCINOMAS - A HISTOPATHOLOGICAL STUDY

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Background: Carcinoma is the commonest canine gastric tumor, accounting for around 90% of gastric malignancies, with reported predisposing factors of diet and breed. Histological classification, based on WHO and Lauren classifications, is based on

predominant growth pattern, architectural and cytological features. *Helicobacter* are reported frequently in the canine stomach but their role in disease, including neoplasia, is uncertain.

Objective and Methods: To characterize and histologically classify a large series of canine gastric carcinomas and to evaluate possible risk factors for tumor development. To assess the presence of *Helicobacter* in relation to gastric inflammation, as scored by WSAVA grading, and histological tumor classification.

Results: 137 cases of canine gastric carcinoma (surgical and endoscopically-retrieved samples) from the archives of the Royal Veterinary College and VPG Histology were included. Carcinomas were most frequently present in Staffordshire bull terriers, Collies and Labrador retrievers. Mean age was 9 years but no sex predilection was identified. Signet-ring cell type carcinoma was the most frequent histological type (38%, 52/137), followed by undifferentiated (26%), tubular (24%), mucinous (9.5%) and papillary (1.5%) types. Signet-ring cell types were more frequently diagnosed in endoscopic samples. Mean gastric inflammatory score (MGIS) was 3.3 for Helicobacter-positive cases and 2.6 for negative cases. MGIS was greater (3.7) in cases with high bacterial numbers compared to those with few (3.0) and mucinous carcinomas were most often positive (53.8%, 7/13).

Conclusions: This study contributes further to our understanding of the potential role of *Helicobacter* spp. inducing inflammation and possible tumor formation in canine gastric carcinomas.

NY-93: THE EFFECT OF DEXAMETHASONE ON HEMATOLOGICAL PROFILES, HAEMOSPORIDIAN INFECTION, AND SPLENIC HISTOLOGY IN HOUSE FINCHES (HAEMORHOUS MEXICANUS)

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Background: The emergence and rapid spread of *Mycoplasma gallisepticum* (MG) in house finches (*Haemorhous mexicanus*) has prompted both experimental and field studies investigating the effects of mycoplasmosis on house finch survival. In other bird species, the administration of glucocorticoids has induced immunosuppression with increased susceptibility to infectious agents, involution of immune organs, and lymphopenia and heterophilia.

Objective: Dexamethasone was used to induce stress-like immunosuppression in house finches following recovery of an experimental MG infection, allowing characterization of alterations in hematological profiles, haemosporidian infection, and splenic histology.

Methods: Eighteen birds were injected subcutaneously with either dexamethasone (experimental group, n=7) or saline (control group, n=8) daily for 8 days. Blood smears were prepared at day 0, 4, 8 and 9 of treatment, and birds were then euthanized. Necropsies and histopathologic examination were performed. Sections of spleen were graded on the histologic presence of follicles and CD3 immunoreactivity. White blood cell differential counts and analysis for hemoparasites were performed on blood smears.

Results: A significant decrease in lymphoid follicles in dexamethasone-treated birds was noted ($p < 0.001$). Dexamethasone induced a relative lymphopenia and heterophilia, severe on day 4 and almost absent by day 9. Increased Leucocytozoon spp. and Plasmodium spp. parasitism in the dexamethasone-treated birds was detected. No difference in intestinal coccidiosis, splenic CD3 immunoreactivity, and ocular lesions were seen between the control and treatment group.

Conclusions: Dexamethasone is capable of inducing changes consistent with immunosuppression in house finches, likely comparable to that of stress-induced immunosuppression. The role of MG infection is unclear.

Veterinary Student Poster Session

SP-01: EXPRESSION AND FUNCTION OF THE COSTIMULATORY CHECKPOINT MOLECULE OX40 BY T CELLS IN DOGS

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OX40 (CD134) is a transiently expressed costimulatory checkpoint molecule associated with T cell activation in mice and humans. Agonistic antibodies targeting OX40 have demonstrated an ability to induce antitumor immunity in rodent cancer models and are currently being assessed in human clinical cancer trials. We generated monoclonal antibodies against recombinant canine OX40 with the intent of developing clinical grade immunotherapeutics for dogs. A preliminary screen was completed to identify candidate antibodies that recognize native canine OX40 on T cells and tumor cell lines. Specificity of the anti-OX40 antibodies was validated by western blot. Characterization of OX40 expression by T cells from dogs was assessed by flow cytometry. We found resting T cells from dogs expressed little or no OX40, however, upon activation with Concanavalin A, OX40 expression was significantly upregulated. Upregulation was apparent as early as 12 hours and remained elevated for at least 5 days. *In vitro* studies to determine functional properties revealed that anti-OX40 antibody exposure prolonged T cell activation, as reflected by increased survival, Granzyme B expression, and Interferon gamma secretion. These findings indicate that OX40 is a functional costimulatory checkpoint molecule in dogs and suggest that OX40 targeted immunotherapy is a promising cancer therapeutic. Based on our findings we initiated a

clinical study in dogs with oral melanoma to evaluate the effect of local OX40 immunotherapy on antitumor immunity.

SP-02: CHARACTERIZATION OF ENDOGENOUS FELINE LEUKEMIA VIRUS (ENFELV) LONG TERMINAL REPEAT (LTR) INTEGRATION SITE DIVERSITY

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Endogenous retroviruses (ERV) offer a unique perspective of evolutionary history, and play important parts as homeostatic regulators. Though ERVs no longer encode infectious virus, long terminal repeats (LTRs) act as promoters for *cis*-activation of host genes and enhancer elements for *trans*-activation of host genes up to 1Mb from their insertion site. Endogenous murine leukemia virus LTRs integrated near host anti-viral genes promote the expression of proteins that restrict against exogenous retroviral infections. We previously documented a negative dose-dependent correlation between endogenous feline leukemia virus (enFeLV) LTR and exogenous infection, hinting at LTR-mediated viral restriction. Here, we assess the enFeLV-LTR integration site diversity and examine associated genes in close proximity to enFeLV-LTR integration that may act as anti-viral genes. We use a targeted linker-mediated PCR approach to deep sequence LTR integration sites in 20 domestic cats from three populations with varying degrees of inbreeding. We identified few commonly shared LTR integration sites among individuals, though closed colonies had fewer unique integration sites than outbred cats. One of the gene families identified proximal to LTR integration sites were zinc regulatory functions. This study identifies a finger proteins that have a variety of new technology to assess ERV integration sites in individual animals, associates similarity in integration sites with degree of relatedness, and provides a baseline for inquiry into endogenous retroviral control of host genes in the domestic cat.

SP-03: IN VITRO EFFECTS OF INTEGRIN SIGNALING INHIBITION IN CANINE AND HUMAN OSTEOSARCOMA CELL LINES

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Osteosarcoma (OSA) is the most common primary malignant bone tumor. New therapeutic strategies for OSA are desperately needed, as overall survival rates of OSA patients have remained unchanged for 30 years, primarily due to an inability to treat relapsed disease, with only ~20% of patients alive 4 years after tumor recurrence. Spontaneous tumors in pet dogs represent a valuable surrogate for evaluation of novel cancer therapies. Canine and human OSA share many similarities including primary tumor location, response to conventional therapies and the presence of micrometastases at diagnosis. We show that periostin, an integrin-binding matricellular protein linked to multiple tumor-promoting functions including cancer cell survival, migration and enhanced metastasis, is significantly upregulated in canine and human OSA and associated with gene expression enrichment for integrin signaling. Therefore, we assessed the in vitro effects of two integrin signaling-targeted drugs, Cilengitide, an αvβ3/5 inhibitor, and a Focal Adhesion Kinase Inhibitor (FAKi14)

in canine and human OSA cell lines via western blot, flow cytometry, cell survival/proliferation and migration assay. FAKi14 demonstrated significant dose dependent inhibition of proliferation/survival in all cell lines at pharmacologically relevant concentrations. Necrosis was determined to be the mechanism of death. Canine OSA cells were more sensitive to FAKi14 than human OSA cells (canine IC50=5.34µM; human IC50= 10.9µM). Cilengitide demonstrated significantly less anti-proliferative effects than FAKi14, with clinically relevant IC50 values not reached in half of the cell lines.

SP-04: EFFICACY OF FOUNTAIN FLOW CYTOMETRY FOR RAPID DETECTION OF SEPSIS IN FLUIDS

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Bacterial sepsis is a life-threatening condition, and prognosis is highly dependent on the time taken to initiate effective treatment. Bacterial culture and cytology are the two standard techniques currently used in the veterinary field for detecting sepsis, but these methods are far from ideal. Culture results can take days to obtain, which significantly delays the diagnosis and thus the timely administration of appropriate treatment. Cytology can lack sensitivity, increasing the risk that sepsis will go undetected. This pilot study investigated the efficacy of Fountain Flow Cytometry (FFC) as a new diagnostic technique for detecting bacterial sepsis. FFC is a form of high-volume flow cytometry in which LED illumination is used to enumerate fluorescently labeled bacteria. It is both fast (30 minute total analysis time) and adaptable. In this clinical diagnostic trial, mammalian pleural and peritoneal effusions were tested for bacterial sepsis using FFC, and the results were compared to those obtained from culture (the gold standard test). Sixty total cases were enrolled, of which 3 were positive by both FFC and culture and 51 were negative by both methods. Three samples had disparate results. The calculated sensitivity, specificity, positive predictive and negative predictive values were 60%, 94.4%, 50%, and 96.2%, respectively. Preliminary data indicates that with further optimization to increase test sensitivity, FFC promises to be a clinically useful technology for the rapid diagnosis of bacterial sepsis in veterinary patients.

SP-05: THE VALUE OF DISTANCE MENTORING IN PATHOLOGY: A STUDENT PERSPECTIVE

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Mentorship greatly facilitates career development, especially for students interested in specialties like pathology. Mentors provide career advice, create networking and skill development opportunities, and help set career or research goals, creating a successful career foundation. Although mentorship is important, establishing an in-person relationship can be challenging. Pathology might be underemphasized in the curriculum or career programs. A student might also be interested in a field that is uncommon at their institution. Additionally, school and work commitments for both parties could hinder

developing an in-person relationship. Distance mentoring allows students to contact pathologists in their areas of interest and increases communication flexibility. Pathology greatly interests me, but because pathology classes have just begun, I have not met my institution's pathology faculty. However, since a chance encounter, I have been in a distance mentoring relationship with a pathologist for 6 months, communicating via email and FaceTime. My distance mentor offers a broad perspective on career paths and recommends career development and networking opportunities. Our electronic communication can also occur at any time, without requiring coordination of schedules. The distance can feel more impersonal, and the relationship more difficult to maintain than a traditional mentorship. But establishing a communication schedule, defining specific goals, and utilizing videocalls can strengthen the relationship. Investing in a distance mentorship produces the same positive career outcomes, including networking and career opportunities, as a traditional mentorship. The flexibility and limitless possible mentors make distance mentoring ideal for students seeking a particular mentor.

SP-06: HOW TO GET THE MOST OUT OF A MENTORSHIP: THE MENTEE'S PERSPECTIVE

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A successful mentorship can provide benefits to both the mentor and mentee, such as increased job satisfaction, enhanced productivity, continued knowledge of advancements in the field, and moral support. However, there are steps that must be taken to ensure that a mentorship will be fruitful. We explore the mentor-mentee relationship through the lens of the mentee and offer "10 tips" for promoting a worthwhile relationship. These include (1) setting goals and expectations, (2) having self-motivation, (3) acknowledging personal limitations, (4) being flexible, (5) providing feedback to the mentor, (6) leaving room for mistakes, (7) having the ability to take constructive criticism, (8) expanding discussions to include work-life balance and personal growth, (9) being open and honest about emotions, and (10) allowing the mentorship to evolve with time. As a 1st year veterinary student, I have used these tips to capitalize on my interest in pathology and help further my professional development in this area. This has included securing an externship as a summer research fellow at Massachusetts Institute of Technology and submitting an abstract for a conference poster presentation. The greater rewards given to the field of veterinary medicine following successful mentorship are also explored, including the establishment of a culture of collaboration, a tendency for increased communication among colleagues, and the cultivation of a community where it is encouraged to ask for help. In the long run, optimizing the mentor-mentee relationship will ignite proliferation along multiple facets within the field of veterinary medicine for years to come.

SP-07: EFFICACY OF A NRF2 AGONIST TO INCREASE LONG BONE STRENGTH IN A RODENT MODEL OF OSTEOARTHRITIS

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Primary osteoarthritis (OA) is a multi-factorial, age-related disease that causes progressive damage to structural components within a joint, particularly bone. OA pathology is associated with chronic production of inflammatory mediators resulting in tissue remodeling, pain, and decreased mobility in affected individuals. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor present in many cell types and considered a master up-regulator of antioxidant/anti-inflammatory enzymes. Our study aimed to investigate a novel Nrf2 activator, PB125, as a therapeutic option for treatment of OA. We hypothesized that one potential benefit of PB125 would be the mitigation of OA through increased strength of long bones associated with knee joints. Five-month-old male and female Dunkin-Hartley guinea pigs, an animal model of primary OA, were orally administered PB125 or vehicle control for ten months. Femoral bones were collected and four-point bending tests were performed to measure ultimate bending stress (UBS), which is correlated to bone strength. Statistical analyses between groups were performed using a student's t-test. PB125 treated females showed a statistically significant ($p=0.005$) increase in UBS compared to control females. In contrast, there was no difference between PB125 treatment and control males. Additionally, PB125 treated females had a greater UBS ($p=0.05$) than PB125 treated males. PB125 treated females had stronger bones than control females, as well as PB125 treated males. Increased bone strength could result in better joint stability, thereby mitigating degenerative lesions from OA. We are currently evaluating knee joints to determine where OA was influenced from the treatment.

SP-08: UNUSUAL OCCURRENCE OF PARAMPHISTOMUM SP. IN A BISON IN THE NORTHEAST REGION OF THE USA

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Background: Paramphistomosis is an important disease in ruminants responsible for economic losses in tropical and subtropical regions. It is caused by *Paramphistomum* spp., a trematode parasite located in the rumen as an adult, and in the small intestine when immature. Freshwater snails are the intermediate hosts, where it replicates asexually. The ruminant becomes infected via ingestion of encysted metacercariae in the grass.

Objective: Reporting an unusual occurrence of *Paramphistomum* sp. in a bison in the northeast United States.

Methods: A female, nine-month-old, 72 kg bison was submitted to necropsy with a history of severe emaciation and death. Other 9 out of 10 young animals were lost over the past year. Necropsy and histopathology were routinely performed, and feces was submitted for parasitology using Quantitative Sugar and Zinc Sulfate Flotation methods.

Results: The body presented severe emaciation. Fecal flotation revealed severe infection by *Trichuris discolor*, *Capillaria* sp., and Strongyles including *Nematodirus* sp., and mild infection by *Eimeria* spp., supporting a diagnosis of intestinal parasitism. Additionally, attached to the ruminal mucosa and within ruminal contents were dozens of pink, flat, 0.1 x 0.2 cm, ovoid organisms. Histologic examination revealed a trematode attached to a ruminal papilla by its large oral sucker. It has a 10 µm thick tegument, spongy parenchymatous body with paired ceca, vitellaria, and testis.

Conclusions: This calf suffered from severe intestinal parasitism, which was likely the cause of emaciation and death. *Paramphistomum* spp. may have contributed to ill thrift and is an unexpected finding in the northeast United States.

SP-09: COMPARING MITOTIC INDEX TO KI67 IMMUNOLABELING IN CANINE MENINGIOMA

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Background: Meningiomas are the most common primary brain tumor in dogs. Meningiomas arise from arachnoid cap cells and are variably neuroinvasive. In human meningiomas, tumor grade correlates with mitotic index determined by light microscopy or immunohistochemistry for Ki67; however, no equivalent grading scheme exists for canine meningioma.

Objective: This study aims to compare mitotic index determined by light microscopy versus Ki67 immunolabeling in canine meningioma.

Methods: Forty cases of canine meningioma from the Anatomic Pathology archive at Cornell University College of Veterinary Medicine were reviewed histologically. Immunohistochemistry for Ki67 was performed. For histologic analysis, mitotic index was determined manually over ten fields at 400x magnification. Ki67 immunolabeling was assessed over ten fields using proprietary Ventana software scanned at 400x magnification.

Results: Using HE staining, the mitotic index ranged from 0 to 4.2 per HPF (mean=0.88, median=0.65). For Ki67, immunolabeled cells ranged from 0 to 17.8 per HPF (mean=2.82, median=1.7). Immunolabeling for Ki67 resulted in increased detection of mitotically active neoplastic cells than simple mitotic index. In nine cases, immunolabeling revealed a substantial number of mitotically active cells even though no mitotic figures were noted on the corollary HE slide.

Conclusion: Determining mitotically active cells in canine meningioma biopsies with Ki67 is more sensitive than HE based mitotic index. When a grading scheme for canine meningioma is developed, utilizing Ki67 immunohistochemistry rather than mitotic index may provide more nuanced information regarding prognosis.

SP-10: DETECTION OF BOVINE PAPILLOMAVIRUS DNA IN EQUINE CUTANEOUS SCHWANNOMAS AND SARCOIDS

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Compared to sarcoids, which are estimated to account for up to 50 percent of all equid skin lesions, cutaneous schwannomas are considered a rare neoplasm in horses. While classical sarcoids can be easily differentiated from schwannomas by their distinct epidermal-dermal histomorphological features, the absence of rete pegs, frequent ulceration, and high variability in sarcoid dermal components can make this diagnosis challenging. Consequently, polymerase chain reaction (PCR) and in situ hybridization (ISH) assays have been described for detection of Bovine Papillomavirus (BPV) DNA in sarcoids, while positive S100 immunostaining supports a diagnosis of schwannoma. However, recent contradictory reports on the sensitivity and specificity of BPV detection methods prompted reevaluation of the diagnostic utility of these approaches and association between BPV and equine cutaneous schwannonoma. Based on histomorphologic classification, 19 schwannomas and 9 sarcoids were selected from archived formalin-fixed, paraffin wax-embedded (FFPE) surgical tissues for detection of BPV1/2 DNA sequences using ISH and PCR assays. While 9/9 sarcoids had strong BPV1/2 nucleic acids signal by ISH, only 4 were positive by PCR assay (44.4% correlation). By contrast, of the 19 schwannomas, 11 (57.9%) had strong BPV1/2 signal by ISH and none were positive by PCR assay. The data suggests that ISH is more sensitive than PCR for detection of BPV in FFPE tissue samples, and BPV1/2 is more broadly associated with equine cutaneous neoplasms than previously reported.

SP-11: ROLE OF SPI-1 AND SPI-2 (SALMONELLA PATHOGENICITY ISLAND 1 AND 2) OF SALMONELLA TYPHIMURIUM IN ENTERIC COLONIZATION AND SYSTEMIC DISSEMINATION USING THE AVIAN MODEL

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Salmonella Typhimurium has two clinical manifestations: enteritis and systemic infection. Most of the genes necessary for *Salmonella*'s virulence are located in two regions known as *Salmonella* pathogenicity islands 1 and 2 (SPI-1 and SPI-2). SPI-1 allows the bacteria to invade the intestine, while SPI-2 is important for the intracelullar survival and replication, although it is also necessary for the intestinal disease. Poultry is a good experimental model for *Salmonella* infections as it shows the two clinical manifestations.

To assess the role of SPI-1 and SPI-2 in intestinal colonization and systemic dissemination, three groups of fifteen chickens were orally infected with the wild type strain of *S*. Typhimurium, as well as its derivative mutants ΔSPI1 and ΔSPI2. At 24, 48- and 72-hours post infection, five chickens from each group were euthanized and

examined post mortem. Samples of cecum, small intestine, liver, and spleen were taken for histopathological analysis and CFU determination.

Wild type strain was recovered from all the organs, while no colonies were recovered from any of the sampled organs infected with SPI-1 and SPI-2 mutants. The wild strain infected organs showed multifocal hepatic necrosis and periportal lymphocyte infiltrate, fusion and atrophy of intestinal villi, with epithelial hyperplasia and intraluminal bacteria, and granulocyte hyperplasia in the spleen. Those organs infected with the mutant strains did not present any alterations compared to the wild strain infected organs.

Our results confirm the importance of the genes present in pathogenicity islands 1 and 2 for the intestinal colonization and systemic dissemination in chicken

SP-12: COMPARATIVE IMMUNOHISTOCHEMICAL EVALUATION OF PSMA MONOCLONAL ANTIBODIES

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Prostate-specific membrane antigen (PSMA) is a type-II integral membrane protein that is expressed in both normal and malignant prostatic epithelia. In prostate cancer its expression positively correlates with disease stage and progression resulting in strong clinical interest for utilizing PSMA as a diagnostic and/or therapeutic target in cancer management. Despite its name, which suggests exclusive prostatic expression, PSMA is also expressed in other tissues where it has alternate nomenclatures: CNS (NAAG peptidase, NAALADase), GI tract (folate hydrolase; FOLH) and kidney (glutamate carboxypeptidase II; GCPII)]. Outside of the CNS and the prostate, PSMA has been relatively unstudied and discrepancies exist in the literature regarding its normal tissue distribution. Herein, we evaluate 3 monoclonal PSMA antibodies for their cross-species tissue reactivity. Paraffin-embedded sections of healthy renal tissue from Macaca, Canis, Felis and Rattus genera were stained for PSMA using antibodies 1A11, 3E6, D7I8E and signal intensity and tissue localization were compared. All species exhibited the same general PSMA localization, characterized by staining in a subset of renal cortical tubules with minimal-to-absent expression within the glomeruli or medullary structures. However, interspecies variation was noted with intensified signal at the brush border of tubular epithelial cells in feline and rat tissues compared to diffuse cytoplasmic staining of tubular epithelial cells in macaque and canine tissues. Antibodies yielding the most robust and specific signal for each species were as follows: 3E6 (macaque and canine), D7I8E (rat) and 1A11 (feline). Results from this study will inform antibody selection for upcoming PSMA comparative tissue distribution studies.

SP-13: NON-STEROIDAL ANTI-INFLAMMATORY DRUGS REDUCE VAS DEFERENS EPITHELIAL MRP4 EXPRESSION AND PROSTAGLANDIN EXPORT

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Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit prostaglandin (PG) synthesis and can compromise epithelial barriers. The present study attempted to characterize whether NSAID-induced alterations occurred in the male reproductive tract, which typically expresses high levels of PG synthase 2 (PTGS2). Immortalized porcine vas deferens epithelial cells (PVD9902) were grown on Snapwell® permeable supports in the absence or presence of NSAIDs (Ibuprofen, Celecoxib, Indomethacin) and transepithelial electrical resistance (R_{te}) was measured daily. On day 14, NSAIDs were withdrawn, and tests were performed on days 14, 16 and 18. Mucosal and serosal media were harvested to determine PGE2 concentrations. Electrophysiological assessments were conducted followed by harvesting total cell protein and RNA. Monolayers in all conditions developed a 'tight' epithelial barrier with R_{te} > 4000 Ω cm^2. NSAID exposure increased time to half-maximal barrier formation by 13-35 hours, and reduced the overall barrier development rate. Absent stimulation, PG accumulates preferentially in the mucosal medium, although serosal accumulation can be detected. NSAIDs reduced PGE2 in both mucosal and serosal media to undetectable levels. We speculated whether PG export from the cell was due to the multidrug resistance-like protein (MRP4). Immunoblot and RT-PCR confirmed MRP4 expression, which was reversibly reduced by NSAID exposure. Our results show that NSAID exposed male reproductive duct epithelia exhibit decreased PG secretion (> 95%), delayed barrier formation and reduced expression of a major PG export protein. These results suggest that therapeutic NSAID treatments may have a negative effect on male reproductive tracts, thus affecting fertility.

SP-14: PATHOLOGY AND EMERGENCE OF CYTAUXZOON FELIS IN THE SOUTH EASTERN US

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Cytauxzoonosis is an emergent, tick-borne protozoal infection of domestic housecats caused by *Cytauxzoon felis*. Cytauxzoonosis has traditionally been endemic to the southeast United States, although its cause of emergence in recent years has been questioned. This is likely due to the relationship with its tick vector and the widespread distribution of its reservoir host, the bobcat. In this study, we discuss the pathology and emergence of *C. felis* in south eastern US. Two unrelated domestic short hair felines were presented at two different clinics in Kentucky with pyrexia, anemia, lymphopenia, neutropenia, and thrombocytopenia. Both cats died/euthanized despite intensive care and treatment. On necropsy, both cats were markedly icteric, and had marked splenomegaly and lymphadenomegaly. The liver and lung had multifocal tan, and dark red areas, respectively. Histologically, large numbers of schizont-infected macrophages occluded blood vessels leading to thrombosis and ischemic necrosis in multiple organ systems. We also examined the distribution and emergence of *C. felis* through an epidemiological survey. We observed that, in general, case numbers have increased from 2012-2018. More interestingly, we observed that *C. felis* distribution has recently expanded northward into states where the disease has not been previously documented.

SP-15: PATHOLOGIC FEATURES AND GENETIC CHARACTERISTICS OF EXTRA-INTESTINAL PATHOGENIC ESCHERICHIA COLI IN CATS AND DOGS

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Background: Extraintestinal pathogenic Escherichia coli (ExPEC) is a common Gram-negative bacterial pathogen associated with meningitis in infants and septicemia, cystitis, and pneumonia in humans and animals. Cytotoxic necrotizing factor 1 (CNF1) is a conserved virulence factor of ExPEC that allows bacterial penetration to multiple organs and targeted to molecularly identify ExPEC. Little is known about other virulence factors associated with disease in dogs and cats. The aim of this study was to define the epidemiology and pathology of ExPEC and identify genotypic characteristics of ExPEC in dogs and cats.

Methods: Isolates of ExPEC from necropsied dogs and cats were evaluated via PCR for the virulence factors known to be associated with bacterial isolates of human ExPEC. Clinical data and client history were used to define the prevalence and epidemiology. Tissues were collected to characterize the pathology associated with infection.

Results: Forty-four dogs and eleven cats necropsied in 2013-2019 at MSU VDL were diagnosed with ExPEC. These animals ranged from 0-days-old to 14-years-old with a median age of 2 months. Most pathologic lesions were observed in the lungs followed by brain and liver. Over 80% of bacterial isolates evaluated expressed virulence genes including *papGIII*, *hlyD*, *hlyC-A*, *sfa*, *fimH*, and *malX.*

Conclusion: In ExPEC infected animals, pneumonia was commonly diagnosed and often associated with degrees of necrosis. This study demonstrates the majority of virulence factors associated with human ExPEC infections can be detected in companion animal isolates. Preliminary data provides evidence for shared microbial genetics in ExPEC affecting companion animals and humans.

SP-16: OPHIDIOMYCES OPHIODIICOLA EPIZOOTIC IN A CAPTIVE COLLECTION OF SNAKES IN ARIZONA

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Background: In May 2018, a sanctuary received a snake collection including 42 *Lachesis* spp. adults and 24 *Lachesis melanocephala* eggs from North Carolina. A received *L. melanocephala* laid an additional 12 eggs. Between October 2018 and February 2019, hatchlings (n=25) developed lethargy, anorexia, crusting dermatitis, pit organ swelling, and death (100%). Adults remained asymptomatic. Three months after the first bushmaster hatchling and over the course of six months, skin lesions (crusting dermatitis and subcutaneous nodules) developed in 18 snakes from 12 species.

Results: Antemortem skin swabs and postmortem affected skin were PCR positive for *Ophidiomyces ophiodiicola* in 100% of bushmasters (5/5) and in 15 other snakes. Thirteen postmortem exams (10 dead; 3 euthanized) documented fungal dermatitis, and bacteriology and mycology of skin and liver from 6 snakes yielded 33 bacteria and > 6 other fungi suggesting co-infections. Two snakes had concomitant fungal pneumonia consistent with *O. ophiodiicola*. Three bushmasters had granulomatous hepatitis that was PCR negative for *O. ophiodiicola* in two that were tested.

Conclusion: Death was attributed to combined septicemia, nephrosis, pneumonia, and fungemia. *Ophidiomyces ophiodiicola* compromised the epithelial barrier and favored opportunistic co-infections, while pulmonary and systemic ophidiomycosis was uncommon (4 snakes). This epizootic is ongoing and surviving snakes are receiving antifungal therapy. This is the first report of *O. ophiodiicola* in Arizona and the first time diagnosed in 12 novel species. It is hypothesized that the fungus entered the collection with an animal or fomite from NC, reaffirming the risk of the anthropogenic spread of emerging pathogens.

SP-17: CHARACTERIZING THE EXPRESSION AND FUNCTIONALITY OF ASPH IN MURINE AND CANINE OSTEOSARCOMA

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Background: Aspartate β-hydroxylase (ASPH) is a highly conserved, Notch pathway-activating enzyme implicated in cancer progression. ASPH is also overexpressed in numerous human tumor histologies. Given the overall lack of effective therapeutics for osteosarcoma (OS), the similarities observed in both canine and human OS tumorigenesis, and the conserved nature of ASPH, we chose to investigate whether ASPH may be a druggable therapeutic target for OS.

Objective: To characterize the expression of ASPH in OS and investigate whether small molecule inhibitors of ASPH influence cell viability and migration in OS cell lines.

Methods: Basal levels of ASPH were characterized in murine and canine OS cell lines (n=4) via western blot analysis. *In vitro* experiments assessing inhibition of cell viability (MTS reduction) and cell migration (scratch assays) with ASPH inhibitors were also performed.

Results: ASPH exhibited differential protein expression between cell lines, with the most abundant expression noted in the less aggressive murine OS cell line. All other OS lines investigated showed little to no ASPH expression. High levels of ASPH inhibitor treatment significantly decreased cellular viability in a subset of canine OS. The scratch assay noted negligible difference between cells treated with inhibitor and those without.

Conclusions: ASPH expression may be inversely correlated with OS tumor aggressiveness and ASPH inhibition does not consistently decrease OS function *in*

vitro. While these findings suggest ASPH may not be a therapeutic target for OS, further investigation into an expanded panel of OS cell lines and evaluation of ASPH expression in spontaneous canine OS is warranted.

SP-18: HISTOPATHOLOGICAL CHARACTERIZATION AND LIFE-CYCLE ELUCIDATION OF TREMATODES CAUSING OCULAR DIPLOSTOMIASIS IN ICTALURID CATFISH

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Farm-raised catfish remains the largest sector of the United States' aquaculture industry. Predation by piscivorous birds and infections with their trematode parasites represent significant threats to the sustainability of the industry. These trematodes develop in avian, molluscan and fish hosts; many of which have unknown life-cycles which can preclude targeted control. Ocular diplostomiasis in fish is associated with multiple trematode genera, cataractous changes, and impaired vision. Two novel diplostomids in the genera *Bursatintinnabulus* and *Bursacetabulus* from the intestine of American white pelicans, *Pelecanus erythrorhynchos,* were morphologically and molecularly characterized. Larval cercariae of these species and *Austrodiplostomum compactum* were collected from naturally infected *Biomphalaria havanensis* snails and used in experimental infections of fingerling channel catfish *Ictalurus punctatus*. Fish were sampled at 7, 21, and 35 days post infection (dpi) to evaluate infectivity and pathogenicity using histopathology. By 7 dpi, *Bursatintinnabulus* metacercariae were evident in the ventricular system of the brain and *Bursacetabulus* and *A. compactum* metacercariae were present in the eyes. At 21 dpi, all species were evident in the eyes, with marked inflammation associated with *A. compactum*. Mild ependymitis was evident in the brain of *Bursatintinnabulus* infected fish. Metacercariae numbers and ocular inflammation increased by 35 dpi; *A. compactum* infected fish had severe anterior uveitis with accumulation of parasites in the iridocorneal angles. Inflammation was associated with migration of the annular ligament across the inner surface of the cornea, anterior synechia, hyalitis, and dorsal loss of pigmented epithelium with melanosis. This data suggest ocular diplostomiasis in catfish warrants further study.

SP-19: BILATERAL PYOSALPINX IN 2 JUVENILE CATS

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Pyosalpinx is an uncommon condition that is most frequently noted in the bovine and porcine species, and rarely in other domestic animals. Pyosalpinx often occurs secondary to metritis, ovarian manipulation, neoplasms, or luminal obstruction. The current report documents 2 juvenile cats with bilateral pyosalpinx that were incidentally found during elective ovariohysterectomy surgery. Both cats were estimated to be roughly 12 weeks old, resided in different animal shelters, and were clinically normal at the time of surgery, through the MSU-CVM spay and neuter community service. Gross

lesions in both cats comprised of marked bilateral uterine tube ectasia that were filled with purulent material, associated with dark red velvety vascular proliferation along the broad ligament and the serosa. Chronic bilateral suppurative and lymphoplasmacytic salpingitis, perisalpingitis, and peritonitis were present in both cases, with numerous intralesional gram-negative rod-shaped bacteria and proteinaceous exudate. Moderate endometritis was only noted in one cat, and ovaries of both animals were unaffected. Bacterial culture yielded *Escherichia coli* in one cat, and the same cat developed unilateral pyonephrosis 5 weeks after the initial surgery. This finding suggests the salpingitis may have originated from a lower urinary tract infection. No other pathology associated with the reproductive tract or underlying immunosuppressive condition was noted, and both cats recovered with antimicrobial therapy and surgical intervention. To the authors' knowledge, there have been no prior documented cases of pyosalpinx in feline species.

SP-20: IMMUNE INFILTRATION OF OSTEOSARCOMA AND METASTASIS IN A GENETICALLY ENGINEERED MOUSE MODEL

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Osteosarcoma (OSA) is a malignant neoplasia of bone that naturally occurs in humans and large dog breeds. Genetic and phenotypic presentation of OSA are similar in humans and canines suggesting relevance of intraspecies studies. Prognosis in canines is poor and have not seen improvement in recent decades. Success of immune therapy in other cancers has sparked an interest in applying it to osteosarcoma. Immune infiltration of tumors is important for cell mediated control and targetability and have prognostic capabilities. Our study aims to gain an understanding of immune infiltration of OSA and lung metastasis in development to improve treatment.

OSA is notoriously resistant to immune infiltration, inhibiting the effectiveness of immunotherapy, however there have been mixed success in trials suggesting a greater need to understand the role of the immune system in OSA. We purpose to examine the immune infiltration of osteosarcoma using a Genetically Engineered Mouse Model (GEMM), SP7.Cre x p53Flux. Using immunohistochemistry, we stained primary tumor and lung metastases from spontaneous arising OSA. We found differences in T-cell ($CD4^+$ and $CD3^+$) infiltrate by region, the greatest occurring in primary facial and pelvic osteosarcoma. Additional markers included macrophage ($CD68^+$) and myeloid ($CD11b^+$). We found no significant differences between primary osteosarcoma and lung metastasis, while differences have been noted in specific regions of osteosarcoma by phenotype.

SP-21: INTRAOCULAR OSTEOSARCOMA IN A RABBIT (ORYCTOLAGUS CUNICULUS)

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An 8-year-and-9-month-old, male, lop-ear rabbit (*Oryctolagus cuniculus*) presented with gradual enlargement and exophthalmos of the left eye over a three month period followed by spontaneous death. At necropsy, the left eye measured 4 × 4 × 4 cm, and the cornea was diffusely covered by a thick layer of crust. On cross section, the intraocular tissue was diffusely effaced and occupied by a hard, light gray mass. Histologically, the intraocular mass comprised a highly cellular, invasive neoplasm composed of spindle to angular cells arranged in interlacing bundles with abundant production of osteoid and cartilage. The neoplastic cells had small amounts of eosinophilic cytoplasm with indistinct cell borders. Nuclei were round to oval with coarsely stippled chromatin and distinct nucleoli. Anysocytosis and anisokaryosis were moderate and there were 20 mitoses per ten 400× fields. These histological findings were consistent with an osteosarcoma. Although the neoplasm showed invasion over the sclera, there was no connection between the neoplasm and the orbit. No metastatic lesions of the osteosarcoma were detected. Other representative necropsy findings included multiple masses in all lung lobes; the diagnosis of a neuroendocrine carcinoma was made histologically for all lung masses. Intraocular osteosarcomas are extremely rare in domestic animals and very few cases have been reported. To the best of our knowledge, this is the first time intraocular osteosarcoma has been documented in a rabbit.

SP-22: MALIGNANT MESENCHYMAL TUMORS OF THE SKIN IN PET HAMSTERS; A RETROSPECTIVE STUDY

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Hamsters, which are popular pets all over the world, have a relatively high incidence of neoplasia. The purpose of this study was to characterize common malignant mesenchymal tumors of the skin in pet hamsters, and to describe the histopathologic findings of each tumor. We characterized 18 tumors from 13 Russian hamsters (*Phodopus sungorus*) and five Syrian hamsters (*Mesocricetus auratus*) that were submitted for histopathologic examination from 2014 to 2019. H&E sections were reviewed by all authors and additional staining using special stains and immunohistochemistry analysis were performed as necessary. In Russian hamsters, the most common tumor was soft tissue sarcoma (n = 10), which included fibrosarcoma (n = 5), pleomorphic sarcoma (n = 2), malignant peripheral nerve sheath tumor (n = 2) and liposarcoma (n = 1). Other infrequent tumors consisted of histiocytic sarcoma (n = 1), extraskeletal osteosarcoma (n = 1), and rhabdomyosarcoma (n = 1). The mean and median age of onset was 18.4 months and 20 months, respectively. In Syrian hamsters, the all malignant mesenchymal tumors were soft tissue sarcomas (n = 5), including myxosarcoma (n = 2), fibrosarcoma (n = 1) and pleomorphic sarcoma (n = 1). The mean and median age of onset was 20.3 months and 24 months, respectively. There was a bias toward males in this study (11 males, six females, and one unknown). To the best of our knowledge, this is the first pathologic study of skin sarcomas in domestic hamsters.

SP-23: RETROSPECTIVE ANALYSIS OF HISTOLOGIC LESIONS IN CAPTIVE ARACHNIDS FROM NORTH CAROLINA

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Invertebrate medicine is an emerging veterinary subspecialty, with arachnids being a common invertebrate taxon kept in zoologic collections. However, there are few published reports detailing causes of morbidity and mortality. We reviewed the histopathology from postmortem and biopsy tissue submissions from 21 captive arachnids (17 tarantulas and four scorpions) from three institutions in North Carolina over nine years. Lesions were most common in midgut diverticula (18/21), skeletal muscle (13/21), book lung (12/21) circulatory sinuses (11/21), heart (10/21), midgut (9/21), central nervous system (9/21), chitin (8/21), and circulatory vessels (8/21). Lesions were uncommon in the foregut, Malpighian tubules, coxal gland, or silk gland. Inflammation was most common in the midgut divertica (14/21), skeletal muscle (11/21), and book lung (11/21). Inflammation was often associated with infectious agents, including fungi (9/21), bacteria (6/21), nematodes (1/21), or arthropod parasites (1/21). Fungal and/or bacterial septicemia were suspected as contributing to death in 6/21 cases. Inflammatory foci surrounding infectious agents were often associated with melanin pigment deposition. Depleted eosinophilic globules in the midgut diverticula interstitial cells (8/21) and skeletal muscle atrophy (6/21) suggested decreased nutritional status, and were present in conjunction with infection, pregnancy, or occasionally in isolation. Other contributors to death included molting-associated exoskeleton trauma. No animals had evidence of neoplasia. In conclusion, infectious agents were common contributors to morbidity and mortality, and studies are needed to characterize these agents in arachnids.

SP-24: CNS ASPERGILLOSIS IN A 4 YEAR OLD GERMAN SHEPARD DOG

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Case Description: A 4 year old spayed female GSD presented for ataxia and was diagnosed with presumptive central nervous system aspergillosis. Despite a grave prognosis, long term anti-fungal therapy successfully improved the dog's symptoms.

Clinical Findings: On neurologic exam, a right-sided head tilt was noted along with ataxia in all four limbs, with the forelimbs being more effected than the pelvic limbs. MRI showed multifocal midbrain/brainstem lesions consistent with inflammation or edema. CSF analysis revealed a mixed cell pleocytosis (WBC= 111/ul, RI=0-3) with a significant eosinophilia. Urinalysis demonstrated mixed neutrophilic and eosinophilic inflammation with many fungal hyphae. Given the breed, systemic aspergillosis was suspected. Urine culture yielded *Aspergillus spp*., verified by fungal 28S PCR.

Treatment and Outcome: Amphotericin B was administered IV, and the patient was discharged with oral terbinafine and posaconazole. To date, the patient continues to improve and her neurological signs have resolved.

Clinical Relevance: *Aspergillus*spp. is a fungus ubiquitous in the environment. With German Shepard Dogs specifically, aspergillosis can disseminate systemically, possibly due to an inherited deficiency in IgA and mucosal immunity. The prognosis for CNS aspergillosis is grave, and treatment requires long-term anti-fungal therapy. The authors are unaware of prior reports of eosinophilic pleocytosis with CNS aspergillosis. This case also demonstrates the importance of a minimum diagnostic database, as the hyphae in the urine identified the presumptive causative agent and directed further testing and treatment.

SP-25: INVESTIGATING THE PREVALENCE OF GERMLINE METHYLATION OF BRCA1, BRCA2, AND ATM IN PATIENTS WITH FAMILIAL PANCREATIC DUCTAL ADENOCARCINOMA

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Pancreatic cancer is a devastating disease with few treatment options and high mortality, with pancreatic ductal adenocarcinomas being the most common pancreatic malignancy. Up to 10% of pancreatic ductal adenocarcinomas are identified as familial, meaning two or more first-degree relatives are affected, but only a fraction of those are caused by inherited pathogenic variants in established pancreatic cancer predisposition genes such as *BRCA1*, *BRCA2*, and *ATM*. Recently, an inherited non-coding region variant that results in methylation-associated silencing of *BRCA1* has been identified in two families with hereditary breast and ovarian cancer syndrome. We hypothesized that a similar mechanism would account for a proportion of patients with familial pancreatic ductal adenocarcinoma previously unexplained by pathogenic variants in the coding region of established pancreatic cancer predisposition genes. To investigate the prevalence of CpG island methylation in *ATM*, *BRCA1*, and *BRCA2*, peripheral blood lymphocyte DNA from 81 patients in the National Familial Pancreas Tumor Registry was digested with methylation-dependent and methylation-sensitive enzymes and subsequently quantified by qPCR with gene CpG island specific primer pairs using the Qiagen Epitect Methyl II PCR Assay kit. All of the samples were >96% unmethylated at each CpG island tested. A total of 700 patients and 300 control DNA samples will be assayed going forward to give 80% power to detect CpG island methylation at 1% in patients and 90% in controls assuming a type 1 error rate of 0.05%.

SP-26: HISTOPATHOLOGY OF RAT VOCAL FOLDS FOLLOWING SYSTEMIC DEHYDRATION

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Hydration treatments are frequently recommended for optimal voice even though the impact of dehydration on the vocal folds is not fully understood. Body weight loss due to water withholding is a common measurement of dehydration. However, laboratory rats have been shown to lose weight due to the stress of handling alone. The objective of this study was to: 1) determine adjunct biomarkers of systemic dehydration; and 2) determine if hydration state alters vocal fold histopathology. The dehydration biomarkers tested included hematocrit, osmolality, packed cell volume (PCV), and renal renin mRNA expression and protein synthesis. The dehydration biomarkers of hematocrit, osmolality, and PCV were subject to too much variability to be utilized. Renal renin mRNA expression and protein synthesis were significantly increased in dehydrated rats. To determine histopathologic changes in the vocal fold tissues of rats, larynges were prepared for histological staining with hematoxylin and eosin (HE), Masson's trichrome, Verhoeff-van Gieson, and Alcian Blue (pH 2.5) pre- and post-hyaluronidase incubation. Dehydration did not significantly affect tissue morphology based on an adapted semi-quantitative assessment. Quantitative histopathologic analysis of vocal folds showed significantly lower levels of hyaluronan present in dehydrated rat vocal folds and a quantifiable increase in percent stained area in the vocal fold lamina propria. This increase in percent stained area suggests that dehydration decreased the non-stained (i.e. fluid) area of the lamina propria. Collectively, these results indicate that systemic dehydration may induce changes in the rat vocal folds that potentially affect their function.

SP-27: MICRORNA EXPRESSION AS A DIAGNOSTIC TOOL FOR UROTHELIAL CARCINOMA IN CANINE URINE SEDIMENT

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Urothelial carcinoma (UC) is a malignant tumor that develops from the epithelial cells that line the urinary tract, accounting for approximately 2% of all tumors in dogs. Most dogs develop the high grade, invasive form of UC that rapidly proliferates and has a high metastatic potential. Dogs with UC typically present with clinical sign of similar to those from inflammatory and infectious lower urinary tract disease (LUTD). Many cases of UC also have an inflammatory component. In the presence of inflammation, cytology of the urine sediment cannot differentiate reactive from neoplastic transitional cells. Tissue biopsy via surgery, cystoscopy, or urinary catheter is the gold standard for the diagnosis; these are invasive and expensive exams. A potential non-invasive alternative to diagnose UC is through microRNA expression signatures in biofluids, such as urine. In this study, urine sediment samples were classified as UC or LUTD based on the patient's medical records. MicroRNA was extracted, and spectrophotometry was used to determine concentration and purity. The average total amount obtained was 219 ng/µL (SD± 162.3). RT-PCR was performed to assess the expression of microRNAs that may serve as biomarkers for a non-invasive diagnostic test for UC. RT-qPCR revealed upregulation of mir-34 in UC compared to LUTD samples. No significant changes were detected in let-7a, let-7b, let-7c, and let-7e expression. Additional microRNA markers and samples are being tested to add to these results. This test could

be performed at first presentation when dogs are suspect of having UC by examination of urine sediment.

SP-28: HEMOTROPIC MYCOPLASMAS IN WILD BOARS, HUNTING DOGS, AND HUNTERS IN BRAZIL

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Background: Hemotropic mycoplasmas are small pleomorphic bacteria that parasitize erythrocytes of several mammalian species, including humans. The risk of exposure of these bacteria amongst humans and animals, especially if sharing environments with high presence of ticks, is of public health concern.

Objectives: The purpose of this study was to determine the potential risk of transmission between populations of wild boars, hunting dogs, and hunters from Brazil.

Methods: Blood samples were collected from 65 free-range wild boars, 165 hunting dogs, and 25 hunters from southern and central-western Brazil (Atlantic Forest and Cerrado biomes). DNA was extracted using the Blood Genomicprep Mini Spin kit (Promega). A Pan-qPCR developed to detect hemotropic mycoplasma was performed. Conventional PCR followed by sequencing was carried out for 15 of the positive samples by qPCR to identify the specific mycoplasma species within the samples.

Results: A total of 36/65 (55%) of wild boars and 85/164 (52%) of dogs were positive (Ct≤ 30) for at least one mycoplasma species by qPCR. The human samples were all negative 0/25 (0%). Sequencing of four wild boars samples identified *Mycoplasma suis* (three samples) and *Mycoplasma parvum* (one sample). Five hunting dog samples sequenced were identified as *Mycoplasma haemocanis*.

Conclusion: Although exposure to the mycoplasma species is present, the study revealed there is no evidence to suggest they were transmissible amongst the population of wild boars, hunting dogs, and hunters tested. Additional samples will be analyzed to explore the possibility of infection across species.

SP-29: MENINGITIS ASSOCIATED WITH DRACUNCULID NEMATODES IN A SPOTTED EAGLE RAY (AETOBATUS NARINARI) ON ST. KITTS

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This study describes a case of meningitis in a spotted eagle ray (*Aetobatus narinari*) associated with dracunculid nematodes. The ray was found dead on the beach of Dieppe bay, St. Kitts. Postmortem examination showed poor body condition, hepatic atrophy, empty gastrointestinal tract, and around 100mL of serosanginous fluid within

the cranium. Analysis of cranial fluid showed the majority of cells were erythrocytes with a few leukocytes, consistent with hemorrhage or blood contamination. Histopathology demonstrated regional mild to moderate increase in mononuclear leukocytes associated with occasional nematodes in the meninges of the caudal mesencephalon, cerebellum, and brain stem. Nematodes were within venous lumina and meningeal stroma, were 10-20 um diameter, and had prominent bright eosinophilic digestive tracts, consistent with sprirurids. Sequencing of the 18S small subunit ribosomal DNA revealed a 94% identity with *Philonema oncorhynchi* (order Spirurida; superfamily Dracunculoidea; family Philometridae). Philometrids are of the most important dracunculoid nematodes of fishes and typically parasitize gonad, but meningeal spirurid infections have also been documented in nurse sharks (*Ginglymostoma cirratum*). This case adds to the limited data addressing pathology and phylogeny of spirurids in elasmobranchs and is exceptional because of the involvement of a batoid host.

SP-30: PATHOLOGY SURVEY OF CALLINECTES SAPIDUS ON ST. KITTS

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The blue crab *Callinectes sapidus* is endemic to the western Atlantic seaboard and comprises one of the most valuable fisheries of the United States. Yet, little is known regarding populations in the Eastern Caribbean. The aim of this survey is to describe the lesions present in crabs fished on St. Kitts. Fifty-one crabs, representing three females and 48 males with mean (std) carapace width of 11.0 (+/-1.0) cm, were harvested from two salt ponds using baited crab traps from November 2018 to March 2019. Comprehensive postmortem examinations were conducted, and tissues were preserved in Davidson's solution and processed routinely for histopathologic examination. Lesions were identified in 23/40 (58%) of crabs and included cuticle degeneration or ulceration (i.e. shell disease, 31%), lamellar *Lagenophrys* sp. infestation (34%), skeletal muscle degeneration (7%), lamellar nematodes (5%), cardiac hemocytosis (5%), and excretory calcinosis (2%). Apart from shell disease, lesions were seldom grossly apparent. Systemic reserve inclusion cell hypertrophy and hepatopancreas epithelial calcium spherules were commonly present. Lesions to indicate the presence of commercially important viral or protist diseases known to impact *Callinectes* in North America were otherwise lacking. It is hoped that baseline pathology data will facilitate *Callinectes* mortality investigations in the Eastern Caribbean.

SP-31: PREVALENCE AND DEGREE OF HCT/PCV MISMATCH IN PATIENTS WITH ABNORMAL SERUM SODIUM CONCENTRATION

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Abnormal serum sodium concentrations can be associated with artefactual changes in RBC values, including mean cell volume (MCV), hematocrit (HCT), mean cell hemoglobin concentration (MCHC) when determined by automated haematology analysers, which can interfere with clinician's interpretation of the hemogram.

Blood samples collected from cats (n=2154) and dogs (n=5379) that were presented to the Royal Veterinary College's Queen Mother Hospital for Animals were categorised based on hyponatremia, normonatremia and hypernatremia. Mismatch was calculated by the difference between values of packed cell volume (PCV) and automated hematocrit (HCT).

There was a significant mean difference in HCT/PCV mismatch between hyponatremic, normonatremic and hypernatremic dogs ($P<0.001$) and similarly in cats ($P<0.001$). It was found that there is a statistically significant weak correlation between sodium levels and mismatch in normonatemic and hypernatremic cats and dogs. However, the correlation in hyponatremic cats and dogs there was not statistically significant. Reference intervals (RI) for the difference between PCV and HCT were established in normonatremic dogs and cats with PCV values within RI. Prevalence of hyponatraemic and hypernatraemic cases in which the mismatch fell outside these reference limits were calculated and the prevalence was found to be low.

The study concludes that significant difference in HCT/PCV mismatch is present between hyponatraemic, normonatraemic and hypernatraemic states. However, the weak correlation makes it hard to quantify the true proportion of the mismatch according to sodium levels. Surprisingly, there was low prevalence where the mismatch was prominent, suggesting that there are other confounding factors that may affect HCT/PCV mismatch.

SP-32: PATHOGEN SURVEILLANCE AND BLOOD-MEAL ANALYSIS OF CULEX QUINQUEFASCIATUS IN ST. GEORGE PARISH, GRENADA, WEST INDIES

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Background: The *Culex quinquefasciatus*, commonly known as the southern house mosquito, was introduced to Grenada and the Caribbean from the southern USA. Because there is a dearth of information known about this important disease vector in Grenada, its feeding range and pathogen burden are under investigation.

Methods and Results: During 2017 and 2019, mosquitoes were collected using BG-Sentinel traps at various sites in St. George Parish, Grenada, West Indies. After morphological identification and sex determination, *C. quinquefasciatus* females were used for further testing. Using previously validated primers, PCR was conducted on the DNA extracted from single blood-fed mosquitoes to determine the vertebrate hosts on which the mosquitoes fed. Reverse-transcriptase PCR was conducted on the RNA extracted from pools of up to fifty female mosquitoes to detect arboviruses. Sanger sequencing of potential positive amplicons and comparison of sequences to those in NIH GenBank were conducted to determine blood-meal hosts and to ascertain the prevalence of disease-causing agents. Findings indicate that blood-fed *C. quinquefasciatus* in Grenada received most of its blood meals from indigenous birds, such as the eared dove, curved-bill thrasher, bananaquit, Carib grackle, and house wren. To date, no viruses have been conclusively detected by RT-PCR, but analysis is ongoing.

Conclusion: Based on the blood-meal analysis, the *C. quinquefasciatus* predominantly fed on indigenous birds. Moreover, research is ongoing in order to confirm whether the local *C. quinquefasciatus* harbors pathogens (e.g. *Dirofilaria immitis*, flaviviruses, *Plasmodium*spp.) that cause disease and death to animals and humans in Grenada.

SP-33: PSEUDOMONAS LUTEOLA INFECTION IN A DOMESTIC SHORTHAIR

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An 11-month-old, spayed female domestic shorthair cat was presented for investigation of a left ventral abdominal mass 1 inch in diameter with draining tracts and ulcerations on its surface. Previous cytology and histopathology revealed organisms 12µm in diameter with a clear, colorless capsule and a small (2µm by 4-6µm), dark oval nucleus, resulting in a severe, chronic, pyogranulomatous dermatitis and panniculitis. These unusual organisms were cautiously interpreted by numerous pathologists as fungi, possibly an atypical presentation of *Cryptococcus* or *Sporothrix* spp. Fungal culture of the organism yielded no growth of pathogenic fungus after three weeks. Panfungal PCR was negative for fungal DNA. Due to the unclear appearance of the organisms observed histologically, as well as the negative fungal PCR and culture results, PCR targeting the bacterial 16S rRNA region was performed. DNA extracted from formalin-fixed paraffin-embedded tissue was used as template in 16S PCR. Gel electrophoresis yielded a single band at around 500 bp, and PCR product was sequenced. The resulting sequence was queried with the NCBI BLAST database. The 357 bp sequence matched *Pseudomonas luteola* with 100% identity. Previously, *Pseudomonas luteola* organisms with similar morphology to those observed in this cat have been described in ferrets as the causative agent of pleuropneumonia and mediastinitis as well as panniculitis. *Pseudomonas luteola* may be interpreted incorrectly as fungal organisms, given that the capsule surrounding the organisms stains strongly positive with PAS stain. This report expands the list of species in which infection with *Pseudomonas luteola* may occur.

SP-34: SYSTEMIC TOXOPLASMOSIS IN A HORSE

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An adult American Quarter Horse gelding with a history of weight loss presented with an acute onset of colic, fever, soft feces, and elevated liver enzymes. Following euthanasia, a necropsy performed by the referring veterinarian revealed necrosis of the cecum and masses on the gastric serosa. Histologically, the masses were lymph nodes with granulomatous inflammation and large areas of liquefactive necrosis. Within and surrounding necrotic areas, were free and intrahistiocytic clusters of basophilic, round to ovoid, 2 µm protozoal tachyzoites. Similar but milder inflammation was evident in the spleen, lung, and liver. Necrotizing typhlitis was evident, but other parts of the gastrointestinal tract were within normal limits. Tachyzoites were observed only in the

lymph nodes and lung. Immunohistochemical staining for *Toxoplasma gondii* was positive, and staining for *Neospora caninum* was negative. *Toxoplasma gondii* infects humans and a wide variety of animal species. Although studies have shown seropositivity to *Toxoplasma gondii* in horses throughout the world, clinical toxoplasmosis is extremely rare in this species. To the best of the authors' knowledge, this is the first reported case.

SP-35: MENINGOENCEPHALITIS IN A GRAY FOX DUE TO CONCURRENT CANINE DISTEMPER VIRUS INFECTION AND PUTATIVE SARCOCYSTIS SPP. INFECTION

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A 9-week-old male gray fox (*Urocyon cinereoargenteus*) died after a brief history of decreased ambulation and diarrhea. Significant gross findings were not evident at necropsy. Histologically, the cerebrum had mild, multifocal, necrotizing meningoencephalitis. Affected areas of the brain had gliosis, a sparse mononuclear inflammatory cell infiltrate, occasional protozoal schizonts and merozoites, and both intracytoplasmic and intranuclear viral inclusion bodies in the glial cells. The cerebellum and brain stem were within normal limits. The fox also had mild pneumonia with intralesional fungi consistent with Histoplasma capsulatum. Immunohistochemistry (IHC) for canine distemper virus in the cerebrum was positive, while IHC for *Sarcocystis neurona* was negative. The morphology of the protozoa was consistent with *Sarcocystis* spp., and *Sarcocystis canis* or a novel *Sarcocystis* species was suspected. *Sarcocystosis* is rare in canids. Both *S. neurona* and *S. canis* have been reported in domestic dogs, but this is apparently the first report of sarcocystosis in a fox. Immunosuppression induced by canine distemper virus infection likely predisposed the animal to *Sarcocystis* infection.

SP-36: SUBCUTANEOUS IMPLANTATION OF HEPATOCELLULAR CARCINOMA IN A DOG

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A liver mass was discovered on abdominal ultrasound in a 15-year old male castrated Cocker Spaniel mix, and a fine needle aspirate indicated hepatocellular neoplasia. The mass was later surgically resected and diagnosed as hepatocellular carcinoma. Two years later, a subcutaneous mass was discovered at the cranial end of the abdominal surgical incision. Biopsy of this mass was also consistent with hepatocellular carcinoma. HepPar and cytokeratin immunohistochemistry were both positive, confirming the cells were of hepatocyte origin. This mass could have seeded from the initial fine needle aspirate, could have implanted during the surgical resection of the primary tumor, or represent true metastasis. True metastasis of hepatocellular carcinoma in dogs is rare and tends to occur to lymph nodes, lungs, and the gastrointestinal tract. Seeding of hepatocellular carcinoma following percutaneous aspiration or needle biopsy has been

reported in humans, but it has not been reported in dogs to the best of the authors' knowledge.

SP-37: SPECIFIC IN SITU DETECTION OF TRYPANOSOMA CRUZI IN CHAGAS DISEASE

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Trypanosoma cruzi is the causative agent of Chagas disease and affects nearly 8 million people worldwide. Cardiac Chagas disease occurs when the parasite changes from a blood (trypomastigote) to tissue (amastigote) form and infiltrates the heart, forming pseudocysts within cardiomyocytes. The inflammatory response is lymphoplasmacytic myocarditis. However, lymphoplasmacytic myocarditis is sometimes observed without pseudocysts, making the confirmation of a clinical diagnosis via histology difficult. The purpose of this study was to: (1) develop a specific *in situ* technique to identify amastigotes for use as a diagnostic tool, and (2) investigate the presence and distribution of *T. cruzi* amastigotes in the tissue of animals with Chagas disease. We performed a fluorescent in situ hybridization (FISH) assay using an oligonucleotide probe specific to the *T. cruzi* kinetoplast and identified *T. cruzi* DNA within the cardiac tissue of fifteen dogs, a coyote, and a horse previously diagnosed with Chagas disease by histology, PCR, or indirect-fluorescent antibody assay. All dogs had lymphoplasmacytic myocarditis; ten dogs had readily visible amastigotes seen on H&E, and five had no visible amastigotes on H&E. We also determined our probe's specificity for *T. cruzi* by confirming a lack of cross-reactivity against other protozoa including *Leishmania* sp., *Neospora* sp., *Sarcocystis neurona*, *Trypanosoma evansi*, and *Toxoplasma* sp. This study will allow for further exploration of *Trypanosoma cruzi* infections in both domestic and wildlife populations.

SP-38: THE ASSOCIATION BETWEEN ACUTE INFLAMMATION AND REDUCED ANTITHROMBIN IN CATS

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Reduced concentrations of the endogenous anticoagulant antithrombin increases the risk of thrombosis. In most species, antithrombin is a negative acute phase protein that is downregulated during inflammation but this is disputed in cats. Antithrombin has been recommended as a marker of disseminated intravascular coagulation in cats, but to avoid misdiagnosis of consumptive coagulopathy, it is important to determine if inflammation is associated with reduced antithrombin in cats. In this case-control study, electronic medical records were searched to identify cats with below reference interval antithrombin (n = 77) and breed-matched controls (n = 154) with within or above reference interval antithrombin that presented to a referral hospital between 2009 and 2019. Acute inflammation was considered present if band neutrophils were above reference interval, and using pre-specified criteria, cats were classified as affected or unaffected by other conditions known to reduce antithrombin: severe blood loss, synthetic liver failure, and protein losing disorders. Increased band neutrophils were positively associated with low antithrombin (odds ratio (OR): 2.9, 95% confidence

interval (CI) 1.4-5.9, P = 0.005), as was consumptive coagulopathy (OR: 3.4, CI: 1.8-6.6, P = 0.0002) and severe blood loss (OR: 3.2, CI: 1.2-8.7, P = 0.02). Protein losing disorders were not significantly associated with low antithrombin (OR: 0.4, CI: 0.1-1.1, P = 0.069), possibly reflecting difficulty in identifying protein loss retrospectively. These findings provide evidence for an association between acute inflammation and reduced antithrombin, although further prospective studies are needed to definitively determine if antithrombin is a negative acute phase protein in cats.

SP-39: SEPTICEMIA/ENDOTOXEMIA SECONDARY TO FIBRONECROTIZING CLOSTRIDIAL ENTEROCOLITIS AND COELOMITIS IN AN EMU

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A 25-year-old emu (*Dromaius novaehollandiae)* presented for necropsy with a history of lethargy and anorexia. During gross examination, a moderate amount of tan to green, mucoid, opaque, foul smelling fluid was found within the coelomic cavity coating the serosal surfaces. The large intestine was diffusely dilated and filled with dark red fluid and strands of fibrin. A thick yellow to green, opaque, friable diphtheritic membrane covered the diffusely rough, thickened, dull mucosa of the small intestine and most severely, the colon. The distal colon was diffusely, markedly distended and focally contained a 50x7x5 cm, semi-firm accumulation of feces coated by a 4-5 mm diameter layer of fibrin located 21 cm from the opening of the cloaca suspected to be an impaction. Histologically, the mucosa was diffusely ulcerated and replaced by a dense accumulation of fibrin, necrotic debris, and degenerate heterophils. Gram-positive bacilli were diffusely distributed throughout the necrotic mucosa. The definitive diagnosis of *C. difficile* was achieved by detection of A/B toxins in the intestine. This report describes a case of fibrinonecrotizing enterocolitis and coelomitis with impaction secondary to *Clostridioides difficile* infection. Ratites are predisposed to gastrointestinal impaction due to an indiscriminate diet. *C. difficile* gastrointestinal infections in ratites have lead to high mortality rates in flocks and can be prevented by improved management practices.

SP-40: BABESIOSIS IN A POT-BELLIED PIG

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Babesiosis is a globally distributed, zoonotic disease caused by *Babesia* spp., which are protozoa that infect erythrocytes and cause hemolytic anemia, hemoglobinuria, and other associated clinical signs. While it is more often observed in certain domestic species, such as cattle, sheep, goats, and dogs, babesiosis is uncommon in swine, with outbreaks being especially rare in Southern Africa. Although a few outbreaks have been observed in Southern Europe, Russia, and China, only three outbreaks of porcine babesiosis have ever been reported in Africa. A 12-year old, female intact pot-bellied pig from Gauteng province, South Africa was sent to the University of Pretoria upon dying after a two-day history of anorexia and reluctance to rise. A necropsy with histopathologic examination, blood smear cytology, and PCR of splenic samples were performed. Significant icterus and marked splenomegaly were noted upon necropsy.

Histopathologic evaluation revealed hemoglobin pigment casts, extramedullary hematopoeisis in the kidney and splenic red pulp, and intrahepatic cholestasis. Urine dipstick testing indicated 4+ hemoglobinuria. On the blood smear, 2-3 µm, round to piriform merozoites consistent with *Babesia* spp. morphology were observed within erythrocytes, and PCR confirmed the presence of *Babesia* sp. Overall, this is a complete description of a case of porcine babesiosis that emphasizes the potential emerging nature of babesiosis by describing an affected pig from Southern Africa, from which reports remain rare.

SP-41: COMPUTED TOMOGRAPHY AS A NOVEL METHOD TO EVALUATE HEPATIC PATHOLOGY IN THREE SPECIES OF FRESHWATER TURTLES

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Freshwater turtle species are suffering from anthropocentric-caused population declines, making preservation of managed populations increasingly important. Turtles under human care have an increased risk to develop hepatic lipidosis, potentially resulting in early death. Computed tomography (CT) provides a reliable and detailed approach to screen disease and provide antemortem diagnosis of increased fatty liver composition. Hepatic attenuation values (measured as Hounsfield units (HU)) of Vietnamese Pond turtles (10) and Northern Snake-Necked turtles (6) under human care and wild Blanding's turtles (95) were measured and recorded using CT studies. There were significant differences in hepatic attenuation between Vietnamese Pond, Northern Snake-Necked, and Blanding's turtles, with median HU values (95% confidence interval) of 5.39 HU (-6.45-61.50), 71.74 HU (59.44-94.49) and 95.42 HU (78.55-116.37), respectively, suggesting that hepatic fat content may vary between species and management practices. Bloodwork was performed in the two species under human care. The AST values were significantly higher in Vietnamese Pond turtles, and the HU were significantly lower. The lower attenuation values negatively correlated to higher AST. Blanding's turtles undergoing folliculogenesis presented with higher HU, suggesting a potential use of hepatic lipid stores for energy. Overall, the findings demonstrate species variation, with CT analysis that demonstrated a negative correlation of HU and AST in two managed species; CT can therefore be used as an alternative screening tool to assess hepatic pathology. Moreover, the results indicate further need to assess natural species variation on CT, which will aid in more accurate diagnosis of hepatic lipidosis.

SP-42: COMPREHENSIVE EVALUATION OF KIDNEY TISSUE FROM YORKSHIRE TERRIERS WITH RENAL DISEASE

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Comprehensive evaluation of kidney tissue from Yorkshire terriers with renal disease has revealed glomerular basement membrane (GBM) lesions. This retrospective study characterizes these lesions and associated clinical features. Eighty-six Yorkshire Terriers (4% intact females, 49% spayed females, 4% intact males, and 44% castrated males), ranging in age from 8 to 198 months were included. Clinical parameters, including but not limited to: signalment, blood pressure, serum creatinine, serum albumin, and urine protein to creatinine ratio (UPC) were assessed by a board-certified veterinary internist. Median serum creatinine was 1.0mg/dL (range: 0.3 to 7.1mg/dL; n=77) and median UPC was 8.1 (range: 0.08 to 28.1; n=74). Light microscopy, transmission electron microscopy, and immunofluorescence assessed the presence and severity of glomerular, vascular, and tubulointerstitial lesions. 33.3% of dogs were diagnosed with immune complex glomerulonephritis (ICGN), 21.0% with podocyte-driven disease (e.g. podocytopathy and focal segmental glomerulosclerosis), 30.9% with GBM-mediated disease, 3.70% had renal maldevelopment, and the remaining 11.1% had other lesions. No dogs were diagnosed with renal amyloidosis. In 90% of the cases, ultrastructural evaluation revealed one or more lesions in their GBM, independent of the primary disease process, including: multilamination, electron-lucent vacuoles in the GBM, and club-like projections toward the urinary space without associated immune complexes. Although the clinical significance and pathogenesis of these GBM lesions is unknown, these GBM abnormalities might be the sole diagnostic finding in Yorkshire Terriers with renal disease. Also ICGN might be less prevalent in proteinuric Yorkshire Terriers compared to previous reports of all dogs biopsied for proteinuric renal disease.

SP-43: INTERROGATING THE ROLE OF WWOX IN CANINE MAST CELL TUMORS AND CELL LINES

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Mast cell tumors (MCT) are the most common skin tumor in dogs with behavior varying from benign to aggressive, metastatic disease. While cKIT mutations are present in 30% of high grade MCTs, the genetic alterations driving tumorigenesis in the 70% of MCTs that do not possess cKIT mutations remains unclear. The WW domain-containing oxidoreductase (WWOX) tumor suppressor is frequently lost in cancer and plays a role in regulating DNA damage repair (DDR). The overarching hypothesis of this study is that loss of WWOX impairs DNA damage response and repair pathways, thereby contributing to genomic instability in MCs. WWOX expression was assessed by IHC in paired normal dermal MCs (N=19), low grade MCTs (N=15), and high grade MCTs (N=11). qRTPCR and Western blotting showed that WWOX is decreased in MC lines and primary MCTs compared to bone marrow-cultured MCs, suggesting that loss of WWOX is a frequent event in this disease. To better define the functional consequences of WWOX loss on MC behavior, MCs transduced with control or WWOX lentiviral vectors were treated with ionizing radiation and cell survival/viability was assessed by MTT & clonogenicity assays. Overexpression of WWOX in the C2 MC line did not alter DDR or cell viability; however, enforced WWOX expression significantly decreased the plating efficiency of C2 cell suggesting that WWOX may alter the expression of cell

surface proteins that contribute to cellular adhesion. These findings provide insight into the functions of WWOX in MCs with the ultimate goal of identifying novel targets for therapeutic intervention.

SP-44: NECROTIZING ENTERITIS IN A CAPTIVE FLOCK OF LORIKEETS

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Lorikeet enteritis is an ongoing issue for many zoological institutions, including the Columbus Zoo and Aquarium (CZA). Over the past five years, many lorikeets of various species (Trichoglossus moluccanus, haematodus, and capistratus) at the CZA have died or have been euthanized. Clinical presentations included lethargy, anorexia, weight loss and/or sudden death. The goal of our study was to characterize the main cause of death in this flock. We characterized the histologic characteristics of these cases, as well as analyzed clinical histories, and compared to 16s next generation sequencing data on cloacal swabs collected from 2018-2019. Histologic examinations revealed a 72% prevalence of necrotizing enteritis, with 70% of these cases having intralesional bacteria. Fifty five percent of necrotizing enteritis cases also presented with coelomitis and 33% with lesions consistent with septicemia/ bacteremia. The gut microbiota of CZA lorikeets afflicted with enteritis had significantly increased levels of Clostridium perfringens when compared to the same birds at previously heathy time points and to other healthy lorikeets. There was no significant difference in age between lorikeets that had necrotizing enteritis (mean of 5.4 years) and those with another illness (mean of 7.8 years). Seasonality of deaths, association with previous treatments, and further characterization of the bacteria present in the postmortem lesions as well as environmental sources are ongoing areas of study. The purpose of the study is to assist in clinical management and husbandry practices in order to mitigate enteritis-related deaths in captive lorikeet populations.

SP-45: CD31 IMMUNOHISTOCHEMISTRY IN FELINE KIDNEYS TO CHARACTERIZE PERITUBULAR CAPILLARY DENSITY IN CKD

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Feline chronic kidney disease (CKD) is characterized by tubulointerstitial inflammation, tubular atrophy and fibrosis. Hypoxia is a key driver of fibrosis and is associated with capillary rarefaction (reduction in vascular density) in humans. It is unknown whether similar pathophysiologic mechanisms occur in cats. CD31 immunohistochemistry was assessed in formalin-fixed paraffin-embedded kidney tissue collected at autopsy from 21 CKD cats and 5 normal controls. Consecutive high power fields (40x), ten from the cortex and five from the corticomedullary junction (CMJ), were examined and an observer (blinded to clinical data) counted and colored capillary area. Image analysis was used to determine capillary number, average capillary area (ACA), and percent capillary area (PCA). Differences between normal and CKD cats were assessed with Mann-Whitney and correlation with serum creatinine was assessed with Spearman

correlation. No significant difference in capillary number was found between normal and CKD cats in either region. No significant difference in ACA was found between normal and CKD cats in the CMJ region of the kidney, but CKD cats tended ($p = 0.06$) to have smaller ACA in the cortex than normal cats. ACA was significantly negatively correlated to serum creatinine ($p = 0.03$, $r = - 0.42$). There was no significant difference in PCA between normal and CKD cats in either region, but the lowest PCAs were found only in CKD cats (7/21 CKD cats versus 0/5 normal cats having a PCA <2%). Reduction in ACA and density may be present in later stages of CKD in cats.

SP-46: HEMOABDOMEN AS AN INDICATOR OF CANINE SPLENIC HEMANGIOSARCOMA: A RETROSPECTIVE STUDY OF 449 SPLENECTOMIES (2012-2019)

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Introduction: Following splenectomy, hemangiosarcoma (HSA) has a 3 month median survival time compared to benign conditions that are effectively cured. Recommendation to pursue splenectomy is complicated due to unclear correlation between clinical presentation and prognosis. This retrospective study evaluated whether dogs with splenic lesions and hemoabdomen were more likely to have HSA than dogs with splenic lesions and no hemoabdomen.

Methods: 449 records representing dogs splenectomized at a teaching hospital between January 2012 and May 2019 were reviewed. Signalment, presence or absence of hemoabdomen, splenic gross morphology, and splenic histopathology were recorded. An odds ratio was used to assess how much more likely dogs with splenic lesions and hemoabdomen were to have HSA than dogs with splenic lesions and no hemoabdomen.

Results: Of 449 splenectomized dogs, 164 dogs (36.6%) had hemoabdomen and 285 dogs (63.5%) presented with other clinical signs, most commonly lethargy. Of the 164 dogs with hemoabdomen, the most common diagnoses included HSA (65.9%, n=108), hematoma (10.4%, n=17), and nodular hyperplasia (9.1%, n=15). Of the 285 dogs without hemoabdomen, 17.5% (n=50) were diagnosed with HSA while 82.5% (n=235) were diagnosed with non-HSA malignant lesions (n=49) or benign lesions (n=186).

Conclusion: While dogs with hemoabdomen were nine times more likely to have splenic HSA than dogs without hemoabdomen, only slightly more than half (65.9%) of those dogs with hemoabdomen had HSA. It therefore may be more useful to use absence of hemoabdomen as a positive prognostic indicator than to use the presence of hemoabdomen as a negative prognostic indicator.

SP-47: IDENTIFYING EFFECTIVE ANTIVIRAL COMPOUNDS AGAINST FELINE INFECTIOUS PERITONITIS (FIP) VIRUS

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Feline infectious peritonitis (FIP) is a severe and fatal disease of cats that results from an infection with a ubiquitous feline enteric coronavirus that is capable of mutating into the FIP virus (FIPV), allowing systemic spread. Cats with FIP have an extremely poor prognosis as there is no current commercially available cure for FIP and current treatment protocols are palliative. Recent research has indicated that a small molecule nucleoside analog, GS-441524 (Gilead), is highly effective at treating cats with wild type FIPV infections but patent restrictions have precluded the commercialization of this compound for veterinary use. The aim of this study is to identify novel, safe, effective, and commercially available small molecule compounds that demonstrate antiviral activity against FIPV *in vitro* at physiologically achievable concentrations. Tissue culture-based viral plaquing assays were used to screen 40 compounds from commercial suppliers and research collaborators for antiviral efficacy against FIPV and cytotoxicity in feline kidney cells. Six of the compounds demonstrated antiviral activity against FIPV with 50% effective concentrations (EC50) between 7.5 uM to 13.5 uM. One compound, a commercially available replication complex inhibitor was determined to be highly effective in blocking FIPV replication *in vitro*, with an EC50 of 158 nM. We also determined this compound to be non-cytotoxic at more than 50 times the EC50. Clinical trials in naturally FIPV infected cats will follow the *in vivo* determination of compound metabolism.

SP-48: CUTANEOUS AND SUBCUTANEOUS LYMPHOHISTIOCYTIC NODULES IN DOGS

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Background: Lymphohistiocytic nodules, which arise in canine skin and subcutis, pose a diagnostic challenge to pathologists due to lack of well-defined diagnostic criteria as well as unknown etiology and clinical outcome. Differential diagnoses include regressing histiocytoma, arthropod bite reactions, reactive histiocytosis, or an idiopathic inflammatory process.

Objectives: To better characterize the histopathologic features and gain prognostic information for lymphohistiocytic nodules.

Methods: 143 cases were identified from the PennVet Biopsy database (2004-2018) via a computer search for lympho- or fibrohistiocytic nodules/proliferations. H&E stained slides were reviewed and medical records or surveys were available for 47 cases. IHC was performed on 34 cases: Iba-1, CD204, E-Cadherin, CD3 and CD79b.

Results: Median age at biopsy was 6 years (<1-14). No breed or sex predispositions were identified. In nearly all cases, lesions were solitary. Recurrence was rare (1 case) and not clinically significant when present. Per survey responses, 30 cases had no history of tick exposure, and 19 cases received monthly tick prevention. No specific histologic features were associated with a different clinical course. Six of 34 cases characterized with immunohistochemistry were classified as regressing histiocytoma based on E-cadherin positivity. The remaining cases were composed of predominately

CD204+ infiltrates (non-Langerhans cell histiocytes or macrophages) with intermixed CD3+ T-cells and fewer CD79b+ B-cells.

Conclusions: Lymphohistiocytic nodules are typically solitary, benign, and non-recurrent lesions, for which surgical excision is curative. Tick-bite reactions appear unlikely as a putative cause. Immunohistochemistry is helpful in defining a subset of diagnostically challenging regressing histiocytomas. These lesions afford a favorable prognosis.

SP-49: CHIARI I DEFECT AND SYRINGOMYELIA ASSOCIATED WITH MENINGIOMATOSIS IN A BULL TERRIER

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A 5-year-old female dog with a stiff neck, depression, and hypomotility showed cervical kyphosis, bilateral hydrocephalus, cerebellar protrusion through the foramen magnum, and syringomyelia by magnetic resonance. Due to poor prognosis, the animal was euthanized and submitted for postmortem evaluation. On gross examination, lateral ventricles were expanded by abundant cerebrospinal fluid; dorsoventral thinning of the cerebellar vermis was observed, as well as protrusion of tonsils towards the foramen magnum (without spinal compression). At the level of vertebrae C2, C3 and C4, the spinal cord was expanded by a cystic cavity containing abundant cerebrospinal fluid (syringomyelia). Multiple foci of tumor with a granular appearance were identified in the cerebellum flexure and the central canal of the spinal cord. Histologically, similar tumor foci were observed in the cerebellar aqueduct, leptomeningeal space of the oblongata medulla, and the dura mater; these foci were also infiltrating the white matter of the spinal cord. Tumor cells were polyhedral to fusiform and they were arranged in a solid pattern with moderate cytoplasm and hyperchromatic nuclei with some atypia; anisocytosis, anisokaryosis, and high mitotic activity were observed. The tumor morphology and multifocal distribution were consistent with meningiomatosis. Hydrocephalus, syringomyelia, and Chiari defect were associated with cerebrospinal fluid obstruction due to meningiomatosis.

SP-50: SWAYBACK AND ENZOOTIC ATAXIA IN TWO KIDS

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Two French Alpine goats, a 2-day-old female and a 20-day-old male, were referred to the Pathology Department of the National Autonomous University of Mexico. Their clinical history referred they were premature and they were fed by probe. Clinical examination of both animals revealed ataxia and prostration. Due to their poor prognosis, they were euthanized. Postmortem examination revealed loss of white matter in the brain which was related to porencephaly, polymicrogyria and hydrocephalus. Histological examination showed degeneration, necrosis, edema, vascular proliferation, presence of glial nodules and bilateral symmetric neuroaxonal degeneration in the white matter of the brain and spinal cord. Copper detection was

performed by atomic absorption spectrometry. Serum copper deficiency was confirmed in the female, whose copper value was 2,074 µg/dL; while in the male the copper deficiency was corroborated in the liver (1.1 ppm). These results are consistent with enzootic ataxia which is a disease related to copper deficiency in small ruminants and is characterized by neuroaxonal degeneration and demyelination. Copper is a component of several enzyme systems, including cytochrome-c oxidase, superoxide dismutase and the protein ceruloplasmin. Therefore its deficiency interferes with energy generation by mitochondria in the brain, oxidative stress, catecholamine synthesis and modification of peptide neurotransmitters. We must highlight the importance of copper for reproduction, fetal development and growth in neonates, so it is important to add it to the diet of pregnant animals and to evaluate copper levels in the food, as well as evaluate antagonisms with other minerals such as molybdenum, sulfates, iron and zinc.

SP-51: WATERHOUSE-FRIDERICHSEN SYNDROME IN A WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS), SECONDARY TO PODODERMATITIS AND EMBOLIC PNEUMONIA CAUSED BY FUSOBACTERIUM NECROPHORUM AND TRUEPERELLA PYOGENES

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A six-year-old white-tailed deer (*Odocoileus virginianus*), from Mexico City, was submitted to the Pathology Department of the Facultad de Medicina Veterinaria y Zootecnia, UNAM in Mexico City, with a history of edema in the pelvic and thoracic left limbs and hoof detachment from the left limb. The animal was found dead and referred for postmortem study. Gross examination revealed a severe pododermatitis in the distal fourth phalanx of the pelvic and thoracic left limbs, a severe multifocal embolic pneumonia, and severe bilateral adrenal cortical hemorrhages. On histology, an extensive necrosuppurative dermatitits and severe multifocal necrosuppurative pneumonia, both with septic thrombi and abundant colonies of gram-positive cocci, and moderate number of gram-negative bacilli were also identified. The cortex of the adrenal glands had multiple areas of hemorrhage and coagulative necrosis. DNA was extracted from paraffin embedded tissues of the skin and lungs, and PCR was carried out in which *Fusobacterium necrophorum* and *Trueperella pyogenes* were identified in skin lesions. The hemorrhage and necrosis in the adrenal cortex were attributed to the Waterhouse-Friderichsen Syndrome which is an entity in large animals that is related to septicemic events, abdominal trauma, ACTH overstimulation, congenital coagulation disorders, and thrombosis of adrenal afferent vessels. This condition is poorly understood but it has been proposed that adrenaline release may induce platelet aggregation and adrenal vasoconstriction, predisposing to adrenal thrombosis and infarction in association with concurrent disseminated intravascular coagulation.

SP-52: MYCOBACTERIUM AVIUM SPP. HOMINISSUIS INFECTION IN A WILD MULE DEER (ODOCOILEUS HEMIONUS)

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Cattle in Canada are regarded as free of *Mycobacterium bovis*, the causative agent of bovine tuberculosis and a zoonotic pathogen of global importance. The bacterium is endemic in wood bison (*Bison bison athabascae*) and elk (*Cervus canadensis*) in 2 national parks. The presence of wildlife reservoirs combined with the recent identification of 2 *M. bovis*-infected cattle herds has led to concerns over surveillance in wildlife. In November 2018, a yearling male mule deer (*Odocoileus hemionus*) was found dead in Banff National Park, Alberta and was submitted to the Canadian Wildlife Health Cooperative Alberta Region at the University of Calgary for diagnostic investigation. The deer died of blunt-force trauma, presumably from a vehicle strike. There were mineralized granulomas in the right caudal lung lobe and suppurative lymphadenitis of the tracheobronchial lymph node. Both lesions contained acid fast bacteria. Routine culture yielded a mixture of bacterial species that were likely post-mortem contaminants. A generic *Mycobacterium* sp. PCR of lung and lymph node was positive; sequencing identified *Mycobacterium avium* spp. *hominissuis*. This is a rare opportunistic environmental pathogen of humans, pigs, rabbits and other species that typically infects the lungs and intestinal tract, presumably by inhalation and ingestion respectively. The lesions in this case are similar to those described in *M. bovis*-infected cervids. This case demonstrates that routine disease surveillance activities in wildlife populations, including molecular investigations, are crucial to providing ongoing assurance to agricultural and public health sectors of the absence of *M. bovis* in wild cervid populations outside known endemic areas.

SP-53: SARCOCYSTIS SPECIES PROTOZOAL ENCEPHALITIS IN A WILD BLACK BEAR (URSUS AMERICANUS)

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The health of wild black bear populations is affected by multiple factors, including habitat loss and human-wildlife conflict. The role of disease in these challenged systems remains relatively unexplored. A juvenile male black bear was found dead in October, 2018 in a provincial recreational area in southwestern Alberta and was submitted to the Alberta Region of the Canadian Wildlife Health Cooperative at the University of Calgary for diagnostic investigation.

Lesions included suppurative cellulitis, fasciitis, and myositis of the left lower hindlimb and lymphoplasmacytic encephalitis. Numerous intralesional protozoal schizonts were present in the affected muscle and brain. These were identified as *Sarcocystis* sp. with immunohistochemistry. Immunohistochemistry for canine distemper virus and rabies virus were negative. Bacterial culture yielded a pure culture of *Streptococcus halicoeri* from the leg lesions, which is probably secondary bacterial infection. Although the intermediate stage of *Sarcocystis* sp. is frequently observed in the muscle tissues of a

variety of wildlife, it is rarely associated with disease. *Sarcocystis* sp. is a known cause of encephalitis in birds and fatal hepatic sarcocystosis has been described in black bears (*Ursus americanus*) and polar bears (*U. maritimus*). Its role as a fatal brain pathogen of wild bears remains undescribed, making this case highly unusual. *Sarcocystis* sp. and other infectious diseases may be an important and underrecognized cause of wild bear mortality.

SP-54: CONGENITAL CATARACT LEADING TO APPARENT ABANDONMENT AND STARVATION IN A WILD NEONATE MUSKOX (OVIBOS MOSCHATUS) FROM THE NORTHWEST TERRITORIES, CANADA

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Congenital ocular defects are infrequently reported in wildlife, despite the profound impact on individual survival. In May 2019, a male neonate muskox (*Ovibos moschatus*) in the barren land ecosystem of the North Slave region, Northwest Territories, Canada was observed alone. Following an attempt to reintroduce it into a muskox herd, it was found dead and was submitted to the Canadian Wildlife Health Cooperative Alberta Region, University of Calgary, for diagnostic investigation. The carcass was severely emaciated with abnormal forestomach contents (hair, leaves and sand) indicative of pica. Locally-extensive alopecia extended over the dorsum and there was systemic lymphoid depletion. The left eye was affected by cataract, mild lymphocytic panuveitis and neutrophilic keratitis. The right eye had been scavenged. Based on the age of this calf, the cataract was likely congenital with secondary uveitis and keratitis. Congenital cataracts have been reported secondary to *in utero* viral infections, toxin exposure, nutritional deficiencies and genetic mutations, and they are often associated with additional developmental abnormalities. Immunohistochemical labelling of skin for bovine viral diarrhea virus was negative. Muskoxen have low genetic diversity, suggesting a genetic defect as a potential cause. Based on the extent of the cataract, vision would have been severely compromised and likely contributed to abandonment, starvation, and subsequent death. The etiology is unknown, however this report stresses that ocular pathology in wild neonates maybe associated with death. In addition to climate change and pathogen emergence, factors that influence calf survival could be significant to wildlife populations in the Canadian Arctic.

SP-55: GENETIC DIVERSITY OF AMDOPARVOVIRUSES IN STRIPED SKUNKS (MEPHITIS MEPHITIS) AND DEMONSTRATION IN TISSUE BY IN SITU HYBRIDIZATION

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Background: Amdoparvoviruses (APVs, family Parvoviridae) cause persistent infections in several small carnivore species. The archetype virus, Aleutian mink disease virus, causes immune-mediated disease and chronic nephritis. A

phylogenetically related skunk amdoparvovirus (SKAV) has been described in striped skunks from Canada. Recent work has identified SKAVs with high prevalence in California striped skunks, but viral phylogeny and disease association have not been established.

Objectives: Determine prevalence, phylogenetic relationships, and disease association of APVs in CA skunks.

Methods: Spleen from 23 free-ranging skunks, submitted for routine necropsy, were tested by conventional PCR targeting 400nt of the SKAV capsid-coding (VP) gene. From positive samples, kidney and feces were also screened, and a 1300nt segment of the non-structural (NS) gene was PCR-amplified from individual animals and sequenced for phylogenetics. SKAV-specific in situ hybridization (ISH) probes targeting VP were used to confirm specific distribution in histologic sections.

Results: SKAV was detected in spleen of 20/23 skunks (87%), kidney of 15/20 (75%), and feces of 4/15 (27%). By partial NS sequence, CA SKAVs clustered with SKAVs previously reported in Canadian skunks. ISH demonstrated both nuclear and cytoplasmic viral nucleic acid in renal tubular epithelium and in scattered interstitial leukocytes in a skunk that died with severe tubulointerstitial nephritis.

Conclusions: Phylogenetically distinct SKAVs infect California skunks at high prevalence (87%). In situ hybridization demonstrates infection of renal epithelium in regions of tubulointerstitial nephritis, which supports viral replication and cytolysis in renal tubular epithelium, and a spectrum from mild to severe disease association in infected skunks.

SP-56: USE OF MESENTERIC LYMPH NODE CYTOLOGY FOR THE DIAGNOSIS OF FELINE INTESTINAL SMALL CELL LYMPHOMA

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Background: Intestinal lymphoma is the most common lymphoma in cats. Mesenteric lymph node (mLN) aspirates are often used as a surrogate sample when there is intestinal thickening. It is currently not known how many small granular lymphocytes (GLs) are present in normal feline mLNs, and how many are indicative of underlying intestinal lymphoma.

Objectives: To determine the lower and upper limits of the GL % present in mLNs of cats with normal intestine, inflammatory intestinal disease, small cell non-granular lymphoma, and small cell granular lymphoma.

Methods: Granular lymphocytes in Wright-Giemsa stained impression smears and aspirates of mLNs were differentially counted. Results were correlated with T-cell molecular clonality PCR status and accompanying intestinal histopathology when available.

Results: The percentage of small GLs in mLNs of cats with histopathologically confirmed normal (n=16) or inflamed intestine (n=9) ranged from 0 - 1.11%. The percentage of small GLs in mLNs of cats with confirmed intestinal small cell lymphoma (n=38), or a clonal mesenteric node (n=47), or both, ranged from 0.07% - 57.51%. More than 1.5% small granular lymphocytes in mesenteric lymph node aspirates was always associated with either confirmed intestinal small cell lymphoma or a clonal molecular clonality PCR result.

Conclusions: Granular lymphocytes are present in very low numbers in the mesenteric nodes of cats with either normal or inflamed intestines. An increased percentage of nodal small granular lymphocytes (>1.5%) is a marker of underlying intestinal small cell lymphoma and provides useful guidance for the diagnostic workup of cats with intestinal disease.

SP-57: INJECTION SITE REACTIONS FOLLOWING ADMINISTRATION OF SUSTAINED RELEASE MELOXICAM IN THREE STRAINS OF MICE: BALB/CJ, C57BL6/J, AND ICR(CD1).

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Meloxicam is a non-steroidal anti-inflammatory drug commonly used in mice. One dose is effective for up to 12-24 hours of analgesia in mice (administration routes can be subcutaneous, intraperitoneal, per os). A sustained release formulation (MSR) is available through a compounder (Zoopharm, Windsor, CO) that may be effective for up to 72 hours, which can reduce animal handling by providing longer duration analgesia. However, injection site reactions have been documented in some species. To determine if reactions occur in mice, and if there are strain differences, we evaluated injection sites following MSR treatment of three commonly used strains of mice; BALB/CJ, C57BL/6J, and ICR(CD1). Ten week-old, sex-matched mice were injected (subcutaneous) with either a single dose of MSR (n = 60) or a control dose of meloxicam (n = 24) or saline (n = 24). Mice were examined daily for 7 or 14 days, and lesion erythema and mass characteristics were scored using a described system. Histologic samples were evaluated and scored for inflammation severity. Of MSR-treated mice, 55% developed erythematous lesions and 82% developed mass lesions (both more often than controls, $P < 0.001$, $P < 0.001$, Fisher's exact). Between strains and sexes, severity of inflammation was not statistically significantly different except in female ICR(CD1) mice compared to males at day 14 ($P = 0.0476$, Kruskal-Wallis ANOVA with Dunn's post-hoc multiple comparisons test). MSR injections result in frequent injection site reactions in these three strains of mice.

SP-58: INVESTIGATING THE ROLE OF FELINE FOAMY VIRUS IN FELINE CHRONIC KIDNEY DISEASE

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Chronic kidney disease (CKD) is fatal in 30% of the feline population and culminates in loss of renal parenchyma and function. No specific cause for CKD has been identified. Based on the pattern of histologic changes, a lack of correlation with hypertension or salt (human causes for kidney disease), and the finding that older and immunosuppressed cats are more likely to be affected, we propose that feline CKD could be caused by a chronic, persistent viral infection. Furthermore, there is recent precedent in other species, since a newly discovered parvovirus causes chronic, progressive nephritis in immunosuppressed mice. This study explores whether a retrovirus, feline foamy virus (FFV), is 1) present in feline renal tissues and 2) causes tubulointerstitial nephritis in cats. DNA was extracted from kidney and urine samples from a cohort of random cats; a 221 nucleotide region of the viral LTR was amplified by PCR. DNA extracted from a second cohort of feline kidney samples from retrospective necropsy cases with mild to severe nephritis were also tested for FFV by PCR. FFV was present in kidney tissue in ~27% of cats. By PCR, FFV is detectable in normal cats and those with mild to severe nephritis, so there is no correlation with kidney damage and FFV presence. Direct viral detection within tissue sections by in situ hybridization will establish the microanatomical and cellular target of FFV. If FFV causes CKD, we expect to detect the viral genome in areas of renal tubular epithelial loss and inflammation.

SP-59: MYELOSUPPRESSION IN A DOG RECEIVING LEFLUNOMIDE FOR TREATMENT OF IMMUNE-MEDIATED THROMBOCYTOPENIA

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Case Report: A 10-year-old male neutered Labrador retriever was diagnosed with immune-mediated thrombocytopenia (ITP) on routine CBC by a primary veterinarian. Following the diagnosis, immunosuppressive doses of Prednisone and Leflunomide were initiated. A CBC performed three weeks following the initial dose of Leflunomide revealed a mild decrease in hematocrit which was within the reference interval. Five weeks post Leflunomide administration, the patient developed a severe non-regenerative anemia which progressed to pancytopenia a week later. Bone marrow findings suggested potential myelosuppression, which has been anecdotally reported in dogs treated with Leflunomide. Given the pancytopenia possibly secondary to myelosuppresion, Leflunomide administration was discontinued. Administration of a different immunomodulatory agent (cyclosporine) was initiated for treatment of ITP. Within one week of discontinuing Leflunomide, the leukocyte count began to improve and evidence of an erythroid regenerative response was observed with an increased reticulocyte count.

Conclusion: This case provides strong evidence of reversible bone marrow suppression caused by Leflunomide administration. The presence of a pancytopenia and the cytologic and histologic interpretation of the bone marrow samples were consistent with myelosuppression. Cell counts in all three lineages improved shortly after discontinuation of Leflunomide therapy. There is limited information in the veterinary literature about Leflunomide administration in dogs, however there have been anecdotal reports of myelosuppression associated with the use of this medication. This case further supports the potential consequence of myelosuppression with Leflunomide

administration, which was reversible after discontinuation of the medication in this patient.

SP-60: SPURIOUS BIOCHEMICAL CHANGES IN A DOG WITH SEVERE RHABDOMYOLYSIS

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A two-year-old female spayed, mixed breed dog presented on emergency with acute vomiting and persistent, diffuse tremoring. On physical exam, the patient was obtunded and exhibited severe tachycardia, hyperthermia, ataxia, and muscle tremoring and rigidity. A chemistry panel revealed several abnormal analytes. Specifically, the AST and anion gap resulted in negative values below analytical measurement range suggestive of an abnormal reaction. After initial review, additional testing (CK, troponin I, and LDH) was performed by the pathologist. The AST reaction speed, due to the marked increase, likely caused the analyzer to report a value lower than the dynamic range. After a 1:80 dilution, the analyzer reported an AST of 66,956 (Reference interval= 15-52 U/L). The anion gap was negative for similar reasons; LDH released from damaged myocytes led to additional oxidization of NADH, decreasing absorbance and falsely increasing the total CO2 used to calculate the anion gap. LDH was 65,416 U/L after a 1:80 dilution. Given concern for rhabdomyolysis, CK and troponin, more specific enzymes assessing acute skeletal and cardiac muscle damage, were measured. The serum was diluted to 1:2,560 resulting in a CK value of 944,640 (Reference interval 49-244 U/L), and a troponin of 3.97 (Reference interval 0.00-0.12 ng/mL). The laboratory data and clinical presentation confirmed rhabdomyolysis, suspected to be secondary to drug intoxication. Rhabdomyolysis can lead to renal, myocardial, and respiratory failure. Early recognition in this case was crucial for starting supportive care and improving prognosis. This dog recovered over a period of approximately two weeks.

SP-61: ESTABLISHMENT OF A CANINE B-CELL AND T-CELL LYMPHOMA XENOGRAFT MODEL TO CHARACTERIZE THE IN VIVO EFFICACY OF NEW TREATMENT STRATEGIES

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Lymphoma is a leading cause of cancer-related death in dogs. Though initial response rates to treatment are high, the development of drug resistance is a major barrier to long-term survival, indicating the need for alternative treatment approaches. Our *in vitro* investigations revealed that several canine lymphoma cell lines, when exposed to a selective Bcl-2 inhibitor (venetoclax, ABT-199), underwent cell death linked to key features of apoptosis. To evaluate venetoclax' *in vivo* anti-lymphoma activity, we established a murine xenograft model. The canine B-cell and T-cell lymphoma cell lines CLBL-1 and Ema, respectively, were grown in cell culture and each was subcutaneously engrafted into 8 Rag 2-/-γc-/- immunodeficient female mice (n=16 total). Mice were monitored for weight, overall health status (grimace scale), and, once visible, tumor volume was estimated using caliper measurements. Mice were treated with oral

venetoclax or vehicle control for 21 days (d) until euthanasia, necropsy and tissue collection. All mice engrafted with CLBL-1 developed subcutaneous (SC) tumors within 3-20 d (mean=14 d). Neoplastic lymphocytes also variably infiltrated the liver, spleen, bone marrow, and intrabdominal organs. Six of eight mice engrafted with Ema developed SC tumors (20-34 d, mean=25 d) whereas the remaining two mice developed intrabdominal disease only (100% engraftment). Cytology and histopathology for both tumor types revealed large-cell lymphoma and intermediate to high grade lymphoma, respectively. The established canine lymphoma xenografts are highly suitable to model local and disseminated disease and we are currently determining the impact of oral venetoclax on canine lymphoma growth inhibition.

SP-62: ORGAN WEIGHTS IN RELATION TO AGE, SEX, AND DISEASE IN CYNOMOLGUS MONKEYS (MACACA FASCICULARIS)

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Lab animal research is an important contributor to both human and animal medicine. Currently, there is extensive use of cynomolgus monkeys (*Macaca fascicularis*) in research to study pathology and toxicology. The purpose of this study was to define reference values for absolute organ weights in *Macaca fascicularis* of different ages and sex, and to provide a resource for organ weight to body weight percentages. Organ weights were obtained from necropsies of cynomolgus monkeys at the Wake Forest School of Medicine from 1997-2018. Data were compared using scatter plots. Percent body weights for each organ were analyzed with one-way ANOVA. This evaluation showed that male body weights and absolute organ weights were greater for all age groups, however, female organ to body weight percentages were greater for most organs studied. Effects of dietary macronutrient composition were significant. This information will be useful for further toxicology and pathology studies concerning cynomolgus monkeys.

SP-63: A RETROSPECTIVE STUDY OF MORBIDITY AND MORTALITY IN SQUIRRELS IN THE SOUTHEASTERN USA FROM 1975-2019

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Rodents represent a substantial component of terrestrial biota, with over 43% of known extant mammalian species in the order Rodentia. Particularly, wild rodents are a key part of the ecosystem, serving both as prey species and seed dispersers. A 44-year retrospective study was performed to assess causes of morbidity and mortality in squirrels, primarily in the southeastern USA. The analysis included results of diagnostic evaluations of squirrel carcasses or select tissues collected at field necropsy (n=323) performed at the Southeastern Cooperative Wildlife Disease Study (SCWDS) from 1975 to 2019. This study examined the primary and secondary causes of morbidity and mortality in these animals; major categories included infectious and non-infectious; these categories were further divided into bacterial, viral, and parasitic for the former

and trauma, toxicosis, nutritional, neoplasia, and miscellaneous for the latter. Five species of squirrels were represented, including the Eastern gray squirrel (*Sciurus carolinensis*; n=229), Fox squirrel (*S. niger*; n=71), Northern flying squirrel (*Glaucomys sabrinus*; n=4), Southern flying squirrel (*G. volans*; n=3), and Western gray squirrel (*S. griseus*; n=1). Non-infectious causes of death accounted for the majority of fatalities (n=146; 58%) with trauma being the most common diagnosis (n=89; 61%), followed by toxicosis (n=32; 22%). The main infectious cause of mortality was bacterial infection (n=28, 44%), mainly due to pneumonia or septicemia. Less common infectious causes included squirrel fibroma virus, West Nile virus, and *Toxoplasma gondii*. These results provide insight into the role of various anthropogenic factors in squirrel mortality, as well as other less commonly diagnosed causes.

SP-64: RANDOM, REAL TIME SEQUENCING OF AVIAN AVULAVIRUSES ISOLATED FROM WILD BIRD SAMPLES

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Wild animals are known reservoirs for many viruses that can transfer into domestic animals or humans. For example, wild fowl have been observed to carry virulent and non-virulent strains of avian avulavirus 1 (AAvV1), an economically impactful disease within the poultry industry. Therefore, the monitoring of wild animal populations for potential transfer of virulent viruses is imperative to protect domestic animal populations. Current methods for diagnosing viruses rely on agent-specific assays, such as PCR. While effective, the inherently high specificity of these assays can result in failure to detect mutants or novel viruses. MinION sequencing of samples is emerging as an effective way to quickly sequence whole genomes of viruses and to genotype viruses, without prior knowledge of the virus present in the sample. This novel technology could potentially provide both the accuracy and speed required to diagnose important viruses, such as avian AAvVs. The goal of this project was to determine the ability of MinION to effectively identify RNA viruses from hemagglutination-positive, influenza-negative viral cultures, isolated from wild bird populations using a random hexamer strand switching protocol. MinION sequencing was used to genotype egg-cultured viral isolates derived from cloacal/oropharyngeal swabs from North American ducks and shorebirds. This study shows that the MinION results align with the pan-AAvV and AAvV1 PCR results, while also detecting avian avulaviruses other than AAvV1 including AAvV2 and AAvV4. Additionally, MinION sequencing allowed for the detection of multiple viruses in several of the samples which would normally require multiple PCRs.

SP-65: RETROSPECTIVE STUDY OF BIOPTIC DIAGNOSES IN PSITTACINE BIRDS SUBMITTED TO THE ONTARIO VETERINARY COLLEGE

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Background: Surgical pathology, commonly known as “biopsy”, is a diagnostic tool that is becoming increasingly common in avian patients. Data regarding the use of surgical pathology in psittacine birds is lacking and limited to individual case studies.

Objective: To retrospectively describe the demographic characteristics and disease prevalence in a large cohort of psittacine birds submitted for biopsy procedure.

Methods: The database included, for each case, taxonomic data, age groups, biopsy sample and location, and final diagnoses submitted to the Ontario Veterinary College and Animal Health Laboratory from 1998-2017. Final diagnoses were further categorized by aetiology and body system. Logistic regression was performed to determine associations between demographic characteristics and disease processes.

Results: Data were retrieved from 347 birds, representing 20 genera, including most commonly: macaws (18%), cockatoos (17%), and Amazons (14%). The most common locations that biopsies were sampled from include skin (23%), liver (12%), crop (10%), eye (5%), and kidney (4%). Disturbances in growth (26%), idiopathic causes (21%), non-diagnostic (18%), degenerative (9%), bacterial (8%), and viral (5%) were the most frequent disease processes. The genus *Nymphicus* had a positive association with disturbances in growth and neoplasia, with an odds ratio of 5.11 and 3.91, respectively. The genus *Amazona* had a significant association with viral diseases, with an odds ratio of 5.23.

Conclusions: This study is one of few summaries of biopsy data in a large cohort of psittacines, and provides useful correlation data between genera and disease processes.

SP-66: THE EFFECT OF FORMALIN FIXATION TIME AND FORMALIN-FIXED, PARAFFIN-EMBEDDED TISSUE AGE ON RNASCOPE IN SITU HYBRIDIZATION SIGNAL AMPLIFICATION

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RNAscope® *in situ* hybridization (ISH) is a new technique for detection of RNA targets and studying gene expression in formalin-fixed, paraffin-embedded (FFPE) tissues. While the RNAscope® protocol suggests that prolonged FFPE tissue storage and formalin fixation time may limit signal, past studies have successfully detected RNA targets in archival tissue. To better understand the effects of storage time on RNA detection, we compared RNAscope® ISH in archival raccoon FFPE tissues (n=13) positive for Canine Distemper Virus (CDV) via immunohistochemistry (IHC) that were fixed for less than 5 days but stored in paraffin from 6 months to 18 years. To determine the effect of prolonged formalin exposure, detection of housekeeping gene mRNA expression was compared in tissues fixed in formalin for up to 28 days prior to embedding in paraffin. RNA target signal amplification was scored in Image J software by measuring the intensity (integrated density) and percent area of staining in tissues. RNAscope® ISH successfully detected CDV in FFPE tissue samples stored up to 15 years; staining intensity (ANOVA, p=0.661, F=0.2022) and percent area of staining

(ANOVA, p=0.7288, F=0.1318) did not vary significantly over time in paraffin. Housekeeping gene mRNA signal intensity (ANOVA, p=0.2934, F=1.1207) and percent area of staining (ANOVA, p=0.5396, F=0.38) also did not vary significantly in tissues over 28 days in formalin. These findings indicate that archival FFPE tissues and those with fixation times up to at least 28 days may be suitable for use in RNAscope® *in situ* applications.

SP-67: THE ROLE OF MITOCHONDRIAL OXIDATIVE STRESS IN THE DEVELOPMENT OF AGE-RELATED OSTEOARTHRITIS

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Osteoarthritis (OA) is the most common joint disease of both humans and animals, but treatment options to slow its progression are limited. Previous theories suggest that OA is caused by the random accumulation of cellular damage by free radicals. Recent studies propose that disturbances in oxidation and reduction (redox) signaling due to excessive mitochondrial reactive oxygen species (ROS) play a larger role in the development of disease. Peroxiredoxins (Prxs) are the major regulators of redox signaling and Prx3 is the primary mitochondrial Prx. The aim of this study was to determine the effects of inducible transgenic overexpression of Prx3 (iPrx3) on the development of OA in mice, with the hypothesis that Prx3 overexpression would reduce age-related OA.[LR1] Stifle joints from 18-month and 24-month-old control and iPrx3 mice were processed for histology with sectioning and staining of a representative mid-coronal section with hematoxylin and eosin (H&E). The medial tibial plateau was semiquantitatively evaluated for articular cartilage (AC) structural damage using a previously established murine grading scheme, and measurements were made of the thickness and area of AC, calcified cartilage, and subchondral bone using imaging software. The summed ACS scores of the 18-month-old mice were significantly lower in the iPrx3 group than the control group (p=0.003), and no significant difference was observed between treatment and control groups in the 24-month-old mice.[LR2] These studies support the role of mitochondrial ROS in the development of early age-related OA and suggest that therapeutic targeting of mitochondrial ROS would be of benefit in slowing disease progression.

SP-68: PROGNOSTIC FEATURES OF CANINE GLIAL TUMORS

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The dog is proving to be useful as a translational model for human beings with brain tumors. Hitherto, canine glioma histopathologic diagnosis and prognosis have been based on criteria developed for human glioma, an approach which is less than ideal given our increasing awareness of differences in this disease between the two species. Here we report, for the first time, histopathologic features of canine gliomas that correlate with long-term clinical outcome as defined by survival. Histologic sections of tumor biopsies and whole brains (when available) were reviewed for 37 dogs with

glioma, all of which had been treated with cytoreductive surgery and immunotherapy. Tumors were diagnosed as astrocytic, oligodendroglial or undefined glioma (using Comparative Brain Tumor Consortium criteria). Putative features of malignancy were evaluated, viz. mitotic counts, glomeruloid vascularization, necrosis, and diffuse infiltration of brain. Mitotic counts were graded on a 0 to 4 basis; other features were noted as present or absent. For biopsies, dogs with astrocytic tumors live longer than those with oligodendroglial or undefined tumor types (median survival 734, 205, 130 days respectively). Low-grade gliomas had better outcomes than high-grade gliomas (median survival 734, 194 days, respectively). Low mitotic counts, absence of glomeruloid vascularization and of necrosis correlated with increased survival (median 293, 223, 220 days, respectively). High mitotic counts, glomeruloid vascularization, necrosis and diffuse infiltration correlated with poor outcomes (median 190, 170, 154, 212 days, respectively). Whole brain analysis had similar and more robust correlations. These findings will facilitate more accurate prognosis for canine glioma.

SP-69: CLINICOPATHOLOGIC FINDINGS OF A SUSPECTED PRIMARY MYELOID SARCOMA IN A STANDARD POODLE

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Myeloid sarcoma is a rare neoplasm of extramedullary immature myeloid cells diagnosed commonly with acute myeloid leukemia. We studied a 3.5-year-old, female spayed Standard Poodle with a mass extending along the left mandible to the cranial mediastinum with enlarged regional lymph nodes and no clinically significant findings on CBC and serum chemistry. Cytology, flow cytometry, and histologic examination were used in the evaluation of the mass. Left prescapular lymph node and cranial mediastinal mass cytology revealed large round cells containing round to irregular eccentric nuclei 2-3x the diameter of an RBC, 0-4 poorly defined nucleoli, and small to moderate amounts of medium blue cytoplasm which sometimes contained fine diffuse pink granules or coarse pink granules resembling those of eosinophils. Morphology suggested myeloid origin. Approximately 30% of cells on a lymph node aspirate displayed alkaline phosphatase activity following NBT/BCIP application. Histopathology of the mass revealed large polygonal cells that failed to stain with toluidine blue and failed to label with antibodies for cKIT, tryptase, thyroglobulin, cytokeratin, CD3, CD79a, and CD18. Left prescapular lymph node flow cytometry characterized a class II MHC-, CD34+ leukocytosis with CD14+ and CD18+ cells. Bone marrow cytology revealed no atypical blasts. The mass was diagnosed as a myeloid sarcoma, likely myelomonocytic in origin considering morphologic evidence in conjunction with ALP staining and flow cytometry results. Although complete necropsy was not performed and follow up period for this patient was relatively short, this case is suspected to be a rare example of primary extramedullary myeloid sarcoma.

SP-70: DISSEMINATED PNEUMOCYSTOSIS IN A TOY POODLE

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Background: Species belonging to the genus *Pneumocystis* are fungi that infect many mammalian species, including humans. They are primarily found in mammalian lungs and cause opportunistic pneumonia. A one-year, seven-month-old spayed toy poodle was presented with persistent respiratory distress and gradual weight loss and melena. Severe infectious disease with disseminated intravascular coagulation (DIC) was suspected from radiographic and laboratory findings. The patient showed no improvement in respiratory state and DIC and died. Necropsy revealed disseminated *Pneumocystis carinii* (*P. carinii*) infection.

Objective: Our objective was to identify the pathological findings of a dog with disseminated pneumocystosis.

Methods: Histopathologic sections of multiple organs were stained for hematoxylin and eosin (HE), Grocott methenamine silver staining and immunohistochemistry (IHC) for *P. carinii*. Formalin-fixed paraffin-embedded tissue sample were subjected for PCR test and sequence analysis.

Results: Histopathological examination revealed the organisms in the lungs, lymph nodes, liver, heart, kidneys, spleen, gastrointestinal tract and pancreas. In the lungs, the organisms were present not only in the alveolar space but also in the interstitial tissue, and calcifications containing *P. carinii* were observed. IgA and IgG depletion, consistent with possible immunodeficiency, was confirmed by IHC.

Conclusions: This report describes disseminated pneumocystosis in a toy poodle suspected with immunodeficiency. The infection in nonthoracic lymph nodes provided evidence of the lymphogenous route of infection of *P. carinii*. The calcifications in the lungs may represent dystrophic calcification induced by *P. carinii* infection. This is the second report of the disseminated form of disease in canines.

SP-72: SEQUELAE OF FETAL INFECTION IN A NON-HUMAN PRIMATE MODEL OF LISTERIOSIS

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Listeria monocytogenes (Lm) is a common environmental bacterium that thrives on vegetation and soil, but can infect humans if ingested resulting in severe disease in immunosuppressed populations, including pregnant women and newborns. To understand how the immunological milieu of pregnancy increases susceptibility to infection, we study listeriosis in cynomolgus macaques (nonhuman primate species), because they closely resemble humans in placentation and immunology of pregnancy. Nonhuman primates are susceptible to Lm infection, and spontaneous abortions due to listeriosis occur in outdoor macaque colonies, making them ideal models.

In this study we inoculated a cohort of macaques with a dose of 10^6-10^8 CFU Lm and found evidence of inflammation and fetal distress, despite infection not resulting in fetal demise. Animals that were reinfected with an equivalent or higher dose of the same strain resulted in approximately half of cases continuing to term and half ending in fetal demise. These cases had inconsistent bacterial colonization of the fetal compartment, suggesting that Lm doesn't need to directly infect the placenta to cause adverse pregnancy outcomes. Timed collection of tissues following inoculation demonstrated that transplacental transmission can occur as soon as 5 days post-inoculation. Collectively, our studies demonstrate that common laboratory culture tests may not always recover Lm despite known maternal ingestion. Notably, we also find it is possible for maternal infection to resolve in some cases with no discernible adverse outcome; however, such cases had evidence of sterile intrauterine inflammation, with unknown consequences for fetal development.

SP-73: IMMUNE-MEDIATED DERMATITIS, THYMOMA, AND NASAL TUMOR IN A CAPTIVE BLACK BEAR (URSUS AMERICANUS)

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Thymomas have been reported in several domestic species, but few reports in non-domestic mammals exist. Thymomas can be associated with immune-mediated and paraneoplastic conditions, including exfoliative dermatitis in cats. Studies in humans also indicate that thymoma patients have an increased risk for other malignancies.

A 19-year-old male captive black bear developed progressive alopecia starting at the head and neck and progressively lost weight despite maintaining a normal appetite. In addition, two episodes of epistaxis occurred during this time period. After approximately 3 months of medical management, the animal rapidly declined and was euthanized. At necropsy, approximately 80% of the animal's body was alopecic, and remaining hair was easily epilated. The skin was thickened and scaly with areas of hypo- and hyperpigmentation.

Histopathology of the skin revealed orthokeratotic hyperkeratosis, lymphocytic interface dermatitis, scattered apoptotic keratinocytes, basal cell degeneration, superficial dermal fibrosis, and follicular atrophy with loss of sebaceous glands. A lobulated mass was present in the cranial mediastinum and histologically consistent with a thymoma. Additionally, a multilobulated, invasive soft tissue mass was present in the nasal cavity.

The dermatopathological findings in this bear are similar to those reported in cats with thymoma-associated exfoliative dermatitis. This is the first reported case of thymoma and presumptive thymoma-associated paraneoplastic syndrome in a bear.

SP-74: NASAL GANGLIONEUROMA IN A DOG

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An 11-year-old, intact male Shilo Shepherd presented with a one-month history of right-sided epistaxis, frequent face rubbing, and decreased airflow through the nasal cavity. Physical exam revealed a soft tissue swelling on the right lateral aspect of the muzzle, and airflow was decreased through the left naris and absent through the right naris. Computed tomography identified a destructive nasal lesion that occupied the entire right nasal cavity and most of the left nasal cavity. Histopathology of the biopsied mass was consistent with a ganglioneuroma. Despite treatment with intensity-modulated radiation therapy (IMRT), the local disease remained stable and progressed, and the patient was humanely euthanized. On necropsy, the nasal cavities contained a 15 cm x 5.5 cm x 5 cm, expansile, pink, fleshy-to-gelatinous mass that expanded from the rostral nasal passage, caused lateral deviation of the caudal nasal septum, and effaced the caudal nasal septum and turbinates. Histologically, the normal architecture of nasal turbinates was widely replaced by a poorly cellular proliferation of neoplastic neurons, the large cell bodies of which were arranged in clusters widely separated by abundant neuropil and large numbers of round to spindloid peripheral glial cells. Cell bodies of neoplastic neurons were pyriform, had distinct cell borders, and contained a moderate amount of amphophilic cytoplasm with stippled basophilic Nissl substance. Anisokaryosis was moderate and there were 0-1 mitoses observed in 10 high-power (400x) fields. To the authors' knowledge, this is the first reported case of a ganglioneuroma in the nasal cavity of a dog.

SP-75: UNIQUE CLINICAL PRESENTATION OF MANDIBULAR OSTEOMYELITIS IN A WALLABY

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A 4-year-old female red-necked wallaby (*Macropus rufogriseus*) was euthanized after a nine-month history of progressive bilateral enlargement of the mandible. On radiographs there was bilaterally symmetrical smooth osseous proliferation along the ventral surface of the mandible. Repeated bone biopsies had no growth on culture and initial biopsy samples contained only reactive bone. There was no evidence of dental disease on examination and radiographs, and she failed to respond to long-term antibiotic treatment. On postmortem, the left and right mandible were focally expanded by firm, smooth, rounded masses. On cut section, both mandibular masses were white to tan with scattered miliary light brown foci. Histologically, the sections of mandible contained abundant trabeculae of woven bone lined by osteoblasts and numerous reversal lines interspersed with abundant fibrosis and multifocal aggregates of neutrophils, macrophages, multinucleated giant cells, lymphocytes, and plasma cells which often surrounded radiating, eosinophilic, globular material (Splendore-Hoeppli). Bacterial cultures revealed numerous bacteria including *Actinomyces hyovaginalis* and *Clostridium septicum*. Mandibular osteomyelitis is a leading cause of morbidity and mortality in macropods. The terminology of macropod mandibular osteomyelitis has

been inconsistent and often implies specific agents such as “lumpy jaw” suggestive of actinomycosis and “oral necrobacillosis” suggestive of *Fusobacterium necrophorum* infection. The most current term, Macropod Progressive Periodontal Disease (MPPD), considers compounding factors like polymicrobial infections, plaque-mediated gingivitis, and abnormal dentition that predispose macropods to this progressive and debilitating disease. This case was further complicated as the clinical presentation was not suggestive of osteomyelitis.

SP-76: ROLE OF TUMOR-ASSOCIATED MACROPHAGES IN CANINE SOFT TISSUE SARCOMA

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Canine soft tissue sarcomas (STS) are a heterogenous group of tumors arising from tissues of mesenchymal cell origin. They are a common neoplasia in dogs and most arise in the subcutaneous tissues. While complete surgical excision can be curative, some tumors display highly infiltrative and potentially metastatic behavior. A better understanding of the mechanisms of tumor development and progression are necessary for better diagnostic, prognostic and therapeutic options. Tumor-associated macrophages (TAMs) have recently been identified as being recruited by tumor cells to help with survival, progression and metastasis. Thus, we hypothesized that TAMs would play a role in the progression of canine STS. To investigate this, we designed a pilot study to evaluate presence, distribution, and gene expression profile of macrophages in canine STS samples. Immunohistochemical staining for the canine macrophage marker IBA-1 was performed on 16 archived, formalin-fixed, paraffin embedded (FFPE) canine STS samples. Images were taken of each sample and analyzed with imaging software to determine percent area taken up by IBA-1 stain. The results show that Grade II tumors appear to have the highest degree of macrophage infiltration compared to Grade I and Grade III tumors. In addition, the distribution of macrophages within tumor samples appears to be random with no predilection for specific sites within the tumor. Real-time PCR results showed no significant gene expression differences of our chosen pro-inflammatory and anti-inflammatory cytokines. Future studies using larger patient numbers, fresh tissue samples and evaluating patient outcome data are necessary to build upon the results obtained here.

SP-77: SYSTEMIC MYCOBACTERIOSIS IN TWO MAREMMA SHEEPDOGS

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An approximately one-year-old, female spayed Maremma Sheepdog presented with a history of vomiting, diarrhea, anemia, and pyrexia. On abdominal ultrasound, a generalized lymphadenopathy and splenomegaly was discerned, and aspirates of the spleen and lymph nodes showed granulomatous inflammation with numerous intracellular non-staining bacilli within macrophages. Euthanasia was performed and necropsy showed diffusely enlarged lymph nodes up to 20 x 10 x 10 centimeters with cortices expanded and diffusely tan to yellow, and a mildly enlarged spleen with

multifocal, 0.1 centimeter diameter, white foci scattered throughout the sinuses. Histopathologic evaluation showed severe, multifocal to coalescing, granulomatous hepatitis, lymphadenitis, and splenitis with intralesional acid fast bacilli. Culture, PCR, and DNA sequencing confirmed the diagnosis of *Mycobacterium avium*. Two weeks prior an approximately one-year-old, intact male, Maremma Sheepdog presented to a private practice with a history of lethargy, weight loss, anorexia, and vomiting. Similar findings were discerned on abdominal ultrasound, cytology of the spleen and abdominal lymph nodes, and subsequent necropsy and histopathologic evaluation. Systemic mycobacteriosis is rare and mostly reported in younger dogs. The pathogenesis is poorly understood, though mechanisms involving immunosuppression or exposure to the organism early in life have been suggested. Treatment is often unrewarding, and infected animals may pose a zoonotic risk.

SP-78: MULTI-ORGAN LYMPHOMA SECONDARY TO ATOXOPLASMOSIS IN A FINCH

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A young adult finch of uncertain age was submitted for necropsy at VMCVM after being found emaciated and lethargic the day prior, with a clinical history of atoxoplasmosis. There was no evidence of pathology or abnormality on gross necropsy. Sections of lung, liver, intestines, ventricles, kidney, spleen, and heart were collected for histopathology. Histopathology of the small intestine revealed sheets of neoplastic round cells effacing the lamina propria, isolating crypts, and variably extending into the submucosa and serosa. Low numbers of associated heterophils, rare plasma cells, and rare neoplastic cells contained a large cytoplasmic vacuole containing low numbers of 2 um, round, basophilic merozoites. Remaining glands were markedly hyperplastic with piling up epithelium and prominent mitotic figures. Many of the epithelial cells contained macrogamonts, microgamonts, and schizonts. Histopathology of the liver contained a moderate number of the neoplastic cells that were identified in the intestine effacing the portal areas and filling the sinusoids. As seen in the intestines, large cytoplasmic vacuoles containing a low number of merozoites were found within neoplastic cells. These findings are consistent with the diagnosis of systemic atoxoplasmosis and concurrent multi-organ lymphoma. Atoxoplasmosis is associated with severe disease in young passerine birds and systemic infection mainly affects the liver. Neoplastic transformation due to *Atoxoplasma* spp. has previously been described, potentially due to the chronic inflammation or direct damage from the parasite. While the organism and pathogenesis are poorly understood, this case further supports the potential link between atoxoplasmosis and concurrent neoplasia, particularly lymphoma.

SP-79: HEPATIC LISTERIOSIS IN A SUGAR GLIDER

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A 5-year-old, intact, female sugar glider (SG) was presented to the Virginia-Maryland College of Veterinary Medicine Veterinary Teaching Hospital for necropsy after spontaneous death. Clinical history included that the SG was the second out of two SGs

who previously presented with weakness, loss of appetite, and inactivity. Prior to death, the SG was previously treated with subcutaneous fluids and Baytril. Gross observation of the SG included a body condition score of 9/9 with an abundant amount of body fat and 2cc of clear fluid in the thorax. The liver was enlarged, moderately pale with a fine, irregularly mottled pattern and pale tan areas throughout varying from 1 mm across to much finer granulation. Histologically, the liver revealed irregularly vacuolated hepatocytes with smooth vacuoles, infiltration of neutrophils and lymphocytes, and multifocal areas of necrosis. Additionally, the spleen, esophagus, pharynx, and kidney all displayed lymphocyte infiltration. *Listeria monocytogenes* was isolated on culture of the liver. These findings are compatible with the diagnosis of hepatitis likely due to listeriosis. Listeriosis is a bacterial infection commonly caused by *L. monocytogenes* associated with consumption of contaminated food. Literature searches for sugar glider listeriosis revealed one case of *L. monocytogenes* causing sepsis. Although there are limited reports on specific diseases found in SGs, this case expands our differential considerations when considering an ill SG.

SP-80: HIPPOCAMPAL NECROSIS AND PSEUDO-NEGRI BODIES IN A CAT

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An adult previously-stray female spayed American domestic short-haired cat presented to a primary care veterinarian with anorexia, anuria, ileus, and neurologic signs. Approximately 11 months prior to presentation the cat was found to be FIV positive by ELISA, given rabies and FVRCP vaccines, and treated with oral orbifloxacin for upper respiratory infection. At presentation she was under-conditioned, dehydrated, and non-visual. CBC and chemistry were consistent with hemoconcentration, thrombocytopenia, hyperglycemia, hyperproteinemia, and elevated alanine aminotransferase. The cat progressively worsened despite treatment, and humane euthanasia and necropsy were elected. Histopathology revealed bilateral neuronal necrosis throughout the hippocampus, characterized by shrunken, angular, hypereosinophilic neurons with nuclear pyknosis. Within the thalamus, mesencephalon and leptomeninges there were multifocal, bilateral and asymmetrical perivascular infiltrates of lymphocytes and few plasma cells. Neurons adjacent to foci of inflammation multifocally contained one or more round to ovoid, 2-6 micrometer diameter eosinophilic cytoplasmic inclusion bodies resembling Negri bodies. Brain samples submitted to the Virginia Department of Health were negative for rabies by direct fluorescence antibody testing. Additional brain samples were submitted to the CDC Infectious Diseases Pathology Branch – no rabies virus antigen was detected with IHC and no viral particles were observed within cytoplasmic inclusions by transmission electron microscopy. This supports a diagnosis of pseudo-Negri bodies, which are reported to be an incidental finding in older dogs and cats. The pathogenesis of hippocampal necrosis in cats is incompletely understood, but various factors have been proposed including autoimmune targeting of voltage-gated potassium channels, epileptic seizures, and environmental or toxic etiologies.

SP-81: PANCREATIC ENDOCRINE CARCINOMA IN A DOG

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A 9 year old, male castrated, mixed breed dog presented to the Virginia-Maryland College of Veterinary Medicine Veterinary Teaching Hospital with a history of vomiting and inappetence of a week duration. The dog had previously been diagnosed with hyperadrenocorticism. Abdominal ultrasound revealed an irregular poorly defined mass in the left pancreatic lobe, diffusely enlarged liver with multiple variably sized masses, and bilaterally enlarged adrenal glands. Fine needle aspirates of the pancreatic and liver masses were obtained and were found to be consistent with an islet cell carcinoma. Due to poor prognosis and decline in the hospital the patient was euthanized and presented for necropsy. Necropsy revealed a mass effacing most of the duodenal lobe of the pancreas, an enlarged friable liver effaced by multiple raised nodules, and a necrotic mass in the right axilla. Histologically the pancreatic mass displayed nests and packets of polygonal cells, characteristic of neuroendocrine tumors. Similar neoplastic cells were observed in the liver. The most common pancreatic islet cell tumors are derived from insulin-secreting beta cells,however, non-beta cell neoplasms can occur as well.

SP-82: CESTODES AND THEIR CALCAREOUS CORPUSCLES: USING SPECIAL STAINS CAN HELP!

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Identification of parasites in tissue sections is often challenging: recognizing key features of different taxa is critical for accurate diagnoses, yet such features may be difficult to see. One such dilemma is presented by calcareous corpuscles of cestodes, which are distinct to this group of helminths. The corpuscles are difficult to identify: they are concentrated in the anterior of the worm, can be hard to distinguish on standard H&E, and information about additional staining characteristics is concentrated in old literature, (neither readily known nor commonly accessed). Previous studies report that calcareous corpuscles (~20-30 µm) contain calcium, glycogen, alkaline phosphatases, mucopolysaccharides, and magnesium. Therefore, we were curious: what are the staining characteristics of corpuscles using common special stains? Formalin-fixed, paraffin embedded tissues containing adult cestodes from five cases (fish, avian, and mammal) were sectioned at 5 µm, and six special stains were applied: (i) Alizarin Red (free Ca^{2+}), (ii) Von Kossa (calcium phosphate), (iii) Prussian Blue (Ferric iron $Fe3^{+}$), (iv) Periodic acid–Schiff (PAS, polysaccharides), (v) Gomori Methenamine-Silver (GMS), and (vi) Gram stain. In four of the five cases, Alizarin Red and Von Kossa highlighted the corpuscles, with the former having less background staining and better visibility. Although PAS and GMS did not stain calcareous corpuscles, they did distinguish the spongy parenchyma and outer integument of the cestodes, (and provided excellent definition of the spiny hooks on the proboscis of an acanthocephalan). Our study has shown the value of the special stains for identifying distinguishing features of helminths in tissue sections.

Late-Breaking Poster Session B

LB-1: NRF2 AS A THERAPEUTIC TARGET FOR PRIMARY OSTEOARTHRITIS

Kendra Andrie[1], Rob Musci[1], Sydney Bork[1], Maggie Campbell[1], Joseph Sanford[1], Daniel Palmer[1], Owen Wahl[1], Christian Puttlitz[1], Benjamin Miller[2], Karyn Hamilton[1], Kelly Santangelo[1]
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Chronic inflammation and oxidative stress are key contributors to the pathogenesis of osteoarthritis (OA). Nuclear factor-erythroid 2-related factor-2 (Nrf2) is a transcription factor that serves as a master regulator of anti-inflammatory agents and phase I xenobiotic and phase II antioxidant enzymes, as well as enzymes involved in autophagic and proteasomal activity. Previous work using the Hartley guinea pig model of spontaneous OA has demonstrated a reduction in Nrf2 gene expression within knee joints of aging animals. Further, chondrocytes exposed to a Nrf2 activator *in vitro* demonstrated increased Nrf2 expression and decreased intracellular reactive oxygen species production. Therefore, we hypothesized that oral administration of a Nrf2 activator delays the progression of primary OA via activation of the Nrf2-pathway. Male (N=28) and female (N=28) Hartley guinea pigs, aged 5-months, were randomly assigned to receive daily oral treatment with either the Nrf2 activator PB125 or vehicle control. Animals were treated for 10-months and sacrificed at 15-months, a point when advanced OA changes were expected in control animals. Treatment with PB125 resulted in: increased long bone strength as determined by ultimate bending stress (P=0.006); a decreased total knee joint histology score for OA (P=0.0095); and increased total distance traveled during a 10-minute trial as assessed by overhead cage monitoring (P=0.04). Further, Nanostring gene expression profiling unveiled significant changes in inflammatory transcripts locally within knee joint tissue. Collectively, this work provides insights into the pathogenesis of OA and aids in the development of a novel therapeutic for managing disease.

LB-2: SEVERE VERMINOUS PERITONITIS IN A JUVENILE BLACK CROWNED NIGHT-HERON (NYCTICORAX NYCTICORAX HOACTLI) OF THE VALLEY OF MEXICO

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Eustrongylides spp. infection in many species of nestling and adults Ardeids has been reported; however, there's no epidemiological and pathological studies of this parasite infection in birds of Mexico. A juvenile Black crowned night-heron (*Nycticorax nycticorax hoactli*), was submitted for postmortem examination to the Diagnostic and Research Bird Diseases Laboratory of the Faculty of Veterinary Medicine of the National an Autonomous University of Mexico. At necropsy, the pectoral muscles were moderately atrophied, and the keel was prominent. The ventral serosal surface of the proventriculus, ventriculus and intestines was focally overlaid by multiple, tortuous

intertwining tubules (parasite tracts), that contained few nematodes 0.5 mm in diameter and up to 10 cm long; as well, the proventriculus had a 3 cm wide perforation. On histopathology the wall of the proventriculus contain multiple variably sized granulomas surrounding multiple well-preserved nematodes which had a thick, eosinophilic cuticle, polymyarian-coelomyarian musculature, pseudocoelom, esophagus, intestinal tract and male/female reproductive tracts. The parasitological analysis identified *Eustrongylides* spp. as the causal agent. Polymerase chain reaction (PCR) for the nuclear large subunit ribosomal RNA gene (LSU rDNA) and sequencing are in process for complete identification of the parasite. Owing to his migratory habit, species of this group of birds are highly important epidemiologically in the spread of several pathogens in different geographical areas. Also, this disease is considered the most likely cause of death in chicks of wading birds in North America and could have public health implications.

LB-3: PRELIMINARY SEROLOGICAL EVIDENCE OF SELECTED ARBOVIRUS IN FREE-RANGING BATS AND BIRDS FROM ENDEMIC AREAS IN COSTA RICA

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Arbovirus are considered important causes of emergent zoonotic diseases with public health significance. The potential role of wildlife in their transmission cycle is not fully elucidated. Thus, free ranging bats and birds were analyzed for arboviral infection. A total of 144 bats (26 species) and 140 birds (43 species) were sampled in two Costa Rican different regions. Blood and tissue samples were collected. Molecular (RT-PCR), serological and histological assessment were performed. Neutralizing antibodies were detected by plaque reduction neutralizing tests against Dengue (DENV 1-4), Zika Virus (ZIKV), Yellow Fever Virus (YFV), West Nile Virus (WNV), Saint Louis Encephalitis Virus (SLEV), Eastern Equine Encephalitis Virus (EEEV) and Venezuelan Encephalitis Virus (VEEV). In bats, 34.95% (43/123) for DENV-1, 16.26% (20/123) for DENV-2, 5.69% (7/123) for DENV-3, 4.87% (6/123) for DENV-4, 2.43% (3/123) for WNV, 4.87% (6/123) for SLEV, 7.31% (9/123) for EEEV and 0.81% (1/123) for VEEV were found positive by PRNT. Antibodies to ZIKV and YFV were not found. In birds, PRNT resulted positive against WNV in 0.80% (1/124), SLEV in 5.64% (7/124), EEEV in 8.4% (6/71) and VEEV in 5.63% (4/71). No significant histological findings were observed. Results for RT-PCR are still pending. Although this is the first serological evidence of SLEV, WNV, EEEV and VEEV in bats and birds in Costa Rica, this does not demonstrate an active role in transmission, since viremia or acute infection was not measured. Therefore, further studies are needed to elucidate their potential role.

LB-4: BOVINE RESPIRATORY SYNCYTIAL VIRUS FATAL INFECTION IN A YOUNG GOAT

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Background: Bovine respiratory syncytial virus (BRSV) is a pneumovirus causing bronchointerstitial pneumonia primarily in calves under 6 months of age and in yearlings and adult cattle. Lesions and demise in small ruminants are rare.

Objective: Reporting a very unusual fatal infection of BRSV in a kid.

Methods: A one-week-old, 4.15 kg, male goat that died after brief severe respiratory distress, was submitted for pathological examination. The adult does had mild coughing and recovered in one week. Necropsy and histopathology were performed and tissue samples were submitted for direct fluorescent immunohistochemistry and bacteriological aerobic culture.

Results: Lungs had a cranioventral and sparse lobular polygonal red discoloration. Histologically, there was necrosis of bronchial and bronchiolar epithelium and distal airways were expanded by degenerate neutrophils, cellular debris, macrophages, sloughed epithelial cells, fibrin, edema and there were sparse syncytial cells with eosinophilic oval intracytoplasmic inclusion bodies. Other changes included type II pneumocyte hyperplasia, macrophages infiltrating the alveolar septa and necrosis. Direct immunohistochemistry and bacteriological culture of lung tissue revealed BRSV and *Mannheimia haemolytica*.

Conclusions: BRSV is a constant threat to the bovine industry and can sporadically cause fatal acute severe lesions in young small ruminants. *M. haemolytica* was also identified in this case, but the gross and histologic lesion morphology seems to indicate that BRSV was the significant and fatal etiologic agent in this kid. The adults were mildly affected and quickly recovered.

LB-5: ONGOING INVESTIGATION INTO THE CAUSE OF SEASONAL HINDLIMB PARALYSIS SYNDROME IN CARNABY'S COCKATOOS IN WESTERN AUSTRALIA

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The preliminary results of an ongoing investigation into an emerging disease of leg paralysis and mortality, termed Carnaby's Hindlimb Paralysis Syndrome (CHiPS), in endangered Carnaby's black cockatoos (*Calyptorhynchus latirostris*) from Western Australia are presented. Current clinical and epidemiologic findings are most suggestive of a toxicosis as the cause, particularly an organophosphate toxicosis. To date, necropsies have been performed on 17 case animals and 14 controls. No significant findings have been demonstrated with gross examination and histopathology; including serial sectioning of the brain, spinal cord, sciatic nerves, brachial plexus and multiple skeletal muscle bodies, as well as acetylcholine esterase assays ($p>0.05$ and within published reference interval). Initial results from an assay used to investigate delayed organophosphate neuropathy (NTE) in brain tissue has demonstrated a significant difference between case and control birds ($p<0.001$). The median brain NTE activity for control birds was 0.1172 mU/g of brain tissue (min-max = 0.05-0.21) compared to the median brain NTE activity of 0.0226 mU/g of brain tissue in CHiPS cases (min-max =

0.00-0.10). The authors are currently working on validation of this assay and production of a reference interval for NTE in the brains of Carnaby's black cockatoos. Toxicology screening is pending.

LB-6: HEMATO-BIOCHEMICAL AND HISTOPATHOLOGICAL OBSERVATIONS IN SUBEPIDERMAL VESICULAR AND PUSTULAR DERMATITIS IN A DROMEDARY (CAMELUS DROMEDARIUS) HEMANT DADHICH1; MANISH AGRAWAL1; ABHILASHA DADHICH1 AND MANISHA MATHUR1 1DEPARTMENT OF VETERINARY PATHOLOGY, COLLEGE OF VETERINARY AND ANIMAL SCIENCE, BIKANER- 334001 RAJASTHAN INDIA

Hemant Dadhich, Manish Agrawal, Abhilasha Dadhich, Manisha Mathur Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

The dromedary tolerates high temperature, solar radiation and water deprivation as it has ability to support a very high degree of water loss. Due to the extremely concentrated urine and insignificant sweat, the camel does not loose water. The temperature of skin remain cool due to coarse and well ventilated hairs on its back which allow evaporation to take place on the surface of the skin. The skin infections are caused by bacteria, viruses, parasites and fungi. Among bacterial infections, staphylococcal dermatitis, contagious skin necrosis or skin wound and abscesses have been reported to occur the most. In the present study, a dromedary aged about 6 years suffering from skin lesions on neck region reported to the veterinary clinic. A biopsy was taken for histopathology and blood was collected for clinical pathological examination. On the basis of histopathological changes, the Sub-epidermal vesicular and Pustular Dermatitis was identified in a dromedary suffering from skin lesions primarily of dermatitis. Hematological examination revealed significant increase in TEC, MCHC, TLC and neutrophils count with significant decrease in MCV and lymphocyte count. There were significant increase in total protein and serum globulin and a mild increase in serum glucose and albumin levels were also observed. Microscopic findings revealed dermo-epidermal separation, severe subepidermal edema and cellular infiltration. There were formation of subepidermal vesicle and pustules formed due to hydropic degeneration of basal cells. There was also severe intercellular edema with blowout of basement membrane zone.

LB-7: LOCALIZED HISTOPLASMA CAPSULATUM OSTEOMYELITIS IN THE HUMERUS OF A DOG

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Histoplasmosis is a common fungal infection that affects animals worldwide caused by the saprophytic fungus *Histoplasma capsulatum.* Infection occurs via inhalation of macroconidia present in contaminated soil and can present as a subclinical, pulmonary or systemic granulomatous disease. Case description: A 12-month-old, male, mixed-breed dog presented to the Small Animal Clinic of St George's University with a history of unknown duration of non-weight bearing lameness, pain and severe swelling in the

right upper forelimb. The radiographic exam revealed a severe generalized osteoproliferative and osteolytic lesion affecting the humerus, distal scapula, and proximal radius/ulna. Additional lesions were not identified on screening radiographs. Limb amputation was performed followed by cytology, bacterial and fungal culture, and surgical biopsy. Macroscopic examination of the humerus revealed a focally extensive, transmural, hard, white, solid, 5cm in diameter mass effacing the proximal epiphysis; and diffusely roughening of the cortical surface. Lymph nodes were enlarged, with prominent lymphoid follicular hyperplasia. Histopathology revealed severe chronic pyogranulomatous cellulitis, pyogranulomatous eosinophilic lymphadenitis and severe chronic pyogranulomatous osteomyelitis and osteonecrosis with numerous intrahistiocytic, PAS-positive, 2-4um, narrow-based budding, round, fungal organisms. Cytology, bacterial and fungal cultures, and fungal PCR were negative. The patient recovered uneventfully with no reoccurrence of clinical signs. Here, we describe a rare presentation of localized *Histoplasma capsulatum* osteomyelitis in the humerus of a dog diagnosed via histopathology and radiology. We hypothesize that infection occurred secondary to contamination of a focal injury. Negative results from ancillary tests were interpreted as a result of inadequate tissue sampling

LB-8: MOUSE KIDNEY PARVOVIRUS PRODUCES CHRONIC SHEDDING IN IMMUNOCOMPETENT MICE

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Mouse kidney parvovirus (MKPV) was recently identified as the etiology underlying inclusion body nephropathy in immunocompromised mice. MKPV has since been identified in wild and laboratory mice but is controlled by the adaptive immune system and is subclinical in immunocompetent mice. MKPV positive CD-1 mice (n = 30) were identified via PCR and individually housed for clinical observation and diagnostic sampling. Cage swabs, fecal pellets, urine, and blood were evaluated over 200 days for all mice. PCR of the urine identified chronically infected mice (n = 27), the majority of which were viremic at 5 months of age (n = 22). Histologic lesions associated with MKPV in these mice reveals a progressive lymphoplasmacytic tubulointerstitial nephritis with tubular degeneration and karyomegalic cells; inclusion bodies were not observed. Although inclusion bodies were absent, intralesional MKPV mRNA was observed via chromogenic RNA *in situ* hybridization. Evaluation of diagnostic testing was also pursued on both colony animals and dirty bedding sentinels and indicates that PCR of a swab passed through wet and dirty bedding may prove effective at identifying positive animals or cages; fecal PCR occasionally failed to identify positive cages. MKPV can be detected in a variety of sample types from immunocompetent mice including urine, feces, cage swabs, and formalin-fixed paraffin-embedded renal tissue. Immunocompetent mice are susceptible to persistent infection and MKPV shedding. Intranuclear inclusion bodies are not a consistent feature of MKPV infection in immunocompetent mice.

LB-9: PATHOLOGY OF PSEUDORABIES VIRUS CLASSICAL STRAIN BRISTOL AND THE VARIANT JS-2012 STRAIN IN EXPERIMENTALLY INFECTED PIGS

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Background: Vaccination of pigs has been widely performed in China to reduce losses caused by pseudorabies virus (PRV) infection. In 2011, PRV outbreaks were reported in the vaccinated swine population in China which were caused by a new PRV variant. Based on phylogeny, these PRV variants have been assigned to genotype II whereas "classical" PRV strains are genotype I. Classical PRV is fatal in neonatal pigs, however the mortality rate decreases as the pigs age. In contrast, several experimental infection studies have concluded that when compared to classical PRV strains the variant PRV strains showed higher mortality rates in all age groups.

Objective: The primary objective of this study was to generate positive clinical samples for assay validation by infecting pigs of 2 different age groups (3 and 7 weeks) with a classical PRV strain (Bristol) and a variant PRV strain (JS-2012). A secondary goal was to determine if there were clinical and pathological differences between the groups

Results: In both age groups there was increased mortality in pigs infected with Bristol (3/3, 3/3) compared to those infected with JS-2012 (6/8 3 weeks, 0/8 7 weeks). In the 3 week group, histological lesions were widespread affecting many organs in animals infected with Bristol whereas pigs infected with JS-2012 had lesions in brain, trigeminal ganglion and lung.

Conclusion: Both PRV strains resulted in neurological signs, mortality and lesions in pigs. In contrast to previous studies, under our experimental conditions, the classical Bristol strain appeared more virulent than the variant JS-2012 strain.

LB-10: FUNCTIONAL AND PHENOTYPIC CHANGES IN BOVINE MONOCYTES DURING LETHAL THEILERIA PARVA INFECTION

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The tick-born, apicomplexan hemoparasite, *Theileria parva*, is the leading cause of death in cattle of sub-Saharan Africa. Immune protection against *T. parva* involves $CD8^+$ cytotoxic T cell responses to parasite-infected cells and humoral immune responses to sporozoite surface proteins. However, knowledge regarding the role played by innate immune cells in ECF pathogenesis and *T. parva* control is lacking. Here we demonstrate an increase in intermediate monocytes ($CD14^{++}CD16^+$) with a concomitant decrease in the classical ($CD14^{++}CD16^-$) and non-classical ($CD14^+CD16^+$) subsets at 12 days post-infection (DPI) during lethal, but not non-lethal, *T. parva* infection. Monocytes from lethally infected cattle demonstrated *ex vivo* up-regulation of IL-1β and TNFα mRNA and increased nitric oxide production. Furthermore, *in vitro* stimulation with *T. parva* schizont-infected cells or *Escherichia coli* LPS resulted in

significant up-regulation of IL-1β production by monocytes from lethally infected cattle compared to those from non-lethally infected animals, and monocytes from lethally infected animals produced significant amounts of IL-10 mRNA after stimulation with *T. parva* schizont-infected cells. In conclusion, we demonstrate that lethal *T. parva* infection results in functional and phenotypic changes in bovine monocytes. These changes can serve as biomarkers for ECF progression and severity, thereby aiding in the standardization of protection assessment for *T. parva* candidate vaccines.

LB-11: DEVELOPMENT OF A SIMPLE PROTOCOL FOR DIAGNOSTIC-QUALITY HISTOPATHOLOGIC SECTIONS OF THE EUROPEAN HONEY BEE (APIS MELLIFERA) AND A SURVEY OF CLINICALLY NORMAL BEES WITH SPECIAL STAINS FOR USE AS DIAGNOSTIC CONTROLS

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Background: Following the implementation of the Veterinary Feed Directive (VFD) in 2017, it is anticipated that honey producers and veterinarians may reach out to diagnostic centers for help with disease management. There are few practical protocols available in the literature describing the preparation of bees for diagnostic histology, and a survey of clinically normal bees with special stains used routinely in diagnostic pathology does not exist in the English literature.

Objective: 1) To develop a simple protocol for diagnostic-quality histopathologic sections of the European Honey bee (*Apis Mellifera*) omitting prior organ isolation, or chemicals or equipment beyond routine equipment in diagnostic histology laboratories, 2) To describe normal anatomy in clinically healthy bees, 3) To establish normal staining patterns for a suite of standard special stains.

Methods: Clinically normal, adult bees were euthanized and preserved in formalin. Softening of exoskeletons was followed by routine processing to paraffin-embedded blocks, and a suite of special stains was applied.

Results: All organ systems described in the available literature were visualized, except for the reproductive tract. Gram and Giemsa staining highlighted a mixed population, mostly gram positive, variably sized rods and coccobaccili, with highest concentrations in the rectum. PAS highlighted the peritrophic membrane and secretory vesicles in hypopharyngeal glands. Fungal entities were not visualized in these clinically normal bees (PAS or GMS).

Conclusions: A protocol utilizing chemicals and equipment routinely used in diagnostic histology laboratories was developed, produced diagnostic-quality sections of honey bees, and supported generation of normal controls for a suite of special stains.

LB-12: AN UNUSUAL DIAGNOSIS: OSTEOPETROSIS IN A 3-YEAR-OLD CAT

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A 3-year-old, spayed female, 2.65 kg, orange and black, domestic medium hair cat was submitted to the Animal Disease Diagnostic Laboratory. The cat first presented on October 9, 2018 to the Purdue Small Animal Hospital for a one month history of weight bearing lameness of the right rear limb and mandibular swelling. Radiographs revealed a right hind limb patellar fracture and osteopetrosis in the long bones. Eleven months later, the cat's quality of life had significantly decreased and it was euthanized. Histopathological findings included osteopetrosis of both long and flat bones (femur, tibia, rib, patella), left mandibular osteoma, right patellar fracture, retinal ganglion cell degeneration and loss, persistence of deciduous teeth, and splenic and hepatic extramedullary hematopoiesis. Osteopetrosis is defective bone resorption and persistence of primary spongiosa due to lack of or impaired function of osteoclasts. Osteopetrosis has been described in humans and animals; however, it has very rarely been described in cats. Osteopetrosis has been associated with gene mutations including ATP6i (osteoclast proton pump), ClCN7 (osteoclast chloride channel), SLC4A2 (osteoclast anion exchange), and RANKL (needed for osteoclast differentiation). Rarely, acquired osteopetrosis in cats has been associated with FELV infection, and diffuse osteosclerosis has been associated with lymphoma, myeloproliferative disorders or systemic lupus erythematosus, none of which were present in this case.

LB-13: FELINE PERIPHERAL ODONTOGENIC FIBROMA: HISTOLOGIC AND CLINICOPATHOLOGIC DESCRIPTION OF 50 CASES

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Background: Oral neoplasms are common in domestic feline species, representing between 3 to 12% of overall neoplasms in the cat. Peripheral odontogenic fibromas (POFs) are classified under this category, but are relatively uncommon, compromising only 0.3% of feline oral neoplasms. The long term prognosis, age distribution, gender and characteristics of POFs have never been, to the knowledge of the authors, fully described in the literature. Furthermore, POFs may be challenging to distinguish from other common malignant tumors such as oral squamous cell carcinomas.

Objectives: To describe the clinical, histologic features, histologic variants and prognostic correlates of feline POFs as well as establish guidelines for differentiating POF from malignant oral neoplasms.

Methods: Fifty cases were collected. All cases were verified as POFs based on an established histomorphological case definition. Clinical follow up was obtained on 17 cases to evaluate survival times and recurrence.

Results: Histopathology of feline POF identified two variants; a fibroblastic variant with immature collagen and prominent odontogenic ectomesenchyme, and a mature form with large swaths of mature collagen and islands of odontogenic mesenchyme. Clinical follow-up was obtained for 17 cases and demonstrated rare mass recurrence, no metastasis, and no mortality related to the oral neoplasm. Clinical outcomes were not correlated with the histological variant, location or with presence of matrix.

Conclusions: Peripheral odontogenic tumors in cats are a benign entity. Two histologic variants of feline POFS have been identified, which have similar prognostic outcomes. Clinical follow up demonstrates a favorable prognosis via complete or incomplete surgical excision.

LB-14: FURTHER CHARACTERISATION OF BRONCHOPNEUMONIA WITH INTERSTITIAL PNEUMONIA (BIP) IN BEEF FEEDLOT CATTLE

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Bronchopneumonia with interstitial pneumonia (BIP) is a unique and yet uncharacterised form of bovine respiratory disease in North American beef feedlot cattle that is described as a concurrent caudodorsal interstitial pneumonia (AIP) and cranioventral bronchopneumonia (BP). Presented here is a continuation of disease characterisation utilising histologic and microbiological data with comparison to its constituent forms of BRD (i.e. AIP and BP). 39 Cases were blindly diagnosed by histology as BIP (n = 16), AIP (n = 7) or BP (n = 16). Sensitivity and specificity of post-mortem (gross) diagnosis of BIP was 88% and 61%, respectively (PPV = 31%, NPV = 96%; Prevalence = 17%). The prevalence of key histological lesions of respiratory disease in BIP cases is discussed; comparative frequency of these lesions did not reveal significant differences between BIP and BP or AIP in cranioventral or caudodorsal lung samples, respectively. In cases of BIP, cranioventral lung lesions were more likely to be chronic (88%; $p = 0.004$) while caudodorsal lung lesions were more likely to be acute (64%; $p = 0.004$). Pasteurella multocida was isolated from a greater proportion of BIP cases compared to AIP (63% vs 14%; $p = 0.04$). This data provides a framework for characterisation of this potentially novel disease and will act as the foundation for further investigative work into the pathogenesis of BIP. Initial hypotheses include dysregulation of 3-methylindole metabolism (the causative agent of AIP) by a primary inflammatory episode.

LB-15: UTILIZATION OF ADVANCED DIAGNOSTIC TECHNIQUES FOR DETECTION OF A UNIQUE ADENOVIRUS IN A PACIFIC PARROTLET (FORPUS COELESTIS)

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Approximately 4 months post adoption, a young adult, male, Pacific parrotlet (*Forpus coelestis*) became acutely lethargic and huddled at the bottom of the cage. Within 24 hours of the development of initial clinical signs and in spite of supportive care, the bird developed seizure activity and was humanely euthanized. Gross autopsy findings were mild serous coelomic effusion and a diffusely pale tan liver. Histopathology showed that the hepatocytes, along with the histiocytes of the spleen and intestinal tract, contained large, homogenous, amphophilic to eosinophilic, intranuclear inclusion bodies.

Additionally, there was marked lymphocytolysis within the spleen. Electron microscopy performed on the liver revealed numerous, small, round to polyhedral, electron-dense viral particles ranging in size from 58 to 74 nm in diameter within the hepatocyte nuclei. In situ hybridization (ISH) was performed at the University of Georgia on the liver utilizing standard viral probes and was negative for avian adenovirus, Pacheco's disease virus, generic and psittacine circovirus, and polyomavirus. Deep sequencing was performed for detection of viral DNA and found adenoviral DNA not typically detected by standard probes. This virus is similar to a siadenovirus previously detected in budgerigars (budgies) and a sun conure. An ISH probe developed from this similar siadenovirus strain was positive in this parrotlet. This is the first reported case of this variant of avian siadenovirus within the parrotlet genus. To date, the three other birds (another parrotlet and two budgies) in the household are clinically healthy.

LB-16: A RETROSPECTIVE STUDY OF PIGEON HERPESVIRUS (COLUMBID HERPESVIRUS-1) INFECTION IN DOMESTIC PIGEONS (COLUMBIA LIVIA F. DOMESTICA) DIAGNOSED AT THE CALIFORNIA ANIMAL HEALTH AND FOOD SAFETY LABORATORY SYSTEM (1991-2014)

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Background: Pigeon herpesvirus (PiHV) infection plays an important role in respiratory disorders in domestic pigeons such as the multifactorial respiratory disease complex.

Objective: This 23.5-year (1991-2014) retrospective study characterized the naturally occurring PiHV infection in domestic pigeons diagnosed at the CAHFS of the University of California-Davis.

Methods: A total of 62 pathology reports, involving a total of 97 domestic pigeons, were retrieved and analyzed. The number of birds in each accession, location at county level, breed/production type, affected organs, microscopic lesions, diagnostic methods used, and concurrent infections were evaluated. Detection of eosinophilic intranuclear inclusion bodies was the *sine qua non* condition on which histopathologic diagnosis was based.

Results: The diagnosis of PiHV infection was mainly based on histopathology alone (71%) or on histopathology combined with viral isolation (12.9%), transmission electron microscopy (4.8%) or PCR (1.6%). The disease affected one system in 54.8% of cases, and the digestive system was most commonly affected (88.2%). The liver (62.9%), crop (27.4%) and esophagus (22.6%) were the most commonly affected organs. The disease was a secondary diagnosis or incidental finding in most (56.5%) cases. Most (88.7%) cases had one (33.8%), or up to four (54.8%) other concurrent infections, pigeon circovirus infection (41.9%), trichomoniasis (38.7%), aspergillosis (17.7%) and colibacillosis (16.1%) being the most common. Unusual microscopic presentations of PiHV infection were also noted.

Conclusions: This study is the first on natural PiHV infection based on a large set of domestic pigeon cases under field conditions, highlighting certain disease patterns, awareness of which may aid diagnosis.

LB-17: ENCAPSULATING PERITONEAL SCLEROSIS CAUSED BY ABDOMINAL DISSEMINATION OF PANCREATIC DUCTAL ADENOCARCINOMA IN A DOG

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Background: Progressive fibrosis of the peritoneum with small intestinal encasement (encapsulating peritoneal sclerosis, EPS) is a condition of diverse etiology with low incidence but with high mortality. In dogs, mesothelioma and poorly-differentiated carcinoma have been reported to be the cause of EPS. Pancreatic ductal carcinoma (PDC), a highly aggressive human neoplasm of pancreatic duct origin, does not seem to have been investigated in detail in dogs or in animals in general.

Objective: Our aim is to broaden the differential diagnoses for EPS by presenting an autopsy case of canine PDC.

Methods: An 11-year-6-month old neutered male French Bulldog exhibiting repeated vomiting was presented to a regional veterinarian. Physical examination, CBC, blood chemistry, CT imaging, exploratory laparoscopy, and biopsy of the peritoneum were performed by regional and referring veterinarians. The animal died despite a 3-month symptomatic treatment. A full autopsy was followed by histopathology, histochemistry (Masson trichrome, PAS), and immunohistochemistry (CK AE1/AE3, CEA, vimentin, WT-1, Muc1, Muc2, S-amylase, chromogranin A, Ki-67, TGF-b).

Results: CT, laparoscopy, and biopsy of the small intestinal serosa revealed EPS of unknown etiology. Results of histopathology and immunohistochemistry on samples obtained at autopsy supported the diagnosis of PDC. The neoplasm never formed a discrete nodule. The entire peritoneum and all laparoscopy ports were hardened with marked desmoplasia. Some tumor cells showed anaplasia. Hematogenous metastasis was detected in the lung and right adrenal. Suspected cause of death was septicemia and pulmonary edema.

Conclusions: To our knowledge this is the first report of canine PDC to have caused EPS.

LB-18: BLOOD SMEAR EXAMINATION ALONGSIDE AUTOMATED HEMATOLOGY ANALYSIS IN FIRST-OPINION PRACTICE-HOW FEASIBLE IS IT?

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The practice of microscopic blood smear examination does not appear to be keeping up with the proliferation of in-house automated analyzers. Likely causes for the omission include lack of training and available time. An online survey was distributed to first-opinion veterinary clinics across the United Kingdom to investigate the use of

hematology analyzers, the frequency of blood smear examinations, and the reasons for not utilizing the latter. Majority of respondents (138/182) were veterinary surgeons, with lower numbers being veterinary nurses, technicians, laboratory coordinators. Automated analyzers were present in the practices of 89.6% of the respondents. Only about a quarter of respondents look at blood smears in significant numbers; others indicated that smear examination was rare or not part of their hematology analysis. Majority of respondents who don't have a hematology analyzer also tend not to examine blood smears. Common reasons to look at blood smears included error flags on hematology analyzer, platelet clumping, checking for neutrophil left shift and toxicity, checking for erythrocyte regeneration, and if analyzer results did not match clinical picture. Many respondents had an adequate to excellent microscope available, and typically the veterinary surgeons made the smears (vs. veterinary nurses). Main reasons for not examining smears with the automated analysis were lack of time, confidence/skill, and possibility to send samples to external laboratories. There appears to be a need to increase awareness of the importance of smear examination when using automated hematology analyzers, in combination with further training in both university and continuing education.

LB-19: CHARACTERIZATION OF A MURINE T-CELL TRANSFER MODEL OF INFLAMMATORY BOWEL DISEASE.

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Introduction: Inflammatory bowel diseases (IBD) are multifactorial disorders characterised by chronic and relapsing inflammation. The T-cell transfer model is used in preclinical efficacy studies for IBD. CD4+CD45RBhigh T-cells injected intraperitoneally into immunodeficient mice migrate to the intestinal mucosa and drive a Th1/Th17 immune response against the gut microbiota. As the host animals lack regulatory T-cells, chronic colitis develops, mimicking the human disease. We describe a simple, reproducible histopathology scoring system based on a semiquantitative assessment of mucosal changes, and the extent of the lesion, which showed good correlation with other study endpoints.

Study Design: 8-week old SCID and Rag2-deficient mice were evaluated, over 6 weeks, for the development of colitis, following transfer of naive T-cells. PBS-injected animals were used as controls. Endoscopy, colon length and weight measurements and microscopic examinations were carried out. Separately, SCID animals with induced colitis were treated with anti-IL-12 p40, to test for a demonstrable therapeutic effect.

Results: T-cell transfer resulted in increased weight loss, colon density (ratio weight/length), macroscopic disease activity index and endoscopy index, when compared with controls. Histopathological changes were consistent with chronic colitis. Anti-IL12 (p40) treatment ameliorated experimental T-cell-dependent colitis, both macroscopically and microscopically.

Conclusion: This confirms the validity of the histopathology scoring system and the value of this model in investigating the efficacy of therapeutic agents acting via modulation of T-cell function.

Animal studies were ethically reviewed and carried out in accordance with the Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals.

LB-20: SUBCUTANEOUS SARCOMAS RELATED TO CHRONIC SUBCUTANEOUS ADMINISTRATION OF 30% (W/V) AQUEOUS SULFOBUTYL ETHER 7-B-CYCLODEXTRIN (CAPTISOL™) IN WISTAR HAN RATS

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Cyclodextrins solubilize and stabilize drugs and improve drug delivery. A polyanionic variably substituted sulfobutyl ether of β-cyclodextrin [sulfobutyl ether 7-β-cyclodextrin (SBE-β-CD; Captisol™)] interacts well with neutral drugs to facilitate solubility and chemical stability, and is used in products approved by the Food and Drug Administration. Five groups of 60 male and 60 female Wistar Han rats were administered a dose volume of 3 mL/kg saline control (Group 1), 30% (w/v) SBE-β-CD in sterile water for injection, final pH 7.2±0.5 (vehicle control/Group 2), or SBE-β-CD combined with low, mid, and high doses of a test article (Groups 3-5) by once daily subcutaneous administration in a 2-year carcinogenicity study. Due to rapidly expanding subcutaneous tumors in all groups except the saline control, males were terminated early during Week 60 and females were terminated early during Week 83. The subcutaneous tumors were initially observed after approximately 9 months of dosing and required humane euthanasia after approximately 4-6 weeks of growth. The tumors consisted primarily of pleomorphic fibrosarcomas and fibrosarcomas and were attributed to chronic irritation in the subcutaneous tissue related to SBE-β-CD administration. There was no exacerbation of tumor development by the test article. SBE-β-CD-related non-neoplastic findings included cytoplasmic vacuolation of epithelial cells in the kidney and prostate, parathyroid chief cells, and macrophages in the subcutaneous injection site. Chronic subcutaneous administration of 30% (w/v) SBE-β-CD causes malignant sarcomas at the administration site that result in high mortality and preclude its use as a vehicle for subcutaneous administration in 2-year carcinogenicity studies in rats.

LB-21: POLYARTHRITIS ASSOCIATED WITH A NOVEL MYCOPLASMA SPECIES IN TWO BIG BROWN BATS (EPTESICUS FUSCUS)

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Two Big Brown Bats (*Eptesicus fuscus*) with swelling of multiple joints were presented to a rehabilitation center in May of 2019. The bats were found separately in two townships in Western Ohio. The bats were initially treated with antibiotics for seven

days and anti-inflammatory drugs for five days, but no improvement was seen. The bats were euthanized after 5 weeks when they were no longer able to roost. Necropsy and histologic examination demonstrated severe suppurative polyarthritis and cellulitis. Polyarthritis was characterized by destruction of the synovium, with cellular debris in the joint space and degenerate cells on the roughened articular surfaces. Osseous roughening, suppurative periostitis, and severe suppurative and necrotizing cellulitis were also seen. There were occasional pathologic fractures of long bones adjacent to the articular spaces. Bacterial and viral cultures of swabs and tissues from multiple joints were negative. Pan-Mycoplasma-specific PCR targeting the intergenic spacer region was positive on swabs of the affected joints of both bats. DNA sequences of portions of the intergenic spacer region and 16S rRNA gene of the Mycoplasma detected in the bats shared only 77% and 91% identity, respectively, with other described species of Mycoplasma; thus, it likely represents a novel species.

LB-22: DIET AND GENETICS IMPACT ZONAL DISTRIBUTION OF LESIONS IN MOUSE NAFLD MODELS

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Introduction: Human NAFLD subtypes have zonal lesion distribution pointing at metabolic zonation as main determining factor of NAFLD subtype-specific hepatic phenotype. Here, we compared histopathology data from several mouse models with the histopathology of human NAFLD. Design and

Methods: Microscopic examination was performed on livers from mice fed various diets: standard high fat diet, ALIOS or AMLN diet, human PNPLA3 I148M expressing mice on regular chow, and ob/ob mice on regular chow.

Results: Mild to moderate steatosis was observed in the ob/ob model known for high *de novo* lipogenesis and insulin resistance and in mice on standard high fat diet. The lesion distribution resembled human adult NAFLD with steatosis in Zone 3. In addition to mostly microvesicular steatosis in Zone 3 due to high fat diet, high fructose in ALIOS and AMLN diets produced macrovesicular steatosis in Zone 1, which is also often observed in pediatric NAFLD. HuPNPLA3 I148M mice fed regular chow diet had random or diffuse distribution of steatosis, also reported in human carriers of the huPNPLA3 I148M variant.

Conclusion: Diet and genetics have similar impact on lesion distribution in human NAFLD and mouse models, which is likely explained by evolutionary conservation of metabolic zonation of the hepatic metabolism. Even though listed mouse NAFLD diets poorly resemble human diets because of non-physiological amounts of fats, cholesterol and fructose, lesion distributions in these mouse models mimic human NAFLD subtypes. Impact statement: This emphasizes the importance of appropriate selection of mouse models for efficacy studies targeting different human NAFLD subtypes.

LB-23: EVALUATION OF RAT ACUTE PHASE PROTEINS AS INFLAMMATORY BIOMARKERS OF ADJUVANT ADMINISTRATION

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Background: The development of vaccines often includes the use of adjuvants to enhance the immune response, thus it is important to understand the acute phase protein (APP) response and microscopic finding of common adjuvants used in vaccine studies.

Objectives: The purpose of this investigation was to characterize the APP response and microscopic correlates after injections of common adjuvants into rats.

Methods: The responses to intramuscular administration (IM) of $AlPO_4$, $Al(OH)_3$, CpG/$Al(OH)_3$, or QS21 to Wistar rats were compared with TdaP vaccine, saline or a positive control intravascular injection of LPS. A time course study with serial assessment of APP and microscopic correlate study with assessment of APP 48 hours post dose (HPD) were done. Alpha-2-macroglobulin (A2M) and alpha-1 acid glycoprotein (A1AGP) were measured using MSD analyzer. Fibrinogen (FIB) was measured on STA Compact.

Results: The time course of APP responses was characterized. IM of TDaP vaccine or CpG/$Al(OH)_3$ resulted in inflammatory changes and muscle degeneration/necrosis at injection site and accumulation of macrophages with granular material in draining lymph node associated with higher FIB and A1AGP 48 HPD. The IM of $AlPO_4$ or $Al(OH)_3$ resulted in inflammatory changes at the injection site and accumulation of macrophages with granular material in draining lymph node, but no changes in FIB, A1AGP or A2M. The IM of QS-21 resulted in inflammatory changes and coagulation necrosis of skeletal muscle at injection site associated with higher FIB, A1AGP or A2M.

Conclusion: APP can be used to monitor the inflammatory response to common vaccine adjuvants.

LB-24: OMENTAL MILKY SPOTS REACTION TO INTRAPERITONEALLY-DELIVERED METALLIC AND CARBONACEOUS NANOPARTICLES

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Background: Omental Milky-spots (OMS) are clusters of leukocytes embedded in the omental tissue, having an essential role in peritoneal inflammatory and immune processes. Interestingly, OMS are also the major implantation site for malignant cells during peritoneal dissemination.

Nanoparticles (NPs) have become widely used in biotechnology research. The potential toxic effect is the main limiting factor for NPs use in medicine.

Objective: To evaluate the OMS reaction in CD1 mice after a single intraperitoneal administration of silver and gold metallic-NPs (AgNPs and AuNPs) and two forms of carbon nanotubes (MWCNT and SWCNT).

Methods: Following complete characterization of tested-NPs, at 48 hours after administration, the major organs and omentum were examined by classical histopathology, high-resolution dark-field microscopy with hyperspectral imaging and transmission electron microscopy. The number and size of OMS were analyzed using the whole-mount technique.

Results: For all tested NPs, the OMS were the major tissue of accumulation, followed by peritoneal lymph nodes, reflecting the mainly-lymphatic absorption of NPs from the peritoneum. For the MWCNT and SWCNT groups, a significant increment in both number and size of OMS was observed. These were associated with granulomatous changes within OMS, consisting of macrophage accumulation (often NPs-laden) and mild, mainly submesothelial fibrosis. Except for the histologically-visible accumulation of Ag and AuNPs within the OMS, no significant findings were observed in the metallic NP groups.

Conclusion: These data support that OMS are the main sites of accumulation for both tested metallic and carbonaceous NPs, and the main site of reaction following CNT intraperitoneal administration.

LB-25: FINDING THE DIAGNOSIS FOR A MYSTERY MASS IN THE ORAL CAVITY OF A DOG

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A two-year-old, neutered male, German Shepherd dog presented to his referring veterinarian for evaluation of a 4 cm mass on the hard palate. Cytologic evaluation of a fine needle aspirate from the mass revealed a population of large (40-100 microns) round cells with abundant basophilic cytoplasm containing numerous small lavender granules. Their nuclei were round to oval with finely stippled chromatin pattern and a single large prominent nucleolus. The mass was surgically excised, and histologic evaluation revealed a solid mesenchymal neoplasm with cells cytomorphologically similar to those seen by cytology. Differential diagnoses included granular cell tumor (GCT), oncocytoma, rhabdomyoma, and melanoma. These tumors have similar morphology; however, complete surgical excision is generally curative for GCT, oncocytomas, and rhabdomyomas, but melanomas may have aggressive biologic behavior. Special stains were performed to differentiate these tumors. The neoplastic cells were positive for Periodic Acid Schiff (PAS), S-100, neuron-specific enolase (NSE), lysozyme, and vimentin. They were negative for cytokeratin, sarcomeric actin, desmin, melan-A, and PNL-2. Negativity for melan-A and PNL-2, and desmin and actin ruled out melanoma and rhabdomyoma, respectively. Positivity for mesenchymal and neural markers and negativity for cytokeratin suggests a GCT. Electron microscopy evaluation was inconclusive. GCT are tumors derived from neural tissue characterized by their granular appearance from abundant cytoplasmic lysosomes. Ultimately, the

mass was diagnosed as a GCT and recurred a few months later. In this report, the oral GCT was unusual in its diagnostic challenge and clinical regrowth at the excision site, possibly suggesting an aggressive variant.

LB-26: SYSTEMIC TOXOPLASMOSIS IN AN OSPREY (PANDION HALIAETUS)
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Toxoplasma gondii (T. gondii) is an apicomplexan parasite capable of causing disease in a wide variety of vertebrate animals. Birds of prey appear resistant to toxoplasmosis with few cases been reported. An adult, intact female Osprey in poor body condition (1/5) was found weak, unable to fly and was submitted for examination. The bird died shortly after the presentation and was submitted for necropsy. Grossly, the liver was diffusely and moderately swollen, mottled with multifocal to coalescing, flat, variably sized, pale tan foci extending into the parenchyma. The spleen was swollen, measuring 3 cm x 2 cm x 2 cm, and was diffusely mottled pale tan to red-brown. Microscopically, there was severe, multifocal to coalescing hepatocellular necrosis with numerous intralesional intracytoplasmic tachyzoites within hepatocytes, endothelial cells and macrophages, and lymphoplasmacytic perivascular cuffings. Occasionally tissue cysts filled with bradyzoites were observed. Additional tissues with similar microscopic lesions and the organisms include the spleen, lung, heart, proventriculus and sciatic nerves. Identification of the organisms as *T. gondii* was confirmed by immunohistochemical staining in tissue sections. In addition, sequencing of PCR amplicons produced from liver and spleen with primers specific for the *T. gondii* B1 gene yielded 100% sequence identity to two isolates (GenAccession #: KX270373.1, LN714499.1) within the NCBI database. No bacteria and fungi were identified by bacterial and fungal culture. The osprey tested negative for West Nile virus and for Avian Influenza virus. Herein, we described the pathologic and molecular findings of a severe case of systemic toxoplasmosis in an Osprey.

LB-27: CHRONIC COPPER TOXICITY IN A SHEEP
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Case Description: A 5-month-old, female intact, Katahdin hair sheep presented with a history of heat distress, followed by a 24-hour neurologic status including opisthotonus, ataxia and abnormal mentation. Before the onset of the neurologic signs, the sheep was suspected having a heat distress. The humane euthanasia was elected due to the little improvement with the treatment and was submitted for post-mortem examination.

Results: Grossly, the carcass was icteric and the liver was diffusely dark orange to dark brown. Bilaterally, the kidneys were diffusely dark brown to black. The urinary bladder contained approximately 30 mL dark brown urine. Histopathologically, the liver exhibited a primarily centrilobular to midzonal, multifocal to bridging necrosis and lipid-type vacuolar degeneration with megalocytosis, bile duct hyperplasia, cholestasis and fibrosis. Multifocally, the renal tubules were filled with orange, globular to hyalinized hemoglobin casts. Spongiosis and Alzheimer type II astrocytic gliosis were noted in the

thalamus and arbor vitae of cerebellum. The level of copper in the submitted liver sample had a high range of copper (240 ppm), which neared the documented toxic range (250-1000 pm), and few copper in the hepatocytes and Kuffer cells were confirmed by Rhodanine stain.

Conclusion: Herein, we described the clinical, pathologic and toxic findings of a chronic copper toxicity in a sheep. Although there was no history of exposure to toxic substance, the possibility of ingesting toxic plants, pyrrolizidine alkaloid, was highly suggested with the findings of megacytolosis in the hepatocytes.