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Clinical Pathology Focused Scientific Sessions

THE KAPPA TO LAMBDA LIGHT CHAIN RATIO IN DOG SERUM CAN IDENTIFY IMMUNOGLOBULIN SECRETING NEOPLASMS

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Background: The ratio of κ to λ light chains (κ:λ) in serum is a biomarker of immunoglobulin secreting neoplasia in humans but has not been evaluated in dogs.

Objective: Investigate the ability of a mass-spectrometry (MS)-based canine κ:λ assay to identify immunoglobulin secreting neoplasms.

Methods: Samples from control dogs, dogs with an infectious etiology, dogs with secretory plasma cell tumors (sPCT) and dogs with non-secretory B cell neoplasia were evaluated using an MS-based canine κ:λ assay and anti-human κ-light chain and anti-human λ-light chain immunofixation.

Results: The MS-based κ:λ assay on whole serum identified 5 κ-predominant sPCT (mean κ:λ=3.307)and 5 λ-predominant sPCT (mean κ:λ=0.023). Ranked ANOVA with Tukey's multiple comparison documented differences between the 2 sPCT groups and between the sPCT groups and all other groups ($p < 0.05$ for all). The infectious etiology group had a lower mean κ:λ ratio (mean κ:λ=0.069) than control samples (mean κ:λ=0.103, Tukey's multiple comparison p=0.035). Similar results were obtained when samples were enriched for free light chains using size exclusion chromatography, except for the statistical difference between the control and infectious etiology group. All λ-predominant cases had only anti-human λ light chain immunofixation labeling. Three κ-predominant cases had only anti-human κ-light chain immunofixation labeling and the remaining two sPCT cases did not label with either antisera by immunofixation.

Conclusion: The MS-derived serum κ:λ may be a useful biomarker of immunoglobulin secretory neoplasia in dogs with the ability to distinguish secretory neoplasia from infectious or other causes of immunoglobulin secretion.

IDENTIFICATION OF REGENERATING ISLAND-DERIVED PROTEIN 3E IN DOGS

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Background: Regenerating islet-derived protein (REG) 1A (aka pancreatic stone protein) and REG3A (aka pancreatitis-associated protein) are upregulated in humans with sepsis, pancreatitis, and gastrointestinal diseases, but little is known about this protein family in dogs.

Objective: To identify REG1 and REG3 family members in dogs.

Methods: REG-family genes were computationally annotated in the canine genome (ROS_Cfam_1.0) and proteome (UniProtKB), with verification of gene expression using RNA-seq databases (Barkbase). The presence of the protein in canine pancreatic tissue and plasma was investigated with Western blot and immunohistochemistry using anti-human REG1A and REG3A antibodies. Protein identity was confirmed with mass spectrometry.

Results: Two members of the *REG3* subfamily were found in the canine genome, *REG3E1* and *REG3E2*, both encoding for the same 176 AA protein, subsequently named REG3E. Anti-human REG3A antibodies demonstrated cross-reactivity with the canine REG3E protein in pancreas homogenate and canine plasma, yielding a protein band of approximately 17 kDa. Mass spectrometry confirmed this protein to be the product of the annotated *REG3E* genes. Strong immunoreactivity to anti-human REG3A antibodies was found in sections of canine pancreas affected with acute pancreatitis, and weak expression in healthy pancreatic tissue. Recombinant canine REG3E protein underwent a selective trypsin digestion as described in other species. No evidence for the presence of a homolog of REG1A was found in any of the investigations in dogs.

Conclusions: Dogs express REG3E in the pancreas, whose role as biomarker merits further investigations. Homologs to REG1A were not found, and therefore are not likely to exist in dogs.

EVALUATION OF ELECTROPHORETIC URINE PROTEIN BANDING PATTERNS IN DOGS WITH URINARY TRACT INFECTIONS

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Background: Urine gel electrophoresis can characterize renal proteinuria in dogs. While urinary tract infection (UTI) can cause proteinuria, its influence on urine protein banding patterns is unknown.

Objective: Determine if UTIs in dogs interfere with interpretation of urine protein banding patterns and whether a UTI-specific banding pattern can be identified.

Methods: Proteins in 51 urine samples collected from 49 dogs with culture-confirmed UTI and no known kidney disease were separated using 4-12% sodium dodecyl sulfate Bis-Tris gels. Protein banding patterns were evaluated for presence and severity of glomerular and tubular damage based on an established scoring system. Correlations were performed between gel scores and degree of hematuria or pyuria.

Results: Samples included 14 with pyuria, 9 with hematuria, 14 with both, 13 with neither, and 1 without urinalysis data; 38 samples had bacteriuria. Most samples exhibited a glomerular or mixed (tubular and glomerular) banding pattern (43% and 27%, respectively); 28% were consistent with normal urine. Primary tubular banding

patterns were rarely observed (2%). Of samples with glomerular patterns, 80% were consistent with mild damage. Weak to moderate correlations were observed between magnitude of pyuria or hematuria and glomerular [r=0.46 (pyuria), r=0.26 (hematuria)] or tubular [r=0.34 (pyuria), r=0.48 (hematuria)] damage severity scores. A 14 kDa band was strongly indicative of hematuria.

Conclusion: The presence of a UTI can interfere with accurate urine protein gel electrophoresis interpretation, and magnitude of pyuria and hematuria do not reliably predict gel interpretation. A band at 14 kDa suggests hematuria and likely represents a hemoglobin monomer.

EVALUATION OF FOLATE RECEPTOR BETA IN ACTIVATED SYNOVIAL MACROPHAGES OF DOGS WITH STIFLE OSTEOARTHRITIS ASSOCIATED WITH CRANIAL CRUCIATE LIGAMENT RUPTURE

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Background: Osteoarthritis (OA) is a progressive and destructive joint disease. The activated phenotypes of macrophages with folate receptor-ß (FRβ) are involved in OA-related inflammation in humans and are potential targets for early detection and therapeutic interventions. The presence of FRβ in activated synovial macrophages in dogs with OA has not been previously demonstrated.

Objective: To evaluate the expression of FRβ in synovial macrophages and determine the difference in polarization of macrophages into inflammatory phenotype (M1) and anti-inflammatory phenotype (M2) between OA and control dogs.

Methods: Synovial fluid from the knee was collected from ten dogs with OA due to CCL rupture and ten controls. Straight preparations were made to estimate total nucleated cell count, and cytocentrifuge preparations fixed with acetone. Immunocytochemical staining for FRβ, M1 marker inducible nitric oxide synthase (iNOS), and M2 marker arginase 1 (ARG1) were performed. Positive large mononuclear cells were counted.

Results: The number and percentage of FRβ-positive cells in the OA samples were increased compared to controls ($p < .05$). The number of iNOS-positive cells was increased in the OA compared to control samples ($p < .05$). The percentage of iNOS-positive cells was higher than ARG1-positive cells in the OA samples.

Conclusion: Activated macrophages with FRβ are present and detectable in synovial fluid of dogs. Higher expression of FRβ in activated macrophages in canine OA may be further explored as potential therapeutic targets. A higher percentage of iNOS-positive cells in canine OA may indicate a polarization of synovial macrophages toward an M1 proinflammatory phenotype.

TRANSCRIPTOMIC ANALYSIS TO IDENTIFY GENES ASSOCIATED WITH THROMBOSIS AND COAGULATION IN LIPOPOLYSACCHARIDE-EXPOSED BOVINE MONOCYTE-DERIVED MACROPHAGES

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Background: Dysregulation of the inflammatory and hemostatic systems in patients with septicemia often leads to disorders of coagulation and thrombosis, which may be associated with higher morbidity and mortality. Thus, there is a critical need to better understand the processes that contribute to a procoagulant and prothrombotic state in patients with septicemia. **Objective:** This study aimed to investigate the differential expression of genes associated with thrombosis and coagulation in macrophages exposed to E. coli lipopolysaccharide (LPS) in vitro relative to a control to identify prognostic markers or therapeutic targets in patients with sepsis. **Methods:** Bovine monocyte-derived macrophages (mdMac) from Holstein steers were exposed to E.coli LPS or phosphate buffered saline (PBS) for three hours. RNA was then isolated, a DNA library constructed, and sequencing and annotation was performed using RNA-seq technology. **Results:** Bovine mdMac exposed to E. coli LPS displayed statistically significant upregulation of pro-inflammatory genes (interleukin 6, interleukin 1 alpha) and genes associated with thrombosis and coagulation (factor V, heparan sulfate-glucosamine 3-sulfotransferase 1, nitric oxide synthase 2, prostaglandin I2 receptor, p-selectin, tissue factor). In addition, significant downregulation for genes protective against thrombosis and coagulation (protein C, thromboxane A2 receptor) was observed. **Conclusions:** Further in vivo studies investigating changes in the above-identified genes in patients diagnosed with septicemia are warranted to determine if upregulation or downregulation of specific genes is linked to changes in platelet function, parameters of hemostasis, and prognosis.

GENE EXPRESSION PROFILING OF CANINE ACUTE LEUKEMIA

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Background: The transcriptome of human acute leukemia (AL) has been extensively characterized, revealing distinguishing prognostically significant features. Evaluating the transcriptome of canine AL could reveal similarities with human AL, prognostically significant genes and therapeutic targets.

Objective: Characterize the gene expression profile of canine AL and compare to human AL.

Methods: RNA was extracted from 10 healthy blood, 5 healthy bone marrow, and 110 CD34+ AL samples classified via immunophenotyping. AL blood samples were tentatively classified as 52 myeloid, 33 lymphoid, and 24 were unclassifiable. Transcriptome sequencing was performed on an Illumina Novoseq 6000 and read counts were aligned to the CanFam3.1 genome, post quality assessment. Differential

gene expression analysis and gene set enrichment/variation analysis (GSEA/GSVA) were performed. Curated leukemia/lymphoma gene sets were referenced from MsigDB.

Results: Unsupervised hierarchical clustering revealed 2 major transcriptomic groups with multiple subgroups within the AL cohort. Group 1 overexpressed genes traditionally associated with myeloid lineage (e.g. *ANPEP, BPI*) and was enriched for human acute myeloid leukemia gene sets. Group 2 over expressed genes associated with lymphoid (e.g. *DNTT*) lineages. Group 1 presented with lower leukocyte counts, higher hematocrit, higher platelet counts, and lower proportions of CD34+ cells compared to group 2 (p value <0.05).

Conclusions: Gene expression profiling revealed two major groups which have overlapping, and unique features compared to human AL gene signatures. Significant differences in clinical presentation were observed between the major canine AL groups. Focused analysis of the minor subgroups could provide insight into additional overlapping features with specific human AL subtypes.

PREVALENCE OF FEA 6 AS A NOVEL, IMMUNOGENIC FELINE ERYTHROCYTE ANTIGEN : AN UPDATE ON THE MAPPING OF NON-AB BLOOD GROUPS IN CATS

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Background: The identification of 5 novel Feline Erythrocyte Antigens (FEAs) based on naturally occurring alloantibodies (NOAb) prompted in-depth research regarding their respective immunogenicity, notably by carrying out targeted sensitizations in FEA-negative cats. Recently, sensitization experiments led to the production of unexpected post-transfusion antibodies directed against an unknown FEA, namely FEA 6.

Objectives: To establish the prevalence of FEA 6 as a presumably new antigen, to identify anti-FEA 6 NOAb and estimate their prevalence in FEA 6-negative cats. Secondarily, to confirm the prevalence of FEAs 1-5 and corresponding NOAb, while assessing the association between FEA 6 and other FEAs.

Methods: Blood typing for FEAs 1-6 was performed prospectively in 193 type A, transfusion-naive cats. The presence of NOAb was evaluated using extensive crossmatching within pools of 4-6 cats. When NOAb were detected, the FEA against which they were directed was inferred based on the extensive blood typing of the cats included in the same pool.

Results: The prevalence of FEA 6 was 67%. Agreement analyses (McNemar's test, Kappa statistic and Gwet's coefficient) were supportive of FEA 6 being distinct from FEAs 1-5. Incompatibilities were detected in 2.8% of crossmatches, accounting for eleven cats (5.7%) having NOAb. No anti-FEA 6 NOAb were identified. Only FEA 1-negative status was associated with a higher risk of having NOAb (OR=6.6, p<0.005).

Conclusions: Although no anti-FEA 6 NOAb were identified, the relative prevalence of FEA 6 and its potential immunogenicity raise concerns regarding its clinical significance and role in post-transfusion sensitization that warrant further investigations.

THE EFFECTS OF SEDATION ON BASAL AND STIMULATED CONCENTRATIONS OF CORTISOL AND OTHER ADRENOCORTICAL HORMONES IN DOGS

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Background: Accurate diagnosis of disease of the hypothalamic-pituitary-adrenal axis (HPA) relies on compatible clinical, biochemical and ultrasonographic changes in conjunction with reliable adrenal function tests. To ensure pet wellness and personnel safety, dexmedetomidine, trazodone, and butorphanol are commonly used in clinical practice for their sedative or anxiolytic effects.

Objective: Objectives of this study were to evaluate the effects of these three sedatives on both basal and post ACTH stimulated adrenocortical hormones as compared to a saline control.

Methods: Twelve purpose bred healthy beagles were enrolled in this prospective, randomized repeated measure 4-way, 4-period crossover design study.

Results: Only the effect of the anesthetic was found to significantly alter both baseline and post-ACTH cortisol values (P<.001). Specifically, butorphanol significantly increased baseline and post-ACTH cortisol (P<.001; p=.048 respectively) while dexmedetomidine only significantly increased basal cortisol concentration. Butorphanol significantly increased basal levels of androstenedione (P <.001), estradiol (P =.005), progesterone (P =.016), 17-hydroxyprogesterone (P <.001), and aldosterone (P <.001) while dexmedetomidine significantly increase basal levels of androstenedione (P<.001), estradiol (P=.004), and aldosterone (P=.018). Only butorphanol significantly increased post-ACTH 17-hydroxyprogesterone levels (P <.001). Trazodone did not significantly impact measured values of basal or post-ACTH adrenocortical hormones.

Conclusions: Based on the results of the present study, caution should be used when interpreting basal or ACTH stimulation test results after sedation when evaluating the HPA, particularly when butorphanol or dexmedetomidine are used prior to HPA evaluation.

ARTIFICIAL INTELLIGENCE-ASSISTED CYTOLOGIC DIAGNOSIS OF CANINE LARGE B-CELL LYMPHOMA

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Background: Lymphoma is a common cancer in dogs, with the multicentric form usually presenting as generalized peripheral lymphadenopathy. Fine needle aspiration (FNA) is generally used to diagnose peripheral lymphadenopathy in dogs and requires manual microscopic examination of cytology smears by board-certified clinical pathologists. However, emerging artificial intelligence (AI) technology holds promise for automated microscopic diagnosis.

Objective: To apply an AI algorithm on photomicrographs to test its ability to accurately classify non-neoplastic lymph nodes and lymph nodes from dogs with large B-cell lymphoma.

Methods: In this retrospective study, non-neoplastic lymph node aspirates (n=3) and large B-cell lymphoma aspirates (n=8) were selected based on cytologic interpretation and confirmatory flow cytometry test results. In each case, slides were placed under a 100x oil objective and 15-21 photomicrographs were captured using a hand-held iPhone 13 and default camera application. A convolutional neural network-based object detection algorithm was developed for the identification of neutrophils and small, intermediate, and large lymphocytes in captured images. The AI-assisted canine lymphoma diagnosis was automatically performed by calculating the proportion of intermediate to large lymphocytes.

Results: In total, 8,340 intact cells were detected in 240 photomicrographs. In the clinical testing set, the average precision was 0.837 for detection and classification of lymphocytes, and the classification accuracy for diagnosing lymphoma was 0.9714.

Conclusions: The AI algorithm showed an excellent classification performance in diagnosing large B-cell lymphoma based on static images captured by a smartphone. The result supports the future development of applications for automated microscopic diagnosis of canine large B-cell Lymphoma.

COMPARISON OF VARIOUS SAMPLING METHODS ON VISCOELASTIC TEST RESULTS USING A POINT-OF-CARE DEVICE (VCM VET™) IN HEALTHY DOGS

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Background: A point-of-care viscoelastic test (VCM Vet™) was recently marketed for cage-side functional measurement of clot formation and degradation. However, veterinary studies are limited.

Objectives: To investigate the impact of sampling site/method on VCM results in healthy dogs, and find out if results are affected by ease of blood sampling and various hematological parameters.

Methods: Two VCM assays were performed on 52 healthy dogs. Uncoagulated whole blood was collected from a direct jugular venipuncture on all dogs to run the first VCM

assay (n=52), establish reference intervals for each parameter, perform a complete blood count, and measure fibrinogen concentration. Blood collection for the second assay performed an hour later was randomized as follows: contralateral jugular using a Vacutainer (n=17), direct stick in a saphenous vein (n=17) or cephalic catheterization (n=18). Intra-class correlation indice, Kruskal-Wallis or non-parametric ANOVA, Spearman test or Pearson test were performed (significance $p < 0.05$).

Results: Reference intervals were established for each VCM parameter. The intra-class correlation (ICC) between methods was poor (<0.5). Difficulty of blood sampling was associated with an increase in clot formation time ($p=0.03$). An increase in hematocrit was associated with an increase in clot time ($p=0.042$), whereas an increase in platelet count was associated with increased alpha angle ($p<0.02$), amplitudes at 10 and 20 minutes ($p<0.01$), maximum clot formation ($p<0.01$), and decreased clot formation time ($p<0.01$).

Conclusions: Method and sampling site influence VCM results. Therefore, patient coagulation profile should be monitored using the same protocol, and hematological parameters should be considered when interpreting results.

ASSESSMENT OF FIXATION AND DECALCIFICATION METHODS FOR CANINE BONE MARROW

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Background: Bone marrow (BM) biopsies are obtained routinely to investigate unexplained leukocytosis, leukopenia, non-regenerative anemia, thrombocytopenia, circulating atypical or neoplastic cells, or infectious agents. There is a paucity of standardized methods for fixation and decalcification that consider subsequent sample morphological quality and suitability for immunologic or molecular testing.

Objective: This study aimed to identify an optimal method of BM fixation and decalcification.

Methods: Replicate pieces of marrow tissue from the sternum of 20 dogs euthanized for reasons unrelated to the study were obtained with the aid of a miter saw within 24 hours of euthanasia. Marrow tissues were submerged in acid-zinc-formalin (AZF) fixative and 10% neutral buffered formalin. Another 21 samples were fixed in AZF and placed in fast decalcifier (10% HCl), slow decalcifier (5.5% HCl/0.12% EDTA) or 14% EDTA for 1, 12, and 24 hours, respectively. Samples were then routinely processed, sectioned at 3 µm and stained with H&E. The quality of sections was scored by 4 assessors on a scale from 1 to 4.

Results: The mean scores of samples fixed with formalin and AZF were significantly correlated ($p=0.003$) but there was no significant difference between fixatives ($p=0.566$).

Different decalcification methods yielded mean morphology scores of 2.273, 2.416 and 2.511 for fast, slow and EDTA methods, respectively. Differences between decalcification methods were not statistically significant.

Conclusions: Two types of fixative and three methods of decalcification yielded BM sections of comparable morphological quality. The suitability of the different methods for immunologic and molecular assessment remains to be determined.

ESTIMATION OF TOTAL NUCLEATED CELL COUNT IN EFFUSION FLUID SMEAR COMPARED TO THE AUTOMATED HEMATOLOGY ANALYZER

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Background: Total nucleated cell count (TTNC) is an important variable for fluid classification. However, evaluation in stained effusion fluid smear is subjective and refers to a rough estimation when the slides are submitted to the diagnostic laboratory.

Objective: To compare the results of the TTNC evaluated microscopically in stained smears with those obtained by the Advia 120 Hematology System and validate appropriate correction factors for the manual technique.

Methods: 139 canine direct smears made of thoracic and abdominal effusions (70 abdominal and 69 thoracic) were retrieved from the case record of the Clinical Pathology Laboratory. The smears were evaluated and counted by two observers and the average number of nucleated cells in 20 microscope fields (400x) along the two axes of the slide was calculated for all samples. The agreement between both methods was assessed via Passing-Bablok test and Bland-Altman plots.

Results: The concordance correlation coefficient (CCC) between the two TNCC methods for the abdominal fluid was 36.78%, the precision 36.78%, and accuracy 99.56%; for thoracic fluid, CCC was 76.79%, and the precision 77.98% and accuracy 98.47%, and in the total fluid samples CCC was 65.85% and the precision and accuracy were 66.19% and 99.49%, respectively.

Conclusions: The microscopic method for the determination of TNCC in abdominal and thoracic fluid samples can be used interchangeably to the ADVIA with acceptable clinical bias.

COMPARISON OF THE IDEXX PROCYTE ONE® TO TWO HEMATOLOGY ANALYZERS

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Background: The IDEXX ProCyte One® Hematology Analyzer is a new benchtop flow cytometric analyzer targeted to veterinary practices.

Objective: To determine if results from the ProCyte One analyzer are comparable to the IDEXX ProCyte DX® and the Siemens ADVIA® 120 Hematology System.

Methods: Fifty-two anticoagulated canine blood samples submitted to the Clinical Pathology Laboratory of the Texas A&M Veterinary Medical Teaching Hospital and containing a minimum of 0.5 mL were prospectively run on all three analyzers. Bias was determined using Passing-Bablok regression and Bland-Altman plots.

Results: Negative proportional bias was observed for platelet count between the ProCyte One and ProCyte DX analyzers (b=0.786; 95% CI 0.746–0.827) and between the ProCyte One and Advia analyzers (b=0.859, 95% CI 0.794 – 0.933). For reticulocyte count, negative proportional and constant bias was observed between the ProCyte One and ProCyte DX (b=0.856, 95% CI 0.759 – 0.918; a= -3.26 10^3/uL, 95% CI -6.78 – -0.19) and negative constant bias was found between the ProCyte One and Advia analyzers (a=-10.71 10^3/uL, 95% CI -19.84 – -6.87). No bias was demonstrated between ProCyte One vs. ProCyte Dx and ProCyte One vs. Advia for RBC, WBC, HCT, neutrophil percentage, and lymphocyte percentage.

Conclusions: Overall, the IDEXX ProCyte One® results were directly comparable to two other commonly used hematology analyzers for most parameters and, where biases were detected, differences were unlikely to affect clinical decision making.

STORAGE-RELATED CHANGES IN EQUINE LEUKOCYTE MORPHOLOGY AND ADVIA 120 AUTOMATED DIFFERENTIAL CELL COUNTS

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Background: To minimize storage-related artifacts in leukocyte morphology and counts, EDTA-anticoagulated whole blood should be evaluated promptly or refrigerated at 4°C, a challenge for equine ambulatory practice. The effects of suboptimal blood storage on equine leukocytes are not well-documented.

Objective: To document changes in equine leukocyte morphology and automated differential count in equine blood stored at four temperature conditions for up to five days.

Methods: Equine EDTA-anticoagulated whole blood was stored at room temperature (22°C), 37°C (simulate high ambient temperature), refrigerated (4°C), or in a cooler with icepacks (3 - 19°C). Blood smears were prepared at 0, 2, 4, 12, 24, 48, and 120 hours. Automated differential cell count was performed using the ADVIA 120 at 0, 8, 24, 48, and 120 hours. Leukocyte integrity and morphologic changes including nuclear swelling,

Döhle body-like inclusions, vacuolation, granulation, and cytoplasmic appearance were assessed and quantified.

Results: Pyknotic leukocytes were noted within 2 hours in blood stored at 37°C, and by 12 hours in blood stored at 22°C. Nuclear swelling was the predominant morphologic change observed in blood stored for ≥ 24 hours at 22°C, 4°C, or in a cooler with ice packs. Morphologic changes including Döhle body-like inclusions, and cytoplasmic vacuolation were observed in blood stored at all temperature conditions; full quantification results are pending. Automated neutrophil count tended to decrease with time, and occasionally decreased below reference interval (pseudoneutropenia).

Conclusions: Storage-related artifactual changes to leukocytes could alter clinical interpretation of blood smears and automated CBC results.

A POMERANIAN WITH STOMATOCYTOSIS

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Background A ten-year-old, intact female Pomeranian was referred to Purdue University Veterinary Hospital in May 2022 for evaluation for abdominal pain, anorexia, and vomiting. A segment of necrotic jejunum was surgically removed. Incidentally, a remarkable stomatocytosis was identified on blood smear evaluation.

Objective To interpret the results of osmotic fragility tests (OFT) and to discuss the possible association between stomatocytosis and findings on complete blood count (CBC), serum biochemistry, and blood gas analyses.

Methods CBC (ADVIA®2120i), serum biochemistry (Vitros®4600), blood gas analyses (Stat Profile Prime Plus®), blood smear evaluation, and OFT.

Results Marked stomatocytosis was present during the first six days. Compared to that of a control dog, the OFT plot of the patient displayed a positive shift with a roughly linear relation between the degree of hemolysis and the concentration of phosphate-buffered saline. The tests demonstrated increased fragility of patient's erythrocytes, consistent with overhydrated stomatocytes in humans and dogs. On day 24, however, the stomatocytosis subsided, and a concurrent, remarkable reticulocytosis developed. Evaluation of sequential CBCs revealed markedly elevated MCV with gradual increase over time and consistently decreased MCHC. Blood gas analyses revealed a gradual resolution of alkalemia, contradictory to a previous finding that stomatocytosis is more prominent in an acidic environment. Serum biochemistry demonstrated a resolving hyponatremia and hypoosmolality.

Conclusions: The abnormal MCV and MCHC changes are similar to those reported in miniature and standard Schnauzers and Alaskan malamutes. However, hypotonicity, pH

changes, and electrolyte abnormalities may have contributed to the appearance of stomatocytes in this dog.

ANALYTICAL VALIDATION OF AN ELISA FOR MEASUREMENT OF FELINE PANCREATIC LIPASE IMMUNOREACTIVITY

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Background: The diagnosis of feline pancreatitis can be challenging. Clinical presentation often includes mild, nonspecific clinical signs, such as vomiting, anorexia, and weight loss. Measurement of feline pancreatic lipase immunoreactivity (fPLI) in serum has been shown to be highly sensitive and specific for a diagnosis of pancreatitis in cats. However, no peer-reviewed analytical validation for a commercial assay for the measurement of fPLI has been published.

Objective: To analytically validate the Spec fPL® assay (IDEXX Laboratories, Westbrook, ME), a commercially available ELISA for the quantitative measurement of fPLI.

Methods: Dilutional parallelism, spiking recovery, precision, reproducibility, and the effect of interfering substances were assessed. The upper limit of the reference interval was calculated based on the 95th percentile of results from clinically healthy cats (n=107) and a decision point for diagnosing pancreatitis was calculated for an expected specificity of 99%.

Results: Analytical validation showed good linearity, accuracy, precision, and reproducibility (intra- and interassay coefficients of variation < 9%) as well as absence of interference from lipemia, hemolysis, or icterus. The upper limit of the reference interval for Spec fPL was determined to be 4.4 µg/L based on the 95th percentile of results from 107 clinically healthy cats and a decision point for diagnosing pancreatitis was determined to be 8.8 µg/L with an expected specificity of 99%.

Conclusions: The Spec fPL assay was shown to be analytically valid. The results of this study suggest that the use of a decision point of 8.8 µg/L results in a high diagnostic specificity for pancreatitis.

UTILITY OF LDH MEASUREMENT FOR DETERMINING THE ETIOLOGY OF MODIFIED TRANSUDATE PLEURAL EFFUSION IN CATS

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Background: Fluid analysis is part of the initial approach for determining pleural effusion etiology. Feline pleural effusions are often classified as modified transudates (MT), but this classification provides limited diagnostic information regarding causation.

Objectives: To investigate whether there is an association between effusion classification and etiology, and to evaluate the diagnostic utility of fluid lactate dehydrogenase measurement (fLDH) for identifying feline pleural MT etiologies.

Methods: Pleural effusion samples from 114 cats, with known etiologies, were included. Samples were classified as transudates, MTs, and exudates. The correlation between MT fLDH and plasma LDH activity (pLDH) was assessed using Pearson's correlation coefficient. The diagnostic utility of fLDH for identifying MT etiologies was investigated using ROC-curve analysis.

Results: Causes of pleural effusion included neoplasia (38.5%), cardiac disease (CD) (19.7%), FIP (18.0%) and pyothorax (12.3%). MT was the most common classification (44.2%) but was not associated with etiology. There was no correlation between fLDH and pLDH. MT fLDH was useful at separating CD from non-CD causes (AUC 0.873) with a cutoff value of <535.5 U/L (Sens, 100%; Spec, 66.67%) and separating FIP from non-FIP causes (AUC 0.849) with a cutoff value of >641.3 U/L (Sens, 88.24%; Spec, 80.0%).

Conclusions: fLDH does not appear to be affected by pLDH. fLDH may be a useful adjunctive marker for differentiating some causes of pleural modified transudate in cats.

EOSINOPHILIC CAVITARY EFFUSIONS IN CATS: 49 CASES (2010-2020)

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Background: Eosinophilic effusions are commonly defined as effusions with ≥10% eosinophils. Eosinophilic cavitary effusions are infrequently observed in cats, with sporadic case reports comprising the majority of the recent literature.

Objective: To review disease associations of cats with eosinophilic cavitary effusions and to assess if a lower threshold of eosinophils (5-9%) may warrant consideration of similar etiologies associated with effusions with ≥10% eosinophils.

Methods: Cytology reports were retrospectively reviewed for all feline cavitary effusions submitted for fluid analysis from 2010-2020. Cases were selected if the reported manual leukocyte differential included ≥5% eosinophils and separated into 5-9% eosinophils and ≥10% eosinophils. Medical records were reviewed for associated medical conditions.

Results: In a total of 669 effusions, 50 (7.4%) effusions from 49 cats were identified with ≥5% eosinophils. The eosinophil proportion was ≥10% in 24 effusions from 23 cats. The most common underlying cause was neoplasia (10/23, 43%), followed by unknown/idiopathic (8/23, 35%), cardiac disease (3/23, 13%), and inflammatory disease (2/23, 9%). The underlying causes for the 26 effusions with 5-9% eosinophils were similar: neoplasia (8/26, 31%), unknown/idiopathic (8/26, 31%), cardiac disease (6/26, 23%), and inflammatory disease (4/26, 15%). No statistical difference in the disease category was observed between the 2 groups (P = 0.64).

Conclusions: Consistent with current literature, neoplasia remains a primary consideration for cats with eosinophilic effusions, with several previously unreported associated diseases identified. Preliminary results suggest an eosinophil differential of 5-9% may be seen with similar diseases considered for classically defined eosinophilic effusions.

Clinical Pathology Posters

C1: IMMUNOPHENOTYPING OF LEUKEMIA FROM 76 DOGS BY FLOW CYTOMETRY

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Background: Immunophenotyping by flow cytometry (FCM) enables identification, quantification and maturity staging of malignant cell lineage.
Objective: To summarize CBC and FCM results of leukemic populations (Vetmeduni Vienna, 2010-2021). Samples were selected by WBC count, cell morphology, scatterplots and/or >1% large unstained cells.

Methods: FCM employed multi-color staining with canine-specific and human cross-reactive monoclonal antibodies against CD3, CD4, CD5, CD8, CD11a, CD11d, CD14, CD21, CD34, CD45, CD79, and MHC-II. Samples were classified by lineage (B cell, T cell, myeloid) and/or as acute leukemia (CD34+), NK-like (CD11d+ and large granular lymphocyte morphology), undifferentiated (CD45, CD45RA, and/or CD11a+ without specific lineage markers), or unclassified (<50% pan-markers).

Results: Of 76 cases, 60 (79%) had leukocytosis, 6 (8%) had WBC within Reference Interval (WRI), 7 (9.2%) were leukopenic, and 3 (3.8%) had no CBC results available. Fifty-four of 76 (71.1%) were of lymphocytic origin: 25/76 (32.9%) B-cell, of which 80% were CD34+; 19/76 (25.1%) T-cell, of which all were CD34-; 8/76 (10.5%) mixed B- and T- cell phenotype, of which 37.5% were CD34+; and 2/76 (2.6%) acute NK-like. Thirteen of the 76 (17.1%) were of myeloid origin, of which 53.8% were CD34+. Six (7.9%) were undifferentiated, of which 83.5% were CD34+. Three (3.9%) were unclassified, all of which were CD34-. 50% of cases with WBC WRI and 71.4% with leukopenia were CD34+.

Conclusion: 3.9% of cases remained unclassified. A high ratio of $CD34^+$cases among patients with normal and subnormal WBC count underlines the importance of thorough blood smear and scattergram evaluation in routine hematology.

C2: QUANTIFICATION OF PROGNOSTIC BIO-MARKERS VIA FLOW CYTOMETRY IN CANINE CUTANEOUS MAST CELL TUMORS

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Background: Canine cutaneous mast cell tumor (ccMCT) has a wide range of biologic behavior. Prior studies have shown that immunohistochemistical expression levels of Ki-67 and phosphorylated c-kit (pKIT) correlate with both histological grading and median survival time of patients with this tumor. Therefore, quantification of these markers via flow cytometry (FC) may improve the pre-surgical prognostication ccMCT.

Objective: To validate a multicolor FC panel that targets c-kit, pKIT, Ki-67 and viability.

Methods: We utilized the NI-1 ccMCT cell line to validate a FC panel that includes a viability dye (FVS620, BD Biosciences) and the following primary conjugated antibodies: c-kit-PE (ACK45, BD Biosciences), pKIT-A647 (polyclonal bs-3242R BIOSS) and Ki-67-FITC (20Raj1, eBioscience). We first validated single antibodies with appropriate isotype and negative controls prior to multiplexing. For the multiplexed panel, appropriate compensation beads were used to compensate for fluorescence spillover and fluorescence minus one (FMO) controls were used to identify appropriate gates. Data was acquired using FACScalibur and analyzed using FlowJo.

Results: C-kit and pKIT produce an appropriate (positive) staining pattern when applied to the NI-1 cells, but Ki-67 show only slight nonspecific binding. We have confirmed that Ki-67 activity can be identified in canine peripheral blood leukocytes (PBMCs).
Conclusions: FC can be used to identify concurrent c-kit and pKIT expression in the NI-1 line. Additional anti-Ki-67 antibodies and staining buffers are being tested using the NI-1 line, PBMCs, and primary ccMCT aspirates. Preliminary data suggest that our FC panel is valid when applied to aspirates from patients with ccMCT.

C3: GASTROINTESTINAL LYMPHOMA IN 33 CATS – RESULTS OF A MULTIMODAL DIAGNOSTIC APPROACH

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Background: Gastrointestinal lymphoma is the most common form of lymphoma in felines having an unfavorable prognosis. A multimodal diagnostic approach is required to improve lymphoma classification and identification of subtypes targeting patient tailored therapeutic strategies.

Objective: In this cohort study, all currently available routine diagnostic methods including histopathologic World Health Classification (WHO) were applied.

Methods: Thirty-three patients were included. Cytology, Flow cytometry, lymphocyte clonality testing and histopathologic classification including immunohistochemistry (IHC) were performed.

Results: Eight cats (24%) suffered from gastric and 25 (76%) from intestinal lymphoma. All gastric lymphomas were of B-cell origin, whereas 15 (60%) intestinal lymphomas were of T-cell and 10 (40%) were of B-cell immunophenotype. The most common T-cell phenotype (46%) was $CD3^{+}CD5^{-}CD4^{-}CD8^{-}$. The most frequent B-cell phenotype in the intestines (60%) and gastric (87%) was $CD21^{+}CD79^{+}$. IHC results were in line with FCM and lymphocyte clonality testing as no cross-lineage phenomenon was observed. Ten out of the 15 intestinal T-cell lymphomas were enteropathy associated T-cell lymphomas type-I (EATL), 3 were peripheral T-cell lymphoma not other specified (PTCL-NOS) and two enteropathy associated T-cell lymphomas type II (EATL-II). All B-cell lymphomas were diffuse large cell lymphomas (DLBCL).

Conclusions: In this case series the multimodal diagnostic approach showed consistent results. However due to the small sample size the multimodal diagnostic approach has to be still recommended.

C5: EVALUATION OF THE URINE DIPSTICK PROTEIN AND SULFOSALICYLIC ACID TESTS FOR CONFIRMATION OR EXCLUSION OF PROTEINURIA IN DOGS

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Background: A urine dipstick colorimetric test is the typical first-line screening test for the detection of proteinuria. The Sulfosalicylic Acid Test (SSA) is an adjunctive method to confirm proteinuria. However, some studies have shown the SSA adds little to the diagnostic value of a greater than trace positive urine dipstick result.

Objective: This study aims to compare the urine dipstick with the SSA method, using the UPC ratio as the reference method to determine proteinuria in dogs.

Methods: We performed urine dipstick, SSA, and UPC ratio from 221 dog urine samples submitted to the clinical pathology laboratory at Washington State University (WSU).

Results: SSA showed higher accuracy (SSA, 88% and dipstick, 73%), sensitivity (SSA, 85% and dipstick, 65%), negative predictive value (NPV) (SSA 76% and dipstick, 59%), and better values to likelihood ratio negative (LR-) (SSA, 0.18 and dipstick, 0.40). However, the dipstick test revealed higher specificity (dipstick, 88% and SSA, 79%), positive predictive value (PPV) (dipstick, 90% and SSA, 87%), and better results for likelihood ratio positive (LR+) (dipstick, 5.31 and SSA, 4.13).

Conclusions: Our results indicated that SSA was better at determining true-positives whereas dipstick colorimetric test was better at determining true-negatives. Therefore, SSA and dipstick seem to be complementary tests, and their association is important in the semiquantitative evaluation of proteinuria in dogs.

C6: COMPARISON OF TWO METHODS FOR MICROSCOPIC DETECTION OF EQUINE BAND NEUTROPHILS

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Background: Detection of band neutrophils is vital in horses with acute disease and may provide prognostic information regarding morbidity and death, however, automated analyzers are unreliable for detection of band neutrophils. Additionally, agreement amongst pathologists for detection of band neutrophils in equine blood smears is only moderate. This may be due to the lack of a standardized approach to the initial blood smear scan.

Objective: To better define a standardized procedure for the initial detection of band neutrophils.

Methods: Two methods of microscopic detection were compared, 1) a standardized initial scan examining 20 fields at 200X magnification, and 2) a manual 100 cell leukocyte differential count (LDC). Three raters of differing experience evaluated 50 equine blood smears, and agreement between each method and a pre-determined clinical reference standard (CRS) was evaluated using a statistical approach that takes into account the inherent imprecision of the 100 cell LDC.

Results: There was moderate to strong agreement between the CRS and the LDC method for the two more experienced raters (Fleiss' Kappa = 0.62-0.80), however, there was minimal to no agreement with the standardized initial scan (FK = 0.28-0.31). For the less experienced rater, there was weak to no agreement for both methods (FK = 0.17-0.46).

Conclusions: For more experienced raters, detection of band neutrophils was improved by using the LDC method rather than a standardized initial scan. The less experienced rater had poor detection of band neutrophils regardless of method and submitting blood smears to a veterinary laboratory for evaluation is recommended.

C7: WBC SCATTERGRAMS IN IMMUNODEFICIENT MICE: EXAMINE THE BLOOD FILM

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Background: In immunodeficient mice, laser-based hematology analyzers validated for murine species; provide white blood counts (WBC) consistent with leukopenia with both normal and abnormal leukocyte scattergrams.

Objective: To demonstrate the need for blood smear review when WBC numerical data are outside reference intervals, in mice.

Methods: Forty blood specimens from 8-week old male and female immunodeficient NSG mice were analyzed with two laser-based hematology instruments (Idexx ProCyte Dx, Sysmex XT-2000iV) and by microscopic evaluation of blood smears. White blood cell scattergrams were reviewed, and automated and manual differential WBC percentages were compared using ANOVA.

Results: Severe leukopenia was observed with both analyzers. Abnormal WBC differential scattergrams and analytical flags warning of low reliability of the differential WBC numerical count with the XT-2000IV prevented comparison with the manual differential count. Automated and manual differential WBC percentages were compared for the ProCyte Dx, which displayed interpretable WBC scattergrams. A statistically significant effect of the method (analyzer compared with manual; $P < 0.001$) on mononuclear WBC counts was observed. Lymphocytes and monocytes were 10-fold over- and under-estimated respectively when comparing automated to manual counts, even though the ProCyte Dx analyzer displayed normal-looking scattergrams.

Conclusions: Murine differential WBC counts should be determined by manual rather than automated methods when total WBC count results are leukopenic or when abnormal scattergrams are observed.

C8: INTRACYTOPLASMATIC CORPUSCLES IN BASOPHILS FROM THREE GARDEN TREE BOAS (CORALLUS HORTULANUS) NEGATIVE FOR REPTARENAVIRUS

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Background: The growing demand for maintaining snakes in captivity for research purposes or the pet trade contributes to improvements in the health and well-being of these animals. In this context, laboratory tests such as blood counts aid in the control and prevention of diseases. Knowledge of blood cell morphology in target species is essential for complete hematological evaluation.

Objective: To evaluate the blood cells of Garden Tree Boa (*Corallus hortulanus*) and to report abnormal morphology.

Methods: Between the years of 2021 and 2022, blood samples collected in EDTA from three healthy female *C. hortulanus* were evaluated. RBC and WBC counts were determined manually using Natt and Herrick stain. Blood smears were stained with Wright-Giemsa stain and evaluated by a trained veterinary clinical pathologist using light microscopy. To rule out Inclusion Body Disease (IBD) two samples were screened by RT-PCR for reptarenavirus infection.

Results: CBC results showed no abnormalities. On the blood smears, rounded, refractile and light blue structures were observed in basophils from all samples. These structures measured between 0,75 and 3,58 µm (average 1,37 µm) and presented as single or multiple (up to 13) distinct corpuscles in the cell cytoplasm amid a background of fine punctate basophil granules. All tested samples were negative for reptarenavirus.

Conclusion: This study reports a description of distinct pale blue intracytoplasmic corpuscles in basophils from *C. hortulanus*. Further immunocytochemical, molecular, and/or ultrastructural studies are needed to elucidate and differentiate the corpuscle etiology and composition.

C9: THE DISAPPEARING ACT- A CURIOUS CASE OF ACUTE LEUKEMIA IN A DOG

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Background: Acute leukemia is an aggressive disease that is poorly responsive to treatment and historically associated with a grave prognosis.

Case description: A 5-year-old neutered-male golden retriever was referred to the Oregon State Veterinary Teaching Hospital for consultation following diagnosis of acute leukemia. The dog had presented to his primary veterinarian 14 days prior for lethargy. Complete blood count revealed marked leukocytosis (147K/uL) with large, immature round cells often containing magenta cytoplasmic granules. Flow cytometry of peripheral blood revealed a CD34+ leukocytosis without expression of lymphoid or myeloid-specific antigens, consistent with unclassified acute leukemia. An immunosuppressive dose of prednisone was prescribed 7 days prior to referral. On presentation, the lethargy had resolved and CBC revealed a normal leukocyte count with rare neoplastic cells. Prednisone was gradually tapered to 0.5 mg/kg every other day. The dog remained asymptomatic and CBC monitoring did not reveal a return of neoplastic cells until 149 days later. Repeat flow cytometry was consistent with unclassified acute leukemia. The patient was treated with a modified CHOP chemotherapy protocol and achieved a transient partial response. He was euthanized 42 days later for declining quality of life. Necropsy with histopathology revealed neoplastic infiltrates in the liver, spleen, kidneys, stomach, bone marrow, and lymph

nodes. Attempts to induce *in-vitro* myeloid differentiation of neoplastic cells were unsuccessful, which may suggest cytotoxic T cell or natural killer cell origin.

Discussion: The rapid and prolonged response of this dog's acute leukemia to prednisone alone was unexpected, however, anecdotal reports of similar cases exist.

C10: CYTOLOGIC, HISTOPATHOLOGIC AND IMMUNOHISTOCHEMICAL FEATURES OF A KELOIDAL FIBROMA IN A DOG

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Background: A 5-year-old English Bulldog presented with a firm, well-circumscribed, 1 cm in diameter mass on the left flank.

Objective: Determine cytologic, histopathologic and immunohistochemical features of the mass.

Methods: Fine-needle aspiration (FNA) biopsy samples were collected for cytologic analysis. Mass was excised and submitted for histopathologic examination.

Results: Cytology revealed highly cellular sample, consisting of spindle cells, numerous bundles of thick glassy eosinophilic material (hyalinized collagen) and inflammatory cells. Spindle cells showed moderate anisocytosis and anisokaryosis, had oval nuclei with coarsely stippled chromatin, 1-3 prominent round nucleoli and moderate amounts of wispy cytoplasm. Cells sometimes associated with eosinophilic extracellular matrix. Binucleated and trinucleated spindle cells were often noted. Low numbers of small lymphocytes and individual well-granulated mast cells were also present. On histopathologic examination, the dermis and subcutis were expanded by a well delineated, partially encapsulated and moderately cellular mass. The mass was composed of hyalinized collagen fibers separated by spindle-shaped mesenchymal cells. Anisocytosis and anisokaryosis were mild and rare mitoses were present. The mass was completely removed. Histopathologic evaluation was consistent with keloidal fibroma, a rare benign variant of fibroma. High numbers of neoplastic cells showed positive immunoreactivity for vimentin and small to moderate numbers showed positive immunoreactivity for smooth muscle actin.

Conclusion: This is a rare cytologic description of keloidal fibroma correlated with histopathologic findings and immunolabeling. Since moderate cellular atypia was present on cytology, in cases in which keloidal neoplasia is suspected, histopathologic examination is necessary to differentiate between keloidal fibroma and keloidal fibrosarcoma.

C11: CLINICOPATHOLOGICAL FINDINGS IN A DOG WITH PULMONARY ALVEOLAR MICROLITHIASIS-LIKE DISEASE

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Background: Pulmonary alveolar microlithiasis (PAM) is a genetic disorder caused in people by mutations in the SLC34A2-gene encoding a sodium-phosphate carrier protein, and resulting in the formation of intra-alveolar calcium-phosphate microliths. CT findings of reticular pattern with ground glass opacity are characteristic. Histological findings consist of lamellar, calcified, periodic acid-Schiff (PAS) and Von Kossa (VK) positive, alveolar microliths. Rare cases with findings consistent with PAM are described in domestic species, although the genetics have never been investigated. A 10-year-old, male neutered Shih Tzu dog presented with chronic cough. CT revealed diffuse ground glass attenuation. Broncho-alveolar lavage (BAL) cytology revealed mild, chronic inflammation and amorphous, pink, polarizing, crystalline material. Prednisolone treatment was ineffective. CT was repeated 2 months later revealing progressive changes, and BAL showed worsening inflammation with the same crystalline material. The dog deteriorated further, necessitating euthanasia.

Objective: To identify the nature and etiology of the crystalline material.

Methods: BAL was submitted for culture and PCR. Necropsy was performed and PAS, VK, and Alizarin red (AR) were applied to cytology and histology slides.

Results: No pathogens were detected. On cytology, crystals were PAS and unevenly VK positive, and AR negative. At necropsy the lungs were firm, gritty, and failed to collapse. Histologically, many alveolar spaces, and occasional alveolar walls, were distended by smooth-margined deposits of grey, lamellar, often crystalline material, which was PAS, AR and VK positive. A diagnosis of PAM-like disease was made.

Conclusions: PAM-like disease should be considered when crystalline material is found in BAL samples.

C12: CASE REPORT: NASAL ENTAMOEBA SP. INFECTION IN A DOG

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A 12-year-old female spayed Boston Terrier presented to a local veterinarian for chronic nasal discharge of approximately seven months duration. She was initially treated with clindamycin, cefovecin (Convenia), and prednisone with mild improvement. A slide

preparation of nasal discharge was submitted to the Antech Laboratory at Oklahoma State University Veterinary Teaching Hospital for evaluation. Cytologic findings included marked suppurative inflammation, numerous bacterial rods and cocci, and the presence of frequent individually oriented structures suspected to be amoeba organisms. Organisms were round, approximately 15-20 micron diameter, with large amounts of lightly basophilic, vacuolated cytoplasm, and a single, round, approximately 3-4 micron diameter, eccentric nucleus. Numerous small, elongated, eosinophilic structures with pseudopod-like projections were present within the background, which may be trophozoites. Amoebazoa PCR and 16S rRNA sequencing performed at the University of Georgia showed organisms were most similar to *Entamoeba gingivalis* (91%) and *Entamoeba suis* (90%), suggesting a novel *Entamoeba* sp. Entamoeba infrequently cause infection in humans and domestic animals including dogs, non-human primates, and cattle. Infection in dogs is most commonly associated with *Entamoeba histolytica,* and typically causes ulcerative colitis. A case of nasal colonization and complication of periodontal disease by *Entamoeba gingivalis* in a 13-year-old Italian Greyhound has been recently reported in the literature, which resolved within six weeks following a dental cleaning, tooth extractions, and treatment with clindamycin. In the present case, no periodontal or intestinal disease was reported. Due to worsening of nasal congestion and decreased quality of life, the patient was euthanized three months after presentation.

Diagnostic Pathology Focused Scientific Sessions

SELENIUM TOXICOSIS CAUSING POLIOMYELOMALACIA IN GROWER PIGS

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In regions with selenium deficient soils, selenium is supplemented in livestock diets to prevent against selenium deficiency-associated diseases such as reduced fertility and nutritional myopathy. Selenium intoxication can occur due to miscalculation of feed supplementation or by ingestion of selenium accumulating plants. Chronic intoxications present with dermatitis and coronitis. Acute intoxications present with acute paresis that may progress to paralysis and death.

Grower pigs on eight farms in Michigan developed acute hind-limb paresis that progressed to paraplegia. Affected animals had been fed 18% protein grower feed supplied by the same feed mill. Two affected 3-month-old pigs from one farm were submitted alive to MSU VDL. The pigs were non-ambulatory and in lateral recumbency, but were otherwise quiet and alert. The pigs were humanely euthanized and necropsies were performed.

No gross lesions to suggest a cause for the clinical signs were observed. Histopathologic examination revealed severe bilaterally symmetrical poliomyelomalacia affecting the ventral horns of the cervical, lumbar, and sacral spinal cord. Liver mineral

analysis from each pig revealed 47.13 ug/g and 18.75 ug/g of selenium by dry weight with the expected range being 1.60-3.20 ug/g.

Based on the combination of clinical findings, lesions within spinal cord, and demonstration of high liver concentrations of selenium, selenium toxicosis was diagnosed. Follow up with the referring veterinarian revealed that excess amounts of selenium had been added to the feed mix. Though an uncommon differential in areas with selenium-poor soil, selenium toxicosis should be considered in regional outbreaks of acute paresis and paralysis in pigs.

MACROSCOPIC AND MICROSCOPIC CHARACTERIZATION OF LOBAR HOLOPROSENCEPHALY IN TWO DOGS WITH ALTERED DRINKING BEHAVIOR

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Background Holoprosencephaly (HPE) is an uncommon congenital brain malformation affecting humans and animals, characterized by a total or partial failure of separation of the prosencephalon into two separate hemispheres. Three forms of HPE with decreasing severity have been described: alobar, semi-lobar and lobar. Lobar HPE in two male entire dogs which presented with altered drinking behavior is described.

Materials and Methods Dog 1, an 11-week-old Akita, presented with hypernatremia, seizures, and oligodipsia. Dog 2, a 3-year-old Jack Russell Terrier, presented with adipsia. Both dogs were humanely euthanized and complete post-mortem examination (PME) was performed. The CNS and samples of major organs were fixed in 10% neutral-buffered formalin and processed for histopathology.

Results At PME macroscopic lesions were restricted to the brain in both cases. There was absence of the septum pellucidum, septal nuclei, fornix, fornix columns, and dorsal portions of the hippocampus, bilaterally. This resulted in ventral dislocation of the cingulate gyrus and corpus callosum, which appeared markedly thinned. Moreover, there was absence of the ventral portion of the interhemispheric fissure. The diencephalic structures caudal to the optic chiasm, as well as the mesencephalic and rhombencephalic structures, were unremarkable. On histopathology there was focal fusion of the midline ependyma and diencephalic structures.

Conclusions Macroscopic and histologic findings in these two cases are consistent with lobar HPE. The reported clinical signs can be explained by a defect in hypothalamic osmoreceptors, and therefore, although rare, HPE should be considered as a differential in cases of altered drinking behavior and sodium imbalances.

CONGENITAL LYMPHANGIOMATOSIS IN A 2-YEAR-OLD BEAGLE

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A 2-year-old, female intact, Beagle dog was presented to the Texas A&M University Veterinary Teaching Hospital for evaluation of pleural effusion, dyspnea, and coughing. Abdominal ultrasound showed numerous variably sized cysts in the liver, spleen, pancreas, abdominal lymph nodes, dorsal abdomen, and cranial mediastinum. At necropsy, a single thymic cyst, numerous hepatic cysts, and several solid splenic nodules were identified. Histopathology showed cystic vascular channels lined by a single layer of flattened spindle cells with minimal cellular atypia in the liver, spleen, mesentery, and an abdominal lymph node. The flattened spindle cells were negative for cytokeratin and von Willebrand's factor immunohistochemical staining, ruling out a polycystic disease and making blood vessel origin less likely, respectively. Immunohistochemistry for prospero homeobox protein-1 (PROX-1) showed positive intranuclear staining of the flattened spindle cells lining the vascular channels in the liver, spleen, and mesentery, confirming a lymphatic cell origin. Based on the young age of the dog and presence of lymphatic-lined vascular proliferations in several organs, a diagnosis of congenital lymphangiomatosis was favored. Congenital lymphangiomatosis in domestic animals is rare and histologic features are not well-described, making differentiation between lymphatic neoplasms and congenital lymphangiomatosis challenging. As in humans with the analogous disease generalized lymphatic anomaly, the pathogenesis is unknown. To our knowledge, congenital lymphangiomatosis involving the spleen and abdominal lymph nodes of a dog has not been previously reported.

CHRONIC INTESTINAL PSEUDO-OBSTRUCTION DUE TO INTESTINAL LEIOMYOSITIS IN A DOG TREATED WITH HUMAN INTRAVENOUS IMMUNOGLOBULIN, MYCOPHENOLATE, AND PREDNISOLONE

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Chronic intestinal pseudo-obstruction (CIPO) is a severe motility disorder of the intestinal tract in humans and animals. A 10-year-old, spayed female, Dachshund dog presented for gastrointestinal signs suggestive of ileus. On abdominal exploratory, the gastrointestinal tract was atonic without peristalsis. Physical obstruction was ruled out. Full-thickness intestinal biopsies of the duodenum, jejunum and ileum had similar histologic features. The tunica muscularis was infiltrated by a T cell predominant lymphocyte infiltrate, plasma cells and neutrophils. Smooth myocytes were vacuolated, pale, fragmented, and disrupted by plump fibroblasts amid fine collagen fibers, resulting in loss of architecture of the circular and longitudinal muscularis. The clinical history, microscopic findings, and the absence of mechanical intestinal obstruction, warranted the diagnosis of CIPO due to intestinal leiomyositis. This is an uncommon

disease with a poor prognosis and a reported median survival time of days to weeks after diagnosis in dogs. The pathogenesis is poorly understood, and there are no specific treatment guidelines for this disease. This patient was treated with a single injection of human intravenous immunoglobulin (IVIG-GAMUNEX© 1 g IV constant rate infusion [CRI]) combined with a conservative steroid therapy regimen and mycophenolate. The patient improved within one week and remained clinically normal 4 months after initial diagnosis. Human IVIG has been used in dogs as an experimental treatment for immune-mediated hemolytic anemia, thrombocytopenia, and a few other autoimmune diseases with variable success, but has not been reported to be used as an adjunctive therapy for intestinal leiomyositis.

CYTOLOGIC AND HISTOLOGIC FEATURES OF PULMONARY HYALINOSIS IN A DOG WITH CONCURRENT NEUROENDOCRINE CARCINOMA

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Background: Multiple pulmonary nodules were noted within the right middle and left caudal lung lobes of a 13-year-old spayed female mixed breed dog. The pulmonary nodules were serially imaged and fine needle aspirates of the nodules were obtained at two different time points. Three months following discovery of the nodules, a right middle and left caudal lung lobectomy was performed. The lung lobes were submitted for histopathology.

Methods: Cytologic specimens were stained with modified Wright-Giemsa stain. Tissue specimens were processed routinely and stained with histochemical (hematoxylin and eosin and periodic acid-Schiff [PAS]) and immunohistochemical (pan-cytokeratin, vimentin, chromogranin A [CgA], synaptophysin, neuron specific enolase [NSE], and thyroid transcription factor-1 [TTF-1]) stains.

Results: Cytology revealed numerous birefringent hyaline concretions accompanied by macrophagic to granulomatous inflammation. Histopathology revealed multiple foci of moderately-differentiated carcinoma. Neoplastic cells were positive for TTF-1 and NSE, supportive of a diagnosis of primary pulmonary neuroendocrine carcinoma. In close proximity to neoplastic foci were multifocal to coalescing intra-alveolar aggregates of acellular hyaline material. This material was birefringent under polarized light, PAS-positive, and associated with histiocytic and granulomatous inflammation, consistent with pulmonary hyalinosis.

Conclusion: To our knowledge, this is the first description of the cytologic features of pulmonary hyalinosis in a dog or other species. Diagnostic cytopathologists should be aware of pulmonary hyalinosis as a potential cause of radiographically identifiable pulmonary nodules and that pulmonary hyalinosis may accompany other respiratory diseases.

MIRNOME EXPRESSION ANALYSIS IN CANINE DLBCL

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Background: Lymphoma is a prevalent malignancy in dogs. Diffuse large B-cell Lymphoma (DLBCL) is the most common subtype, representing about 50% of the clinically seen lymphoma cases. Thus, searching for additional biomarkers capable of early detection and monitoring DLBCL is essential for improving and sustaining remission rates. Next-generation sequencing provides innovative information about biomarkers and the differential expression of genes, including microRNAs. Non-coding microRNAs negatively influence gene expression by attaching to the 3'-untranslated region of protein-coding mRNA, causing targeted RNA degradation or translational repression. MicroRNAs' stability and easy accessibility make them promising biomarkers for identifying and sub-classifying patients.

Objective: We aim to broaden the understanding of microRNAs' role in the molecular biosynthesis of DLBCL.

Methods: We isolated and sequenced microRNAs from ten samples of fresh-frozen lymph node tissue (six DLBCL and four healthy dogs) and validated them by RT-qPCR. The average expression fold-change ($2^{-\Delta\Delta Cq}$) of each microRNA in the DLCBL and healthy groups were compared to find significant differences using the unpaired parametric Welch's 2-sample t-test and false discovery rate (FDR). The geometric expression levels mean of the most consistently expressed candidates were used as data normalizers (miR-361-5p, miR-101, and miR-29c-3p).

Results: Small RNA sequencing (sRNA-Seq) analysis identified 35 differentially expressed miRNAs (DEMs) in DLBCL. RT-qPCR confirmed 23/35 DEMs; 9 were downregulated, and 14 were upregulated.

Conclusions: Our results demonstrate the potential to harness microRNAs as unique diagnostic and therapeutic targets in DLBCL**.** Confirmatory prospective studies on large populations are planned.

RELATIONSHIP BETWEEN UVEAL INFLAMMATION AND VIRAL DETECTION IN 30 CATS WITH FELINE INFECTIOUS PERITONITIS

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Background: Feline infectious peritonitis (FIP) is caused by a mutated enteric coronavirus that infects macrophages. It causes systemic pyogranulomatous phlebitis including uveitis.

Objectives: The aims of this study were to evaluate the sensitivity of viral detection in cases of FIP-induced uveitis using immunohistochemistry (IHC), RT-qPCR, and RNAscope® *in situ* hybridization (ISH), evaluate the agreement between these diagnostic tests, and evaluate the correlation between the type of inflammatory cells (pyogranulomatous versus plasmacytic) and the likelihood of viral detection. To date, the use of RNAscope® ISH for FIP diagnosis has not been thoroughly evaluated; thus, we evaluated its performance and agreement with RT-qPCR and IHC.

Methods: Eyes with FIP-induced uveitis from 30 cats were histologically evaluated, and the type of inflammatory cells was graded from 1 (predominantly pyogranulomatous) to 5 (predominantly plasmacytic). IHC, RT-qPCR, and RNAscope® ISH were performed on these cases.

Results: Viral RNA or antigen was detected in 8/30, 9/30, and 10/30 cats using RT-qPCR, IHC, and RNAscope® ISH, respectively, with high agreement between each test. A weak to moderate but significant negative correlation between the degree of plasmacytic uveal inflammation and the likelihood of detecting FIP antigen and RNA was identified.

Conclusions: RNAscope® ISH had high agreement with IHC and RT-qPCR and had the highest detection rate. This study suggests that the likelihood of confirmatory diagnosis of FIP by IHC, ISH or RT-qPCR in ocular tissues is low in those cases in which there is intense plasmacytic ophthalmitis.

MYOSIN HEAVY CHAIN MYOPATHY IN TWO QUARTER HORSES

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A 1.5-year-old American Quarter Horse (AQH) gelding had a 10-day history of pelvic lameness, nasal discharge, and 3-day onset of myoglobinuria. Endoscopy revealed bilateral abscesses in the retropharyngeal lymph nodes, suggestive of strangles. An 11-month-old AQH filly had a 5-day history of lethargy and rapid muscle atrophy. Thoracic auscultation and ultrasound suggested pneumonia. Both patients had markedly elevated creatine kinase (60,000 U/L and 49,275 U/L, respectively) and aspartate aminotransferase (8,000 U/L and 14,450 U/L, respectively) enzymes. Both horses had severe polyphasic histiocytic and lymphoplasmacytic myositis with necrosis, atrophy, mineralization, and regeneration. The gelding also had renal lesions of purpura hemorrhagica and myoglobinuric nephropathy, and suppurative lymphadenitis by

Streptococcus equi spp. e*qui*. The filly had a 7x4x3 cm pulmonary abscess, caused by *Actinobacillus equuli*. Hair roots were submitted for a five-panel genetic disease test for the AQH. The gelding had one mutated copy and the filly had two mutated copies of the Myosin Heavy Chain 1 (MYH1) gene, supporting the diagnosis of myoglobin heavy chain myopathy (MYHM). A nonsynonymous E321G mutation in the MYH1 gene encodes a hypercontractile myosin heavy chain in type 2X myofibers. Hypothetically, the immune system recognizes the mutated fibers leading to immune-mediated myositis (IMM). Approximately 40% of MYHM horses had exposure to respiratory or gastrointestinal pathogens or vaccinations, particularly *S. equi* spp. *equi*, which suggestively trigger IMM due to epitope mimicry, as seen in one of our cases. In the second case, myositis was possibly triggered by *A. equuli,* which has not been previously linked to IMM.

GM2-GANGLIOSIDOSIS IN A SHIBA INU DOG

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A 1.5-year-old, female, Shiba Inu dog had a clinical history of progressive ataxia, vestibular signs, and neurologic deficits. Magnetic resonance imaging (MRI) showed poor gray-white matter definition in the cerebrum, subjective thickening of the cortical grey matter and thinning of the white matter, and dilation of the cavities of the olfactory bulb with cerebrospinal fluid. The findings were interpreted for a probable metabolic encephalitis. DNA testing for GM2-gangliosidosis revealed this animal was homozygous for the disease variant genotype. Histopathology of the cerebrum and spinal cord revealed swollen neurons with finely granular, eosinophilic cytoplasm containing eosinophilic vacuoles. The white matter had demyelination and dilated myelin sheaths with swollen axons. The swollen neurons were also present in the peripheral spinal nerves and myenteric ganglia. The histopathologic findings support the diagnosis of the lysosomal storage disease, GM2-gangliosidosis. GM2-gangliosidosis is a rare, lipid storage disease caused by a deficiency in hexosaminidase or the activator protein GM2A. This deficiency results in fusion of vesicles with lysosomes to form phagolysosomes, which decreases enzymatic breakdown leading to lysosomal retention of ganglioside and globoside.

CRYPTOBIA IUBILANS INFECTION IN LAKE MALAWI CICHLIDS

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Background: *Cryptobia iubilans* is a flagellate protozoan that causes primarily fatal gastric ulceration and granulomas in cichlids.

Methods: One hundred and fifty Lake Malawi cichlids were introduced to Baton Rouge zoo. While in quarantine, some of them acutely died or had buoyancy issues,

hyperoxia/anorexia, and loss of body condition with a mortality of 12% (18/150). Six cichlids were submitted for necropsy to the Louisiana Animal Disease Diagnostic Laboratory. Histopathologic examination of H&E-stained slides was performed after formalin fixation and decalcification. Gram, Fite-Faraco acid-fast, PAS, and Steiner's silver stains were also performed on selected sections.

Results: The cichlids had multiple granulomas in the gastric wall without apparent causative agents. In one cichlid the granulomas were more numerous, involving multiple areas of the gastrointestinal tract, the mesentery, and few on the liver; the most severely affected portion (stomach) had ruptured with subsequent bacterial coelomitis. No microorganisms were noted in the granulomas with Gram, Fite's acid-fast, PAS, or Steiner's silver stains.

Conclusions: In this family of fish (cichlid), distribution of the granulomas primarily affecting the stomach and lack of infectious agents visualized by special stains are sufficient for a presumptive diagnosis of *Cryptobia iubilans* infection. *Cryptobia iubilans* is an important pathogen in cichlids and can cause high mortality linked with environmental factors or coinfections. This disease may be underdiagnosed or misdiagnosed because the organism is difficult to visualize in granulomas. It should be considered as the primary differential diagnosis for granulomas predominantly affecting the stomach in cichlids.

INCLUSION BODY DISEASE (ARENAVIRIDAE, REPTARENAVIRUS) WITH CONCURRENT PAX5 IMMUNOPOSITIVE DIFFUSE LARGE B-CELL LYMPHOMA IN A RED TAIL BOA

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A 15-year-old, male, euthanized captive red tail boa was submitted for postmortem examination after it became nonresponsive and laterally recumbent. The snake had a history of respiratory disease, coelomic swelling, leukocyte intracytoplasmic inclusion bodies, and a negative reptarenavirus PCR. Grossly, the snake had oral mucoid discharge; multifocal masses in the lungs and intestines; pericardial effusion; multifocal firm nodules on the pericardium and adventitia of heart base vessels; hydrocoelom; and, a segmentally dilated coelom in the cranial third corresponding to a transmural 10x4x4cm esophageal mass that abutted the pericardial sac, tracheal bifurcation, lung, and encompassed the trachea. On cut section, the coelomic esophageal mass was solid, and the portion of esophageal lumen had a diphtheritic membrane. Histopathology revealed lymphoma that infiltrated lungs, alimentary tract, pericardium, myocardium, skin, bone marrow, vessel walls, nerves, and hepatic sinusoids. Neoplastic lymphocytes had marked anisocytosis, anisokaryosis, karyomegaly, multinucleation and 45 mitotic figures in 10 HPF (2.37mm^2). Immunohistochemistry was negative for CD3 and positive for Pax5, consistent with diffuse large B cell lymphoma (DLBCL). Additionally, many cell types, including neoplastic lymphocytes, contained cytoplasmic viral eosinophilic inclusions, and reptarenavirus PCR on the spleen detected *Giessen reptarenavirus.*

This snake was diagnosed with concurrent, potentially anaplastic, DLBCL and Inclusion Body Disease. Whether reptarenavirus predisposes to neoplasia is debated. This case and previous literature describing neoplastic disorders with concurrent reptarenavirus infection contribute to mounting evidence of a direct association.

ALPHAHERPESVIRUS INFECTION IN A VIRGINIA OPOSSUM (DIDELPHIS VIRGINIANA) AND A WATER OPOSSUM (CHIRONECTES MINIMUS)

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Background: Macropodid alpha herpesviruses 1 and 2 (MaHV-1, MaHV-2) are best described in macropods and have been implicated in outbreaks amongst captive marsupial populations in Australia. To the author's knowledge, there have been no reports of MaHV infections in opossum species.

Methods: One Virginia opossum (*Didelphis virginiana*) and one water opossum (*Chironectes minimus*) were submitted for necropsy from a zoo that housed 6 opossums, all of which died within several weeks. Kangaroos and wallabies are also present at the facility. Liver samples from both opposums were submitted for histopathology, negative stain electron microscopy, ultrastructural examination and whole genome sequencing using a GridION nanopore sequencing platform.

Results: Microscopically, both opossums had multifocal areas of necrosis in the liver with intranuclear inclusion bodies within hepatocytes. Other significant findings in the Virginia opossum involved sepsis with isolation of *Streptococcus didelphis* from multiple organs. Ultrastructural analysis of fixed liver tissue identified herpesviral replication complexes in both opossums while negative stain electron microscopy of unfixed liver tissue yielded a negative result. The herpesvirus had > 99% nucleotid identity with MaHV-2.

Conclusions: These cases indicate that both opossum species are susceptible to a MaHV-2 infection. The outbreak has implications particularly in zoo settings with mixed species exhibits.

OUTBREAK OF HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS H5N1, CLADE 2.3.4.4b IN WILD BIRDS IN U.S., 2022: LESIONS AND VIRAL ANTIGEN DISTRIBUTION

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H5Nx Goose Guangdong's highly pathogenic avian influenza (HPAI) viruses caused the largest AI outbreak in North America in 2014-2015. In 2022, a new epizootic associated

with HPAI subtype H5N1 reemerged in U.S. domestic poultry. This strain of HPAI also became highly prevalent in wild bird populations, presenting variable pathogenicity. We describe pathological findings of HPAI in seven wild birds, including: one red-tailed hawk (*Buteo jamaicensis*), one turkey vulture (*Cathartes aura*), and one great-horned owl (*Bubo virginianus*) which were found dead; one Peregrine falcon (*Falco peregrinus*) and one great-horned owl that died in transit or following presentation to a wildlife rehabilitator; and one American green-winged teal (*Anas carolinensis*) and one bald eagle (*Haliaeetus leucocephalus*) that were euthanized. Gross lesions were overall subtle, and only meningeal congestion was observed in turkey vulture, bald eagle and great-horned owl. Cerebral lymphohistiocytic meningoencephalitis was observed in all cases (7/7, 100%), while neuronal and fibrinoid vascular necrosis were observed in the red-tailed hawk and owls (3/7, 42.9%). Coagulative necrosis or lymphohistiocytic/lymphoplasmacytic inflammation was identified in the kidney (5/7, 71.4%), liver (5/7, 71.4%), heart (4/7, 57.1%) and lung (3/7, 42.9%). Influenza A virus nucleoprotein, detected by immunohistochemistry, was usually correlated with the presence of histologic lesions and within the endothelium and parenchyma of the brain (7/7, 100%), kidney (6/7, 85.7%), air sac (5/7, 71.4%), lung (5/7, 71.4%), heart (4/7, 57.1%) and liver (3/7, 42.9%). The microscopic and immunohistochemical features revealed significant endotheliotropism, neurotropism, and nephrotropism of HPAI virus in wild birds in the recent outbreak.

HISTOLOGIC FINDINGS IN NON-CERVIDS ASSOCIATED WITH THE 2021 OUTBREAK OF EPIZOOTIC HEMORRHAGIC DISEASE IN NORTH DAKOTA

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During summer and fall of 2021, there was an increase of epizootic hemorrhagic disease (EHD) cases in North Dakota (ND). All of the 2021 ND EHD cases followed the distribution of *Culicoides sonorensis* along the upper Missouri River.

Cases were initially identified in wild and farmed white tail deer populations and, as the outbreak progressed, began spilling over into elk, cattle and bison herds, and small ruminants. Of 26 total confirmed EHD positive cases that were submitted to the North Dakota State University Veterinary Diagnostic Laboratory, 50% were non-cervids, including eight cattle, four bison, and one goat.

Histologic findings were variable among non-cervids and included lesions in the lung, liver, and vasculature of multiple organs. Pulmonary edema and hemorrhage were most common amongst all species. Additionally, there was myocardial perivasculitis and cerebral and brain stem hemorrhage in one gravid beef cow, acute hepatitis in two other beef cows, focal vasculitis in the omentum and centrilobular hepatocellular degeneration in one bison cow, and fibrinoid necrosis and vasculitis in the rumen of another bison cow.

All cases were identified as serotype 2. Thus far, sequencing hasn't identified mutations in the EHD virus isolates to suggest increased virulence. Instead, the increase of cases has been attributed to two consecutive summers of drought conditions, creating an ideal environment for proliferation of the midge vector.

This report highlights the panoply of histologic lesions associated with the recent EHD outbreak and to raise awareness as a differential for unexpected death in a diverse array of species.

PERICARDIAL EFFUSION ASSOCIATED WITH ANAPLASMA PHAGOCYTOPHILUM IN A DOG

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Background: An 11-year-old female spayed German Wirehair Pointer with a 1-week history of lethargy, hyporexia, diarrhea and coughing presented with pericardial effusion causing cardiac tamponade. The pericardial effusion was an exudate with mixed macrophagic and neutrophilic inflammation. Morulae were found within neutrophils.

Objective: To speciate morulae found within neutrophils in a dog with pericardial effusion.

Methods: Pericardial fluid and whole blood were tested for *Anaplasma phagocytophilum* antibodies via ELISA and IFA (IDEXX, Westbrook, ME) and genetic material by PCR. PCR testing was also performed for Babesia, Apicomplexa, Bartonella, Ehrlichia, Hemotropic Mycoplasma and Rickettsia genera.

Results: Pericardial fluid and blood were PCR positive for *A. phagocytophilum* (NC State Veterinary Hospital Vector Borne Disease Diagnostic Laboratory, Raleigh, NC). Blood was negative by ELISA (Vetscan Flex4 Rapid Test, Zoetis, Parsippany, NJ) for *A. phagocytophilum* antibodies at the time of initial presentation to the referring veterinarian, then tested positive by ELISA (IDEXX SNAP4Dx, Westbrook, ME) seven days later. The IFA was positive for *A. phagocytophilum* antibody at 1:1600 (IDEXX, Westbrook, ME). All other infectious disease testing was negative. An echocardiogram at the time of presentation revealed no structural abnormalities. An echocardiogram performed one month following therapeutic pericardiocentesis and doxycycline treatment for *A. phagocytophilum* showed no recurrence of pericardial effusion.

Conclusions: This case emphasizes the diagnostic importance of thorough cytologic evaluation of effusions. Association of *A. phagocytophilum* with cavitary effusions was previously reported in two equids. This is the first report of *A. phagocytophilum* associated with pericardial effusion in a dog.

GASTROESOPHAGEAL INTUSSUSCEPTION IN A KITTEN WITH DYSAUTONOMIA

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Background: A 6-month-old intact male Russian Blue Kitten presented to the Colorado State University (CSU) Small Animal Internal Medicine Department with a chronic history of ill thrift, lethargy, increased respiratory effort, regurgitation, pilocarpine-responsive mydriasis with absent pupillary light reflexes (direct and indirect), blepharospasm and keratoconjunctivitis sicca. A litter mate exhibited similar yet more mild clinical signs. Thoracic radiographs revealed megaesophagus and a distal esophageal opacity suspected to be a sliding hiatal hernia. Respiratory signs worsened and the patient died at the referral veterinarian 1 month later despite treatment. The patient was submitted to the CSU Veterinary Diagnostic Laboratory for necropsy.

Results: On postmortem exam, the esophagus was markedly dilated with approximately 90% of the stomach inverted into the distal esophagus. The gastric submucosa was expanded by edema. The lungs were diffusely atelectatic, and the left cranial lung lobe sank in neutral-buffered formalin which correlated to aspiration pneumonia microscopically. Histologic examination of the celiac ganglion, and the myenteric and submucosal plexuses throughout the gastrointestinal tract revealed marked neuronal dropout and degeneration with necrosis, satellitosis, and central chromatolysis. Special stains highlighted neuronal dropout and pathologic changes were compared to a 1-year-old healthy cat as a non-age matched control.

Conclusion: The current case is presented as the first reported case of gastroesophageal intussusception in a kitten diagnosed with dysautonomia. The litter mate is alive and doing well at home.

CYTOKERATIN AE1/AE3 IMMUNOLABELING IN DIAGNOSTICALLY CHALLENGING EPITHELIOID HEMANGIOSARCOMA

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Background: Epithelioid hemangiosarcoma is a rare histologic variant of hemangiosarcoma reported in several animal species including dogs, cows, horses, and cats. Histologically, epithelioid hemangiosarcoma can be architecturally arranged as islands and nests, thereby mimicking epithelial cell tumors. In humans, nearly half of epithelioid hemangiosarcomas have been reported to have positive immunolabeling for cytokeratin AE1/AE3 (CK AE1/AE3), which often makes it challenging to distinguish them from carcinomas.

Objective: To determine the presence of CK AE1/AE3 immunolabeling in cases of epithelioid hemangiosarcoma diagnosed in animals.

Methods: A retrospective review of cases (necropsy and biopsy) of animals received by the diagnostic pathology services at five institutions was performed. The criterion for inclusion included a diagnosis of epithelioid hemangiosarcoma based on the following histological features: sheets of spindled, polygonal, cuboidal to round mesenchymal cells, forming, tubular, solid, and sheet-like arrangements with limited vasoformation. Tumors arising from cutaneous, subcutaneous, and visceral locations were included. Immunohistochemistry (IHC) for CD31 and CK AE1/AE3 were evaluated in each case.

Results: A total of 24 cases were evaluated from dogs (23 cases) and an ox. CK AE1/AE3 immunolabeling was observed in 50% (12/24) of the cases. Cytoplasmic CK AE1/AE3 immunolabeling varied from 5% to 100% of the neoplastic cells. All neoplasms had consistent membranous immunolabeling for CD31.

Conclusions: CK AE1/AE3 immunolabeling in epithelioid hemangiosarcomas in the current investigation was similar to that reported in humans and can pose a diagnostic challenge if not paired with CD31immunolabeling.

IN SITU HYBRIDIZATION FOR ESCHERICHIA COLI IN CANINE GRANULOMATOUS COLITIS

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Background: Granulomatous colitis (histiocytic ulcerative colitis) is diagnosed most commonly in boxer dogs and French bulldogs. Microscopically, erosive and/or ulcerative lesions with a mixed inflammatory population dominated by macrophages that often contain intracytoplasmic Periodic acid-Schiff positive material predominate. As enteroinvasive *Escherichia coli* has been implicated as the primary pathogen, current diagnostics rely on a combination of culture and fluorescent *in situ* hybridization which can be time-consuming or technically challenging.

Objective: To utilize RNAscope® *in situ* hybridization for the localization of *Escherichia coli* in canine granulomatous colitis.

Methods: *In situ* hybridization probes for *Escherichia coli* were designed in collaboration with Advanced Cell Diagnostics for their automated RNAscope® *in situ* hybridization platform. The last ten months of archived cases submitted to the New York State Animal Health Diagnostic Center were searched for large intestinal biopsies from dogs that had a clinical suspicion of granulomatous colitis.

Results: Thirty cases were identified. Included were French bulldog (13), boxer (5), Labrador retriever (2), Goldendoodle (2), and individual breeds (8). Average age at time of biopsy was 33.6 ± 6.2 months. 28/30 (93%) of cases had hybridization signal for *Escherichia coli*. 8/28 (29%) were localized only to the lumen/apical portion of

enterocytes. 18/28 (64%) had hybridization signal localized to luminal/apical portion of enterocytes and within macrophages in the mucosa. 2/28 (7%) had transmural hybridization signal.

Conclusions: *Escherichia coli* is readily identified by RNAscope® in situ hybridization in canine granulomatous colitis and this method serves as a quick and specific diagnostic test to confirm enteroinvasiveness.

CUTANEOUS MASTOCYTOSIS IN YOUNG DOGS

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Background: Cutaneous mastocytosis (CM) is a rare condition in young dogs characterized by a multifocal to diffuse proliferation of dermal mast cells.

Objective: To characterize clinical and histopathologic features of CM in dogs.

Methods: Clinical data from 8 dogs that met inclusion criteria (onset <1.5-year-old, >3 lesions) were obtained via a standardized survey. Biopsies from each patient were classified by the Kiupel/Patnaik grading systems. *C-kit* mutation analysis was performed.

Results: The median age of onset was 26 weeks (range: 8-60). All dogs had more than 5-50 lesions characterized as nodules, plaques, and papules. Seven dogs were pruritic. Ultrasonography in 2 dogs did not reveal visceral involvement. No dogs had systemic illness. Histologically, lesions of CM were the same as cutaneous mast cell tumors (cMCT). Two dogs had tumors classified as Kiupel/Patnaik high-grade/grade II while 6 dogs had low-grade/grade II tumors. Mutations in *c-kit* exons 8 and 11 were not detected in all cases. Treatment included antihistamines (8/8), corticosteroids (7/8), and lokivetmab (3/8) with corticosteroids appearing the most effective: complete (1/8), partial (3/8), and no response (3/8). One dog with high-grade/grade II tumors continued to develop lesions at 1622 days post-diagnosis while the other dog was euthanized at 56 days post-diagnosis. Seven dogs are alive at 136-1622 days (median: 747 days) post-diagnosis.

Conclusions: CM occurs in young dogs and is histologically indistinguishable from cMCT. The lesions may persist for years and do not appear to spread systemically. The Kiupel/Patnaik grading systems may not reflect the disease progression and prognosis in CM.

CLOSTRIDIAL DYSBIOSIS AND HIGH MORTALITY IN NSG MICE

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Background While the immunodeficient status of the NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NOD scid gamma, NSG) mouse provides utility for numerous research models, it also results in increased susceptibility to opportunistic pathogens.

Objective Over a nine-week period, a high rate of acute mortality and diarrhea was reported in a single housing room of NSG mice. Diagnostics were performed to determine the underlying etiopathogenesis.

Methods Mice submitted for diagnostic evaluation included those found deceased (n=2), cage mates of deceased mice with or without diarrhea (n=19), or moribund mice (n=6).

Results Mice exhibited small intestinal and cecal dilation with abundant gas and/or digesta (n=18), dark red small intestinal serosa and contents (n=6), or were grossly normal (n=3). Histologically, 24 of 25 mice exhibited dysbiosis of the ileum, colon, cecum, jejunum, and/or duodenum with variable numbers of luminal gram-positive bacilli. Morphologically, there was erosive enterocolitis (n=7) of the distal small and large intestine or widespread epithelial apoptosis with luminal sloughing (n=13) and varying degrees of submucosal edema and mucosal hyperplasia. *Clostridial* spp and *Paeniclostridium sordelli* were identified in 13 of 23 (56.5%) and 6 of 23 (26.1%) mice, respectively. *C. perfringens* (7 of 23, 30.4%) was isolated most frequently. ELISA for *C. perfringens* (alpha, beta, epsilon) and *C. difficile* (A and B) toxins were negative. Luminal immunoreactivity to several *Clostridial* spp was identified within lesioned small intestine. Mice were negative for all other known murine pathogens via PCR.

Conclusions Acute mortality and diarrhea were at least partly attributed to overgrowth of various *Clostridial* spp.

VETERINARY CANCER GUIDELINES AND PROTOCOLS (VCGP.ORG) – AN UPDATE

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Background: There is minimal methodological standardization for assessing tumors in veterinary medicine, or attempts to validate grading schemes. VCGP was founded on

the principle that standard methods must be adopted so that results of investigations can be compared, validated, and applied to clinical cases.

Objective: Engage pathologists (clinical and anatomic) and oncologists in developing standards of tumor assessment, from diagnosis through outcome assessment. VCGP promotes use of standards to evaluate tumors and facilitate communication via the VCGP website which hosts tumor guidelines, protocols, educational platforms and provides a forum for discussion.

Methods: VCGP recommended a standard histologic area (2.37 mm^2) to replace 10 HPF which is not a standardized unit of area. Studies performed prior to the use of a standard area cannot be compared to or applied to current tumor evaluation. Other initiatives include morphologic identification of mitotic figures, use of the term mitotic count and hard criteria for lymphovascular invasion.

Results: Tumor parameters that need investigation to allow standardized assessment and reporting, include: necrosis, margins, ink/dye application, fascial planes, tissue barriers, cytological parameters and parameters that predict treatments. Protocols are needed for aggressive tumors. Standardization of methods and terminology will promote synoptic reporting. These efforts need to be coordinated with journals, diagnostic labs and governing bodies.

Conclusions: All our oncologic studies need to be repeated with standardized methods, and new methods, such that prognoses and predictions for treatment selection are accurate and clinically applicable if our goal to improve pet care of with cancer is to be achieved.

EVALUATION OF SOX-10 IMMUNOHISTOCHEMICAL EXPRESSION IN CANINE MELANOMA AND NON-MELANOCYTIC TUMORS BY TISSUE MICROARRAY (TMA)

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Melanoma frequently presents a diagnostic challenge given the propensity of tumors to lack or contain scant melanin and the variable microscopic phenotype. Previous studies evaluating single IHC markers for diagnosing melanoma have shown unsatisfactory sensitivity and/or specificity for S-100, PNL2, Melan-A, TRP-1, TRP-2 and HMB-45. *Sry-related HMG-Box gene 10* (SOX-10) is a transcription factor acting as a nucleocytoplasmic shuttle protein and is involved in melanocytic, peripheral neural crest and peripheral nervous system development. In humans, SOX-10 expression has been demonstrated in melanoma, breast cancer, gliomas, and schwannomas but has only recently begun to be characterized in veterinary species. In this study, 293 tumors comprised of 165 melanocytic neoplasms and 128 non-melanocytic neoplasms were

evaluated by tissue microarray for SOX-10, PNL2, Melan-A, TRP-1, and TRP-2 expression. SOX-10 had the highest diagnostic sensitivity at 90.7%. Of immunopositive tumors, SOX-10 had the highest average labeling intensity with approximately 82.4% (122/148) of positive melanomas having a labeling intensity of 4/4. Additionally, SOX-10 had the highest percentage 91.9% (136/148) of melanomas label positive for at least 75% of neoplastic cells. Of the 128 non-melanocytic tumors, SOX-10 labeling was observed in mammary carcinoma (6/6), gliomas (4/4), meningioma (1/2) and soft tissue sarcoma (8/28). Therefore, SOX-10 represents a useful immunohistochemical screening marker for the diagnosis of canine melanoma given its extremely high sensitivity and robust staining intensity. This marker may also be beneficial in diagnosing some non-melanocytic neoplasms in dog.

IMMUNOHISTOCHEMICAL INVESTIGATION OF INSULINOMA-ASSOCIATED PROTEIN 1 (INSM1) EXPRESSION IN CANINE AND FELINE NEUROENDOCRINE NEOPLASMS AND COMPARISON WITH CHROMOGRANIN A AND SYNAPTOPHYSIN

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Background: Neuroendocrine (NE) neoplasms can arise at almost any anatomical location and could be histopathologically misdiagnosed as other neoplasms of epithelial origin owing to their complexity and rarity. Insulinoma-Associated Protein 1 (INSM1), a recently demonstrated NE marker, is reported in a growing number of research studies in human medicine. As a transcriptional regulator, highly conserved INSM1 homologues in various species have been confirmed in previous studies.

Objective: Our objective was to investigate the immunohistochemical (IHC) reactivity of anti-INSM1 antibody in dogs and cats and to compare the results of INSM1 with those of Chromogranin A (CGA) and Synaptophysin (SYN) in NE neoplasms.

Methods: We performed INSM1, CGA and SYN IHC on formalin-fixed, paraffin-embedded canine and feline NE normal tissues, 100 hyperplastic and neoplastic lesions, and 72 non-neuroendocrine neoplasms.

Results: We found anti-INSM1 antibody could detect nuclear expression in most canine and feline normal NE tissues, except parathyroid glands. Five parathyroid carcinomas and six parathyroid adenomas/hyperplasia were negative for INSM1. In the rest of specimens, INSM1 was detectable in 95.5% of 89 NE hyperplastic and neoplastic lesions. In contrast, INSM1 was detected in only three of 72 non-neuroendocrine neoplasms. The overall percentage of NE neoplasms that stained positively with all three markers was 78.4%. In addition, INSM1 appears more sensitive to Merkel cells than CGA and SYN.

Conclusions: These findings confirm that INSM1 is a useful IHC marker for diagnosing canine and feline NE neoplasms and are encouraged to be considered as part of immunohistochemical panels to improve the diagnostic capability.

REVISITING THE CORRELATION OF C-KIT MUTATION STATUS AND TREATMENT DECISIONS IN CANINE MAST CELL TUMORS

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Background: Mast cell tumors (MCTs) are the most frequent skin tumors in dogs, with an incidence of 16-21% of all tumors. Mutations in the proto-oncogene *c-Kit*, which encodes for the transmembrane stem cell factor receptor on the mast cells surface, induce constitutive receptor activation.

Objective: Up to 50% of canine MCTs exhibit internal tandem duplications (ITDs) in either exon 8 or 11 promoting cell growth and survival. We aimed to establish an indication for the treatment with tyrosine kinase inhibitors, based on the *c-Kit* mutation status of the canine MCT patients.

Methods: In 53 histopathological confirmed MCT dogs, the *c-Kit* exons 8, 9, 11, 13, 14 and 17 were investigated by isolating genomic DNA from remnant diagnostic material and subsequent sequence comparisons to healthy and malignant reference material.

Results: For exon 8, one ITD was found, and only twelve patients showed ITDs in exon 11. For the latter exon, additional eight dogs exhibited the same silent mutation, had not been detected in the reference material. Interestingly, in one patient, the high-grade (Kiupel)/ grade III (Patnaik) tumor stage did correlate with an amino acid exchange V563D (T>A^{1688}). This driving mutation in human gastrointestinal stromal tumors could therefore also be an activating mutation in canine mast cell tumors.

Conclusions: For *c-Kit* mutation analysis, a time- and cost-efficient work routine was established. The low ITD frequency in exons 8 and 11 together with the absence of other know mutations suggest that the *c-Kit* mutation status alone is not sufficient to make treatment decisions.

IMPROVING ACCURACY IN DIAGNOSING LYMPHOMA IN CHICKENS - IMMUNOHISTOCHEMICAL CHARACTERIZATION OF BOTH NORMAL LYMPHOID TISSUES AND LYMPHOMA

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Background: Lymphoma is a highly prevalent disease in chickens aged 6 weeks and older with most cases arising due to infections by an oncogenic virus. Implicated viruses include Gallid alphaherpesvirus-2 (Marek's Disease), Avian leukosis virus (Lymphoid Leukosis), and Reticuloendotheliosis virus (Reticuloendotheliosis). Despite the abundance of literature concerning the pathogenesis of oncogenic viruses in chickens, there is a relative paucity of literature detailing the histomorphological and immunohistochemical features of normal and neoplastic lymphoid tissues in these animals.

In order to confidently assess lymphoid tissues and accurately diagnose lymphoma in chickens, pathologists must be cognizant of the organizational and morphological differences between the mammalian and avian lymphoid systems. Given that immunohistochemistry can enhance one's ability to confidently assess lymphoid tissues, pathologists must also possess a sound understanding of the immunohistochemical features of both normal and neoplastic avian lymphoid tissues.

Objective: To characterize the immunohistochemical features of both normal and neoplastic lymphoid tissues in chickens.

Methods: Formalin-fixed paraffin-embedded tissues from chickens were processed with both routine hematoxylin and eosin (H&E) staining protocols and immunohistochemical protocols for Paired Box 5 (Pax5) and Cluster of Differentiation 3 (CD3). The distribution of T-cell and B-cell lymphocytes was documented in normal lymphoid tissue and in lymphomas arising from these organs.

Results & Conclusions: Distinction between normal lymphoid tissue, lymphoma, inflammation, and extramedullary hematopoiesis (EMH) can be difficult with H&E stained slides alone. This study provides insight on interpreting Pax5 and CD3 immunohistochemistry in chicken lymphoid tissue and its utility in diagnosing lymphoma.

NOVEL ADOMAVIRUS ISOLATED FROM A PROLIFERATIVE SKIN LESION IN A SAND TIGER SHARK (CARCHARIAS TAURUS)

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Background: Adomaviridae is an emerging viral family in fish with structural and replicative genes sharing a complex evolutionary history with small DNA tumor viruses including papillomaviruses, polyomaviruses, and adenoviruses. Adomaviruses have now been identified from a number fish species and are associated with proliferative skin lesions in a giant guitarfish (*Rhynchobatus djiddensis*) and smallmouth bass (*Micropterus dolomieu*).

Objective: This study histologically describes progressive localized skin eruptions in an aquarium-housed sand tiger shark (*Carcharias taurus*) and molecularly characterizes a novel adomavirus associated with the proliferative lesions.

Methods: Histopathology, transmission electron microscopy (TEM), and next generation sequencing were completed from skin lesion samples.

Results: Lesions were confined to the caudolateral body and peduncle and were pink to red, raised, and mixed gelatinous and granular. Histopathology revealed proliferation of epithelial elements within dermal denticles producing malformed tooth-like structures resembling odontogenic neoplasms in other vertebrates. Nuclear inclusion bodies and viral particles were not observed with histopathology or TEM, respectively. BLAST analysis of Illumina MiSeq sequence data revealed viral sequences with greatest similarity (71.79% identity) to that of the giant guitarfish adomavirus (GAdoV). Lesions in the index animal have since partially regressed but persisted for one year, and four additional sand tiger sharks in the same enclosure have developed similar skin proliferations

Conclusions: This is the second report of an adomavirus characterized from proliferative skin lesions in an elasmobranch and the first virus described from a sand tiger shark. Additional sampling of other affected animals, genome assembly, and RNAscope *in situ* hybridization are underway.

IDENTIFICATION OF UNEXPECTED MYCOBATERIA IN FELINE AND CANINE CUTANEOUS LESIONS BY PCR ON FORMALIN-FIXED PARAFFIN-EMBEDDED TISSUES

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We report cutaneous mycobacterial infections in a cat and a dog with unexpected mycobacteria identified by PCR on formalin-fixed paraffin-embedded (FFPE) skin biopsy tissues. A 2-year-old indoor domestic cat from Pennsylvania, USA had subcutaneous nodules in the axillae, hindlimbs, and along the ventrum. Biopsy showed nodular histiocytic infiltrates with abundant intracellular acid-fast bacilli and mycobacterial immunolabeling, compatible with feline lepromatous leprosy. Culture was not performed, and PCR detected Mycobacterium avium complex (MAC) species. An 8-year-old domestic dog from Colonia, Uruguay had cutaneous nodules on both ear pinnae and left thigh. Ear biopsy showed pyogranulomatous inflammation with rare acid-fast bacilli and mycobacterial immunoreactivity, compatible with canine leproid granuloma. Mycobacterial culture was negative, and PCR detected a member of the Mycobacterium tuberculosis complex (MTBC). Neither animal had known immunosuppression or evidence of extracutaneous involvement. Canine leproid granuloma is typically caused by a nontuberculous *Mycobacterium* sp. of the *M. simiae* clade, and not reported in association with MTBC. Feline leprosy is caused by *M.*

lepraemurium and occasionally other atypical mycobacteria, and lesions are difficult to distinguish clinically from the rare entity of MAC associated opportunistic infection, as in this cat. Species-specific identification of mycobacterial infections is critical for optimal therapy but is difficult due to fastidious growth requirements and variable culturability. PCR is invaluable in the identification of human mycobacterial infections from FFPE tissue, especially when no fresh tissues are available for culture. These cases demonstrate similar value of PCR for accurate mycobacterial identification in FFPE tissues from domestic animals.

DIAGNOSTIC METHODS FOR THE ASSESSMENT OF METABOLIC BONE DISEASE IN RESPONSE TO DIETARY PHOSPHORUS, AND VITAMIN D3 IN NURSERY PIGS

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Background: Metabolic bone disease is an important cause of swine lameness, most often caused by inappropriate levels of dietary phosphorus (P) or vitamin D3. Diagnosis is challenging due to limitations in available diagnostic assays and questions involving the effectiveness and interpretation of assay results.

Objective: Determine the most sensitive method for diagnosing metabolic bone disease in swine and correlate histopathology, bone ash, and density.

Methods: Forty-four-day-old pigs (5 pigs per pen) were randomized to 6 dietary treatments, consisting of; 1) P deficiency, 2) meeting the P NRC requirement, 3) diet 2, including phytase, 4) industry-level P with phytase, and no vitamin D3, 5) diet 4 with 1,653 IU/kg of vitamin D, and 6) diet 5 with an additional 2,000 IU/kg vitamin D3. The 2nd rib, 10th rib, and fibula were quantified histologically in relation to failure of endochondral ossification (FEO) and the presence of infractions.

Results: Higher scores for FEO with increased infractions and thinner medullary bone trabeculae were observed in the pigs fed a P deficient diet. Histologic changes were more significant in the 10th rib compared to the 2nd rib and fibula. Differences in bone ash and density in response to vitamin D3 and P were most apparent with the fibulas and 2nd ribs.

Conclusion: These findings suggest that the 10th rib could be more sensitive in detecting histological changes related to metabolic bone disease than the 2nd rib. The 2nd rib and fibula were considered the most responsive to detecting differences in bone ash and density.

Diagnostic Pathology Posters

D1: COPPER TOXICOSIS IN SHEEP FEED: A RETROSPECTIVE STUDY OF PRODUCT RECALLS, 2016-2022

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Background: The U.S. Food and Drug Administration is the U.S. government agency responsible for regulation and oversight of the U.S. animal food supply. A component of this activity is voluntary recalls for products that are adulterated.
Ojective: A retrospective study of voluntary recalls for sheep food containing excessive copper (> 15 ppm, the maximum tolerated level in complete sheep rations) from 2016 to 2022 was conducted to determine if necropsy information was available to support copper toxicosis.
Method: Information collection included method of identification of the recall, mortalities (if known), histopathologic findings, and copper concentration in the food. There have been 17 recalls for sheep food containing excess copper from multiple manufacturers.
Results: The foods were labeled for adult, growing, or all age classes of sheep. Copper concentrations ranged from 31 ppm to 428.0 ppm. Fourteen firms self-identified excess copper in the animal food either from routine product testing or from complaint investigations by firms; other voluntary recalls occurred following federal or state investigations. Sheep deaths (n=51) were reported in 11 recalls. Necropsy findings were contributory information supporting copper toxicosis. Gross and histopathologic findings were consistent with copper toxicosis including hemoglobinuria, pale mucous membranes, gastroenteritis, blue-black kidneys, splenomegaly, jaundice, centrilobular hepatocellular necrosis, canalicular cholestasis, and renal tubular casts.
Conclusion: Veterinary pathologists can provide information that aids in determination of an animal food-related issue and protecting animal health.

D2: MODIFIED CETACEAN CARDIAC DISSECTION AND SAMPLING FOR CANINES WITH DILATED CARDIOMYOPATHY

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Background: Since 2014, the United States Food and Drug Administration (FDA) has received reports from owners and veterinarians of dogs diagnosed with dilated cardiomyopathy (DCM) and myocardial dysfunction.
Objective: FDA conducted an observational postmortem study of hearts from twelve dogs with suspected or confirmed cardiomyopathies.
Methods: The sampling protocol was adapted from a protocol for cardiac dissection from pygmy and dwarf sperm whales (Kogia spp.). The adapted protocol utilized heart weight and five cardiac cross-sections with measurements of the ventricles and

interventricular septum (dorsally and ventrally) at two levels as well as valvular circumference). Samples of the dorsal and ventral ventricles, interventricular septum, valves, and great vessels were collected and stained with hematoxylin and eosin.
Results: Histopathologic findings included cardiomyocyte atrophy (n = 12), degeneration (n=8) with steatosis (n =9), fibrosis (n=7), interstitial edema (n=6), and attenuated wavy fibers (n=3). Myocarditis (n=4) was mild with lymphocytic to lymphoplasmacytic inflammatory cells.
Conclusions: The protocol, though more time intensive, could be considered for investigations where more extensive cardiac sampling is needed.

D3: NOCARDIA FARCINICA ABORTION IN A GOAT

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A female goat fetus presented to the Colorado State University Veterinary Diagnostic Laboratory following an isolated abortion of twins by a reportedly healthy doe. Postmortem examination did not reveal any gross abnormalities. Histologic evaluation revealed pyogranulomatous and necrotizing bronchopneumonia with intracellular and extracellular gram positive and acid-fast negative filamentous bacilli. Aerobic culture of the stomach contents and pooled lung and liver tissue yielded light growth of *Nocardia* spp. This was further classified as *Nocardia farcinica* by MALDI-TOF MS with a high confidence score of 2.25 and further confirmed by PCR and sequencing. To the authors knowledge, this is the first report of *Nocardia* spp. causing abortion in a goat. Abortions caused by *Nocardia* spp. are uncommonly reported in pigs, cattle and horses, and are primarily caused by *Nocardia asteroides*. *N. farcinica* has been reported as a significant cause of mastitis in goats in Sudan and a rare cause of abortion and stillbirth in cattle. Nocardiosis should be considered as a differential diagnosis in aborted goats with pyogranulomatous pneumonia.

D4: RETROSPECTIVE HISTOLOGIC AND IMMUNOHISTOCHEMICAL CHARACTERIZATION OF CANINE EXOCRINE PANCREATIC ADENOCARCINOMA AND CORRELATION TO CLINICAL FEATURES

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Introduction: Exocrine pancreatic adenocarcinoma (EPAC) is a deadly disease in humans and animals. In humans, histologic subtyping and association to clinical outcomes are critical for clinical decision-making. Six different histologic subtypes of canine EPAC have been described, but defined and reproducible criteria for histologic subtyping, correlation to clinical features, and useful immunohistochemical diagnostic markers are lacking.

Objectives: The goals of this study are to establish reproducible histologic criteria for canine EPAC subtypes; correlate subtypes to antemortem clinical features; and develop subtype-specific immunohistochemical markers for definitive diagnosis.

Methods: Histopathology slides of twenty-five cases of suspected canine EPAC were reviewed.

Results: Fifteen cases of canine EPAC were confirmed on the initial histopathologic review; the most common reason for exclusion was neuroendocrine tumor misdiagnosis. Three different subtypes were identified: acinar-hyalinizing (n=8), acinar-non-hyalinizing (n=4), and mixed acinar-ductal (n=3); no ductal subtypes were identified. Well-differentiated tumors tended to have more abundant intra-tumoral mucin. Metastasis was common (n=11) and most frequently affected the liver (n=9). The mean and median age of dogs was 11 years. Males (n=9) were slightly overrepresented and mixed-breed dogs (n=7) were most common. Concurrent separate neoplasms were present in 6 dogs.

Conclusions: Acinar neoplasms, including hyalinizing and non-hyalinizing, were common (n=12) in this canine EPAC cohort, similar to previous canine studies. This is a striking difference from human EPAC, in which the acinar subtype accounts for <1% of cases. Interestingly, hyalinizing material was found only in conjunction with the acinar cell morphology. Validation of subtype-specific immunohistochemical markers is ongoing.

D5: COLONIC T-CELL-RICH LARGE B-CELL LYMPHOMA ASSOCIATED WITH A TRANSCOLONIC FISTULA AND EQUINE HERPESVIRUS-5 IN A HORSE

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A T-cell rich, large B-cell lymphoma (TCRBCL) with the formation of a transcolonic fistula and concurrent equine herpesvirus-5 (EHV-5) infection was diagnosed in 17-year-old Rocky Mountain. The horse presented with a 4-week history of anorexia, weight loss, lethargy, and fever of unknown origin. Abdominal ultrasound revealed lymphadenomegaly of the abdominal and colonic lymph nodes, thickening of the wall of the right colon, and a mass-like structure associated with that area. On postmortem examination, an approximately 20 cm diameter, firm, white to tan, irregularly shaped mass was found within the mesocolon between the right ventral and right dorsal colon, with a transcolonic fistula through the mass. Histopathology was consistent with lymphoma and immunostains of the mass (CD79a, CD3, and Iba-1) confirmed different cell populations, with a final diagnosis of TCRBCL.
A formalin-fixed and paraffin-embedded section of the mass was analyzed for EHV-5 using *in situ* hybridization (ISH), and the cytoplasm and nucleus of all cells were strongly positive. Alimentary lymphoma (AL) is the third most commonly reported

equine lymphoma, after multicentric and cutaneous lymphomas. EHV-5 infection has been previously associated with cutaneous and multicentric equine lymphoma. This is the first report related to a transcolonic fistula due to AL in a horse. The fistula formation is attributed to multiple factors, including pressure necrosis by the large mesocolonic mass, transmural invasion of the colonic wall by neoplastic cells, and regional inflammation secondary to the mesenteric mass and necrosis therein. Moreover, this is the first diagnosis report of EHV-5 in AL by ISH.

D6: OUTBREAK OF EPIZOOTIC HEMORRHAGIC DISEASE IN CAPTIVE REINDEER (RANGIFER TARANDUS)

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Background: Epizootic hemorrhagic disease (EHD) is best described in white-tailed deer, classically presenting with fulminant hemorrhage and/or edema in multiple tissues. In particular, the gastrointestinal tract (especially abomasum), heart, and skin are involved. EHD has not been reported in reindeer before.
Methods: Four reindeer (*Rangifer tarandus*) were diagnosed with EHD based on history, gross necropsy, histopathology, and polymerase chain reaction and sequencing of the spleen.
Results: All reindeer died or were euthanized after becoming acutely ill over a 12-day period. Affected reindeer displayed abnormal behavior, lethargy, depression and/or lameness. The most consistent gross finding at necropsy was multiple dark red streaks throughout the adrenal gland cortices (4/4). Overt hemorrhage was seen in one case, and involved the subcutis and skeletal muscles over the ventrolateral wall, dorsal musculature and abomasal serosa. Histologically, the most common lesions were adrenocortical congestion and hemorrhage (4/4), adrenocortical coagulative necrosis (3/4) and lymphoplasmacytic meningoencephalitis with gliosis and glial nodules, satellitosis and nonsuppurative perivascular cuffing with vasculitis (4/4). The lesions within the brain were most frequent in the gray matter of the cerebrum, hippocampus and thalamus but were also within the cerebellum and brainstem. Epizootic hemorrhagic disease virus serotype 6 was detected via PCR and sequencing of the spleen in all cases. **Conclusions:** EHD can be clinically relevant and fatal in reindeer. In this cohort of reindeer, the adrenal glands and brains were most severely and consistently affected. In reindeer presenting with neurological signs, EHD should be considered as a differential.

D7: A CASE OF BILATERAL CHOANAL ATRESIA AND PERITONEOPERICARDIAL DIAPHRAGMATIC HERNIA IN A DAY-OLD CRIA

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South American camelids are gaining increasing popularity, with the estimated UK alpaca population reaching 45,000. As such, knowledge of common neonatal diseases is important for both the attending primary veterinary surgeon and diagnostic pathologist.

A one-day-old cria presented with nostril flaring, open mouth breathing, mild epistaxis from nasal capillaries and an inability to suck. Post-mortem examination revealed bilateral choanal atresia and herniation of the right liver lobe through the diaphragm, into the thoracic cavity and into the right side of the pericardium. To our knowledge, this is the first published report of a cria with both **bilateral choanal atresia and congenital peritoneopericardial diaphragmatic hernia (CPPDH)**.

Choanal atresia is a common nasal craniofacial malformation in New World domestic camelids and is also reported in sheep, horses, dogs, and humans. Abnormal development of one or both communications between the nasal cavity and nasopharynx can occur, as a result of persistence of the choanal membrane. Partial or complete obstruction of the nasal passages makes this a particularly debilitating condition for newborn crias that are obligate nasal breathers.

CPPDHs are congenital abnormalities which result from incomplete formation of the septum transversum or lateral pleuro-peritoneal folds. A direct communication between the peritoneal cavity and pericardial sac then persists as the foetus develops, and as it transitions into neonatal and potentially later life. This is a rare condition that typically manifests in dogs and cats. As the diaphragm does not separate the thoracic and abdominal cavities, abdominal organs are able to move into the pericardial sac.

D8: SEVERE ULCERATIVE ENTERITIS ASSOCIATED WITH COPPER DEFICIENCY IN AN AMERICAN BISON

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A 2-year-old, male, American bison (*Bison bison*) presented to the Wisconsin Veterinary Diagnostic Laboratory (WVDL) for postmortem examination. This male originated from a herd of 50 bison managed in Northeastern Wisconsin, experiencing an increase in ill-thrift, wasting, and death over the past year. Hepatic trace mineral analysis of two previously affected herd mates indicated copper deficiency. The herd was supplemented with free choice mineral but continued to sustain losses. Gross and histological examination of this young male revealed findings known to be linked to copper deficiency in ruminants: extreme wasting, diarrhea, myocardial fibrosis and achromotrichia. In addition, there was severe, well-demarcated, fibrinonecrotizing ulceration of the distal jejunum, the pyloric region of the abomasum and the cecum with serosal adhesions. Tissue samples were collected for enteric bacterial culture and molecular PCR testing. *Trueperella pyogenes* was isolated on multiple cultures but was

not considered the causative pathogen of the ulceration. Intestinal samples were PCR negative for *Salmonella sp.*, Bovine Viral Diarrhea Virus, Epizootic Hemorrhagic Disease Virus, Malignant Catarrhal Fever, and *Mycobacteriumavium* ssp. *paratuberculosis.* Hepatic trace mineral analysis indicated severe copper and moderate selenium deficiencies. Postmortem findings indicate a primary copper deficiency with typical lesions; however, ulcerative enteritis has not been previously reported in association with a copper deficiency in ruminants. No infectious cause was found for the ulcerations described.

D9: PATTERNS OF LYMPHOCYTIC INFILTRATES IN FELINE HEPATIC LYMPHOMA AND LYMPHOCYTIC PORTAL HEPATITIS

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Background: Hepatic lymphoma is poorly characterized in cats and differentiating between inflammation and lymphoma is often difficult. Human hepatic lymphoma diagnosis relies on certain patterns of lymphocytic infiltrates and clonality because other features have not proven to be predicative.

Objectives: To define patterns of lymphocytic infiltrates in hepatic biopsies of cats and correlate them with clonality to determine which patterns were predictive of lymphoma.

Methods: A retrospective study was performed using surgical biopsies from 44 cats of which all had been tested by PCR for T-cell receptor gamma gene rearrangements and 24 cats tested by PCR for immunoglobulin heavy chain gene rearrangements. Four patterns of lymphocytic infiltrates were determined: (1) tightly periportal, (2) periportal and centrilobular, (3) nodular, and (4) periportal with sinusoidal extension. A diagnosis of lymphoma was based on microscopic examination, immunophenotyping, and clonality. Various other histomorphologic features were evaluated.

Results: The sensitivity and specificity of the lymphocytic patterns in the diagnosis of lymphoma were determined using Bayesian Hui-Walter analysis against clonality results. Bayesian analysis demonstrated that the different lymphocytic patterns accurately diagnosed hepatic lymphoma with a sensitivity and specificity of 82% (CI 95%: 0.65, 0.96) and 77% (CI 95%: 0.54, 1.00), respectively. None of the other microscopic features evaluated were predictive of lymphoma or inflammation.

Conclusions: Our study identified specific patterns of lymphocytic infiltration that help in differentiating feline hepatic lymphoma from inflammation. We highlight the lack of predictability of some histologic features previously thought to be associated with lymphocytic hepatitis, raising the importance of clonality testing.

D10: ANTERIOR UVEAL MELANOCYTIC NEOPLASM IN A HORSE WITH PIGMENTED CORNEAL STRIAE

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Background: The right globe of a 16-year-old blue roan Quarter Horse mare was submitted for histologic evaluation. The ophthalmologic exam identified a weak menace response and mildly elevated intraocular pressure in the right eye measuring 30 mmHg (reference range: 10-25 mmHg) as well as moderate buphthalmos, corneal edema, pigmented corneal striae, aqueous flare, and an iris root mass with retrocorneal extension.

Methods: The globe was fixed in 10% neutral buffered formalin and bisected vertically. Sections were embedded, processed routinely, and stained with H&E as well as treated with melanin bleach.

Results: On gross examination, there was a black pigmented mass expanding the inferior aspect of the anterior uvea. Histologically, the pigmented mass, which contained a focus of osseous metaplasia, expanded the inferior iris leaflet and occluded the inferior filtration angle. The mass was comprised of neoplastic cells containing dark brown granules consistent with a melanocytic neoplasm. One mitotic figure was identified in 2.37 mm2 in the bleached section. The cornea had multiple breaks of the Descemet's membrane (Haab's striae) lined by heavily pigmented cells. The retina had subjectively decreased numbers of ganglion cells in the inferior (non-tapetal) region.

Significance: There are only sporadic reports of intraocular melanocytic neoplasms in domestic equids. In common with this case, none of the reported cases had documented metastasis and the neoplasms were not judged to be malignant on histopathologic examination. In this case, sloughed neoplastic melanocytes highlighted multiple corneal striae attributed to glaucoma, accounting for a unique clinical appearance of the affected eye.

D11: FUNCTIONAL INSULINOMAS IN A RHESUS MACAQUE

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Background: Pancreatic neuroendocrine tumors **(**PNETs) are rare neoplasms in primates. They arise from the pancreatic islets, and they include insulinomas, glucagonomas, somatostatinomas, gastrinomas, and VIPomas. A 16.7-year-old, Indian

origin, female rhesus macaque presented with neuroglycopenic symptoms to the breeding colony hospital at the Tulane National Primate Research Center (TNPRC). Upon exam and follow-ups, the animal was consistently hypoglycemic, and was treated, and clinically maintained with high sugar food items, and dextrose supplementation. Occasional episodes of seizure and collapse resolved quickly upon administration of dextrose. A functional PNET was the primary differential in this case. Euthanasia and necropsy were performed. A Pancreatic neoplastic mass was found.

Objective: To fully characterize and to determine the cell of origin of the PNET in the 16.7-year-old female Rhesus macaque.

Methods: Gross and microscopic examinations were performed to determine the cause of the clinical signs. Also, several immunohistochemical stains (IHC) were performed to identify the origin of neoplastic cells.

Results: Grossly, the uncinate process of the pancreas had a 2.2 cm diameter, red, round, and firm neoplastic mass. A microscopic neoplastic mass was also noted in another lobe of the head of pancreas. Histologically, neoplastic cells exhibited neuroendocrine packeting, and resembled pancreatic islet cells. Neoplastic cells stained positive for chromogranin A, synaptophysin and insulin immunohistochemical stains (IHC). Somatostatin, gastrin, pancreatic polypeptide IHC were negative. Few cells reacted positively to Glucagon IHC.

Conclusion: The clinical signs, gross and histological findings are consistent with functional insulinomas.

D12: PROLIFERATIVE PARATHYROID LESIONS IN CAPTIVE BRED AMERICAN BULLFROGS (LITHOBATES CATESBEIANUS) WITH METABOLIC BONE DISEASE

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Parathyroid lesions in anurans are infrequently reported and most often in relation to experimental interventions. Husbandry-related parathyroid changes have yet to be documented in this order of Amphibia. Four American bullfrogs (Lithobates catesbeianus) living in a captive colony from the laboratory animal research facilities at North Carolina State University College of Veterinary Medicine were euthanized due clinical concern for metabolic bone disease secondary to inadequate UVB light in their housing. Hyocalcemia was detected in one of the affected frogs on antemortem bloodwork. Antemortem cytology from one of the bullfrogs with a sublingual swelling found a cohesive population of polygonal to spindloid cells most consistent with benign epithelial proliferation. Postmortem examination revealed cystic dilation and variable proliferation of unidentified structures within the cranial coelom corresponding to the anatomic location of anuran parathyroid glands. Histologically, the structures consisted of sheets and whorls of elongated cells with clear central cavitation and a peripheral fibrous capsule. Immunohistochemistry (IHC) for pan-cytokeratin demonstrated strong

cytoplasmic staining in the elongated cells of these structures and a Grimelius stain for neuroendocrine granules identified sparse granules within the cytoplasm of these cells. These findings are supportive of parathyroid origin. Based on the mechanisms underlying vitamin D and calcium metabolism in anurans, we suspect the lack of full spectrum lighting resulted in decreased levels of active Vitamin D, leading to insufficient calcium and metabolic bone disease, ultimately causing parathyroid proliferation in these bullfrogs.

D13: CUTANEOUS HISTIOCYTOSIS IN AN EASTERN GRAY SQUIRREL (SCIURUS CAROLINENSIS)

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A free-ranging, adult male Eastern gray squirrel (*Sciurus carolinensis*) presented for bilateral ulcerated cutaneous masses at the base of both pinnae, one of which was excised for histopathology. The mass was composed of a dense, infiltrative population of pleomorphic round cells with histiocytic morphology and few interspersed multinucleated giant cells forming sheets in the deep to superficial dermis. Large numbers of lymphocytes and plasma cells were also present. Apart from the presence of superficial bacteria in areas of ulceration, no infectious agents were seen with periodic acid Schiff reaction or Ziehl-Neelsen and Steiner stains. The atypical histiocytes and multinucleated giant cells were strongly immunopositive for ionized calcium-binding adaptor molecule 1 (Iba-1). Based on the cellular morphology and immunohistochemical profile, the mass was diagnosed as cutaneous histiocytosis. The contralateral mass resolved spontaneously, and the squirrel was released. Proliferative histiocytic diseases have been well-described in dogs and humans, less so in cats, and rarely in other species. This is the first reported case of cutaneous histiocytosis in an Eastern gray squirrel. While atypical histiocytosis has been described in European red squirrels (*Sciurus vulgaris*) involving the skin and multiple internal organs, the mass in this case exhibited a different immunohistochemical profile. There are implications for squirrel population health and wildlife medicine, as the lesions macroscopically resembled multiple transmissible diseases, including squirrel poxvirus.

D14: FIRST CONFIRMED OREGON CASE OF HIGHLY PATHOGENIC AVIAN INFLUENZA (HPAI) H5N1 DURING THE 2022 UNITED STATES OUTBREAK IN A DOMESTIC GOOSE

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Background A three-year-old, female, domestic goose (*Anser cygnoides*) was found demonstrating seizure-like activity. Two geese were found dead 3-4 days before. The geese are housed in a large flock and free range acreage frequented by wild waterfowl.

There are currently wild Canada geese present. Other birds on the premise include ducks, chickens, peafowl, Guinea fowl, turkeys, and emu.

Objective To provide a detailed report of the first confirmed Oregon HPAI case during the 2022 outbreak for future identification.

Methods An Avian Influenza Matrix PCR, Avian Influenza Virus Subtype H5 rtPCR, Avian Influenza Virus Subtype H7 rtPCR, and Avian Influenza Virus Subtype H5 clade 2.3.4.4 rtPCR were performed on a cloacal swab, which was confirmed by NVSL as HPAI Eurasian (EA) 2.3.4.4 H5N1 goose/Guangdong clade. Routine necropsy and histopathology were performed.

Results *Molecular testing:* Positive for HPAI Eurasian 2.3.4.4 H5N1 goose/Guangdong clade by both OVDL and NVSL testing.

Necropsy: The left stifle is covered by a large subcutaneous hematoma. There are approximately five 1-0.5 cm in diameter subcutaneous hematomas along the length of the neck. The rest of the necropsy is grossly unremarkable.

Histopathology: The liver has acute, moderate lymphoplasmacytic and heterophilic periportal hepatitis with regionally extensive coagulative necrosis. The pancreas has acute, severe, multifocal coagulative necrosis.

Conclusions Avian influenza has established itself as an endemic pathogen worldwide. The 2022 HPAI outbreak in the United States has impacted an estimated 38 million domestic birds. This case constitutes the first confirmed case of HPAI in the state of Oregon during the outbreak.

D15: ARTERIOVENOUS MALFORMATION OF THE LIVER IN FOUR DOGS.

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Background: Hepatic arteriovenous malformations (HAVM) is a rare disorder most commonly seen in young dogs characterized by multiple small communications between the hepatic artery and portal vein. It is often congenital but can be acquired following trauma or ligation of a major vessel. Secondary acquired extrahepatic portosystemic shunts (EHPSS) and hepatic encephalopathy can occur. Clinically, HAVM is often associated with ascites, stunted growth, lethargy, vomiting, and diarrhea.

Objective: To characterize gross and histologic lesions associated with HAVM in dogs and identify any corresponding trends.

Methods: A retrospective study was performed on cases of HAVM within dogs at Texas A&M University between 2005 and 2022 and one case from Cornell University in 2018.

Results: All animals in this study are young dogs (<1-year-old) with no specific breed or sex predilection. Three animals were euthanized due to poor prognosis with necropsy results, and one other was lost to follow-up. Gross findings are characterized by numerous, dilated, tortuous vascular channels embedded within the liver parenchyma. EHPSS with connections between the portal vein and caudal vena cava are also identified. Histopathologic findings are characterized by venous arterialization, arteriolar hyperplasia and hypertrophy, hepatocellular atrophy, bridging fibrosis, biliary ductular reaction, and ductular cholestasis. Findings in the brain are consistent with hepatic encephalopathy.

Conclusion: This case series characterizes gross and histologic findings associated with HAVM in dogs. This is a rare condition that often carries a poor prognosis.

D16: H3N2 IN RESEARCH-USE SWINE DIAGNOSED BY HUMAN RAPID ASSAY PCR INFLUENZA TEST

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Background: Fifteen swine (13 Yorkshire Cross and 2 Durocs) were received from an approved vendor at the United States Army Institute of Surgical Research (USAISR). After a three-day acclimation period, baseline bloodwork was conducted. One pig had bloodwork results indicating an ongoing infectious process. This pig and two additional pigs in one room developed bilateral nasal discharge. Clinical signs were mild to non-existent in all infected pigs. The vendor informed USAISR veterinary staff that the pigs came from a barn with confirmed cases of Influenza A detected via oral fluid samples after shipment.

Methods: Human-use Roche cobas Liat Influenza A/B (nucleic acid) Assay rapid diagnostic pan-influenza tests were obtained from Brooke Army Medical Center to test pigs in the USAISR vivarium.

Results: The 15 new pigs, as well as eight pigs already housed in the vivarium prior to receipt of the shipment, were sampled by nasal swabbing. Test results indicated that seven out of 15 new swine were positive for Influenza A. The positive cases were retested for subtyping to further delineate the specific strain, and the seven positive cases were confirmed as H3N2. The 15 swine were humanely euthanized to prevent further spread within the vivarium and to protect vivarium personnel. Necropsy findings for all euthanized pigs resulted in multifocal consolidation of lung lobes with moderate lymphohistiocytic interstitial pneumonia.

Conclusion: The use of human rapid diagnostic influenza tests may be a convenient, practical way to quickly obtain results for suspected cases of influenza in swine with respiratory clinical signs.

D17: A RARE OVARIAN TUMOR IN A GALAGO (OTOLEMUR GARNETTI)

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Background: A 7-year-old female galago presented for annual physical exam. On abdominal palpation a firm, round, approximately golf ball sized mass was palpated. The animal appeared to be in good health with no other significant findings. Three weeks later, the animal underwent an experimental procedure and was euthanized due to a prolonged anesthetic recovery.

Objective: Animal was submitted for full diagnostic pathology to determine cause of morbidity.

Methods: On necropsy, a 4x6x5cm pedunculated mass was located centrally within the abdomen. It had a mottled appearance, and the stalk was thick, firm and white. The mass contained ~15-20ml of red viscous fluid which encapsulated a solid structure. On histology, the solid structure that was encapsulated in the mass was diffusely necrotic. The stalk of the mass was composed of a mixed cell population, including very large cells resembling oocytes and what appeared to be ovarian stromal tissue intermixed with duct-like structures. The germ cell portion of the tumor was characterized by aggregates of large uniform cells surrounded by large amounts of connective tissue stroma containing few lymphocytes. The sex cord portion consisted of irregular and branching tubules surrounded by large amounts of cellular ovarian stroma.

Results: Tumor was immunopositive for inhibin, SALL4, Oct3/4 and calreticulin diagnosing a mixed germ cell-sex cord-stromal tumor.

Conclusion: This tumor typically occurs in infants and young children, causing abdominal pain, ascites, and uterine bleeding. This is a rare tumor in humans that hasn't been previously reported in any non-human primate species to our knowledge.

D18: ENTEROPATHY WITH INTRALESIONAL BLASTOCYSTIS SP., CRYPTOSPORIDIUM VARANII, PARABASALIDS, AND MIXED BACTERIA IN A BLACK-THROATED MONITOR

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A 4-y-o, captive, male, black-throated monitor (*Varanus albigularis ionidesi*) was submitted for postmortem examination following natural death after a six-month history of lethargy and weight loss. The lizard had bilateral enophthalmia, generalized

sarcopenia, and marked atrophy of fat bodies. The stomach was empty, and there was cholecystomegaly and caudal hydrocoelom. The cranial small intestine had diffusely thickened mucosa with multifocal red foci that transitioned to 15 cm of mid-intestine thickened by a diphtheritic membrane. Histopathology of the cranial intestine revealed proliferative enteritis with apical protozoa that transitioned to a mid-intestine with necroulcerative lymphoplasmacytic-predominant enteritis with intralesional protozoa and myriad bacteria. Additionally, there was interrenal hyperplasia, zymogen granule depletion, and aspermatogenesis. Diagnostics of affected small intestine included mucosal impression smears that demonstrated *Blastocystis* sp.; PCR that was positive for *Cryptosporidium varanii*; 18S metagenomic barcoding that detected *Blastocystis* sp., *Hypotrichomonas acosta*, *Monocercomonas colubrorum*, and *Colpodella* sp.; and, electron microscopy that confirmed *Cryptosporidium* and parabasalids. Multiplex real-time PCR for free-living amoebae and PCR specific for *Blastocystis hominis* were negative. Bacteriology of the diphtheritic membrane isolated 13 bacterial species in addition to *Salmonella* spp. *Cryptosporidium varanii* is a known cause of proliferative enteritis in lizards; however, *Blastocystis* sp. and parabasalids, while infrequently reported in lizard feces, are not associated with necrotizing enteritis. This lizard's decline and death, is attributed to enteritis caused by *Cryptosporidium varanii*, *Blastocystis* sp., and two parabasalids along with secondary bacterial overgrowth.

D19: CHRONIC ACTINOMYCES OSTEOARTHRITIS IN A CAPTIVE SAND GAZELLE (GAZELLA LEPTOCEROS LEPTOCEROS)

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Osteoarthritis is a common cause of morbidity and mortality in geriatric gazelles. *Actinomyces* spp. are well-documented as a cause of osteomyelitis in domestic ruminants, but clinical, gross and histologic descriptions of this bacterial genera infecting exotic hoofstock are lacking. An 8-year-old male castrated sand gazelle was managed for four years for chronic, intermittent and progressive suppurative osteoarthritis of the right tarsus. Culture of joint fluid yielded growth of *Actinomyces* spp. Serial diagnostic imaging (radiographs, computed tomography) of the right tarsocrural joint space identified osseous and soft tissue proliferation with draining tract formation. Treatment included broad-spectrum antibiotics, anti-inflammatories, and eventual joint debridement and infusion with platelet-rich-plasma and stem cells. Despite therapy, lameness persisted and the animal was euthanized. On postmortem examination, the right tarsus was markedly enlarged with multiple superficial draining tracts. Sagittal sectioning revealed expansion of the peri-articular tissue by several discrete pyogranulomas with surrounding fibrosis. Articular cartilage of the joint was multifocally eroded to ulcerated with irregular margination of subchondral bone. Histologically, the

synovium, joint capsule, and overlying soft tissues were markedly expanded by pyogranulomatous inflammation with numerous aggregates of gram-positive, Wright-Giemsa positive, and Fite's negative filamentous bacteria surrounded by Spendore-Hoeppli material, consistent with *Actinomyces*. Inflammation and fibrosis extended into the joint space, where there was regionally extensive cartilage ulceration, osteonecrosis, osteolysis, and pannus formation. *Actinomyces* can be a cause of chronic osteoarthritis in captive gazelles, and should be considered a differential for chronic suppurative to pyogranulomatous osteoarthritis in exotic hoofstock.

D20: PRIMARY INTRAPERICARDIAL EPITHELIOID MALIGNANT MESOTHELIOMA IN A DOG

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Background: A 16-year-old castrated male Cocker Spaniel was necropsied after humane euthanasia. Antemortem morbidities include IRIS Stage II chronic kidney disease and chronic pancreatitis. An echocardiogram performed 8 months prior to necropsy revealed Stage B1 Myxomatous Mitral Valve Disease (MMVD) and 2nd degree atrioventricular (AV) block. On postmortem exam, there was an unexpected intrapericardial mass.

Objective: To further characterize the tissue origin in an unusual occurrence of an intrapericardial mass using routine histopathology and immunohistochemistry.

Methods: Sections of the mass were fixed in 10% formalin, routinely processed, and stained with hematoxylin and eosin (H&E). Alcian blue histochemical staining and cytokeratin AE1/AE3, vimentin, thyroglobulin, and calcitonin immunohistochemistry (IHC) were performed.

Results: Focally, a 2.7 x 2.2 x 0.7 cm, pink-tan, semi-firm, solid mass was fibrously adhered to the pericardium and right ventricular epicardium. Histologically, the neoplasm was predominately composed of branching tubules and papillae lined by cuboidal cells supported by fibrovascular cores. There was mild anisocytosis, mild anisokaryosis, and rare mitoses. Clusters of neoplastic cells with increased cellular atypia were observed in a regional lymph node. Neoplastic cells had patchy cytoplasmic and stromal alcian blue reactivity and frequent positive immunoreactivity for cytokeratin (AE1/AE3) and vimentin. Thyroglobulin and calcitonin immunohistochemistry was negative.

Conclusions: Based on H&E alone, mesothelioma and ectopic thyroid carcinoma were initially considered most likely differential diagnoses. Negative immunoreactivity for thyroglobulin and calcitonin ruled out ectopic thyroid carcinoma. Dual expression of cytokeratin and vimentin, morphology, lymphatic metastasis, and location support the diagnosis of intrapericardial mesothelioma.

D21: BILATERAL OCULAR INVOLVEMENT OF A DISSEMINATED HISTIOCYTIC SARCOMA IN A BICHON FRISE

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Background: A 10-year-old female neutered Bichon Frise dog presented for enucleation of a non-visual, end-stage glaucomatous right eye with subsequent submission of the eye for histopathological examination. At the time of initial presentation, the left eye was unremarkable apart from severe iris atrophy and senile cataracts. Re-presentation for tumor staging three weeks later identified a focal multilobular vascularized mass within the remaining left eye. Euthanasia was elected as there was clinical suspicion of widespread metastasis.

Objective: The objective of the initial biopsy submission and subsequent post-mortem examination was to determine the underlying cause of the end-stage glaucomatous right eye and investigate the intraocular mass affecting the left eye and other clinically suspected organs.

Methods: Histopathology of the enucleated right eye was performed along with immunohistochemical examination with antibodies against multikeratin, vimentin, Iba-1, melan-A and PNL2. A post-mortem examination was performed with histopathologic examination of the left eye and affected major organs.

Results: Examination of the right eye identified the cause of the glaucoma to be a malignant iridociliary round cell neoplasm similar in morphology to the left intraocular mass. Immunohistochemistry revealed positive immunolabelling of neoplastic cells with antibodies against vimentin and Iba-1, leading to a diagnosis of bilateral ocular histiocytic sarcoma. Disseminated neoplastic cells were also identified throughout the viscera, including both kidneys, the lungs and the heart.

Conclusions: This case features a rare presentation of histiocytic neoplasia and highlights the potential diagnostic challenge in differentiating these ocular neoplasms from those with better prognoses, such as (a)melanotic melanomas.

D22: CHRONIC NECROTIZING DISCOSPONDYLITIS IN A PREWEANED MINKE WHALE (BALAENOPTERA ACUTOROSTRATA)

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Background: A preweaned, male minke whale (*Balaenoptera acutorostrata*) was euthanized after live stranding in Norfolk, United Kingdom and recovered for necropsy through the Defra funded Cetacean Strandings Investigation Programme.

Objective: To determine the reason for the live stranding.

Methods: Necropsy, histopathology, microbiology and computer tomography (CT) were conducted.

Results: The animal was in reasonable nutritional condition. In five locations along the spine, vertebral bodies were ankylosed by large, bony proliferations centered over intervertebral spaces. The nucleus pulposus and sometimes the annulus fibrosus of the intervertebral discs were replaced by fibrinous membranes and dense, brown, pus-like material. The CT demonstrated evidence of diffuse spondylitis, multifocal discospondylitis with secondary subluxations in the cervical, thoracic and post-thoracic vertebral column, and luxation in the coccygeal segment resulting in deviation of the tail. There was no evidence of injury to the spinal cord. Microscopic examination of an intervertebral disc showed extensive necrosis with chronic active inflammation. Multiple cultures of the disc material and parenchymatous organs were negative and no organisms were observed with special stains.

Conclusions: The minke whale likely stranded due to reduced mobility. We suspect that this is a case of chronic discospondylitis initiated by a bacterial infection that has since been cleared. Blood supply to the disc is only present in young animals, hence discospondylitis solely occurs in neonates and juveniles. It has been only rarely reported in cetaceans, mostly in museum specimens. To our knowledge, this is the first description of discospondylitis in a minke whale.

D23: MULTISYSTEMIC EOSINOPHILIC EPITHELIOTROPIC DISEASE (MEED) WITH EXTENSIVE HEPATIC INVOLVEMENT IN A HORSE

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Background: Multisystemic eosinophilic epitheliotropic disease (MEED) is a rare equine disease of unidentified cause that most commonly manifests in the skin and gastrointestinal tract. Few reports detail gross and histologic lesions, and extensive hepatic involvement is not documented. An 18-year-old Rocky Mountain Horse mare presented for a week-long history of hyperthermia, anorexia, and intermittent diarrhea. Physical examination revealed tachycardia, tachypnea, multifocal alopecia and crusting, and abdominal distention. Serum biochemistry panel showed moderately to markedly elevated ALP, GGT, and SDH. Antemortem duodenal and rectal biopsies revealed submucosal eosinophilic granulomas with minimal to mild neutrophilic inflammation; MEED or parasitism were considered as differentials. Due to lack of clinical resolution, the mare was euthanized and submitted for postmortem examination.

Objective: Our aim was to characterize gross and histologic lesions leading to this horse's clinical decline.

Methods: Postmortem samples were routinely collected and processed for histologic evaluation.

Results: Gross examination revealed too numerous to count, variably sized, firm, light yellow to tan, green-tinged nodules throughout the liver, mesentery, mesenteric lymph nodes, and to a lesser extent, the lung. Nodules affected approximately 10-20% of the liver parenchyma. The small intestines were transmurally moderately thickened. Histopathology of affected tissues revealed marked eosinophilic and granulomatous inflammation with extensive fibrosis. No etiologic agents were identified with GMS or PAS stains.

Conclusions: These findings are consistent with an atypical manifestation of MEED in a horse with extensive hepatic involvement. MEED may be considered as a cause for nodular hepatopathy and/or elevation of liver enzymes in horses.

D25: AN INFECTIOUS LARYNGOTRACHEITIS OUTBREAK IN COMMERCIAL BROILER CHICKENS

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Over a 32-day time frame, 15 commercial broiler chickens ranging from 43-55 days old, from three farms across east Texas were submitted to the Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL) in College Station, TX for post-mortem examination. The clinical history included increased flock mortality, decreased water consumption, and birds exhibiting gasping, squinting, and head shaking. The tracheal mucosa of all birds was roughened and dark red with variable coverage by diphtheritic membranes (fibrinonecrotizing tracheitis), and the tracheal lumens frequently contained clotted blood (hemorrhage). The larynx of some birds also had adherent fibrin and roughened mucosa (laryngitis). Six birds had dark red, wet, and heavy lungs with fibrin-filled bronchi (fibrinonecrotizing bronchopneumonia). Histopathology performed on the lung and trachea of some birds confirmed a fibrinonecrotizing tracheitis and bronchopneumonia with eosinophilic intranuclear inclusions and syncytial cells in respiratory epithelium. The lesions were consistent with infectious laryngotracheitis (ILT) caused by *Gallid herpesvirus 1* (GaHV-1), which was confirmed by quantitative PCR on tracheal swabs. The outbreak was traced to a poultry trade show near Jewett, TX. Subsequent spread among commercial flocks was secondary to direct or indirect contact of farm personnel with live haul routes as affected birds were control marketed. Strict biosecurity measures were enforced to contain the outbreak, which proved successful. This case represents an example of an ILT outbreak in three commercial broiler flocks with characteristic clinical signs and lesions that was traced to a poultry trade show and successfully contained with controlled marketing of affected flocks and enhanced biosecurity.

D26: CLINICAL LABORATORY EXPERIENCE WITH A NEXT-GENERATION SEQUENCING-BASED LIQUID BIOPSY TEST FOR CANCER DETECTION IN DOGS

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Background Next-generation sequencing-based liquid biopsy testing for cancer detection in dogs became clinically available in 2021 and has since been used in a variety of clinical settings.

Objective This study reviews ordering patterns and outcome data from samples submitted for commercial testing at one clinical laboratory.

Methods Clinical data were analyzed from 1,500 consecutive blood samples submitted for liquid biopsy testing.

Results Over 60% of cases were referred for liquid biopsy as a screening test for dogs with no current suspicion of cancer, another ~25% as an aid-in-diagnosis for dogs suspected of having cancer, and ~10% of cases for “other” indications, including post-diagnosis uses such as minimal residual disease detection and recurrence monitoring. The positivity rate was <10% for the screening population and >25% for the aid-in-diagnosis population. *Indeterminate* results (in which genomic alterations were detected but their significance was uncertain and a complimentary redraw was advised) were uncommon, representing <3% of cases; when a redraw was submitted, the vast majority of cases received a clear positive/negative report on the second test. Outcome data were available for a subset of patients and the observed positive predictive value compared favorably with that of liquid biopsy tests used in human medicine.

Conclusions Once a test is clinically validated and available commercially, it is important for the laboratory to periodically report utilization and performance metrics regarding the test. This observational study provides data demonstrating that most dogs who receive a positive liquid biopsy result are diagnosed with cancer following a confirmatory cancer evaluation.

D27: FATAL PROGENY? YOLK EMBOLISM IN A SAVANNAH MONITOR

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Background: Yolk embolism, which has been reported in female birds, reptiles, and fish, is a fatal condition with disseminated yolk globules within multiple organs and vessels. Pet birds suffering from yolk embolism develop neurological sings, respiratory distress, and sudden death. Wildlife reptiles in reproductive cycle may have a risk of systemic yolk embolism after traumatic injury. The histopathology of yolk embolism is rarely documented in Squamata. A female 5-year-old Savannah monitor (*Varanus*

exanthematicus) was found up-side down before sudden death at the Taipei Zoo. Necropsy was performed.

Object: Our object was to determine the cause of death of the patient by necropsy and histopathological examination.

Methods: Standard H&E and PAS-stained slides were examined.

Results: There was no gross lesion except for bilateral ovaries, where vitellogenic and previtellogenic follicles showed diffusely grey discoloration. Microscopically, there was an increased number of foamy macrophages engulfing yolk-like material infiltrating the follicles. Numerous yolk globules were found in the vessels of multiple organs, including liver, spleen, reproductive tract, central nervous system, and kidney.

Conclusions: The actual cause of yolk embolism is unknown. Several researchers have hypothesized that yolk embolism is the consequence of vascular damage and folliculostasis. Here, we report the first case report of yolk embolism in Savannah monitors. The cause of yolk embolism in the present Savannah monitor may be due to traumatic injury or oophoritis.

D28: VETERINARY FORENSIC INVESTIGATION: AN UNUSUAL AND FATAL ACCIDENT CAUSED BY INJECTION NEEDLE DURING ANESTHESIA INDUCTION IN A DOG

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Background: An adult male street dog was captured and sheltered for castration. During anesthesia induction, the dog was agitated and aggressive. Thus, intramuscular injection failed to perform in one attempt. After the second and successful IM injection in the quadriceps muscle, the dog showed tachypnea and vomited blood, then died 20 minutes later. Severe anesthesia complications were suspected.

Objective: The objective of this case report is to highlight the importance of forensic death examination when investigating unusual death.

Methods: Forensic necropsy was performed to identify the cause of death.

Results: At necropsy, the dog had hemothorax and an 8mm laceration on the left caudal lung lobe. A 2mm spindle-shaped puncture wound was noted in the intercostal muscles between the left ninth and tenth ribs with adjacent subcutaneous to muscular hemorrhage. A mild contusion was on the skin. These findings are consistent with a needle track. Histologically, the pulmonary parenchyma had severe hemorrhage and diffuse edema with fibrin accumulation in the laceration. Skeletal muscles of the

puncture wound in intercostal muscles showed acute necrosis, fibrin accumulation, and hemorrhage.

Conclusions: The cause of death was lung laceration and hemothorax caused by needle penetration of the chest wall, which accidentally happened during anesthesia induction. Injection needles are instruments used in daily veterinary practice; however, they can accidentally penetrate deep organs and result in fatal consequences in a chaotic situation. In this case report, we demonstrate the patterns of a needle track and discuss the approach to identifying it.

D29: DETECTION OF STAT5B MUTATION IN FELINE SMALL CELL T CELL INTESTINAL LYMPHOMA WITH DROPLET-DIGITAL PCR ANALYSIS (DDPCR)

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Background: Signal transducer and activator of transcription 5b (STAT5B) is a transcription factor that transmits downstream signaling through a variety of tyrosine kinase receptors causing uncontrolled cellular proliferation, anti-apoptotic responses, and angiogenesis. An A to C transversion single-nucleotide polymorphism (SNP) causing an Asn to His activating mutation in feline small cell T cell intestinal lymphoma cases has been described. Our laboratory developed a droplet-digital PCR (ddPCR) assay to detect this mutation.

Materials and Methods: Endoscopic duodenal biopsies were collected from 30 cats with chronic enteropathy. Three pathologists blindly categorized samples as lymphoplasmacytic enteritis, small cell T cell lymphoma, or possible lymphoma. PARR (PCR for Antigen Receptor Rearrangement) was completed to assess clonality. ddPCR was performed, which utilizes two competitive probes, one to detect the wild-type variant (STAT5B-) and one to detect the mutant allele (STAT5B+).

Results: 11/30 cases had complete agreement of histologic diagnosis by all three pathologists. Nineteen of 30 cases were diagnosed as lymphoma by at least one or more pathologist; all demonstrated T cell clonality, and 16/19 were STAT5B+. Six of 30 cases were not definitively diagnosed as lymphoma by any pathologist; 2/6 showed T cell clonality, both of which were STAT5b+. Five of 30 were diagnosed as enteritis by all three pathologists, 4/5 were not clonal, and all were STAT5B-.

Conclusion: This study confirms cats diagnosed with small cell T cell intestinal lymphoma frequently carry this STAT5B mutation.

D30: DISSEMINATED HISTIOCYTIC SARCOMA IN A QUARTER HORSE MARE

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A 16-year-old Quarter Horse mare was presented for weight loss, lethargy, and inappetence. Physical examination revealed tachycardia, cyanosis, cool extremities, and a distended abdomen. Euthanasia was elected. Postmortem examination revealed hemoabdomen with a large blood clot adhered to a hepatic fracture. The liver was massively enlarged, friable, and mottled yellow to dark red. There were widely disseminated, 1-4 cm diameter, white, firm nodules throughout the parenchyma. The caudodistal margin of the right liver lobe was continuous with a 47 cm x 48 cm, red to yellow to tan, firm, irregular mass that effaced the right adrenal gland. In the lungs, there were disseminated, 0.3-2 cm diameter, firm, white to red nodules. Histology of the mass, liver, right adrenal gland, and hepatic lymph nodes revealed a highly infiltrative pleomorphic round cell population. Cells had a moderate amount of glassy eosinophilic cytoplasm and large round to ovoid nuclei with vesiculated chromatin and typically one prominent nucleolus. Multinucleated cells were common, and erythrophagocytosis was rare. Neoplastic cells were diffusely positive for vimentin, and approximately 25% of cells were IBA-1 positive. Neoplastic cells were negative for cytokeratin, CD3, CD20, CD18, MUM-1, and CD117. Disseminated histiocytic sarcoma was diagnosed. Histiocytic sarcoma occurs most frequently in dogs and is rare in horses. Primary sites of origination include the spleen, bone marrow, lung, and periarticular tissues with frequent lung and liver metastasis. The site of origination in this horse is suspected to be the liver.

D31: MEDULLOBLASTOMA WITH EXTENSIVE NODULARITY IN A DOG

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Background: A 3-year-old male castrated, Labradoodle presented for a 1-week history of loss of balance, lethargy, and shivering. On examination, the animal had mild vestibular ataxia, thoracic limb dysmetria, and right rotary nystagmus. MRI revealed a mass associated with the cerebellum and a biopsy was taken at the time of surgical debulking. Histology revealed features consistent with medulloblastoma with extensive nodularity. The animal improved after surgery, but the signs progressed, and a month later was euthanized.

Objective: Histologic and immunohistochemical characterization of this cerebellar neoplasm.

Methods: Tissue sections from the biopsy and brain were examined histologically. Immunohistochemistry (IHC) for GFAP, synaptophysin, OLIG2, and MAP2 was employed.

Results: Histologic examination revealed a neoplasm with a diverse population of neuron-like cells and astrocyte-like cells embedded in a neurofibrillary matrix. Intertwined with this population were nests of angular neuroblastic cells. IHC revealed

strong cytoplasmic immunolabeling throughout the neoplastic population for MAP2 and synaptophysin. The astrocyte-like cells had strong immunolabeling for OLIG2 and the primitive neuroblastic cells did not have any immunolabeling.

Conclusions: Medulloblastoma with extensive nodularity is a rare subtype of embryonal brain tumor diagnosed in children, defined by significant neuronal and glial differentiation. This is a unique histologic pattern not previously reported in dogs.

D32: AN OUTBREAK OF TOXOPLASMOSIS OF FOUR RING-TAILED LEMURS (LEMUR CATTA) IN TAIPEI ZOO, TAIWAN

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Background: *Toxoplasma gondii* is an obligated intracellular zoonotic protozoal parasite. New World monkeys, marsupials, and lemurs are highly susceptible animals. In 2019, four ring-tailed lemurs (*Lemur catta*) showed sudden death and acute lethargy in Taipei Zoo, Taiwan. The other two black-and-white ruffed lemurs (*Varecia varigata*) encaged in the same enclosure were clinically healthy. Ringed-tailed lemurs died one after another within five days. Blood examines revealed elevation of ALT, AST, and BUN; thrombocytopenia; and hypocholesterolemia. Hypocholesterolemia was noted in black-and-white ruffed lemurs as well.

Objective: Our objective was to determine the etiology of the acute disease event.

Methods: All sampled tissues were processed to standard FFPE and stained with H&E. Immunohistochemistry (*T. gondii*), conventional PCR, and sequencing were performed.

Results: Microscopically, necrotizing foci could be found in the liver, lymph nodes, and spleen. Alveolar spaces were filled with edematous fluid. Intra-lesion 2-3um nucleated, round apicomplex tachyzoites were stained positive under IHC staining. PCR and sequencing result confirmed systemic toxoplasmosis.

Conclusions: Lemurs are susceptible species to *Toxoplasma gondii*, which seems to be an evolutionary selection. Interestingly, the other two black-and-white ruffed lemurs remained unaffected. The contradiction can be interpreted as different health statuses and adequate immune responses. All six lemurs showed hypocholesterolemia. We hypothesize that serum cholesterol might be consumed by the sheer number of *T. gondii* replicating in the host. Serum cholesterol might be an indicator for *T. gondii* infection in the same outbreak group.

D33: IDIOPATHIC CHOLANGIOFIBROSIS IN A GERIATRIC GUINEA PIG

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An approximately 6-year-old male pet guinea pig presented to the Mississippi State University College of Veterinary Medicine Emergency Service for lethargy, weight loss, anorexia, and abnormal fur pigmentation. Physical exam revealed profound bradycardia, hypothermia, decreased oxygenation saturation, decreased gastrointestinal motility, and 5-7% dehydration. Due to poor prognosis, humane euthanasia was elected. Necropsy revealed a diffusely pale pink to tan liver with a 4 cm x 3 cm, irregularly marginated, focally extensive area of proliferation across the proximal portion of left medial and right medial liver lobes. Histologically, the hepatic parenchyma was replaced by many proliferative bile ductules surrounded by abundant fibrous connective tissue. Associated with the areas of fibrosis were random areas of hepatocellular necrosis and dilated, tortuous bile ducts. Based on the microscopic characteristics and available literature, this guinea pig was diagnosed with idiopathic cholangiofibrosis. Idiopathic cholangiofibrosis has been rarely reported in guinea pigs. In one report of hepatic fibrosis in a colony of guinea pigs, lesions were diagnosed as hepatic cirrhosis, then recently reclassified as “idiopathic cholangiofibrosis” due to the lack of macronodular regeneration, a classic lesion of cirrhosis. In the original report, the etiology was not identified; however, an underlying toxin was suspected. In summary, our guinea pig had lesions consistent with previously but rarely reported idiopathic cholangiofibrosis and an underlying etiology was also not identified.

D34: MUCINOUS ADRENAL CORTICAL CARCINOMA WITH SPINAL, MENINGEAL, AND CEREBRAL VENTRICULAR METASTASIS IN A DOG

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Background: Adrenal cortical carcinoma with myxoid differentiation has been reported in ferrets and humans, composed of tumor cells arranged in cords and clusters separated by a variable amount of mucinous or myxoid substance. They are generally highly malignant in both ferrets and humans and have never been described in canines.

Methods: An 11-year-old male castrated mixed-breed dog presented with clinical signs of seizures and progressive T3 to L4 myelopathy was euthanized and autopsied. The autopsy revealed a multinodular retroperitoneal mass effacing and replacing the left adrenal gland infiltrating the caudal vena cava, regional skeletal muscle, vertebral body, and the right adrenal gland. Formalin-fixed tissues were processed for histopathology. Special staining was performed using Alician blue and Periodic acid-Schiff. Immunohistochemical staining for vimentin, pancytokeratin, α- inhibin, synaptophysin, chromogranin A, neuron-specific enolase, melan A, and smooth muscle actin was also performed for further characterization.

Results: Microscopically, the mass was composed of clusters, cords, and acini of neoplastic cells separated by variably sized lakes of Alician blue and PAS-positive mucinous matrix. Neoplastic cells exhibited strong cytoplasmic labeling for vimentin and occasional intracytoplasmic labeling for pan-cytokeratin. The mass was stained

negative for other markers. The tumor cells had metastasized to the spinal cord, meninges of the brain, and the choroid plexus of the lateral ventricle.

Conclusion: This was a case of poorly differentiated adrenal cortical carcinoma with myxoid differentiation, which has been reported in humans and ferrets. To the best of our knowledge, this is the first report of such a neoplasm in dogs.

D35: HISTIOCYTIC SARCOMA WITH CENTRAL NERVOUS SYSTEM INVOLVEMENT IN CATS

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Background: Histiocytic sarcoma (HS) is commonly diagnosed in companion animals. HS of the central nervous system (CNS) has not been well characterized in cats.

Objectives: To describe the neuroanatomic distribution, neuropathology, and diagnostic immunohistochemistry (IHC) of 6 feline HS of the CNS.

Methods: Cases of feline HS with CNS involvement were retrospectively searched from two academic institutions. Pathology reports and archived glass slides were reviewed. Tissue sections were subjected to IHC for IBA1, E-cadherin, CD3, CD20, and MUM1.

Results: The mean age of affected cats was 9.3 years. Tumors occurred in the brain (4 cases), spinal cord, and brain and spinal cord (1 case each). Tumors were restricted to the CNS in 3 cases. Gross changes in 4 cases consisted of neuroparenchymal swelling (3 cases) or a gray mass (1 case). Histologically, neoplasms were neuroparenchymal with occasional extension to the leptomeninges, and consisted of sheets of neoplastic cells with marked pleomorphism and round to elongate eosinophilic cytoplasm. Nuclei were round to indented and had coarse chromatin with 1-2 nucleoli. Multinucleated neoplastic cells were observed throughout. Mitoses ranged from 1 to 24 in 2.37mm^2 (10 FN22 40× fields). Cytoplasmic immunolabeling for IBA1 was observed in all cases. All other IHCs were negative.

Conclusion: Our findings are similar to those reported in dogs with HS of the CNS. IBA1 immunolabeling confirms the histiocytic origin and is supportive of a diagnosis of HS in cats.

D36: FATAL AIR GUN PROJECTILE INJURIES IN ANIMALS

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Background: Air guns possess serious lethal potential towards humans and animals; however, they are often promoted as toys and are easily obtained due to lack of regulatory guidelines.

Objective: The objective of this study was to review fatal air gun projectile injuries (AGPIs) in a series of animals.

Methods: The autopsy reports of animals that died due to AGPIs were searched from the archives of the university's forensic pathology service (2019 to 2022). Age, sex, weight, location of entrance/exit wounds, number of projectiles, use of postmortem imaging, injuries, and the type of projectile recovered were recorded for each animal.

Results: Three cats, two dogs, and a Virginia opossum were diagnosed with fatal AGPIs. Two of the animals were euthanized. Postmortem radiographs were performed in 5 cases and the projectiles were identified in all instances. All of the cats and dogs had only been shot once; whereas, the opossum was shot 25 times. Four animals were shot with air gun pellets and two were shot with ball bearings.

Conclusions: Even though air guns are marketed as a toy, we highlight how even a single AGPI can prove fatal for an animal. Additionally, we have shown how postmortem diagnostic imaging can be useful in cases of suspected fatal AGPIs.

D37: CHEMILUMINESCENT DETECTION OF DOG BLOOD STAINS: EFFECTS OF SURFACES AND CLEANING AGENTS

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Background: Chemiluminescence is commonly used by forensic investigators to detect trace amounts of suspicious stains including blood at crime scenes. No studies have been performed to evaluate the potential use of this technique for use in the detection of animal blood at animal crime scenes.

Objective: To investigate the use of a chemiluminescent reagent (Bluestar® Forensic) for detection of dog blood that was cleaned up using two different commonly available cleaning agents.

Methods: Dog blood stains (100 uL) were deposited on 5 different surfaces (top and back of carpet, wood, vinyl tile, rubber mat), which are commonly encountered surfaces at animal crime scenes. The samples were then treated with two different cleaning solutions (Clorox Cleaner + Bleach, Lysol All Purpose Cleaner), wiped with a paper towel, and treated with Bluestar® Forensic. The treated stains were visualized and photographed.

Results: Chemiluminescence was observed on 3 of 5 surfaces within both treatment groups.

Conclusions: With the increasing number of veterinary forensic sciences investigators (including veterinary pathologists), it is important for them to be aware that Bluestar® Forensic can be used for the detection of dog blood on multiple surfaces, however, surface characteristics, amount of chemiluminescent reagent applied, and cleaning agents can affect to ability to detect chemiluminescence.

D38: A RETROSPECTIVE STUDY OF FELINE DIGITAL LESIONS SUBMITTED FOR HISTOPATHOLOGY (2010-2022)

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Background: Feline digital lesions are relatively common in the surgical biopsy service of the Athens Veterinary Diagnostic Laboratory (AVDL). The type and frequency of these lesions are infrequently reported in the veterinary literature.

Objective: To characterize the feline digital lesions (digital skin and amputated digits) submitted to the AVDL surgical biopsy service from 2010 to 2022.

Methods: The web-based laboratory archive system was searched for feline digital biopsy cases submitted between January 2010 and January 2022 using the keywords “toe” or “digit”. Selected cases were evaluated, and lesions were characterized as inflammatory (infectious and non-infectious), neoplastic, and other.

Results: Of a total of 11,646 feline biopsies in the studied period, 278 (2.3%) consisted of digital biopsies. Domestic shorthaired cats were most commonly affected (209 cases). The mean age of affected cats was 9.7 years. Neoplasms (185 cases) were more frequently diagnosed than inflammation (82 cases). The most frequent primary neoplasms were basal cell tumor (21 cases), fibrosarcoma (19 cases), mast cell tumor (18 cases), squamous cell carcinoma (17 cases), adenocarcinoma (16 cases), and spindle cell sarcoma, NOS (12 cases). Adenocarcinoma (8 cases) was the most common metastatic neoplasm (pulmonary origin was reported in 2 cases). Inflammatory lesions consisted mainly of eosinophilic granuloma (18 cases) and plasmacytic pododermatitis (13 cases). In 19 inflammatory lesions, intralesional organisms (10 bacterial, 8 fungal, and 1 mixed bacterial and fungal) were observed.

Conclusions: Here we highlight the high frequency of neoplasms when compared to inflammation in the feline digits submitted to our biopsy service.

D39: A RARE VARIANT OF BRAINSTEM HIGH-GRADE UNDEFINED GLIOMA WITH UNUSUAL OLIGODENDROGLIAL DIFFERENTIATION IN A THREE-YEAR-OLD SIBERIAN HUSKY: A CASE REPORT WITH LITERATURE REVIEW

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Background: A 3-year-old intact male Siberian Husky dog was presented with acute onset vestibular signs, continuous pacing with reduced activity level. Magnetic Resonance Imaging (MRI) revealed a well demarcated mass with heterogenous enhancement at medulla oblongata. Concurrently, intralesional hemorrhage, perilesional edema and herniation of cerebellum through the foramen magnum were also observed. Corticosteroids was prescribed after infectious causes had been ruled out. The MRI, performed 3 months later, revealed mild bilateral cerebrocortical atrophy and more severe intralesional necrosis. The dog was euthanized at 8 months after initial presentation because of deterioration of neurological condition.

Objective: We introduced a canine primary high-grade undefined glioma with unusual oligodendroglial differentiation.

Methods: Microscopic examination of brain collected postmortem and a panel of immunohistochemical (IHC) staining were performed.

Results: Histopathology revealed a pleomorphic neoplasm with the presence of serpentine necrosis, microvascular proliferation, cyst formation and mineralization. Two distinct neoplastic cell types with geographical distribution were observed, including nests to clusters of round-shaped cells and pseudopalisading to disorganized spindle-shaped cells. On IHC, the tumor cells tested positive for vimentin, variable positive for glial fibrillary acidic protein (GFAP), Olig2 and SRY-related HMG-box 10 (SOX10) in geographically distinct, and negative for cytokeratin AE1/AE3, E-cadherin, S-100 and synaptophysin. Both astrocytic and oligodendroglial differentiation within the neoplasm was demonstrated by histopathologic morphology and IHC results.

Conclusions: According to the novel revised diagnostic classification of canine gliomas, the brainstem high-grade undefined glioma in a young large-breed dog with a relatively long survival time demonstrate atypical MRI images and unusual biphenotypic features.

D40: ANIMAL ABUSE INVESTIGATION: LIGATURE STRANGULATION AND NON-ACCIDENTAL BLUNT FORCE HEAD TRAUMA IN A DOG

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Background: A four-month-old intact male Collie dog was found dead by the police in the owner's house. The neighbors called the police since they heard the dog screaming. The owner claimed that a Bodhidharma statue accidentally fell on the dog.

Objective: This case report aims to highlight the importance of veterinary forensic investigation in animal abuse cases and demonstrate the features of strangulation and non-accidental blunt force head trauma.

Methods: Postmortem radiological examination and forensic necropsy were performed to identify the cause of death.

Results: At necropsy, the dog had multifocal contusions and abrasions on the dorsal head with massive subcutaneous and muscular hemorrhage. The parietal and frontal cranial bones had comminuted depression fractures. The brain showed destructive injuries. In the neck region, a horizontal ligature mark featured by a central pale zone with hyperemia and abrasions on the edges encircled the neck. There were hemorrhages in the bilateral sternohyoid and thyrohyoid muscles and multifocal petechiae on conjunctivas and thymus. Histologically, diffuse pulmonary edema, multifocal hemorrhage, and emphysema were identified.

Conclusions: In the present case, the depression fractures of the cranium indicated multiple impact areas, suggesting a non-accidental blunt force head trauma. The findings in the neck were consistent with ligature strangulation. The necropsy findings were highly indicative of physical abuse and inconsistent with the owner's allegation. These results allowed the police and animal protection officers to conduct further investigation and served as evidence in trial.

D41: CONGENITAL OCULAR ABNORMALITIES IN A CALF DUE TO INTRAUTERINE BLUETONGUE INFECTION

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CASE DESCRIPTION: A 1-week-old female Aberdeen-Angus calf presented to the UM VTH for euthanasia due to blindness, circling, and neurologic symptoms since birth. On gross examination, there was microphthalmia and a corneal dermoid OS, and dyscoria OD. Histopathologic examination revealed colobomas and retinal dysplasia OU, with OS lens luxation and corneal dermoid. PCR was negative for Bovine Viral Diarrhea Virus (BVD), Bluetongue Virus (BT), Leptospira, Parainfluenza-3, and Neospora caninum on samples of lung, spleen, lymph node, and brain. Serology of peripheral blood was negative for BVD and positive for BT. Intrauterine infection with BT is considered the source of the congenital abnormalities in this patient.

CLINICAL RELEVANCE: Bluetongue is an orbivirus of ruminants spread by Culicoides spp. midges. Clinical disease is most common in sheep, but other ruminants are also susceptible. In cattle, intrauterine infection with BT is associated with early embryonic

death, abortion, stillbirth, and congenital abnormalities such as hydranencephaly and porencephaly. Corneal edema has been associated with BT infection in calves, but to the authors' knowledge, retinal lesions have only been described in lambs.

D42: POLYOSTOTIC PAROSTEAL OSSIFYING LIPOMA IN AN ADULT CAT

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Background: Parosteal ossifying lipoma is a rare form of lipoma associated with the periosteum of bone.

Objective: The goal of this study is to describe a case of polyostotic parosteal ossifying lipoma in an adult cat.

Methods: Radiographic, gross, and histologic evaluation following decalcification of mineralized masses were performed.

Results: A 5-year-old female spayed Domestic Shorthair cat presented in respiratory distress and recent onset of neurological signs. Radiographic evaluation revealed multiple masses of cancellous bone projecting from the bony surfaces of the sternum and ribcage into the external thoracic soft tissue and into the thoracic cavity. Gross findings included multifocal nodular exophytic hard masses on the surface of the left zygomatic arch, ventral sternal midline, intrathoracic sternal midline, external left lateral thorax, and intrathoracic left caudal thorax. Masses were composed of adipose tissue containing microscopic bone deposits lined by primitive mesenchymal stromal cells progressing to cancellous bony trabeculae. Lining the masses was a fibrous layer that was continuous with the fibrous periosteum of the cortical bone. The ossifying fatty tumors continuous with the fibrous periosteum were diagnosed as parosteal ossifying lipomas. Additional findings included pulmonary atelectasis secondary to the intrathoracic parosteal ossifying lipomas and meningoencephalomyelitis confirmed by immunohistochemistry to be caused by feline infectious peritonitis virus.

Conclusions: This report is, to our knowledge, the first in the veterinary literature of parosteal ossifying lipoma affecting multiple bones. The neurological abnormalities are attributed to meningoencephalomyelitis. The relationship between the meningoencephalomyelitis and the parosteal ossifying lipomas in this cat is unknown.

D43: MAXILLARY OSSIFYING FIBROMA IN A FERRET

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Background: A 3-year-old spayed female ferret was presented for a rapidly growing gingival mass that protruded from the rostral surface of the left maxilla, impeding food prehension and mastication. Radiographically, this smoothly contoured protuberant mass had a delicately mineralized opaque appearance. The mass arose from the surface of the left rostral maxilla where it caused luxation of the left lateral maxillary incisors and canine tooth. A fine needle aspirate was inconclusive. The ferret was euthanized due to poor quality of life.

Results: Postmortem examination confirmed a firm, lobular, ulcerated gingival mass arising from the surface of the left maxilla where it protruded beyond the nasal planum. Histologically, this well-demarcated interdental mass minimally invaded the alveolar crest of the maxilla. The mass was composed of random aggregates of small bland ovoid to elongate cells that formed mineralized osteoid matrix. These mineral deposits increased in size to form larger spicules of primitive bone perpendicularly aligned to the ulcerated surface that, from their inception, were lined by osteogenic cells. These features are consistent with an ossifying fibroma. Ossifying fibromas are most commonly reported in young horses but have also been reported in cats, dogs, and sheep. Characterizing features include an expansile and lytic mass with haphazardly arranged bony trabeculae rimmed by osteoblasts intermixed in a fibro-osseous stroma.

Conclusion: To our knowledge, this is the first report of an ossifying fibroma in a ferret.

D44: DOUBLECORTIN IMMUNOLABELING IN CANINE GLIOMAS WITH DISTINCT DEGREES OF TUMOR INFILTRATION

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Background: Increased doublecortin (DCX) immunolabeling at the tumor margins has been associated with tumor infiltration in human and feline glioma. Although DCX immunolabeling occurs in canine gliomas, association with tumor infiltration has not been described.

Objectives: To compare DCX immunolabeling among canine gliomas with distinct tumor infiltration.

Methods: DCX immunolabeling was assessed in 14 diffusely infiltrating gliomas (DIGs, gliomatosis cerebri) and 13 nodular gliomas (NGs) with distinct subtypes

(oligodendrogliomas, astrocytomas, and undefined gliomas) and grades, diagnosed using OLIG2 and GFAP immunohistochemistry. Tumor infiltration was recorded as absent, focal, or diffuse. DCX immunolabeling was classified according to intensity (weak, moderate, strong), distribution (1 = <30% immunolabeling, 2 = 30-70% immunolabeling, 3 = >70% immunolabeling), and location within the neoplasm (random or at tumor margins).

Results: Cytoplasmic DCX immunolabeling was detected in 6/14 (42.8%) DIGs and 7/14 (50%) NGs. All DIGs had moderate and random immunolabeling, with distribution scores of 1 (4 cases) or 2 (2 cases). DCX immunolabeling in NGs was strong (5 cases) or moderate (2 cases), with a distribution score of 1 (3 cases), 2 (3 cases), and 3 (1 case). All DIGs had random DCX immunolabeling. NGs had random immunolabeling in 6 cases, with increased immunolabeling at the tumor margins in 2 cases.

Conclusions: The intensity and distribution of DCX immunolabeling varied among cases. Immunolabeling was not increased at the tumor margins of highly infiltrating DIGs when compared to NGs. Increased DCX immunolabeling was observed in two high-grade NGs (one with focal and one with diffuse infiltration).

D45: GROSS AND MICROSCOPIC SKIN FINDINGS OF THREE GALÁPAGOS IGUANA SPECIES

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The Galápagos archipelago is home to four species of iguanas: the marine iguana (*Amblyrhynchus cristatus*), the common Galápagos land iguana (*Conolophus subcristatus*), the Barrington land iguana (*Conolophus pallidus*), and the Galápagos pink land iguana (*Conolophus marthae*). To our knowledge, no studies have been published on the microscopic anatomy of the skin of Galápagos iguanas. The pink land iguana was only described in 2009 and is a relative newcomer to study. While there were a number of hypotheses on the source of their pink color, we obtained full-thickness skin biopsies from three species (the Santa Fe iguana was excluded) and compared tissue from darkly pigmented surfaces and lightly pigmented surfaces. The results were striking. The “pink” area of the pink iguana is devoid of melanin (melanophores) and the dermis is rich with confluent vascular channels. This was in sharp contrast to the minimally vascular (only capillaries were observed and in some cases were rare) dermal areas of the marine and yellow iguanas. The dermal stratum laxum of every biopsy site contained melanophores except for the pink skin of the pink iguana. Interestingly, the marine iguana has a much thicker epidermis, between three and 10 cells thick depending on location, compared to the thinner epidermis of the land iguanas (one to four cells thick with most areas possessing just one or two cell layers). The microscopic differences might reflect the diversity of habitats and habits. However, the adaptive significance of such a trait for the pink iguana is ground for further investigation.

D46: COLORIMETRIC IN SITU HYBRIDIZATION USING DIGOXIGENIN-LABELED OLIGONUCLEOTIDE PROBES FOR DETECTION OF LEISHMANIA BRAZILIENSIS IN FORMALIN-FIXED AND PARAFFIN-EMBEDDED TISSUES OF HUMANS AND ANIMALS

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Background: American tegumentary leishmaniasis (ATL) is an anthropozoonosis caused by *Leishmania* spp. that occur in the New World and cause lesions in the skin and/or mucous membranes of humans and other mammals.

Objective: The aims of this study were to design and validate digoxigenin-labeled oligonucleotide probes for detection of *Leishmania braziliensis* in formalin-fixed and paraffin embedded samples (FFPE) using a semi-automated colorimetric in situ hybridization (CISH).

Methods: We performed in silico analyses and CISH on FFEP samples from human and animal tissues infected with *L. amazonensis*, *L. guyanensis*, *L. infantum*, *L. martiniquensis*, *L. shawi*, *L. naiffi* and pathogenic fungi (*Cryptococcus* spp., *Histoplasma* spp., *Sporothrix* spp.) and pellets of promastigote forms of *Leishmania* spp. (species above) obtained by culture centrifugation. This study was approved by the Ethics Committee on Human Research of INI, Fiocruz, Brazil (CAAE: 51629615000005262).

Results: Two probes (1 and 2) were developed and clearly detected amastigote and promastigote forms of *L. braziliensis* with a dark blue signal and a good signal-to-noise ratio in the tissues and pellets tested by CISH. In silico, probe 1 cross-hybridized only with *L. guyanensis* and probe 2 was specific for *L. braziliensis*. In CISH, the probe 1 cross-hybridized only with *L. guyanensis*. The probe 2 weakly cross-hybridized with *L. shawi*.

Conclusions: CISH using a combination of two probes was able to discriminate *L. braziliensis* from other *Leishmania* species that cause ATL and can be used as an alternative tool in routine biopsy material to make a diagnosis of this disease.

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D47: MONOCLONAL GAMMOPATHY IN A CASE OF EQUINE INTESTINAL B-CELL LYMPHOMA

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A 12-year-old American saddlebred horse gelding was presented for investigation of progressive weakness and acute weight loss. The CBC and biochemical profile revealed a lymphocytosis (19.4 x 10^9/L; reference interval [RI] 1.3-4.7 x 10^9/L), hypoalbuminemia (20 g/L; RI 30-37 g/L) and hyperglobulinemia (80 g/L; RI 26-41 g/L). Blood film evaluation identified atypical lymphocytes, and mild red blood cell agglutination undispersed by saline dilution. Urinalysis showed a trace of protein and vertebral body radiography disclosed no abnormalities. A presumptive diagnosis of multiple myeloma was made. Subsequently, a serum protein electrophoretogram disclosed a narrow-based, tall peak consistent with a monoclonal gammopathy in the b2 region of the densitometry tracing. Flow cytometric evaluation of whole blood identified a population of $CD3^-CD5^{low}PanB^-$ lymphocytes, consistent with atypical B cells. Euthanasia was elected based on clinical decline and presumed poor prognosis. On postmortem examination, the intestinal and mediastinal lymph nodes were approximately 5-fold enlarged. There were multifocal areas of thickening and nodules in the cecal and colonic mucosa, and the metaphysis and diaphysis of the femoral medullary cavity were reddened. Dense sheets of neoplastic round cells with distinct cytoplasmic borders, scant cytoplasm, moderate anisokaryosis, and a high mitotic rate were identified on histopathologic evaluation. The neoplastic cells were immunoreactive to CD20 but not to CD3, confirming a B-cell lymphoma with involvement of lymph nodes, blood and bone marrow. This case highlights the value of a multimodal investigation of lymphocyte populations when hyperglobulinemia is identified.

D48: PSITTACID HERPESVIRUS 3 AND PUTATIVE ADENOVIRUS COINFECTION IN AN INDIAN RING-NECKED PARAKEET (PSITTACULA KRAMERI) IN THE UNITED STATES

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Background: Psittacid herpesvirus 3 (PsHV-3), a novel psittacid pathogen, has been identified in Bourke parrots, Eclectus parrots, and rose-ringed parakeets. These infected birds had bronchopneumonia and necrosis in the lung, pancreas, and spleen with syncytial cells and intranuclear eosinophilic inclusion bodies. Adenovirus infection in birds is typically multisystemic associated with cellular necrosis and basophilic inclusion bodies in the affected organs. A 6-month-old, intact male, Indian ring-necked parakeet was presented to the Teaching Hospital Avian and Exotic Service for acute onset of lethargy and dull mentation. At admission, clinical observations included horizontal nystagmus, paraparesis, torticollis, lethargy, and bradycardia. Given the rapidly progressing neurologic impairment and poor prognosis, the owner elected euthanasia of the bird.

Methods and Results: At necropsy, there were no significant abnormalities except for a segmental and transmural dark red small intestinal tract. Histologically, the lung had multifocal respiratory epithelial degeneration and necrosis with viral syncytial cells and

intranuclear eosinophilic inclusion bodies. Polymerase chain reaction with pan-herpesvirus primers was performed on frozen lung and the sequence of the amplicon showed a 100% identity with PsHV-3. Severe diffuse necrosis and intraepithelial intranuclear large basophilic to amphophilic viral inclusion bodies were observed in the pancreas. Ultrastructurally, viral particles in the pancreas were 63.5 - 74.4 nm in diameter and displayed intranuclear paracrystalline array, morphologically compatible with adenovirus.

Conclusions: A diagnosis of systemic Psittacid herpesvirus 3 and adenovirus-like coinfection in an Indian ring-necked parakeet was made given the clinical history and pronounced pathologic findings coupled with molecular diagnostic and electron microscopy results.

D49: INTRABILIARY NEMATODIASIS IN A PANTHER CHAMELEON (FURCIFER PARDALIS)

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Background: A 1-year-old male panther chameleon (*Furcifer pardalis*) was submitted for necropsy. The chameleon was in a breeding colony.

Methods: Tissues were fixed in 10% neutral buffered formalin, processed routinely, and stained with H&E. Gastrointestinal parasites were identified using fecal floatation and an intracoelomic nematode was submitted for PCR-based identification.

Results: Numerous nematodes were identified within the coelomic cavity. The gallbladder was grossly distended and histologically, the biliary tract contained intraluminal nematodes as well as hepatic parenchymal loss, fibrosis and biliary hyperplasia. The small and large intestines had an enterocolitis with intralesional coccidia, indicating mixed parasitism. PCR sequencing of the intracoelomic nematode confirmed the presence of *Ascaridoidea* with 91% homology to *Hexametra angusticaecoides* and 82% to *Ophidascaris baylisi*.

Significance: The presence of nematodes in the biliary tract of the panther chameleon has not been previously reported. The chameleon was reported to be captive bred and possible sources of infection include exposure to wild caught reptiles or feeding of infected invertebrates. One month prior to the submission of this chameleon, another male panther chameleon from the colony was submitted for necropsy, which also had hepatic parenchymal loss, fibrosis and biliary hyperplasia. Although no intracoelomic or intrabiliary nematodes were identified in this first submission, the histologic characteristics suggest a similar etiology. Owners and breeders should be encouraged to thoroughly investigate the provenance of acquired animals and to ensure the use of reputable feed sources.

D50: HISTOLOGIC AND IMMUNOHISTOCHEMICAL CHARACTERIZATION OF SPONTANEOUS GASTROINTESTINAL STROMAL TUMORS (GIST) IN FOUR PRAIRIE DOGS (CYNOMS LUDOVICIANUS)

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Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors in the human gastrointestinal tract. They are rarely reported in few animal species, and never before in prairie dogs. We describe four cases of spontaneous GISTs in prairie dogs from one research colony over a 6 year period. Animals were 2 male and 2 female, and all wild-caught adults of unknown exact age. Histories included weight loss (n=3), anemia (n=1), dehydration (n=1), lymphopenia (n=1), alopecia (n=1) and respiratory distress (n=1). All animals were euthanized due to poor prognosis. Intestinal masses were seen at necropsy in 2 animals (1 duodenum, 1 jejunum). One had only cecal wall thickening, and 1 had no gross findings in the GI tract but neoplasia identified in the duodenum on histopathology. Histologically, all tumors had transmural expansion of the intestinal wall by a poorly demarcated, nonencapsulated mesenchymal neoplasm that regionally effaced the intestinal architecture. Masses were mainly composed of dense interwoven bundles of plump spindle cells on a scant fibrovascular stroma. Neoplastic cells had abundant eosinophilic fibrillar cytoplasm, and ovoid to fusiform nuclei. Pleomorphism was mild to moderate, and mitoses were rare. By immunohistochemistry, neoplastic cells had cytoplasmic immunoreactivity for KIT (CD117), and were negative for desmin and S-100. These findings are compatible with GISTs in humans and other animals, and indicate that prairie dogs may be amenable to development of animal models of the human disease.

D51: NECROTIZING MENINGOENCEPHALITIS IN A BINTURONG (ARCTICTIS BINTURONG) CAUSED BY BALAMUTHIA MANDRILLARIS

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A 3-year-old, intact male binturong (*Arctictis binturong*) presented with acute respiratory distress which quickly progressed to respiratory arrest and death. Gross examination of the brain revealed several dark brown, firm nodules up to 1.5 centimeters multifocally expanding the olfactory, parietal, and occipital lobes. The cerebellum was herniated

caudally into the foramen magnum. On histopathology, the cerebral gray matter was extensively effaced and replaced by necrosis, fibrin, and hemorrhage admixed with numerous gitter cells and viable and degenerate neutrophils. Within these areas were moderate numbers of 20–30 micron diameter, round organisms with an undulating, refractile capsule that contained eosinophilic to deeply basophilic, variably sized, granular to globular material. PCR testing of brain was negative for *Neospora* sp., *Toxoplasma gondii*., and canine distemper virus. DNA from fresh-frozen samples of the brain nodules were PCR tested for amoeba using primers which amplify a 136-bp region of the 18S rRNA gene. The nucleic acid sequence of the PCR product was 100% similar to numerous *Balamuthia mandrillaris* sequences in Genbank. *Balamuthia mandrillaris* is a free-living amoeba that is found in soil and stagnant water. Although it has a worldwide distribution, most human and animal cases reported in the United States originate from the southwest region. *B. mandrillaris* most frequently causes a granulomatous amoebic meningoencephalitis, though dissemination to other organs such as the kidneys has been reported. In the present case, no other organ systems were involved. In affected veterinary species, animals present with progressively worsening neurologic disease progressing to respiratory distress, arrest, and death.

D52: A FATAL CASE OF HERPES SIMPLEX MENINGOENCEPHALITIS IN A CAPTIVE BLACK HOWLER MONKEY (ALOUATTA CARAYA)

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A 2-year-old female intact black howler monkey (*Alouatta caraya*) was submitted to Mississippi State College of Veterinary Medicine for postmortem examination. This monkey had a 2.5-day history of anorexia and lethargy progressing to incoordination, disorientation, and death despite hospitalization, supportive care, and antibiotic therapy at the referring veterinary hospital. Gross lesions were limited to mild dehydration and locally extensive consolidation of the cranioventral lungs, consistent with aspiration pneumonia. Histopathology of the brain revealed multifocal necrotizing lymphohistiocytic and suppurative cerebrocortical meningoencephalitis with large eosinophilic neuronal intranuclear inclusion bodies that marginate chromatin. Combined immunohistochemistry for herpes simplex virus (HSV) 1 and 2 of the brain revealed strong nuclear immunoreactivity and mild patchy cytoplasmic immunoreactivity to HSV 1 and/or 2 within cortical neurons, supporting a diagnosis of herpes simplex meningoencephalitis. Herpes simplex virus, or human alphaherpesvirus, causes mild mucocutaneous lesions in its natural host, humans, but can exhibit neurotropism and severe disease in aberrant hosts and, rarely, in neonatal or immunocompromised humans. New World primates are highly susceptible to HSV infection and disease is typically rapidly fatal, manifesting as ulcerative stomatitis and meningoencephalitis. Transmission occurs via direct contact or exposure to infected saliva. Further investigation revealed that an individual caring for this monkey had a history of cold sores, which is the suspected origin of infection in this case. This case report highlights an uncommon but important zooanthroponosis of non-human primates and

demonstrates the importance of taking precautions to protect the safety of these animals during handling.

D53: ODONTOAMELOBLASTOMA WITH ATYPICAL FOCI IN A DOG

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Background: A 7-year-old Labrador retriever dog presented with an ulcerated mass at the lingual aspect of the left mandibular canine tooth. Imaging revealed slight bone involvement.

Objective: Describe an odontoameloblastoma, a rare mixed odontogenic tumor, with atypical foci (rarely described in acanthomatous ameloblastomas).

Methods: Histopathologic evaluation of hematoxylin and eosin stained slides was performed.

Results: Histology revealed a mixed odontogenic tumor with a predominant population of odontogenic epithelial trabeculae along with dental papilla-like ectomesenchyme and mineralized dental matrix, consistent with an odontoameloblastoma. Multiple aggregates of atypical round to polygonal cells were also noted within the odontogenic epithelium, similar to the rarely described atypical foci in acanthomatous ameloblastomas in dogs.

Conclusions: Odontoameloblastomas, ameloblastic fibro-odontomas, and odontomas are mixed odontogenic tumors with three odontogenic components (odontogenic epithelium, dental papilla ectomesenchyme, and mineralized dental matrix), and the classification relies in the relative amounts of these odontogenic components. Although differentiating these three mixed odontogenic tumors may be difficult as they overlap histologically, in this specific patient the preponderance of odontogenic epithelium is most consistent with a diagnosis of odontoameloblastoma. Furthermore, the presence of the atypical foci (atypical cells) observed in this neoplasm is highly unusual. Although the cell of origin of these atypical cells in acanthomatous ameloblastomas remains unclear, atypical foci may also rarely occur in mixed odontogenic tumors and should not be interpreted as a separate poorly differentiated neoplasm.

D54: COMPARISON OF ACID-FAST BACILLI STAINING AND QPCR TO DETECT MYCOBACTERIAL INFECTION IN HUMAN AUTOPSIED PATIENTS WITH A HISTORY OF TUBERCULOSIS AND COINFECTION WITH TIBERCULOSIS AND HIV-1

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Background: Tuberculosis (TB) is an infectious disease distributed worldwide alongside the Human Immunodeficiency Virus (HIV-1), a global burden with high mortality rates in developing countries. The accurate diagnosis of TB is essential for early treatment and prevention of disease spread, especially for HIV-1 patients. TB diagnosis is based mainly on sputum microscopy, rapid molecular tests, and bacterial culture. Acid-fast bacilli staining (AFBS) of sputum is commonly used due to its fast results and affordability. Histopathological evaluation of AFBS in lung tissues is also used, but mainly as a postmortem diagnostic tool. Nevertheless, molecular techniques have been increasingly performed for TB diagnosis because of their higher sensitivity and specificity. They also allow the differentiation between tuberculous and nontuberculous mycobacterial infection, which is essential to prevent unnecessary long-term treatment for TB.

Objective: Here, we compared two AFBS diagnostic tools, sputum microscopy and histopathological evaluation of the lungs, with the molecular approach of qPCR to diagnose mycobacterial infection in archived samples from autopsied human patients.

Methods: *Ziehl-Neelsen*-stained sputum smear and Formalin-Fixed Paraffin-Embedded (FFPE) lung tissue were compared with a qPCR assay of multi-organ FFPE of patients with a history of tuberculosis (11) and coinfection of tuberculosis and HIV-1 (8), targeting the IS1611 and IS1311 regions.

Results: AFBS detected 79% and 74% of cases as positive for the mycobacterial infection in sputum and lung tissue, respectively, while qPCR showed 95% positivity for TB.

Conclusion: The molecular diagnosis allowed higher detection rates of mycobacteria and identified the absence of mixed infection in our archived samples.

D55: PREGNANCY-ASSOCIATED OSTEOPOROSIS IN A COMMON MARMOSET (CALLITHRIX JACCHUS) FOLLOWING MULTIPLE PREGNANCIES

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A 4-year-old intact female common marmoset (*Callithrix jacchus*) presented for veterinary care in April 2022 due to mobility issues and loss of condition. She had given birth to full-term twins in August 2021 and full-term triplets in January 2022, and bloodwork at presentation revealed hypocalcemia (6.8 mg/dL, range 9-11) and hypoalbuminemia (2.8 g/dL, range 3.5-5) and radiographs showed radiolucency along the epiphysis of both humeri and proximal femurs. Despite calcium, vitamin D, and nutritional supplementation and steroids for suspect chronic lymphoplasmacytic enteritis (CLE), her symptoms and hypocalcemia did not resolve, and euthanasia was elected. On histologic examination, all examined bones demonstrated diffuse, marked osteoporosis with loss of medullary trabecular bone and hypertrophy of osteoclasts and

osteoblasts; there was multifocal myelofibrosis affecting the right femur and humerus and a microfracture in the left humerus. Additionally, CLE was observed. Osteopenia, osteoporosis, and fragility fractures are rare sequelae of pregnancies in humans, with no previous pregnancy-associated case reports in common marmosets. The use of marmosets in research has increased, making captive breeding a vital part of marmoset colony maintenance. Bone and gastrointestinal syndrome (BGS), characterized by bone disease with concurrent gastrointestinal disease consisting of CLE and loss of body condition, is a major cause of morbidity in captive populations. Though fibrous osteodystrophy, rickets / osteomalacia, and osteopenia have all been previously associated with CLE in BGS, this case may represent exacerbation of the effects of BGS with subsequent pregnancy-associated osteoporosis by short inter-birth interval pregnancies in a common marmoset.

D56: INTRAOCULAR TUMORS IN THREE COHOUSED MOUTH ALMIGHTY FISH

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Background: Mouth Almighty fish (*Glossamia aprion*) are a freshwater, carnivorous species in the Cardinalfish family that are native to northern and northeastern Australia. Over a 5-month period, three captive adult Mouth Almighty fish housed within the same exhibit at the National Aquarium in Baltimore developed acute exophthalmia, resulting in enucleation of the affected eye in two fish. One of these fish recovered unremarkably, while the other was euthanized after developing exophthalmia in the contralateral eye two months post-enucleation. The third fish was treated supportively, but died despite apparent clinical improvement.

Objective: Enucleated eyes and deceased fish were submitted to the Comparative Pathobiology Department at Johns Hopkins University for histopathologic examination to determine the etiology underlying these fish's acute exophthalmia and mortality.

Results: Histopathology of all affected eyes revealed large intraocular tumors that obliterated the posterior half of the eye, multifocally invaded through the sclera into periocular tissues, and variably infiltrated the optic nerve. Tumors consisted of densely packed, hyperchromatic, small, round to elongate, basophilic cells with scant cytoplasm arranged in sheets, nests, and packets, interspersed with eosinophilic foci of necrosis. Tumor cells frequently palisaded around central areas of necrosis, and occasionally formed rosettes and perivascular pseudorosettes. Due to extensive tumor invasion and advanced disease, it was difficult to determine the tissue of origin, and both choroid and retina were considered possible. Immunohistochemistry for S100, vimentin, GFAP, synaptophysin, and PLN2 were pursued for further characterization.

Conclusions: Differentials include a primitive neuroectodermal tumor, retinoblastoma, melanoma, or a poorly differentiated sarcoma.

D57: CAT LITTER FOREIGN BODY REACTIONS IN THE PAWPADS OF DOMESTIC CATS

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Background: Feline pawpads are susceptible to relatively few, often clinically distinct, skin diseases. Thus, biopsy is rarely undertaken but can be a useful diagnostic tool in complicated cases. A subset of pawpad biopsies at the University of Tennessee contained a characteristic lesion of deep granulomatous to pyogranulomatous inflammation with intrahistiocytic, granular, pale gray-blue, birefringent material (presumptive cat litter).

Objective: To describe the histopathological and clinical characteristics of cat litter foreign body responses in pawpads of domestic cats and investigate underlying or associated conditions.

Methods: The university surgical pathology database was searched for feline paw biopsies containing foreign, birefringent, or refractile material. Eight cases from 2005-2022 were retrieved and histopathology was reviewed.

Results: All 8 cases contained moderate to severe, granulomatous to pyogranulomatous pododermatitis with intralesional presumptive cat litter. Six of 8 lesions contained background aggregates of plasma cells and Mott cells, consistent with plasmacytic pododermatitis. Plasmacytic pododermatitis was clinically suspected in a seventh case, but could not be confirmed microscopically due to overwhelming granulomatous inflammation. Gross lesions included chronic swelling, ulceration, crusting, or hyperkeratosis of the pawpad(s), draining tracts in the pad or adjacent haired skin, and/or discolored tissue. Front paws were more affected in 6 of the 8 cases, possibly due to litter scooping and personal grooming behaviors.

Conclusions: These cases suggest that feline pawpad conditions that compromise the skin barrier, including plasmacytic pododermatitis, can allow for implantation of foreign material such as litter, resulting in a foreign body reaction that can obscure the primary lesion.

D58: ADENOVIRAL NEPHROPATHY IN ZOO BUDGERIGARS (MELOPSITTACUS UNDULATES)

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Background Adenoviruses have been reported to affect multiple species of psittacine birds. A variety of lesions have been described including conjunctivitis, encephalitis, hepatitis and nephritis. In budgerigars, adenovirus infection affecting the kidneys can be detected microscopically by identification of viral inclusion bodies, which can be accompanied by other lesions.

Objective To describe five cases of adenoviral nephropathy in budgerigars.

Methods Histopathology of affected budgerigars was performed along with in-situ hybridization (ISH) to detect viral DNA in renal tissue.

Results Affected birds were aged 4 weeks to 3 years, and were found dead without premonitory signs. The nephropathy consisted of renal tubular epithelial karyomegaly with large amphophilic intranuclear inclusion bodies. Inclusion bodies were only identified in the kidney and were frequent in one budgerigar, but infrequent in the others. They were accompanied by mild lymphoplasmacytic interstitial nephritis (n=2), renal tubular epithelial degeneration and necrosis (n=2), and tubular mineral and protein (n=2). In-situ hybridization (ISH) detected adenovirus DNA in one of the affected birds. Additional ISH for adenovirus DNA testing is pending.

Conclusions An alternative explanation for death was not identified in any case, suggesting that the renal viral infection may be fatal. Renal lesions were similar to a novel *Siadenovirus* reported in budgerigars in Japan and quarantined during importation to USA. Sequencing of the specific strain of adenovirus in these cases for comparison is pending. Further studies on renal adenovirus infection in budgerigars are necessary to understand its pathogenicity.

D59: FOALS WITH GASTRODUODENAL ULCERATION SYNDROME MAY HAVE CONCURRENT HEPATITIS, PANCREATITIS AND ROTAVIRUS A INFECTION

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From 2020 to July 2022 16 foals with gastric-duodenal ulceration syndrome were submitted to the University of Kentucky Veterinary Diagnostic Laboratory for necropsy and further testing. Age ranged from 3 weeks to 5 months (mean 63 days) with 14 thoroughbreds, one warmblood and one unspecific breed. In all cases aerobic and anerobic culture of the large and small intestine, PCR for equine coronavirus, equine rotavirus A, *Lawsonia intracellularis*, *salmonella, clostridium perfringens* and *clostridioides difficile* was performed. The pH of gastric and duodenal fluid was measured in 15/16 cases and a fecal flotation performed in 14/16 cases. Equine Rotavirus A was detected in 8/16 cases, *Salmonella sp.* in 1/16*, C difficile* in 2/16 cases. Gastric pH ranged from 3.22 to 6.87 (mean 5.2) and duodenal pH from 4.49 to 8.1 (6.29). Gross lesions were evident in the stomach all cases, most commonly ulceration (16/16), and perforation (9/16) were observed. Findings within the proximal duodenum and jejunum included enteritis (10/16) and ulceration (3/16) and perforation of the duodenum (3/16). Microscopic findings included severe hyperkeratosis and necro-ulcerative gastritis of the non-glandular stomach, necrosis, ulceration and fibrosis of duodenum and suppurative serositis in cases of perforation. Additional findings included hepatitis (6/16) and pancreatitis in (5/16). These findings highlight the presence of Rotavirus A in 50% of foals with gastroduodenal ulceration syndrome as well as the presence of concurrent hepatitis and pancreatitis in 31% of cases, suggesting the possibility of a syndrome similar to ‘triaditis’ recognized in small companion animals.

D60: HISTOLOGIC AND HISTOCHEMICAL CHARACTERIZATION OF PERIPHERAL NEUROPATHIES IN TWO DOGS

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Background: Peripheral neuropathies (PN) represent a diverse group of disorders affecting the peripheral nerves. PN affect an estimated 2%-8% of humans and are classified as myelinopathy, axonopathy, or neuronopathy. Characterization of these disorders in animals is lacking. Two dogs (Dog 1: 2 year-old male castrated mixed-breed; Dog 2: 8 year-old castrated male Pit Bull Terrier) were submitted for autopsy following development of marked clinical neurologic deficits, failure of response to treatment, and euthanasia.

Objective: Our aim was to determine the cause of clinical signs and characterize the microscopic features in these cases of canine PN.

Methods: Postmortem samples were collected and processed routinely for histologic evaluation. Bielschowsky silver stain (BSS), trichrome, and myelin basic protein (MBP) immunohistochemistry were also performed on spinal cord sections.

Results: The spinal nerve roots of Dog 1 were characterized by marked, multifocal demyelination of axons with axon drop-out, occasional remyelination, and minimal MBP immunostaining. BSS showed patchy axon loss, secondary to demyelination. The spinal nerve roots of Dog 2 were characterized by widespread loss and degeneration of axons with fibrosis. BSS emphasized the paucity of intact axons, MBP showed mild loss of myelin, and trichrome highlighted fibrosis within and surrounding the nerve roots.

Conclusions: Peripheral neuropathies in dogs include both primary myelinopathies (Dog 1) and primary axonopathies (Dog 2) and may manifest with a spectrum of lesions requiring further immunohistochemical and histochemical analysis to delineate pathogenesis. Canine PN is uncommon but should be considered as a differential diagnosis for spinal and peripheral neurologic deficits.

D61: MICROSPORIDIOSIS AS A CAUSE OF SYSTEMIC GRANULOMATOUS INFLAMMATION IN GOURAMIS

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Eight gouramis (red, blue, and dwarf gouramis) housed in three different facilities were submitted with a history of ongoing health issues. Histologically, all eight fish had evidence of systemic granulomatous inflammation affecting the liver, spleen, kidney, coelomic cavity, intestine, heart, skeletal muscle, meninges, and/or fat/connective tissue. The granulomas ranged nodular aggregates of variably pigmented macrophages

to flattened macrophages encircling a central region of eosinophilic cellular debris. The cause of this inflammation was not readily determined using special stains. Electron microscopy revealed microorganisms with the presence of an exospore, endospore, vacuole, nucleus, and polar filament (compatible with microsporidia) within the macrophages. Microsporidia are obligate intracellular eukaryotic parasites most closely relate to fungi. Lesions associated with microsporidiosis vary with a number of factors include the type of organism, tissue tropism, and host response. This case series highlights a unique cause of systemic granulomatous inflammation in gouramis.

D62: PREVALENCE OF SELECT INFECTIOUS DISEASES IN FREE-ROAMING CATS FROM TWO FLORIDA COUNTIES

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Background: Free-roaming cats (FRCs) pose several problems to society including predation, public nuisance issues, and the potential to spread infectious and zoonotic diseases. There is very limited information available pertaining to the prevalence of infectious diseases in FRCs at death.

Objective: To determine the prevalence of select pathogens including Feline Leukemia Virus (FeLV), Feline Immunodeficiency Virus (FIV), *Dirofilaria immitis*, *Mycoplasma haemofelis, Mycoplasma haemominutum, and Cytauxzoon felis* in deceased FRCs from two non-adjacent counties (Volusia and Alachua) in Florida.

Methods: Blood samples from 99 FRCs were analyzed using a combination of a bidirectional flow immunochromatography kit (IDEXX SNAP ® Combo) and PCR assays. Prevalence of infections were calculated, and multivariable logistic regression was used to assess the effect of exposure factors to the pathogens.

Results: The prevalence was highest for *Mycoplasma haemominutum* (10/66 [15.1%]), FeLV (14/98 [14.3%]), and FIV (14/98 [14.3%]. Male cats were at significantly higher risk for infection with FIV (4.5 times) and FeLV (5.4 times). Intact cats were less likely to be infected with FeLV. Male cats in Volusia County were more likely to be infected with FIV.

Conclusions: In these 2 counties, FRCs had similar to higher prevalence rates of infections compared to studies assessing FRCs enrolled in trap-neuter-return programs from Florida.

D63: TRIPLE NEGATIVE (HER2, ERA, PR) INVASIVE MAMMARY DUCTAL CARCINOMA IN A FEMALE RHESUS MACAQUE (MACACA MULATTA)

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A 24-year-old intact female rhesus macaque presented with firm, raised, irregularly shaped, hairless masses arranged in a chain of subcutaneous nodules (with the largest nodule measuring approximately 1 cm X 2 cm X 1 cm) on the chest. The mass was surgically excised and submitted for histopathology with initial clinical differential diagnoses including trichoblastoma, papilloma, and mammary carcinoma. Microscopically, the neoplasm was partially encapsulated, infiltrative, and composed of neoplastic epithelial cells arranged in lobules and acini separated by a dense fibrovascular stroma. Neoplastic cells were polygonal with indistinct cell borders and had round vesicular nuclei with 1-to-3 prominent basophilic nucleoli. Anisocytosis and anisokaryosis were moderate, with more than 15 mitotic figures per 10 high-power (40x) fields. Within lobules, neoplastic glands often contained central accumulations of cell debris and/or eosinophilic secretory material. The stroma contained abundant lymphocytes with fewer plasma cells and neutrophils. Intravascular invasion of the tumor was present and neoplastic cells extended into the deep surgical margins. Immunohistochemistry was performed for human epidermal growth factor receptor 2 (HER2), estrogen receptor alpha (ERα), and progesterone receptor (PR). Neoplastic epithelial cells stained weakly positive for HER2, but negative for both ERα and PR. The final diagnosis was right mammary gland invasive ductal carcinoma, poorly differentiated (high grade). HER2, ERα, and PR biomarkers are commonly evaluated for their prognostic value in predicting hormone therapy outcome and patient survival in breast cancer in human patients. Although mammary gland invasive ductal carcinoma is common in humans, it is rarely reported in nonhuman primates.

D64: OCCURRENCE OF EXTRAGENITAL TRANSMISSIBLE VENEREAL TUMORS IN DOGS IN BRAZIL

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Transmissible venereal tumor (TVT) is a neoplasm transmitted by natural implantation, that commonly affects the canine external genitalia. Eighty-four cases of TVT were diagnosed by the Anatomic Pathology Laboratory of the Federal University of Paraná, Palotina, Paraná, Brazil, from 2014 to 2021. From those, 16/83 (19,27%) dogs had extragenital lesions, being 9 females and 7 males, ranging from 8 months old to thirteen years old. The most affected organ was the skin (8/16, 50%), followed by the oral cavity (4/16, 25%), eye and lymph nodes (3/16 each, 18,75%), and bladder, lung, spleen, pleura, and pharynx (1/16 each, 6,5%). In 6/16 (37,5%) patients, multiple organs were affected. Interestingly, only 3/16 (18,75%) cases had concomitant genital lesions. In most of the cases (11/16, 68,75%), the diagnosis was performed through cytology, while biopsy and necropsy were the main diagnostic methods in 3/16 (18,75%) and 2/16

(12,5%) cases, respectively. Cytologically, the neoplastic cells are round and individually arranged in a monolayer, exhibiting a low nucleus:cytoplasm ratio. The cytoplasm is well-demarcated, pale blue, and vacuolated. Grossly, the masses tend to be tan, friable, and soft. Histologically, the round cells organize in a mantle supported by a fine fibrovascular stroma. The cells have inconspicuous cytoplasmic vacuoles and a single prominent nucleolus. Infiltrates of lymphocytes, histiocytes, and neutrophils are variable. In previous studies, most cases had concomitant genital lesions, contrasting to our population. Therefore, TVT should be included in the differential diagnosis of extragenital round cell neoplasms in regions of high prevalence.

D65: MULTICENTRIC PERIORBITAL BENIGN NERVE SHEATH TUMOR IN A CHICKEN (GALLUS GALLUS DOMESTICUS)

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A three-year-old, intact female, mixed-breed chicken (*Gallus gallus domesticus*) was presented to Auburn University Teaching Hospital for a three-day history of bilateral swelling in the periorbital integument originally thought to be associated with the sinuses. The animal was housed with other chickens and turkeys described as chronic poor doers. The animal had a thin body condition, no nasal discharge, and was otherwise normal at home. Due to patient and flock health concerns, the animal was euthanized and submitted for postmortem examination.

Grossly, the periorbital skin and palpebral conjunctiva were expanded by multifocal to coalescing, white, soft to firm, bulging, multinodular masses with no evidence of invasion into the adjacent tissues. Histopathology revealed discrete masses extending from the periorbital subcutaneous tissue and palpebral stroma with many repetitive, uniform, and bland serpiginous spindle cells within abundant compact undulant bundles that stained dark blue with trichome, supporting a diagnosis of bilateral periorbital benign nerve sheath tumor. No inflammation was observed, and a conjunctival aerobic culture yielded no growth.

The category of benign nerve sheath tumors encompasses neurofibromas, perineuromas, and schwannomas and are not commonly reported in chickens. In veterinary medicine, the distinction between these is not generally made due to lack of established criteria and presumed similar outcomes. Given the history of periorbital swelling, poor body condition, and cohabitation with turkeys, *Mycoplasma gallisepticum* infection was suspected clinically. Interestingly, there was no evidence of infectious disease in this animal to explain the flock's thin body condition.

D66: HEMOPERICARDIUM: NOT ALWAYS HEMANGIOSARCOMA

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While hemangiosarcoma is the most common differential for hemopericardium causing cardiac tamponade in older dogs, the initial assumption during a necropsy may be proven wrong with surprising results. This is a case report of a 13-year-old female Pitbull that died unexpectedly in the care of another person while the owner was out of town. Hemopericardium was the initial finding upon opening of the cadaver. After removal of the pluck and opening of the pericardial sac, a 10 cm long metallic rod with a tapered point was found to penetrate the heart from right to left, ventral to dorsal, entering through the right ventricle and exiting through the left ventricle near the coronary groove to pierce the tip of the left auricle. Upon further examination of the body, a barely perceptible skin perforation was on the mid ventral thorax, with a track and corresponding hemorrhage on the costal pleura. The perforation appeared similar to the numerous pigmented patches on the surrounding skin. Histology of the heart revealed a necrosuppurative myocarditis, indicating that the penetrating rod had been in place for at least 12-24 hours, with 4-12 hours being the time interval for early ischemic necrosis and leukocytic infiltrates appearing within 12-24 hours. This case demonstrates not only the surprising resilience of animals to drastic injuries (and preconceptions to common presentations), but it also demonstrates the necessity of understanding sequential histologic changes to accurately recognize the timeline of events in forensic and clinical settings.

D67: IMMUNE COMPLEX GLOMERULONEPHRITIS IN A FREE-RANGING CALIFORNIA SEA LION (ZALOPHUS CALIFORNIANUS)

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Background: California sea lions (CSLs) are rehabilitated after stranding along the Eastern Pacific coastline and are commonly managed in zoos and aquaria. Urinary diseases comprise approximately one third of the cases at The Marine Mammal Center. Although renal disease is a common cause of morbidity and mortality in CSLs, there has been limited investigation in the variety of diseases that affect them, and the utility of diagnostics commonly used in domestic animals.

Objective: Characterize the clinical and pathologic findings of a free ranging CSL with renal disease in comparison to those without renal disease.

Methods: Histopathology including H&E, JMS, Masson's trichrome and PAS stains, transmission electron microscopy (TEM), urine SDS-PAGE gel electrophoresis, urinalysis and urine protein: urine creatinine ratio (UPC) were performed on two non-diseased free ranging CSLs and one clinical renal diseased CSL.

Results: Histopathology of a stranded subadult male CSL demonstrated chronic active lymphoplasmacytic tubulointerstitial nephritis consistent with leptospirosis, and a glomerulopathy characterized by glomerulosclerosis and crescents. TEM revealed immune complexes likely from active myositis and sarcocystosis. Therefore, the animal was diagnosed with immune complex glomerulonephritis (ICGN) as well as resolving leptospirosis. Urinalysis, UPC and SDS-PAGE gel electrophoresis demonstrated proteinuria consistent with both ICGN-related glomerular injury and *Leptospira*-related tubular injury. The histology, ultrastructure, UPC, and urine SDS-PAGE gel electrophoresis of non-diseased CSLs were like those reported in dogs.

Conclusions: This the first case of ICGN in a free ranging pinniped. Several of the diagnostics used in domestic animals are appropriate to diagnose renal disease in CSLs.

D68: EPIGLOTTIC CHONDROSARCOMA IN AN ENGLISH BULL TERRIER: CASE REPORT

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A case of epiglottic chondrosarcoma in a Bull Terrier bitch was reported, based on the clinical, radiological, and pathological (gross findings and microscopic) approach. Laryngeal chondrosarcoma in dogs is a malignant neoplasm of chondrocytes that may or may not produce non-bone chondroid extracellular matrix. It is rare and has only been reported in two cases; one in the cricoid cartilage and another in the epiglottic cartilage in adult dogs over 6 years of age, being more documented in the trachea in young dogs. Clinically, the patient in this case presented with respiratory distress, cough with sputum, loss of appetite, anorexia, and finally chemical euthanasia was performed.

D69: PNEUMOCOELOM AND HEPATITIS SECONDARY TO PSEUDOMONAS SPP. INFECTION IN AN AXOLOTL (AMBYSTOMA MEXICANUM)

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The axolotl (Ambystoma mexicanum) is a close relative to salamanders and newts, with the characteristic of maintaining its larval features while being able to reproduce. This is an endemic amphibian from Mexico categorized as critically endangered by the International Union for Conservation of Nature and Natural Resources (IUCN). In Mexico, diverse preventive measurements have been taken for the preservation of this species. An axolotl (kept as an exotic pet) was submitted at the “Centro de Diagnóstico Veterinario Bajío” with a history of acute coelomic distention, positive buoyancy lateralized to the right and the skin presented erythema, lacerations, and an excessive mucus production; euthanasia was elected. The most relevant findings to the necropsy were severe pneumocoelom and severe pyogranulomatous hepatitis. *Pseudomonas* spp. was isolated from the granuloma-like lesions of the liver. There are scarce reports

of *Pseudomonas* spp. infections in axolotls, however, some authors report that it is one of the most common pathogens isolated from amphibians.

D70: MULTIPLE NEOPLASIA CASE REPORT: UNDIFFERENTIATED SARCOMA AND INTESTINAL LYMPHOMA IN AN AFRICAN PYGMY HEDGEHOG (ATELERIX ALBIVENTRIS)

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The African pygmy hedgehog (*Atelerix albiventris*) has become more popular as a pet due to its charisma and “fairly simple” husbandry. This increase in the number of individuals in households has been related to the increase of neoplasia reports in this species.

A 2-year-old male hedgehog with a history of a tumor in the cervical area which impeded the mobility of the patient and chronic diarrhea was euthanized and *post-mortem* studies were elected. During the external examination, subcutaneous neoplastic tissue was identified involving the cervical vertebrae and adjacent muscles. Enlarged intestinal loops were described during the internal examination. Histologically the cervical neoplasia was diagnosed as an undifferentiated sarcoma and the intestinal tissue as lymphoma.

There are many reports of multiple neoplasias in hedgehogs, nevertheless, to our knowledge, the concurrence of sarcoma and intestinal lymphoma has not been reported in this species. Further classification via biomarkers and immunohistochemistry (CD3, CD79-a, PAX-5, Vimentin, Factor VIII) is pending.

D71: MACROSCOPIC AND MICROSCOPIC CHARACTERIZATION OF LOBAR HOLOPROSENCEPHALY IN TWO DOGS WITH ALTERED DRINKING BEHAVIOR

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Background Holoprosencephaly (HPE) is an uncommon congenital brain malformation affecting humans and animals, characterized by a total or partial failure of separation of the prosencephalon into two separate hemispheres. Three forms of HPE with decreasing severity have been described: alobar, semi-lobar and lobar. Lobar HPE in two male entire dogs which presented with altered drinking behavior is described.

Materials and Methods Dog 1, an 11-week-old Akita, presented with hypernatremia, seizures, and oligodipsia. Dog 2, a 3-year-old Jack Russell Terrier, presented with adipsia. Both dogs were humanely euthanized and complete post-mortem examination (PME) was performed. The CNS and samples of major organs were fixed in 10% neutral-buffered formalin and processed for histopathology.

Results At PME macroscopic lesions were restricted to the brain in both cases. There was absence of the septum pellucidum, septal nuclei, fornix, fornix columns, and dorsal portions of the hippocampus, bilaterally. This resulted in ventral dislocation of the cingulate gyrus and corpus callosum, which appeared markedly thinned. Moreover, there was absence of the ventral portion of the interhemispheric fissure. The diencephalic structures caudal to the optic chiasm, as well as the mesencephalic and rhombencephalic structures, were unremarkable. On histopathology there was focal fusion of the midline ependyma and diencephalic structures.

Conclusions Macroscopic and histologic findings in these two cases are consistent with lobar HPE. The reported clinical signs can be explained by a defect in hypothalamic osmoreceptors, and therefore, although rare, HPE should be considered as a differential in cases of altered drinking behavior and sodium imbalances.

D72: CONGENITAL LYMPHANGIOMATOSIS IN A 2-YEAR-OLD BEAGLE

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A 2-year-old, female intact, Beagle dog was presented to the Texas A&M University Veterinary Teaching Hospital for evaluation of pleural effusion, dyspnea, and coughing. Abdominal ultrasound showed numerous variably sized cysts in the liver, spleen, pancreas, abdominal lymph nodes, dorsal abdomen, and cranial mediastinum. At necropsy, a single thymic cyst, numerous hepatic cysts, and several solid splenic nodules were identified. Histopathology showed cystic vascular channels lined by a single layer of flattened spindle cells with minimal cellular atypia in the liver, spleen, mesentery, and an abdominal lymph node. The flattened spindle cells were negative for cytokeratin and von Willebrand's factor immunohistochemical staining, ruling out a polycystic disease and making blood vessel origin less likely, respectively. Immunohistochemistry for prospero homeobox protein-1 (PROX-1) showed positive intranuclear staining of the flattened spindle cells lining the vascular channels in the liver, spleen, and mesentery, confirming a lymphatic cell origin. Based on the young age of the dog and presence of lymphatic-lined vascular proliferations in several organs, a diagnosis of congenital lymphangiomatosis was favored. Congenital lymphangiomatosis in domestic animals is rare and histologic features are not well-described, making differentiation between lymphatic neoplasms and congenital lymphangiomatosis challenging. As in humans with the analogous disease generalized lymphatic anomaly, the pathogenesis is unknown. To our knowledge, congenital lymphangiomatosis involving the spleen and abdominal lymph nodes of a dog has not been previously reported.

D73: CHRONIC INTESTINAL PSEUDO-OBSTRUCTION DUE TO INTESTINAL LEIOMYOSITIS IN A DOG TREATED WITH HUMAN INTRAVENOUS IMMUNOGLOBULIN, MYCOPHENOLATE, AND PREDNISOLONE

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Chronic intestinal pseudo-obstruction (CIPO) is a severe motility disorder of the intestinal tract in humans and animals. A 10-year-old, spayed female, Dachshund dog presented for gastrointestinal signs suggestive of ileus. On abdominal exploratory, the gastrointestinal tract was atonic without peristalsis. Physical obstruction was ruled out. Full-thickness intestinal biopsies of the duodenum, jejunum and ileum had similar histologic features. The tunica muscularis was infiltrated by a T cell predominant lymphocyte infiltrate, plasma cells and neutrophils. Smooth myocytes were vacuolated, pale, fragmented, and disrupted by plump fibroblasts amid fine collagen fibers, resulting in loss of architecture of the circular and longitudinal muscularis. The clinical history, microscopic findings, and the absence of mechanical intestinal obstruction, warranted the diagnosis of CIPO due to intestinal leiomyositis. This is an uncommon disease with a poor prognosis and a reported median survival time of days to weeks after diagnosis in dogs. The pathogenesis is poorly understood, and there are no specific treatment guidelines for this disease. This patient was treated with a single injection of human intravenous immunoglobulin (IVIG-GAMUNEX© 1 g IV constant rate infusion [CRI]) combined with a conservative steroid therapy regimen and mycophenolate. The patient improved within one week and remained clinically normal 4 months after initial diagnosis. Human IVIG has been used in dogs as an experimental treatment for immune-mediated hemolytic anemia, thrombocytopenia, and a few other autoimmune diseases with variable success, but has not been reported to be used as an adjunctive therapy for intestinal leiomyositis.

D74: CYTOLOGIC AND HISTOLOGIC FEATURES OF PULMONARY HYALINOSIS IN A DOG WITH CONCURRENT NEUROENDOCRINE CARCINOMA

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Background: Multiple pulmonary nodules were noted within the right middle and left caudal lung lobes of a 13-year-old spayed female mixed breed dog. The pulmonary nodules were serially imaged and fine needle aspirates of the nodules were obtained at two different time points. Three months following discovery of the nodules, a right middle and left caudal lung lobectomy was performed. The lung lobes were submitted for histopathology.

Methods: Cytologic specimens were stained with modified Wright-Giemsa stain. Tissue specimens were processed routinely and stained with histochemical (hematoxylin and

eosin and periodic acid-Schiff [PAS]) and immunohistochemical (pan-cytokeratin, vimentin, chromogranin A [CgA], synaptophysin, neuron specific enolase [NSE], and thyroid transcription factor-1 [TTF-1]) stains.

Results: Cytology revealed numerous birefringent hyaline concretions accompanied by macrophagic to granulomatous inflammation. Histopathology revealed multiple foci of moderately differentiated carcinoma. Neoplastic cells were positive for TTF-1 and NSE, supportive of a diagnosis of primary pulmonary neuroendocrine carcinoma. In close proximity to neoplastic foci were multifocal to coalescing intra-alveolar aggregates of acellular hyaline material. This material was birefringent under polarized light, PAS-positive, and associated with histiocytic and granulomatous inflammation, consistent with pulmonary hyalinosis.

Conclusion: To our knowledge, this is the first description of the cytologic features of pulmonary hyalinosis in a dog or other species. Diagnostic cytopathologists should be aware of pulmonary hyalinosis as a potential cause of radiographically identifiable pulmonary nodules and that pulmonary hyalinosis may accompany other respiratory diseases.

D75: MIRNOME EXPRESSION ANALYSIS IN CANINE DLBCL

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Background: Lymphoma is a prevalent malignancy in dogs. Diffuse large B-cell Lymphoma (DLBCL) is the most common subtype, representing about 50% of the clinically seen lymphoma cases. Thus, searching for additional biomarkers capable of early detection and monitoring DLBCL is essential for improving and sustaining remission rates. Next-generation sequencing provides innovative information about biomarkers and the differential expression of genes, including microRNAs. Non-coding microRNAs negatively influence gene expression by attaching to the 3'-untranslated region of protein-coding mRNA, causing targeted RNA degradation or translational repression. MicroRNAs' stability and easy accessibility make them promising biomarkers for identifying and sub-classifying patients.

Objective: We aim to broaden the understanding of microRNAs' role in the molecular biosynthesis of DLBCL.

Methods: We isolated and sequenced microRNAs from ten samples of fresh-frozen lymph node tissue (six DLBCL and four healthy dogs) and validated them by RT-qPCR. The average expression fold-change ($2^{-\Delta\Delta Cq}$) of each microRNA in the DLCBL and healthy groups were compared to find significant differences using the unpaired parametric Welch's 2-sample t-test and false discovery rate (FDR). The geometric expression levels mean of the most consistently expressed candidates were used as data normalizers (miR-361-5p, miR-101, and miR-29c-3p).

Results: Small RNA sequencing (sRNA-Seq) analysis identified 35 differentially expressed miRNAs (DEMs) in DLBCL. RT-qPCR confirmed 23/35 DEMs; 9 were downregulated, and 14 were upregulated.

Conclusions: Our results demonstrate the potential to harness microRNAs as unique diagnostic and therapeutic targets in DLBCL**.** Confirmatory prospective studies on large populations are planned.

D76: RELATIONSHIP BETWEEN UVEAL INFLAMMATION AND VIRAL DETECTION IN 30 CATS WITH FELINE INFECTIOUS PERITONITIS

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Background: Feline infectious peritonitis (FIP) is caused by a mutated enteric coronavirus that infects macrophages. It causes systemic pyogranulomatous phlebitis including uveitis.

Objectives: The aims of this study were to evaluate the sensitivity of viral detection in cases of FIP-induced uveitis using immunohistochemistry (IHC), RT-qPCR, and RNAscope® *in situ* hybridization (ISH), evaluate the agreement between these diagnostic tests, and evaluate the correlation between the type of inflammatory cells (pyogranulomatous versus plasmacytic) and the likelihood of viral detection. To date, the use of RNAscope® ISH for FIP diagnosis has not been thoroughly evaluated; thus, we evaluated its performance and agreement with RT-qPCR and IHC.

Methods: Eyes with FIP-induced uveitis from 30 cats were histologically evaluated, and the type of inflammatory cells was graded from 1 (predominantly pyogranulomatous) to 5 (predominantly plasmacytic). IHC, RT-qPCR, and RNAscope® ISH were performed on these cases.

Results: Viral RNA or antigen was detected in 8/30, 9/30, and 10/30 cats using RT-qPCR, IHC, and RNAscope® ISH, respectively, with high agreement between each test. A weak to moderate but significant negative correlation between the degree of plasmacytic uveal inflammation and the likelihood of detecting FIP antigen and RNA was identified.

Conclusions: RNAscope® ISH had high agreement with IHC and RT-qPCR and had the highest detection rate. This study suggests that the likelihood of confirmatory

diagnosis of FIP by IHC, ISH or RT-qPCR in ocular tissues is low in those cases in which there is intense plasmacytic ophthalmitis.

D77: MYOSIN HEAVY CHAIN MYOPATHY IN TWO QUARTER HORSES

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A 1.5-year-old American Quarter Horse (AQH) gelding had a 10-day history of pelvic lameness, nasal discharge, and 3-day onset of myoglobinuria. Endoscopy revealed bilateral abscesses in the retropharyngeal lymph nodes, suggestive of strangles. An 11-month-old AQH filly had a 5-day history of lethargy and rapid muscle atrophy. Thoracic auscultation and ultrasound suggested pneumonia. Both patients had markedly elevated creatine kinase (60,000 U/L and 49,275 U/L, respectively) and aspartate aminotransferase (8,000 U/L and 14,450 U/L, respectively) enzymes. Both horses had severe polyphasic histiocytic and lymphoplasmacytic myositis with necrosis, atrophy, mineralization, and regeneration. The gelding also had renal lesions of purpura hemorrhagica and myoglobinuric nephropathy, and suppurative lymphadenitis by *Streptococcus equi* spp. *equi*. The filly had a 7x4x3 cm pulmonary abscess, caused by *Actinobacillus equuli*. Hair roots were submitted for a five-panel genetic disease test for the AQH. The gelding had one mutated copy and the filly had two mutated copies of the Myosin Heavy Chain 1 (MYH1) gene, supporting the diagnosis of myoglobin heavy chain myopathy (MYHM). A nonsynonymous E321G mutation in the MYH1 gene encodes a hypercontractile myosin heavy chain in type 2X myofibers. Hypothetically, the immune system recognizes the mutated fibers leading to immune-mediated myositis (IMM). Approximately 40% of MYHM horses had exposure to respiratory or gastrointestinal pathogens or vaccinations, particularly *S. equi* spp. *equi*, which suggestively trigger IMM due to epitope mimicry, as seen in one of our cases. In the second case, myositis was possibly triggered by *A. equuli,* which has not been previously linked to IMM.

D78: GM2-GANGLIOSIDOSIS IN A SHIBA INU DOG

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A 1.5-year-old, female, Shiba Inu dog had a clinical history of progressive ataxia, vestibular signs, and neurologic deficits. Magnetic resonance imaging (MRI) showed poor gray-white matter definition in the cerebrum, subjective thickening of the cortical grey matter and thinning of the white matter, and dilation of the cavities of the olfactory bulb with cerebrospinal fluid. The findings were interpreted for a probable metabolic encephalitis. DNA testing for GM2-gangliosidosis revealed this animal was homozygous for the disease variant genotype. Histopathology of the cerebrum and spinal cord revealed swollen neurons with finely granular, eosinophilic cytoplasm containing eosinophilic vacuoles. The white matter had demyelination and dilated myelin sheaths with swollen axons. The swollen neurons were also present in the peripheral spinal nerves and myenteric ganglia. The histopathologic findings support the diagnosis of the

lysosomal storage disease, GM2-gangliosidosis. GM2-gangliosidosis is a rare, lipid storage disease caused by a deficiency in hexosaminidase or the activator protein GM2A. This deficiency results in fusion of vesicles with lysosomes to form phagolysosomes, which decreases enzymatic breakdown leading to lysosomal retention of ganglioside and globoside.

D79: CRYPTOBIA IUBILANS INFECTION IN LAKE MALAWI CICHLIDS

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Background: *Cryptobia iubilans* is a flagellate protozoan that causes primarily fatal gastric ulceration and granulomas in cichlids.

Methods: One hundred and fifty Lake Malawi cichlids were introduced to Baton Rouge zoo. While in quarantine, some of them acutely died or had buoyancy issues, hyperoxia/anorexia, and loss of body condition with a mortality of 12% (18/150). Six cichlids were submitted for necropsy to the Louisiana Animal Disease Diagnostic Laboratory. Histopathologic examination of H&E-stained slides was performed after formalin fixation and decalcification. Gram, Fite-Faraco acid-fast, PAS, and Steiner's silver stains were also performed on selected sections.

Results: The cichlids had multiple granulomas in the gastric wall without apparent causative agents. In one cichlid the granulomas were more numerous, involving multiple areas of the gastrointestinal tract, the mesentery, and few on the liver; the most severely affected portion (stomach) had ruptured with subsequent bacterial coelomitis. No microorganisms were noted in the granulomas with Gram, Fite's acid-fast, PAS, or Steiner's silver stains.

Conclusions: In this family of fish (cichlid), distribution of the granulomas primarily affecting the stomach and lack of infectious agents visualized by special stains are sufficient for a presumptive diagnosis of *Cryptobia iubilans* infection. *Cryptobia iubilans* is an important pathogen in cichlids and can cause high mortality linked with environmental factors or coinfections. This disease may be underdiagnosed or misdiagnosed because the organism is difficult to visualize in granulomas. It should be considered as the primary differential diagnosis for granulomas predominantly affecting the stomach in cichlids.

D80: PERICARDIAL EFFUSION ASSOCIATED WITH ANAPLASMA PHAGOCYTOPHILUM IN A DOG

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Background: An 11-year-old female spayed German Wirehair Pointer with a 1-week history of lethargy, hyporexia, diarrhea and coughing presented with pericardial effusion causing cardiac tamponade. The pericardial effusion was an exudate with mixed macrophagic and neutrophilic inflammation. Morulae were found within neutrophils.

Objective: To speciate morulae found within neutrophils in a dog with pericardial effusion.

Methods: Pericardial fluid and whole blood were tested for *Anaplasma phagocytophilum* antibodies via ELISA and IFA (IDEXX, Westbrook, ME) and genetic material by PCR. PCR testing was also performed for Babesia, Apicomplexa, Bartonella, Ehrlichia, Hemotropic Mycoplasma and Rickettsia genera.

Results: Pericardial fluid and blood were PCR positive for *A. phagocytophilum* (NC State Veterinary Hospital Vector Borne Disease Diagnostic Laboratory, Raleigh, NC). Blood was negative by ELISA (Vetscan Flex4 Rapid Test, Zoetis, Parsippany, NJ) for *A. phagocytophilum* antibodies at the time of initial presentation to the referring veterinarian, then tested positive by ELISA (IDEXX SNAP4Dx, Westbrook, ME) seven days later. The IFA was positive for *A. phagocytophilum* antibody at 1:1600 (IDEXX, Westbrook, ME). All other infectious disease testing was negative. An echocardiogram at the time of presentation revealed no structural abnormalities. An echocardiogram performed one month following therapeutic pericardiocentesis and doxycycline treatment for *A. phagocytophilum* showed no recurrence of pericardial effusion.

Conclusions: This case emphasizes the diagnostic importance of thorough cytologic evaluation of effusions. Association of *A. phagocytophilum* with cavitary effusions was previously reported in two equids. This is the first report of *A. phagocytophilum* associated with pericardial effusion in a dog.

D81: GASTROESOPHAGEAL INTUSSUSCEPTION IN A KITTEN WITH DYSAUTONOMIA

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Background: A 6-month-old intact male Russian Blue Kitten presented to the Colorado State University (CSU) Small Animal Internal Medicine Department with a chronic history of ill thrift, lethargy, increased respiratory effort, regurgitation, pilocarpine-responsive mydriasis with absent pupillary light reflexes (direct and indirect), blepharospasm and keratoconjunctivitis sicca. A litter mate exhibited similar yet more mild clinical signs. Thoracic radiographs revealed megaesophagus and a distal esophageal opacity suspected to be a sliding hiatal hernia. Respiratory signs worsened and the patient died at the referral veterinarian 1 month later despite treatment. The patient was submitted to the CSU Veterinary Diagnostic Laboratory for necropsy.

Results: On postmortem exam, the esophagus was markedly dilated with approximately 90% of the stomach inverted into the distal esophagus. The gastric submucosa was expanded by edema. The lungs were diffusely atelectatic, and the left cranial lung lobe sank in neutral-buffered formalin which correlated to aspiration pneumonia microscopically. Histologic examination of the celiac ganglion, and the myenteric and submucosal plexuses throughout the gastrointestinal tract revealed marked neuronal dropout and degeneration with necrosis, satellitosis, and central chromatolysis. Special stains highlighted neuronal dropout and pathologic changes were compared to a 1-year-old healthy cat as a non-age matched control.

Conclusion: The current case is presented as the first reported case of gastroesophageal intussusception in a kitten diagnosed with dysautonomia. The litter mate is alive and doing well at home.

D82: CYTOKERATIN AE1/AE3 IMMUNOLABELING IN DIAGNOSTICALLY CHALLENGING EPITHELIOID HEMANGIOSARCOMA

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Background: Epithelioid hemangiosarcoma is a rare histologic variant of hemangiosarcoma reported in several animal species including dogs, cows, horses, and cats. Histologically, epithelioid hemangiosarcoma can be architecturally arranged as islands and nests, thereby mimicking epithelial cell tumors. In humans, nearly half of epithelioid hemangiosarcomas have been reported to have positive immunolabeling for cytokeratin AE1/AE3 (CK AE1/AE3), which often makes it challenging to distinguish them from carcinomas.

Objective: To determine the presence of CK AE1/AE3 immunolabeling in cases of epithelioid hemangiosarcoma diagnosed in animals.

Methods: A retrospective review of cases (necropsy and biopsy) of animals received by the diagnostic pathology services at five institutions was performed. The criterion for inclusion included a diagnosis of epithelioid hemangiosarcoma based on the following histological features: sheets of spindled, polygonal, cuboidal to round mesenchymal cells, forming, tubular, solid, and sheet-like arrangements with limited vasoformation. Tumors arising from cutaneous, subcutaneous, and visceral locations were included. Immunohistochemistry (IHC) for CD31 and CK AE1/AE3 were evaluated in each case.

Results: A total of 24 cases were evaluated from dogs (23 cases) and an ox. CK AE1/AE3 immunolabeling was observed in 50% (12/24) of the cases. Cytoplasmic CK

AE1/AE3 immunolabeling varied from 5% to 100% of the neoplastic cells. All neoplasms had consistent membranous immunolabeling for CD31.

Conclusions: CK AE1/AE3 immunolabeling in epithelioid hemangiosarcomas in the current investigation was similar to that reported in humans and can pose a diagnostic challenge if not paired with CD31immunolabeling.

D83: IN SITU HYBRIDIZATION FOR ESCHERICHIA COLI IN CANINE GRANULOMATOUS COLITIS

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Background: Granulomatous colitis (histiocytic ulcerative colitis) is diagnosed most commonly in boxer dogs and French bulldogs. Microscopically, erosive and/or ulcerative lesions with a mixed inflammatory population dominated by macrophages that often contain intracytoplasmic Periodic acid-Schiff positive material predominate. As enteroinvasive *Escherichia coli* has been implicated as the primary pathogen, current diagnostics rely on a combination of culture and fluorescent *in situ* hybridization which can be time-consuming or technically challenging.

Objective: To utilize RNAscope® *in situ* hybridization for the localization of *Escherichia coli* in canine granulomatous colitis.

Methods: *In situ* hybridization probes for *Escherichia coli* were designed in collaboration with Advanced Cell Diagnostics for their automated RNAscope® *in situ* hybridization platform. The last ten months of archived cases submitted to the New York State Animal Health Diagnostic Center were searched for large intestinal biopsies from dogs that had a clinical suspicion of granulomatous colitis.

Results: Thirty cases were identified. Included were French bulldog (13), boxer (5), Labrador retriever (2), Goldendoodle (2), and individual breeds (8). Average age at time of biopsy was 33.6 ± 6.2 months. 28/30 (93%) of cases had hybridization signal for *Escherichia coli*. 8/28 (29%) were localized only to the lumen/apical portion of enterocytes. 18/28 (64%) had hybridization signal localized to luminal/apical portion of enterocytes and within macrophages in the mucosa. 2/28 (7%) had transmural hybridization signal.

Conclusions: *Escherichia coli* is readily identified by RNAscope® in situ hybridization in canine granulomatous colitis and this method serves as a quick and specific diagnostic test to confirm enteroinvasiveness.

D84: IMMUNOHISTOCHEMICAL INVESTIGATION OF INSULINOMA-ASSOCIATED PROTEIN 1 (INSM1) EXPRESSION IN CANINE AND FELINE NEUROENDOCRINE NEOPLASMS AND COMPARISON WITH CHROMOGRANIN A AND SYNAPTOPHYSIN

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Background: Neuroendocrine (NE) neoplasms can arise at almost any anatomical location and could be histopathologically misdiagnosed as other neoplasms of epithelial origin owing to their complexity and rarity. Insulinoma-Associated Protein 1 (INSM1), a recently demonstrated NE marker, is reported in a growing number of research studies in human medicine. As a transcriptional regulator, highly conserved INSM1 homologues in various species have been confirmed in previous studies.

Objective: Our objective was to investigate the immunohistochemical (IHC) reactivity of anti-INSM1 antibody in dogs and cats and to compare the results of INSM1 with those of Chromogranin A (CGA) and Synaptophysin (SYN) in NE neoplasms.

Methods: We performed INSM1, CGA and SYN IHC on formalin-fixed, paraffin-embedded canine and feline NE normal tissues, 100 hyperplastic and neoplastic lesions, and 72 non-neuroendocrine neoplasms.

Results: We found anti-INSM1 antibody could detect nuclear expression in most canine and feline normal NE tissues, except parathyroid glands. Five parathyroid carcinomas and six parathyroid adenomas/hyperplasia were negative for INSM1. In the rest of specimens, INSM1 was detectable in 95.5% of 89 NE hyperplastic and neoplastic lesions. In contrast, INSM1 was detected in only three of 72 non-neuroendocrine neoplasms. The overall percentage of NE neoplasms that stained positively with all three markers was 78.4%. In addition, INSM1 appears more sensitive to Merkel cells than CGA and SYN.

Conclusions: These findings confirm that INSM1 is a useful IHC marker for diagnosing canine and feline NE neoplasms and are encouraged to be considered as part of immunohistochemical panels to improve the diagnostic capability.

D85: IMPROVING ACCURACY IN DIAGNOSING LYMPHOMA IN CHICKENS - IMMUNOHISTOCHEMICAL CHARACTERIZATION OF BOTH NORMAL LYMPHOID TISSUES AND LYMPHOMA

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Background: Lymphoma is a highly prevalent disease in chickens aged 6 weeks and older with most cases arising due to infections by an oncogenic virus. Implicated viruses include Gallid alphaherpesvirus-2 (Marek's Disease), Avian leukosis virus (Lymphoid Leukosis), and Reticuloendotheliosis virus (Reticuloendotheliosis). Despite the abundance of literature concerning the pathogenesis of oncogenic viruses in chickens, there is a relative paucity of literature detailing the histomorphological and immunohistochemical features of normal and neoplastic lymphoid tissues in these animals.

In order to confidently assess lymphoid tissues and accurately diagnose lymphoma in chickens, pathologists must be cognizant of the organizational and morphological differences between the mammalian and avian lymphoid systems. Given that immunohistochemistry can enhance one's ability to confidently assess lymphoid tissues, pathologists must also possess a sound understanding of the immunohistochemical features of both normal and neoplastic avian lymphoid tissues.

Objective: To characterize the immunohistochemical features of both normal and neoplastic lymphoid tissues in chickens.

Methods: Formalin-fixed paraffin-embedded tissues from chickens were processed with both routine hematoxylin and eosin (H&E) staining protocols and immunohistochemical protocols for Paired Box 5 (Pax5) and Cluster of Differentiation 3 (CD3). The distribution of T-cell and B-cell lymphocytes was documented in normal lymphoid tissue and in lymphomas arising from these organs.

Results & Conclusions: Distinction between normal lymphoid tissue, lymphoma, inflammation, and extramedullary hematopoiesis (EMH) can be difficult with H&E stained slides alone. This study provides insight on interpreting Pax5 and CD3 immunohistochemistry in chicken lymphoid tissue and its utility in diagnosing lymphoma.

D86: NOVEL ADOMAVIRUS ISOLATED FROM A PROLIFERATIVE SKIN LESION IN A SAND TIGER SHARK (CARCHARIAS TAURUS)

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Background: Adomaviridae is an emerging viral family in fish with structural and replicative genes sharing a complex evolutionary history with small DNA tumor viruses including papillomaviruses, polyomaviruses, and adenoviruses. Adomaviruses have now been identified from a number of fish species and are associated with proliferative skin lesions in a giant guitarfish (*Rhynchobatus djiddensis*) and smallmouth bass (*Micropterus dolomieu*).

Objective: This study histologically describes progressive localized skin eruptions in an aquarium-housed sand tiger shark (*Carcharias taurus*) and molecularly characterizes a novel adomavirus associated with the proliferative lesions.

Methods: Histopathology, transmission electron microscopy (TEM), and next generation sequencing were completed from skin lesion samples.

Results: Lesions were confined to the caudolateral body and peduncle and were pink to red, raised, and mixed gelatinous and granular. Histopathology revealed proliferation of epithelial elements within dermal denticles producing malformed tooth-like structures resembling odontogenic neoplasms in other vertebrates. Nuclear inclusion bodies and viral particles were not observed with histopathology or TEM, respectively. BLAST

analysis of Illumina MiSeq sequence data revealed viral sequences with greatest similarity (71.79% identity) to that of the giant guitarfish adomavirus (GAdoV). Lesions in the index animal have since partially regressed but persisted for one year, and four additional sand tiger sharks in the same enclosure have developed similar skin proliferations

Conclusions: This is the second report of an adomavirus characterized from proliferative skin lesions in an elasmobranch and the first virus described from a sand tiger shark. Additional sampling of other affected animals, genome assembly, and RNAscope *in situ* hybridization are underway.

D87: EVALUATION OF SOX-10 IMMUNOHISTOCHEMICAL EXPRESSION IN CANINE MELANOMA AND NON-MELANOCYTIC TUMORS BY TISSUE MICROARRAY (TMA)

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Melanoma frequently presents a diagnostic challenge given the propensity of tumors to lack or contain scant melanin and the variable microscopic phenotype. Previous studies evaluating single IHC markers for diagnosing melanoma have shown unsatisfactory sensitivity and/or specificity for S-100, PNL2, Melan-A, TRP-1, TRP-2 and HMB-45. *Sry-related HMG-Box gene 10* (SOX-10) is a transcription factor acting as a nucleocytoplasmic shuttle protein and is involved in melanocytic, peripheral neural crest and peripheral nervous system development. In humans, SOX-10 expression has been demonstrated in melanoma, breast cancer, gliomas, and schwannomas but has only recently begun to be characterized in veterinary species. In this study, 293 tumors comprised of 165 melanocytic neoplasms and 128 non-melanocytic neoplasms were evaluated by tissue microarray for SOX-10, PNL2, Melan-A, TRP-1, and TRP-2 expression. SOX-10 had the highest diagnostic sensitivity at 90.7%. Of immunopositive tumors, SOX-10 had the highest average labeling intensity with approximately 82.4% (122/148) of positive melanomas having a labeling intensity of 4/4. Additionally, SOX-10 had the highest percentage 91.9% (136/148) of melanomas label positive for at least 75% of neoplastic cells. Of the 128 non-melanocytic tumors, SOX-10 labeling was observed in mammary carcinoma (6/6), gliomas (4/4), meningioma (1/2) and soft tissue sarcoma (8/28). Therefore, SOX-10 represents a useful immunohistochemical screening marker for the diagnosis of canine melanoma given its extremely high sensitivity and robust staining intensity. This marker may also be beneficial in diagnosing some non-melanocytic neoplasms in dog.

Experimental Disease Focused Scientific Session

SPATIAL TRANSCRIPTOMICS: A CASE FOR CROSS DISCIPLINARY COLLABORATION

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Background: RNA sequencing has revolutionized our understanding of disease and accelerated the development of targeted therapies. Single cell RNA sequencing furthered these advancements by illustrating cellular heterogeneity and allowed for the detection of rare but important clones. New spatially resolved transcriptomic technologies allow for integration of RNA seq data with tissue morphology seen on a traditional histology slide. This allows for interrogation of the immune microenvironment, hypoxia, and proximity to landmarks such as vasculature, tumor boundaries, or pathogens. Production and interpretation of such data requires expertise from several disciplines: histology and pathology, genomics, and computational biology. Many genomics core labs are beginning to offer these technologies without pathology involvement. Pathologists play a vital role in study design, tissue selection and QC, troubleshooting failed runs, annotation, and data interpretation.

Objectives: Offer a roadmap for bringing spatial transcriptomics to an institution with input from histology, pathology, genomics, and computational biology. This includes coordinated scheduling, sample hand-off, billing, and equipment maintenance. Potential pitfalls will also be discussed with emphasis on input from histopathology.

Results: A workflow was developed to facilitate communication between the core labs and investigators. This includes a biweekly meeting of the core labs to discuss upcoming projects, a shared site to store equipment calendars and notes, information to share with investigators during the study planning process, equipment maintenance and reagent ordering protocols, and standardized forms for quote generation. The organization effort ensures a smooth process for investigators with technical expertise from all relevant core lab specialties.

DETECTION OF CYTAUXZOON FELIS IN SALIVARY GLANDS OF AMBLYOMMA AMERICANUM

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Cytauxzoon felis is a tick-borne piroplasmid hemoparasite that causes life-threatening disease in cats. Despite the critical role that ticks play in disease transmission, our knowledge regarding the *C. felis* life cycle remains limited to the feline hosts and no

stage of the parasite has been identified or investigated in ticks. In other tick-borne piroplasmids, sporozoites have played a key role in disease prevention and management. We believe sporozoites have similar potential for cytauxzoonosis. Therefore, the objective of this study is to evaluate different molecular and microscopic techniques to detect *C. felis* sporozoites in tick salivary glands (SG). A total of 140 *C. felis*-infected *A. americanum* ticks were included for this study. Dissected SGs were quartered and subjected to *C. felis* RT-PCR, RNAscope® *in situ* hybridization (ISH), histology, direct azure staining, and transmission electron microscopy (TEM). *Cytauxzoon felis* RT-PCR was also performed on half tick (HT) carcasses after SG dissection. *Cytauxzoon felis* RNA was detected in SGs of 17 ticks. Of these, 7 ticks had microscopic visualization via ISH and/or TEM and 10 ticks had only molecular detection of *C. felis* in SGs via RT-PCR without visualization. *Cytauxzoon felis* RNA was detected solely in HT via RT-PCR in 9 additional ticks. TEM captured rare *C. felis* organisms with characteristic ultrastructural features of piroplasmid parasites. This study describes the first direct visualization of any developing stage of *C. felis* in ticks. Forthcoming studies should employ a combination of molecular and microscopic techniques to investigate the *C. felis* life cycle in *A. americanum.*

SCAVENGER RECEPTOR CD36 MEDIATES PHAGOCYTOSIS OF BORRELIA BURGDORFERI AND DOWNREGULATES SYSTEMIC AND JOINT INFLAMMATION DURING LYME BORRELIOSIS

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Lyme disease, caused by *Borrelia burgdorferi* (*Bb*), is a multisystem inflammatory disease that affects the skin, heart, joints, and CNS in humans. The mouse model of Lyme borreliosis (LB) has been instrumental to understand the immunopathogenesis of Lyme arthritis. The scavenger receptor CD36 is present on various cell types, including monocytes/macrophages. CD36 is involved in many biological processes, including innate immunity against infectious agents, however its function on LB pathogenesis is unknown. This study aims to determine whether CD36 acts as a microbial sensor modulating phagocytosis and inflammation during *Bb* infection. In vitro phagocytosis assays with murine peritoneal macrophage cell line incubated with anti-CD36 antibody and pharmacological inhibitors of scavenger receptors revealed a role for CD36 in *Bb* phagocytosis. Peritoneal macrophages isolated from $CD36^{-/-}$ mice showed impaired *Bb* phagocytosis and enhanced IFNγ and MCP-1 production in response to *Bb*. $CD36^{-/-}$ mice developed more severe tenosynovitis and aortitis than B6 mice at 4 weeks p.i. (w.p.i.). Macrophages were detected by IHC in tibiotarsal joints of infected B6 and $CD36^{-/-}$ mice. Flow cytometry demonstrated an elevated $CD36^{+}$ $F4/80^{+}$ macrophage population in the joints of infected B6 mice. Bacterial burden was significantly elevated in the heart of $CD36^{-/-}$ mice, but similar in the joints of infected mice at 4 w.p.i. Cytokine

and chemokine analysis of sera demonstrated a significant increase in proinflammatory cytokines and chemokines in infected CD36$^{-/-}$ mice. Our findings suggest that CD36 is a novel spirochetal sensor that mediates phagocytosis and downregulates systemic and joint inflammation during late stages of LB.

TIMING OF INFLUENZA COINFECTION PROFOUNDLY ALTERS SARS-COV-2 PHENOTYPE IN SYRIAN HAMSTERS

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The projected endemic patterns of SARS-CoV-2 and influenza are likely to overlap seasonally in the Northern hemisphere. Because respiratory viral interactions may be synergistic or competitive, viral co-exposure may elicit varied clinical outcomes. By using 5 cohorts of 24 animals each (n=120; evenly split by sex and studied at 2, 4, 7 and 10 days after infection), we examined immunopathologic outcomes in Syrian hamsters given influenza A either 3 or 48 hours prior to SARS-CoV-2, compared to either virus alone or mock inoculation. Clearly distinguishable severe (SARS-CoV-2) and mild (H1N1) clinical outcomes were associated with clear patterns of nasal and pulmonary pathology, and pulmonary viral load, cytokine expression and immune cell dynamics. H1N1 given 48 hours prior to SARS-CoV-2 mitigated disease accompanied by reduced SARS-CoV-2 viral load, reduced cytokine expression, earlier interferon-γ expression and more vigorous recruitment of antigen-presenting macrophages. H1N1 given 3 hours prior to SARS-CoV-2 elicited hybrid outcomes. We conclude that in hospitalized individuals, poor outcomes may variably accompany coinfection. However, at the population level, it is likely that SARS-CoV-2 will join the seasonal respiratory virus landscape in which prior exposure to influenza appears to reduce the ability of SARS-CoV-2 to achieve full pathogenicity.

CHARACTERIZATION OF THE CELLULAR EXPRESSION PATTERN OF NECROPTOTIC SIGNALING MOLECULES RIPK1, RIPK3, AND MLKL

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Background: Necroptosis is an inflammatory form of cell death that has been implicated in inflammatory and degenerative diseases. Genetically engineered mouse models and small molecule inhibitors have been used to characterized the roles of RIPK1, RIPK3, and MLKL in inflammatory diseases; however, less data is available on the cell-specific expression these molecules

Objective: The study's goal was to characterize the cellular expression patterns of RIPK1, RIPK3, and MLKL in unchallenged mouse tissues to inform the cellular roles of these molecules in health and disease.

Methods: A combination of immunohistochemistry (IHC) and in situ hybridization (ISH) assays were used to characterize the expression of RIPK1, RIPK3 and *Mlkl*. Due to limitations in individual controls and reagents, cellular expression patterns were cross-validated with secondary assays, which included varied combination of antibodies and ISH probes.

Results: RIPK1, RIPK3, and *Mlkl* are predominately expressed in immune cells, endothelial cells, and mucosal epithelium including intestinal and gastric epithelium, gall bladder, endometrium, stratified squamous epithelium, and urothelium. Minimal to no expression was observed in the neuroparenchyma, hepatocytes, or renal tubules under physiologic conditions. In some tissues, there was a deviation in the co-expression of the molecules. Germinal centers in lymph nodes highly expressed RIPK3, but minimally expressed RIPK1 and *Mlkl* compared to the paracortex and medulla.

Conclusion: Cellular expression patterns of RIPK1, RIPK3, and MLKL provide insights into their cell specific roles in disease. The use of a combination of varied immunohistochemistry and ISH validation methods overcomes intrinsic limitations of controls and individual molecular probes.

DO FUNGICIDES AND HIGHLY PATHOGENIC ISOLATES OF MELISSOCOCCUS PLUTONIUS EXPLAIN OUTBREAKS OF EUROPEAN FOULBROOD DISEASE IN HONEYBEES DURING BLUEBERRY POLLINATION?

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Background: The United States and Canada are the world's largest blueberry producers, with 90% of pollination services provided by honeybees (*Apis mellifera)* each year. However, this symbiotic relationship between blueberry growers and beekeepers is threatened by European foulbrood (EFB), a bacterial disease caused by *Melissococcus plutonius,* resulting in honeybee colony weakness and mortality following blueberry pollination.

Objective: Our objective was to investigate whether fungicide exposure and/or increased pathogenicity of *M. plutonius* are implicated in the surge of reported cases of EFB associated with blueberry-pollination.

Methods: Using an *in vitro* infection model of EFB, honeybee larvae were infected with a pathogenic isolate of *M. plutonius,* exposed to environmentally-relevant

concentrations of four formulated blueberry fungicides, and survival was monitored until adulthood. We also compared survival among larvae infected with different isolates of *M. plutonius* collected from both blueberry-pollinating and non-blueberry-pollinating honeybee colonies within North America.

Results: We found that larvae infected with *M. plutonius* and co-exposed to four fungicidal products combined had a significant 24% decrease in survival relative to infected controls ($p = 0.0038$), whereas larval exposure to individual fungicidal products did not significantly decrease survival from EFB. No difference in survival was found between *M. plutonius* collected from blueberry and non-blueberry-pollinating honeybee colonies.

Conclusions: These *in vitro* results suggest that chronic exposure of honeybee larvae to combinations of four formulated fungicidal products used during blueberry pollination may predispose larvae to EFB; however, increased *M. plutonius* pathogenicity does not appear to explain the increased incidence of EFB during blueberry pollination.

CLASSIFICATION SCHEME FOR SARS-COV-2 (COVID-19) PULMONARY LESIONS IN HAMSTERS AND MICE

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Background: The rapid response to the COVID-19 pandemic required the establishment of animal models by the National Institutes of Health/National Institute of Allergy and Infectious Diseases (NIAID) to evaluate candidate vaccines and therapeutics.

Objective: A classification scheme was developed to provide a consistent baseline grading of major SARS-CoV-2 pulmonary lesions in both Golden Syrian hamsters challenged with SARS-CoV-2 and mice challenged with mouse-adapted variants of SARS-CoV-2.

Methods: Based on the most consistent lesions reported in humans and animal models of SARS-CoV-2, five categories were included: bronchointerstitial pneumonia, type II pneumocyte proliferation, bronchiolar necrosis, bronchiolar hyperplasia, and vasculitis / endotheliitis. An additional category of “global pneumonia” was included to reflect the presumptive functional capacity of the lung as a whole and was graded based on the percentage of the pulmonary parenchyma affected by all pathologies. Sections were evaluated blindly for each separate category and given a grade of 0 (not present) to 4 (marked) based on distribution and severity of the lesions across all lung sections. Additional lesions such as infarction/thrombosis were noted separately.

Results: To date, lung sections from ~600 Golden Syrian hamsters and ~200 mice have been evaluated. The histopathology lesions have been consistent with those published in similar model systems for SARS-CoV-2 and distinct experimental group differences largely agree with virology results. Variations in individual categories have provided insights into therapeutic mechanisms and possible complications such as thrombosis.

Conclusions: This classification scheme is an effective and efficient method of evaluating SARS-CoV-2 pulmonary lesions in animal models.

CHARACTERIZATION OF HOST IMMUNE CELL INFILTRATE IN CAR T-CELL MEDIATED GRAFT VERSUS HOST DISEASE IN A MOUSE MODEL.

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Background: Chimeric antigen receptor (CAR) T cells are a revolutionary cancer therapy technology that is FDA-approved for several hematologic malignancies and under investigation for a wide variety of solid tumors. As this modality becomes more widely employed, the use of allogeneic T cells provides several benefits over autologous transplantation, such as wider availability and decreased cost. However, allogeneic CAR T cells carry increased risk of graft versus host disease (GvHD), which has been observed in human patients as well as mouse models.

Objective: To further understand the mechanism of CAR T cell-mediated GvHD.

Methods: We performed an immunohistochemical characterization of the murine immune cell infiltrate in GvHD lesions in a cohort of NSG mice that received human CAR T cells. CAR T cells were second generation and were targeted against a human tumor-specific antigen without a murine homolog.

Results: A median of 40 percent (range 10-95 percent, interquartile range 30-60 percent) of the immune cell infiltrate in the GvHD lesions is composed of murine cells. The majority of these murine cells are AIF1-positive histiocytes and many are CD11c-positive, implying a prevalent dendritic cell component. Preliminary investigation indicates type 2 polarization of infiltrating macrophages. Finally, there is a negative correlation between the magnitude of the murine immune cell infiltrate and the GvHD severity.

Conclusions: These findings suggest that the composition and activation status of the recipient immune cell infiltrate within GvHD lesions may affect lesion development and severity.

Experimental Disease Posters

E1: PULMONARY FINDINGS FROM MICE IN A SARS-COV-2 SELF-AMPLIFYING RNA VACCINE STUDY

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Objective: A study was performed of immune responses, protection, and pulmonary histopathologic findings after immunization of mice with self-amplifying RNA encoding spike (S) protein followed by SARS-CoV-2 challenge.
Methods: Two variants of the S protein of SARS-CoV-2 were used-wild type (WT) and stabilized pre-fusion conformation (PP); a mock-vaccinated, exposed control group. Mice were assessed for viral load, T-cell response, antibody response, observational changes (weight, mortality), and pulmonary histopathology. Mice were euthanized at Days 2, 4, and 7; lungs were collected in formalin, sectioned routinely, and stained with hematoxylin and eosin. Lungs were scored from 0 – no changes to 4 – severe changes based on bronchiolitis, vasculitis/perivasculitis, interstitial/bronchointerstitial pneumonia, vascular endothelial hypertrophy, Type 2 pneumocyte hypertrophy, and bronchiolar mucosal hyperplasia.
Results: Total score ranges were from 0 to 24. Neutralizing antibodies and T-cell responses were induced in the WT and PP immunized mice. Histopathologic findings included a primarily lymphocytic bronchointerstitial pneumonia and perivasculitis and vascular endothelial hypertrophy at Day 2 for both variants with highest scores at Day 4(x = 12) and diminished scores by Day 7 = 8) . At Day 2 and Day 4, there was perivascular edema. Both immunized groups (PP and WT) had higher histopathologic scores compared to the exposed and unvaccinated control group on Days 2, 4, and 7 (x = 6) and weight loss, despite lower mortality and lower RNA titers.
Conclusions: Utilization of a histopathologic lung scoring method correlated with measures of immune response and could be used for similar experimental models.

E2: OP9-DL4 CO-CULTURE SYSTEM CAN BE EFFECTIVLY USED TO STUDY ONCOGENE COOPERATION

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Background: The OP9 cell line supports hematopoiesis and B lymphopoiesis of bone marrow derived hematopoietic stem cells and embryonic stem cells. The generation of delta-like 4 (DL4) expressing OP9 cells (OP9-DL4) enabled the induction of T cells lineage commitment. **Objective:** OP9-DL4 co-culture system can be effectively used to promotes double negative (DN) thymocytes to differentiate into T cells *ex vivo*. This system can be combined with retroviral transduction of DN thymocytes to study T cell development and leukemogenesis. The combination of OP9-DL4 co-culture system with retroviral transduction of DN thymocyte markedly complicates the laboratory workflow. **Methods:** A series of precisely coordinated steps must be carefully performed to achieve successful outcome. Our established protocol was employed to showcase the ability to use the OP9-DL4 system to study oncogene cooperation in mice. **Results:** DN thymocytes were isolated from the thymus of naïve C57BL/6 mice followed by CD4 and CD8 depletion. DN thymocytes retrovirally transduced with a constitutively active mutant hIL-7Ralpha and RASGRP1, a guanine nucleotide exchange factor and strong activator of Ras, were co-cultured using the OP9-DL4 system and subsequently injected into *Rag1-/-* mice. Transduced DN thymocytes acquired T cell immunophenotype and displayed multisystemic involvement, including bone marrow and blood. **Conclusion:** Routine downstream analysis, including histology, immunohistochemistry, flow cytometry, Western blot, and DNA/RNA sequencing can be employed for individual projects' end goals.

E3: HISTOPATHOLOGIC DIFFERENCES IN GRANULOMAS OF BCG VACCINATED AND NON-VACCINATED CATTLE WITH BOVINE TUBERCULOSIS

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Background *Mycobacterium bovis* (*M. bovis*) is a zoonotic bacterium responsible for bovine tuberculosis. The Bacillus Calmette-Guerin (BCG) vaccine is an attenuated strain of *M. bovis*, which is known to provide variable protection (defined as a decrease in disease severity) in cattle. Disease severity is measured by the number of granulomas, the characteristic lesion of tuberculosis infection. However, the mechanism by which BCG imparts protection remains poorly understood. Understanding the differences between granulomas which form in BCG vaccinates compared to non-vaccinates may help identify how BCG imparts protection and lead to an improved vaccine.

Objective Characterize the differences between granulomas which form in *M. bovis*-infected BCG vaccinates compared to non-vaccinates.

Methods Utilizing special stains and image analysis software, we examined 88 lymph nodes obtained from BGC-vaccinated and non-vaccinated animals experimentally infected with *M. bovis*. We evaluated the number of granulomas, their size, severity, density of multinucleated giant cells (MNGC), and the amounts of necrosis, mineralization and fibrosis.

Results BCG vaccinates had fewer and smaller granulomas with less necrosis than non-vaccinates. The relative number of high- and low- severity lesions was similar as were the amounts of fibrosis, mineralization and density of MNGC between the two vaccination groups.

Conclusions These findings suggest that BCG vaccination reduces bacterial establishment, resulting in the formation of fewer granulomas. In granulomas that form, BCG has a protective effect by containing their size and reducing the relative amount of necrosis, however it does not affect the amount of fibrosis, mineralization or density of MNGC.

E4: INVESTIGATING THE INTERACTIONS OF DOPAMINE RECEPTOR D1 IN LUNG CANCER

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Background: In lung cancer, although mutation-targeting therapies including EGFR inhibitors have improved patient outcomes, resistance is common, so novel and adjunct treatments are still needed. Our lab recently identified dopamine receptor D1 (DRD1) as a novel tumor suppressor in lung cancer and discovered that DRD1 signaling modulates EGFR signaling, cell proliferation, and cell death. However, these mechanisms remain poorly understood.

Objective: Our lab seeks to better understand how DRD1 regulates cancer cell growth and death and if those mechanisms could be co-opted as adjunct lung cancer treatments. In this study we investigate whether DRD1 interacts directly with EGFR and through a protein-protein interaction (PPI) screen identify other proteins that interact with DRD1 and through which DRD1 could regulate cell proliferation.

Methods: We investigated DRD1's PPIs *in vitro* using proximity ligation assay (PLA), immunoprecipitation (IP), and proximity-dependent biotinylation (PDB).

Results: While PLA suggested that DRD1 and EGFR exist in close proximity on the cell membrane, IP did not support direct binding of DRD1 and EGFR. The PDB screen for DRD1's PPIs also supported a lack of direct DRD1-EGFR binding but identified many potential mediators of DRD1's regulation of EGFR signaling and cell proliferation, including GSK3β, an important Wnt/β-catenin regulator.

Conclusions: These results suggest a close but not direct-binding interaction of DRD1 and EGFR. As we continue to evaluate our PPI candidates, we hope elucidation of how DRD1 regulates cancer cell proliferation will lead to novel therapies that alone or paired with EGFR inhibitors result in better outcomes for lung cancer patients.

E5: ALTERED SPINAL CORD NEURONAL SIGNALING IN SIV-INFECTED MACQUES ON ANTI-RETROVIRAL THERAPY

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Background HIV-associated peripheral neuropathy remains one of the most prevalent neurological manifestations of HIV infection. Limited access to human CNS samples including spinal cord makes evaluation of tissues challenging. The CNS can be evaluated in the SIV/macaque model at key time points of infection, with and without ART. Previously, we have shown upregulated interferon pathways in the spinal cord of untreated SIV-infected animals.

Objective The objective was to compare the gene expression profile from the spinal cord of SIV-infected, ART-treated macaques with uninfected animals and identify biological pathways involved.

Methods RNA was isolated from the spinal cord of SIV + ART and uninfected animals. cDNA libraries were prepared followed by sequencing. Relative mRNA expression was compared between groups using DESeq2 in R. Pathway analysis was performed using Enrichr and Gene Set Enrichment Analysis. SeqSeek was used to determine the cell types linked to changes in gene expression.

Results Differential gene expression analysis revealed 476 upregulated genes in SIV + ART animals and 266 downregulated genes compared with uninfected animals (false discovery rate 0.01). Pathways identified include neurotransmitter receptors and post synaptic signaling regulators, and transmission across chemical synapses. SeqSeek analysis revealed that upregulated genes are primarily expressed by neurons.

Conclusions These findings suggest that pathways activated in the spinal cord of SIV + ART macaques are involved in neuronal signaling. This finding provides a basis for further evaluation of mechanisms of SIV infection + ART within the spinal cord, and a potential focus for therapeutic interventions to maintain synaptodendritic homeostasis.

E6: ATTENUATION PHENOTYPES AND PROTECTIVE EFFICACY OF CELL CULTURE ADAPTED PEDV NON-S INDEL STRAIN

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Background: Porcine epidemic diarrhea virus (PEDV) continues to be a threat to the U.S. pork industry. However, a safe and efficacious live attenuated vaccine is lacking.

Objective: We aimed to evaluate attenuation phenotypes and protective efficacy of cell culture-adapted U.S. non-S INDEL PEDV strain USA/IN19338/2013 at different passages in pigs.

Methods and Results: Twelve PEDV naïve pregnant sows were divided into 4 groups (3 sows/group) to farrow piglets. The piglets (3 to 7-day-old) were orogastrically inoculated with PEDV P7 (10^3 $TCID_{50}$/piglet), P100 (10^4 $TCID_{50}$/piglet), P200 (10^4 $TCID_{50}$/piglet), or virus-negative medium, respectively. Selected piglets were necropsied at 4 DPI for pathologic examinations. Remaining piglets were weaned at 21 DPI, orogastrically challenged with PEDV P8 (10^5 $TCID_{50}$/piglet) at 28 DPI and necropsied at 35 DPI. P7-inoculated piglets had severe diarrhea, lethargy, and poor body condition as well as 75% mortality. P100- and P200-inoculated piglets had almost normal body condition and no lethargy; however, P100 inoculation caused severe diarrhea whereas P200-inoculated piglets had minimal diarrhea. P200-, P100-, and P7-inoculation all significantly reduced or prevented fecal virus shedding after challenge at 28 DPI. P200- and P100-inoculated piglets developed similar levels of anti-PEDV IgG antibody and neutralizing antibody in serum. Whole-genome analysis of PEDV P7, P100 and P200 revealed some amino acid mutations and truncation of ORF3 which may be associated with virus attenuation.

Conclusions: Overall, PEDV P200 is a safe and promising vaccine candidate. These data warrants further investigation for vaccination and passive protection in pregnant sows and their suckling pigs.

E8: BREAST CANCER CELL PARACRINE INDUCTION OF CYTOKINE SECRETION BY LUNG FIBROBLASTS IS ASSOCIATED WITH DIFFERENTIAL TUMOR CELL GROWTH AND CHEMORESISTANCE.

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Background: Identifying therapies effective in the metastatic disease setting is a significant unmet clinical need in triple negative breast cancer (TNBC). Cancer-associated fibroblasts play a critical role in promoting breast cancer cell survival, metastasis and chemoresistance within the primary tumor microenvironment, but the contribution of tissue-resident fibroblasts to tumor growth and drug resistance in distant metastatic sites remains largely unexplored.

Objective: To develop a high-throughput primary human donor-derived lung fibroblast (LF) - breast cancer cell (BCC) co-culture model to identify therapeutic vulnerabilities and mechanisms of fibroblast-mediated extrinsic chemoresistance in lung metastatic breast cancer.

Methods: We characterized growth and chemoresistance phenotypes of BCCs co-cultured with primary LFs via bioluminescence and fluorescence imaging, RNAseq, and multiplex cytokine analysis. Using this model, we also performed a high-throughput screen evaluating a library of 900 FDA approved and experimental kinase inhibitor compounds.

Results: Human BCC co-culture with primary LFs resulted in significant subtype-dependent differences in tumor cell growth and drug resistance compared with conventional monoculture. Moreover, we observed significant increases in tumor-promoting cytokines including IL-6, CXCL8, and CCL2 in co-culture vs. conventional monoculture. High-throughput kinase inhibitor library screening defined a subset of compounds, namely inhibitors of the class III PI3K and autophagy regulator VPS34, with increased efficacy in lung fibroblast co-culture, suggesting autophagy induction as a mechanism underlying fibroblast-mediated drug resistance in TNBC.

Conclusions: Breast cancer cell co-culture with primary lung fibroblasts represents a scalable *in vitro* model for therapeutic discovery and mechanistic evaluation of extrinsic modulation of chemotherapy drug response in metastatic breast cancer.

E9: HEPATIC HISTIOCYTIC PROLIFERATION ARISING IN AN IMMUNOCOMPETENT MURINE MODEL OF COLORECTAL CANCER

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Immunocompetent murine tumor models are critical for studying the immunobiology of cancer. Transplantable cell lines in syngeneic models provide a system for predictable, high-throughput testing of novel immunotherapeutic approaches. Limited numbers of such murine cell lines exist for studying colorectal cancer (CRC). Both CT26 and MC38 cell lines have been criticized for lacking mutations frequently observed in human CRC. Cre-LoxP and CRISPR-Cas9 technologies were utilized to induce colorectal tumors for the purpose of generating syngeneic cells lines with clinically relevant mutations. Necropsy and histopathology of two such mice revealed a primary GI tumor in one mouse, with nodular and/or sinusoidal and intravascular accumulations of cells within the liver parenchyma of both mice. Cell lines generated from the liver lesions were negative for pancytokeratin and positive for CD45 suggesting leukocyte lineage. Immunohistochemical staining of liver lesions showed that cells were predominantly, strongly immunoreactive for CD68, IBA-1, and CD11b consistent with histiocytic lineage rather than metastatic CRC. Mouse models for several histiocytic diseases in humans have been developed. The spontaneous occurrence of histiocytic sarcoma, histiocytosis, and histiocyte-associated lymphoma have been reported in mice. It is currently unclear whether the proliferative histiocytic lesions in the livers of our mice occurred spontaneously or were the result of genetic manipulation during CRC tumor model development. This case highlights the importance of pathologic characterization in development of new tumor models.

E10: THERAPEUTIC TRIAL OF FLUVASTATIN IN A CELL LINE XENOGRAFT MODEL OF CANINE MAMMARY GLAND CANCER: A PILOT STUDY

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The first line of treatment for canine mammary tumours (CMTs) is surgical excision and currently, there is no targeted chemotherapeutic options available for inoperable CMTs. Recent studies show the potential involvement of dysregulated Hippo signalling in CMT development and progression. Our recent *in vitro* studies showed that Fluvastatin inhibits GGylation and YAP/TAZ-mediated transcriptional activity via activation of the Hippo pathway in CMTs. In this study, we sought to evaluate the efficacy of Fluvastatin in a xenograft model of CMT. Xenograft mice with CMT were divided into four treatment groups: vehicles (DMSO), Fluvastatin, Doxorubicin or a combination of Fluvastatin and Doxorubicin. On the fourth week, tissues were harvested for morphometric analyses, histopathological and immunohistochemical examination as well as molecular analyses and mass spectrometry. Results showed that the difference in tumour volumes was significant only for the combined Fluvastatin and Doxorubicin group and the final tumour weight was significant only with the Doxorubicin treated group. The percentage of tumour necrosis, activated Caspase 3 or ki-67 did not show any difference between groups. In addition, the expression of Hippo pathway's main effectors, YAP and TAZ, did not demonstrate a statistical difference between groups. Moreover, there was no statistical difference in the mRNA level of key transcriptional target genes (*CYR61*, *CTGF, ANKRD1* and *RHAMM*). Interestingly, results from mass spectrometry detected Fluvastatin in tumour tissues in concentrations comparable to levels with therapeutic effects in other studies. Future research with different statins at higher concentrations and/or with longer treatments could be of interest.

E11: INFLUENCE OF INDOLES AND ITS DERIVATIVES ON A HEALTHY CANINE DUODENUM USING INTESTINAL ORGANOIDS IN A DUAL-CHAMBER PERMEABLE SUPPORT SYSTEM

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Background: Indoles are gut bacteria-derived tryptophan metabolites that have been shown to improve intestinal barrier function in rodents[1].

Objectives: To assess the influence of indole candidates on the physiology of the canine duodenum using a 3D organoid model.

Methods: Canine duodenal organoids were expanded, harvested[3], and plated in a dual-chamber permeable system[4] using 12 replicates in each study group. Indole

analytical standard (IAS, 1 mmol/L), 3-indolepropionic acid (IPA, 1 mmol/L, or 10 mmol/L), and indole-3-carboxyaldehyde (I3A, 1 mmol/L), or experimental vehicular control, were introduced in the apical chamber for 24 hours, and the supernatant was collected for measurement of canine IL-8 using an ELISA immunoassay. Trans-epithelial electrical resistance (TEER) values were measured at 0H and 24H post-incubation.

Results: The T0 TEER values ranged from 128 to 274 Ω.cm^2 with a mean of 195 ± 34 Ω.cm^2. At the 24-hour mark, TEER values decreased in all groups, with a mean reduction of 19 Ω.cm^2, 34 Ω.cm^2, 12 Ω.cm^2, 6 Ω.cm^2, and 19 Ω.cm^2 for IAS, IPA (1 mM and 10 mM), I3A and control, respectively. No significant differences in IL-8 apical concentrations were observed between study groups either.

Conclusions: While no apparent differences in outcome measures could be found between control and indole-treated duodenal organoids, further work involving higher concentrations of indoles and/or stimulation of the innate immune response with TNF-alpha is warranted.

E12: HEPATIC PRO-INFLAMMATORY MYELOID DYSHOMEOSTASIS REPRESENTS A HALLMARK OF KIKWIT EBOLA VIRUS DISEASE (EVD) PROGRESSION IN RHESUS MONKEYS

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Pro-inflammatory hypercytokinemia caused by monocyte/macrophage activation is a hallmark of fatal Ebola viral disease (EVD). In this study, we utilized immunohistochemistry, *in situ* hybridization, multispectral whole slide imaging, and quantitative image analysis to quantify molecular phenotypes of myeloid cells (monocytes, macrophages, and neutrophils) in the liver of rhesus macaques (*Macaca mulatta*; n=21) infected with Zaire Ebolavirus (EBOV-strain Kikwit). Liver samples included uninfected controls (n=3), 3 days post-inoculation (DPI; n=3), 4 DPI (n=3), 5 DPI (n=3), 6 DPI (n=3), and terminal disease (6-8 DPI; n=6). Viral antigen was first observed sporadically in hepatocytes and sinusoidal macrophages of all rhesus monkeys by 3 DPI, which temporally mirrored peak mean peripheral monocyte counts (3-4 dpi) that transitioned to monocytopenia by ≥ 5 DPI. Simultaneously, drastic alterations in hepatic macrophage phenotypes occurred at ≥ 5 DPI characterized by an

abrupt and persistent transition from primarily CD14-/CD16+ and CD68+/163high++ to CD14+CD16- and CD68+163low+ macrophages. Peripheral mean neutrophil counts peaked 3-5 DPI, returning to within normal reference ranges ≥ 6 DPI. In the liver a precipitous increase in the neutrophil chemoattractant and alarmin S100A9 by hepatic macrophages at 5 DPI preceded sinusoidal neutrophil influx ≥ 6 DPI. Findings occurred concurrently with declining liver function, injury, and altered clotting profiles. Further liver dyshomeostasis was reflected by widespread expression of the antiviral Myxoma resistance protein 1 (MxA) and atypical expression of Major Histocompatibility Complex Class II by hepatocytes. Results offer insight into EVD pathogenesis and could inform future therapeutic strategies to prevent and/or modulate the host pro-inflammatory response.

E13: EFFECTS OF PREFERENTIAL WEIGHT BEARING AND REDUCED AMBULATION ON EQUINE LAMELLAE IN AN IN VIVO EXPERIMENTAL MODEL OF SUPPORTING LIMB LAMINITIS

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Background: Equine supporting limb laminitis (SLL) is associated with altered weight bearing and/or increased mechanical load that results in failure of hoof wall lamellae. SLL is a common and serious complication of painful limb conditions such as fractures.

Objective: Examine the effects of prolonged preferential weight bearing (PWB) on equine hoof lamellae using a non-painful *in vivo* experimental model.

Methods: 12 healthy Standardbred horses were housed in stocks with limb weight distribution logged continuously for 92 hours. In 6 horses, a platform shoe placed on the contralateral forelimb (CL) was used to induce increased body weight distribution on the opposite (supporting) forelimb (PWB) (39%:27% difference). Another 6 horses had no intervention besides restricted ambulation (RA) in stocks. Archived tissues from healthy Standardbred horses (n=8) without RA were used as controls (CON). Metabolic analyses, qualitative scoring, histomorphometry and staining for cell death (TUNEL, caspase-3) and proliferation (TPX2) markers were performed on lamellae.

Results: PWB limbs had relative reduced limb offloading with increases metabolic indicators of ischemia compared to SL or RA limbs. Histologic lesions compatible with acute laminitis, including increased numbers of lamellar keratinocytes positive for markers of cell death and proliferation were most severe in PWB lamellae, but were also increased in the CL lamellae, as well as lamellae from RA horses, compared to CON.

Conclusions: Lesions identified in this PWB model confirm that alterations in weight-bearing can induce laminitis but also that restriction of normal ambulation, in the absence of increased weight bearing, can also damage lamellae.

E14: IN SILICO RESEARCH OF DRUGS TO REVERSE GENETIC SIGNATURE FROM CANINE MAMMARY CARCINOMA

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Canine mammary carcinoma is the most frequent tumor in intact female dogs. The treatment choice is radical mastectomy, and there are few options for metastatic disease. Drug repurposing could be a prospective approach for female dogs with metastatic mammary carcinoma. We used an *in silico* strategy to identify drug candidates that could reverse the genetic signature from canine mammary carcinoma based on public data published in the Gene Expression Omnibus (GEO) (Edgar et al. 2002). We used the L1000 platform (Library of Integrated Network-based Cellular Signatures - LINCS), which provides gene expression profiles induced by over 10 000 compounds, shRNAs, and kinase inhibitors. Twenty-one canine mammary carcinomas were used to establish the genetic signature by grouping the individual large-scale gene expression compared to a pool of seven normal mammary tissue samples published by Uva et al., 2009. From these data, 62 genes were selected, 50 upregulated and 12 downregulated, and 50 drugs were listed in the L1000 platform. The three top-downregulated genes were *SFRP2*, *COL2A1* and *DDX60,* and *MMP27*, *EXPH5* and *THRSP were upregulated*. Ketarolac, BRD-K37883585 and vincamine were the three top drugs that could reverse the gene expression signature for canine mammary gland tumors. The in silico strategy is a good model for choosing drugs already approved by the FDA that can be used in further in vitro studies.

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E15: TRANSGENIC MICE EXPRESSING HACE2 AT THE ROSA26 LOCUS EXHIBIT INCREASED SURVIVAL, LIMITED NEURODISSEMINATION, AND LONG-TERM RESIDUAL NEUROLOGICAL AND PULMOANARY HISTOPATHOLOGY FOLLOWING SARS-COV-2 INFECTION

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A widely used transgenic mouse model of SARS-CoV-2 infection features a random, multiple-copy insertion of hACE2 under the cytokeratin 18 promoter into its genome

(K18-hACE2). While highly permissive to infection, K18-hACE2 mice display fatal, near-global neurodissemination of SARS-CoV-2 across a broad range of inoculation doses despite mild-to-moderate pneumonia. Here, we compared infection of K18-hACE2 mice a mouse model with a targeted, single-copy insertion of the K18-hACE2 cassette into the Rosa26 locus of its genome (Rosa26-hACE2). Rosa26-hACE2 mice exhibit reduced mortality (~60%) at 10^4 PFU of SARS-CoV-2 compared to 100% mortality in K18-hACE2 mice and show decreased viral titer in the lung/brain at 10^5 PFU. Several pathological differences between Rosa26 and K18-hACE2 mice are observed: in the lung, Rosa26-hACE2 mice show greater amounts of macrophage/T-cell infiltrate; in the brain, viral replication is geographically restricted to the ventral portions of the brain. Pro-inflammatory ISG profiles are observed through differential gene expression, reflective of distinctive viral dissemination in these mouse strains. Residual histopathology is seen in Rosa26-hACE2 mice at ~1 month-post infection: in the lung, interstitial extracellular stromal matrix deposition with alveolar type 2 (AT2) hyperplasia, and lymphohistiocytic infiltrate with formation of lymphoid aggregates. Lymphocytic infiltrate is present in in the brain where viral antigen was detected during acute disease plus the leptomeninges, choroid plexus, and perivascular spaces. Altogether, our work highlights the importance of gene insertion location/copy number on outcome of transgenic mice and the potential translational relevance of Rosa26-hACE2 mice for characterizing chronic neurological and pulmonary disease seen in long COVID.

E16: TRYPANOSOMA BRUCEI INVADES THE HEART AND CAUSES MYOCARDITIS IN A MURINE MODEL OF AFRICAN TRYPANOSOMIASIS

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Background: *Trypanosoma brucei* is an extracellular kinetoplastid parasite that causes human and animal African trypanosomiases (HAT and AAT). In mammals, *T. brucei* inhabits both hemolymphatics and interstitial tissue spaces, including in the heart. Despite evidence of cardiac disease in HAT and AAT patients, research into host-pathogen interactions in the heart has been limited.

Objective: We used a murine model of African trypanosomiasis to investigate cardiac host-parasite interactions.

Methods: C57Bl/6J mice were infected intravenously with *T. brucei brucei* parasites. On 14 and 28 days postinfection, mice were sacrificed. Plasma NT-proBNP was measured as a biomarker of heart function. Histopathology and immunofluorescence were used to demonstrate extravascular parasites and evaluate myocarditis. Host and parasite gene expression were evaluated via RNA-seq.

Results: NT-proBNP levels were elevated in infected mice, consistent with cardiac dysfunction. By 14 days postinfection, immunofluorescence and histopathology demonstrated numerous parasites occupying extravascular cardiac spaces with

associated mononuclear inflammation. RNA-seq of host transcripts showed that the cardiac immune response was characterized by increased CD8+ T lymphocytes, suggesting that the cardiac host response is polarized toward a type 1 immune response. Compared to bloodstream parasites, intracardiac parasites differentially expressed genes involved in motility, cell cycle, metabolism, and stress responses, suggesting adaptation to the cardiac environment.

Conclusions: Our findings demonstrate that *T. brucei* parasites invade the heart, where they are associated with inflammation and cardiac dysfunction. Research is ongoing to better characterize cardiac dysfunction and investigate host-parasite interactions in the heart which could contribute to parasite invasion and survival.

E17: MURINE MODEL OF THERAPEUTIC INFLUENZA B NEURAMINIDASE MONOCLONAL ANTIBODIES

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Influenza Type B viruses (IBV) cause up to 25% of annual influenza cases, leading to severe respiratory disease in vulnerable populations, primarily pediatric and geriatric individuals. Currently, the only FDA-approved antivirals used to treat influenza are neuraminidase inhibitors, such as zanamivir, which are less effective against IBV than against influenza type A viruses (IAV). Influenza virions express two major surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), the latter of which is of interest due to the lack of current literature concerning its role in humoral immunity. Monoclonal antibodies (mAbs) directed at NA provide an alternative therapeutic candidate to treat IBV. Here we describe mAb FluB-393 and FluB-400, both NA-directed mAbs isolated from a human donor who received the quadrivalent influenza vaccine. Preliminary *in vitro* characterization led to selecting these two mAbs as *in vivo* therapeutic candidates. In this study, we developed an *in vivo* murine model in 8-week-old female BALB/c mice. Mice were administered a 10mg/kg dose of mAb intraperitoneally 18 hours before intranasal challenge with $1x10^5$ PFU/mouse of B/New York/PV01181/2018. Daily weights were measured, with the humane endpoint determined at 80% weight loss. Lungs, trachea, and nasal turbinates were collected at 3- and 6-days post-infection for viral plaque and RT-PCR quantification. Lung tissues were also collected for histopathologic characterization and IHC. This animal model provides a robust capacity to characterize potently neutralizing, cross-reactive IBV antibodies, which in turn supports developing mAbs as preclinical candidates to treat IBV infection and further informs NA-directed vaccine design.

E18: ASSESSMENT OF THE IMPACT OF MOUSE KIDNEY PARVOVIRUS INFECTION ON THE PHARMACOKINETICS OF RENALLY EXCRETED DRUGS AND ON THE ADENINE DIET MODEL OF CHRONIC KIDNEY DISEASE

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Background: Mouse kidney parvovirus (MKPV) was recently discovered and determined to cause murine inclusion body nephropathy. While MKPV causes clinical signs and severe renal lesions in immunodeficient mice, the lesions are mild and subclinical in immunocompetent mice. MKPV's impact on specific murine research models requires investigation.

Objective: To characterize the effect of MKPV on the pharmacokinetics (PK) of renally excreted drugs and a model of chronic kidney disease (CKD).

Methods: C57BL/6NCrl and NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ mice were inoculated with MKPV or sterile PBS. At 14 weeks post-inoculation (PI), methotrexate or lenalidomide was administered intravenously (12 mice per drug, strain, and infection status). Plasma and urine drug concentrations were measured at 8 time points and non-compartmental PK and renal clearance parameters were calculated. For modeling CKD, C57BL/6NCrl mice (30 mice per infection status) received a diet containing 0.2% adenine starting at 15 weeks PI for a period of up to 8 weeks. Blood and urine renal biomarkers and kidney histopathology were assessed 2, 4, and 8 weeks after initiation of adenine diet.

Results: In both strains, MKPV status was associated with differences (1.5 to 1.9-fold) in some non-compartmental PK parameters for methotrexate but not lenalidomide. Renal clearance was not impacted for either drug. In the CKD model, MKPV had no effect on blood and urine biomarkers. Infection was associated with increased lymphoplasmacytic interstitial infiltrates and decreased interstitial fibrosis, but had no impact on tubular degeneration, mineralization and histiocytic interstitial infiltrates.

Conclusion: MKPV infection may affect research outcome in mouse models.

E19: EVALUATION OF TFR-1 EXPRESSION IN CANINE MAMMARY TUMORS AND IN VITRO TESTING OF A FERRITIN DOXORUBICIN-LOADED NANOCAGE

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Background: ferritin (HFn) binds to the transferrin receptor 1 (TFR-1), which is overexpressed in several cancers. In human cancer research, this binding has been studied for targeted anticancer therapies for years. In veterinary medicine, only few studies have evaluated the expression of TFR-1 and its role in cancer therapy.

Objective: to evaluate TFR-1 expression in tissues and cell lines of canine mammary gland tumors and to test the efficacy of an engineered ferritin nanocage loaded with doxorubicin (HFn(DOX)) on canine mammary tumor cell lines.

Methods: immunohistochemistry was used to evaluate TFR-1 expression in 40 canine mammary tissue samples. Western blot, immunofluorescence, immunocytochemistry, and qPCR were employed to assess protein and gene expression of TFR-1 in primary (CIPp) and metastatic (CIPm) canine mammary tumor cell lines. The effect of HFn(DOX) and of free doxorubicin on cell viability was evaluated after 24, 48, and 72 hours using an MTS assay.

Results: TFR-1 expression was significantly higher in malignant mammary tumor tissues compared to paired hyperplastic tissues. Despite qPCR showed a higher expression of TFR-1 in CIPm than in CIPp, HFn(DOX) treatment reduced cell proliferation more efficiently than free doxorubicin only in CIPp at high concentrations after 48 hours.

Conclusions: We demonstrated the higher expression of TFR-1 in canine mammary malignancies and preliminary tested *in vitro* the efficacy of nanocaged doxorubicin in the treatment of mammary tumor. Further studies are needed to better evaluate the efficacy of HFn(DOX) in more heterogenous canine mammary tumor cell lines.

E20: POST-MORTEM STUDY ON THE EFFECTS OF ROUTINE HANDLING AND MANIPULATION OF LABORATORY MICE

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Background: Routine handling and manipulation of laboratory mice are an integral component of most preclinical studies. Any type of handling and manipulation may cause stress and result in physical harm to mice, potentially leading to unintended consequences on experimental outcomes. Nevertheless, the pathological effects of these interventions are poorly documented and assumed to have a negligible effect on experimental variables.

Objective: In that context, we provide a comprehensive post-mortem overview of the main pathological changes associated with routine interventions (i.e., restraint, blood drawing, and intraperitoneal injections) of laboratory mice with an emphasis on presumed traumatic osteoarticular lesions.

Methods: A total of 1000 mice from various studies were included, with 864 animals being heavily manipulated and 136 unmanipulated or being handled for routine husbandry procedures only.

Results: The most common lesions observed were associated with blood collection or intraperitoneal injections, as well as a series of traumatic osteoarticular lesions likely resulting from restraint. Osteoarticular lesions were found in 62 animals (61 heavily manipulated; 1 unmanipulated) with rib fractures and avulsion of the dens of the axis being over-represented. Histopathology and micro-CT confirmed the traumatic nature of the rib fractures.

Conclusion: While these lesions might be unavoidable if mice are manipulated according with the current standards, intentional training of research personnel on appropriate mouse handling and restraint techniques could help reduce their frequency and the impact on animal wellbeing as well as study reproducibility.

E21: FETAL DISEASE PHENOTYPE OF A SHEEP MODEL OF CYSTIC FIBROSIS

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Cystic fibrosis (CF) is a genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. Lung pathology in CF patients is primarily a postnatal phenotype; however, CF pathology is often evident prenatally in other organ systems, particularly within the digestive system. We used an ovine model of CF disease to investigate fetal disease progression, since the sheep closely mirrors critical aspects of human development. Two wildtype (WT) and four *CFTR*$^{-/-}$ sheep fetuses were collected at 50, 65, 80, 100, and 120 days of gestation and at term (147 days). Necropsy and histopathology were used to document the morphologic changes. Tracheal samples were collected for electrophysiological analysis. The earliest gross and microscopic lesions in *CFTR*$^{-/-}$ animals were detected by 80 days of gestation, equivalent to 21 weeks in human. Pancreatic acinar dilation with mucus accumulation, hepatic portal ductular reaction and fibrosis, intrahepatic cholestasis, and intestinal meconium obstruction were observed from day 80 of gestation to term. Concurrently, CFTR-channel activity was evaluated in a Ussing chamber and CFTR-dependent short circuit current in tracheal epithelium was present by 80 days gestation in WT trachea but absent in *CFTR*$^{-/-}$ trachea. *CFTR*$^{-/-}$ sheep fetuses show lesions similar to human newborn, although with a severe phenotype. Nonetheless, the sheep model of CF disease can be used to identify the initiating molecular events in fetal organs with CF-associated pathology, and to study the effect on fetuses of modulator therapies prescribed to women with CF during gestation.

E22: ASSESSMENT OF MOUSE URINARY TRACT PATHOLOGY AND PATHOPHYSIOLOGY DURING EXPERIMENTAL UROPATHOGENIC ESCHERICHIA COLI INFECTION

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Background & Objectives: Urinary tract infections (UTI) are one of the most common infections across species. Various mouse models are utilized to study UTI and many studies use bacterial burden as a predictor of outcome. We aimed to determine if urinary tract bacterial burden correlates with pathology and pathophysiology during UTI.

Methods: Three common UTI model mouse strains (C57BL/6J, C3H/HeOuJ, and C3H/HeN) were infected transurethrally with uropathogenic *Escherichia coli* for 6, 24, 48 hours, 1 week, and 4 weeks. Urine and kidney bacterial burden was enumerated on LB agar. Urine pro-inflammatory markers were assessed via MesoScale Diagnostic immunoassays. The contralateral kidney and bladder were fixed in paraformaldehyde and routinely processed for histopathology. Slides were scored utilizing an established scoring system and a novel system using pertinent histopathologic features.

Results: C3H/HeN and C57Bl/6J mice had acute bladder pathology 6 hours post infection (HPI) with moderate urine bacterial burden. In contrast, C3H/HeOuJ mice had delayed but persistent bladder pathology with higher bacterial burden. Peak bladder pathology scores correlated to significant IL-6, CXCL1, and TNF-α expression in the urine in C57Bl/6J and C3H/HeOuJ mice. Bladder pathology had a fair to moderate correlation to bacterial burden in all strains. C3H/HeN and C3H/HeOuJ mice had moderate to severe kidney pathology, respectively, within 24 HPI which had a moderate correlation to urine burden. C57Bl/6J lacked kidney bacterial burden and pathology.

Conclusions: C3H/HeN and C57Bl6/J mice adequately model acute bacterial cystitis while C3H/HeOuJ have a delayed innate immune response which predisposes to persistent cystitis and pyelonephritis.

E23: PATHOGENESIS OF AQUATIC BIRD BORNAVIRUS-1 (ABBV-1) IN CANADA GEESE

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Background: Aquatic bird bornavirus-1 (ABBV-1) is highly prevalent in wild waterfowl in North America, causing persistent infection of the nervous system. We have recently demonstrated that ABBV-1 can infect poultry species (Pekin ducks, Muscovy ducks, and chickens).

Objective: Investigate the pathogenesis of ABBV-1 in Canada geese, which is hypothesized to be the natural reservoir of this virus.

Methods: Week-old goslings (n=66) were divided into three groups and inoculated with ABBV-1 through one of two routes: intramuscular (IM) and cloacal (CL) ($1.09x10^5$ FFUs/bird). Controls received carrier only. At 1, 8, and 15 weeks post-infection (wpi), 6-9 birds from each group were scheduled to be euthanized, with 3 birds collected for full histopathological analysis. Additional six goslings (week-old) were placed in each group as sentinels and were scheduled to be euthanized at the last sampling point (15 wpi).

Results: No birds showed neurological signs. Infection of the brain and/or spinal cord was detected (RT-qPCR) in IM birds at 8 (3/7) and 15 wpi (7/9), and 1/7 CL bird was positive by 8 wpi. Peripheral tissues (proventriculus, kidneys, and gonads) were positive in 57% (4/9) of IM birds at 15 wpi. Encephalitis/myelitis were identified in 3/3 and 1/3 of the IM birds at 8 and 15 wpi, respectively, and 1/3 CL bird at 8 wpi. All control and sentinel birds were negative.

Conclusions: Intramuscular inoculation of ABBV-1 in Canada geese results in infection, with centripetal and centrifugal spread and systemic virus distribution. Cloacal instillation proved to be an inefficient method of infection.

E24: HOW DO OBESITY AND WEIGHT LOSS ALTER THE MAMMARY GLAND MICROENVIRONMENT?

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Obesity is an epidemic in the United States, with two thirds of women diagnosed as overweight or obese. Obesity is associated with increased breast cancer risk, and it is recommended that obese women lose weight to reduce their risk of cancer. However, the impact of weight gain and loss on the mammary gland is not well understood. Herein, we use a murine model to investigate this phenomenon. We predicted that weight loss would reduce tissue fibrosis and immune cell recruitment to the mammary gland. Mice were fed a low-fat diet, high-fat diet for 16 weeks to induce obesity, or high-fat diet followed by low-fat diet for 5 weeks to induce weight loss. Picrosirius red staining, to label collagen, was coupled with immunohistochemistry and flow cytometry to monitor changes in the mammary gland microenvironment. Collagen deposition and macrophage recruitment were increased in mammary glands of obese mice relative to lean mice, suggesting that obesity contributes to enhanced fibrosis and inflammation. Consistent with increased macrophage recruitment, myeloid progenitor cells were increased in bone marrow of obese mice. Weight loss resulted in reduced macrophage recruitment in mammary adipose tissue and reduced bone marrow myeloid progenitor cells but did not reduce periductal fibrosis. These data suggest that while weight loss may resolve some mammary gland inflammation, it may not reduce all pro-tumorigenic properties of the obese mammary gland. Further exploration of the relationship between

weight loss and inflammation will be integral to improving our understanding of obesity's contributions to breast cancer development and progression.

E26: PSEUDOMONAS AERUGINOSA VOLATILE ORGANIC COMPOUNDS CAUSE MUCUS HYPERSECRETION BY ACTIVATING AHR SIGNALING IN CHRONICALLY DISEASED LUNGS

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Background: *P. aeruginosa* is a major bacterial pathogen infecting chronically diseased human lungs, including cystic fibrosis and chronic obstructive pulmonary disease. Major pathological features include dysregulated airway goblet cell metaplasia, mucus hypersecretion, and failure in clearance. *P. aeruginosa* is metabolically versatile and secretes several secondary metabolites, many of which, display host modulatory effects and contribute to disease pathogenesis. Volatile organic compounds (VOCs) produced by *P. aeruginosa* are detectable in human breath, serving as biomarkers for diagnosis of disease exacerbation in diseased lungs. However, the roles of VOCs in the airway mucus dysregulation is unknown. The aryl hydrocarbon receptor (AhR) has recently emerged as a regulator of mucosal barrier function.

Objective: We hypothesize the VOCs may activate AhR to drive goblet cell differentiation and proliferation, resulting in excessive mucus in diseased lungs.

Methods: We have investigated the impact of several dominant VOCs present in the breath of chronically diseased patients on the goblet cell metaplasia and expression of major mucus glycoproteins MUC5AC and MUC5B using the air-liquid interface 3D culture of 16HBE human bronchial epithelial cells, and in a mouse model of chronic VOCs exposure.

Results: VOCs significantly increase the expression of MUC5B and MUC5AC, while simultaneously down-regulate FOXA2, a key regulator of airway mucus homeostasis. VOCs elevate AhR expression, and pretreatment with AhR inhibitor further attenuates MUC5AC production and restores FOXA2 expression.

Conclusions: The present findings herald the significance of *P. aeruginosa* VOCs on the disruption of mucus homeostasis and the potential pathogenic role of AhR in chronically diseased lungs.

E27: DEVELOPMENT OF MULTIPLEX IMMUNOFLUORESCENT PANELS FOR STUDYING THE IMMUNOBIOLOGY OF METASTATIC COLORECTAL CANCER AND MINIMAL RESIDUAL DISEASE

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Colorectal cancer is the 2nd leading cause of cancer death in people in the U.S. The 5-year survival rate in patients with distant metastasis is a dismal 22%, and the most common site for distant metastasis is the liver, followed by lung and peritoneum. Colorectal cancer progression and metastasis is influenced by the complex interplay between tumor intrinsic factors such as microsatellite instability and characteristics of the tumor microenvironment such as infiltration of cytotoxic and regulatory T cells as well as macrophage polarization. Murine tumor modeling approaches that account for the inherent heterogeneity in immunogenicity of CRC cells as well as variability in the tumor microenvironment and immune function are critical for understanding the process of CRC immune surveillance evasion, escape and immunoediting. We evaluated immune infiltration in syngeneic, murine CRC tumors and found baseline differences in T cell and macrophage infiltration between models. We developed 6-marker, multiplex immunofluorescence (mIF) panels for characterizing immune cell subsets in primary colorectal tumors and micrometastases in orthotopic and experimental metastasis models of CRC in mice. mIF allows for more refined immune cell subset characterization than traditional IHC and important spatial resolution not possible with other classic techniques such as flow cytometry. Advancing current understanding of the immunobiology of metastatic CRC and minimal residual disease (MRD) will lead to improved therapeutic strategies that enhance anti-tumor immune responses for patients with distant metastasis for improved patient outcomes.

E28: DETECTION OF CYTAUXZOON FELIS IN SALIVARY GLANDS OF AMBLYOMMA AMERICANUM

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Cytauxzoon felis is a tick-borne piroplasmid hemoparasite that causes life-threatening disease in cats. Despite the critical role that ticks play in disease transmission, our knowledge regarding the *C. felis* life cycle remains limited to the feline hosts and no stage of the parasite has been identified or investigated in ticks. In other tick-borne piroplasmids, sporozoites have played a key role in disease prevention and management. We believe sporozoites have similar potential for cytauxzoonosis. Therefore, the objective of this study is to evaluate different molecular and microscopic techniques to detect *C. felis* sporozoites in tick salivary glands (SG). A total of 140 *C. felis*-infected *A. americanum* ticks were included for this study. Dissected SGs were quartered and subjected to *C. felis* RT-PCR, RNAscope® *in situ* hybridization (ISH), histology, direct azure staining, and transmission electron microscopy (TEM). *Cytauxzoon felis* RT-PCR was also performed on half tick (HT) carcasses after SG dissection. *Cytauxzoon felis* RNA was detected in SGs of 17 ticks. Of these, 7 ticks had microscopic visualization via ISH and/or TEM and 10 ticks had only molecular detection of *C. felis* in SGs via RT-PCR without visualization. *Cytauxzoon felis* RNA was detected

solely in HT via RT-PCR in 9 additional ticks. TEM captured rare *C. felis* organisms with characteristic ultrastructural features of piroplasmid parasites. This study describes the first direct visualization of any developing stage of *C. felis* in ticks. Forthcoming studies should employ a combination of molecular and microscopic techniques to investigate the *C. felis* life cycle in *A. americanum.*

E29: CHARACTERIZATION OF HOST IMMUNE CELL INFILTRATE IN CAR T-CELL MEDIATED GRAFT VERSUS HOST DISEASE IN A MOUSE MODEL.

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Background: Chimeric antigen receptor (CAR) T cells are a revolutionary cancer therapy technology that is FDA-approved for several hematologic malignancies and under investigation for a wide variety of solid tumors. As this modality becomes more widely employed, the use of allogeneic T cells provides several benefits over autologous transplantation, such as wider availability and decreased cost. However, allogeneic CAR T cells carry increased risk of graft versus host disease (GvHD), which has been observed in human patients as well as mouse models.

Objective: To further understand the mechanism of CAR T cell-mediated GvHD.

Methods: We performed an immunohistochemical characterization of the murine immune cell infiltrate in GvHD lesions in a cohort of NSG mice that received human CAR T cells. CAR T cells were second generation and were targeted against a human tumor-specific antigen without a murine homolog.

Results: A median of 40 percent (range 10-95 percent, interquartile range 30-60 percent) of the immune cell infiltrate in the GvHD lesions is composed of murine cells. The majority of these murine cells are AIF1-positive histiocytes and many are CD11c-positive, implying a prevalent dendritic cell component. Preliminary investigation indicates type 2 polarization of infiltrating macrophages. Finally, there is a negative correlation between the magnitude of the murine immune cell infiltrate and the GvHD severity.

Conclusions: These findings suggest that the composition and activation status of the recipient immune cell infiltrate within GvHD lesions may affect lesion development and severity.

Industrial and Toxicologic Pathology Focused Scientific Session

DIETARY EXPOSURE OF JAPANESE QUAIL (COTURNIX JAPONICA) TO PERFLUOROOCTANE SULFONATE (PFOS) AND A LEGACY AQUEOUS FILM FORMING FOAM CONTAINING PFOS: EFFECTS ON TARGETED ORGAN SYSTEMS, REPRODUCTION, AND CHICK SURVIVABILITY AND GROWTH

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Effects of perfluorooctane sulfonate (PFOS) and a legacy aqueous film forming foam containing 91% PFOS (AFFF PFOS) on liver and kidneys, reproduction, chick survivability and growth of Japanese quail (*Coturnix japonica*) were determined. Briefly, 324 day-old Japanese quail were administered PFOS or AFFF PFOS at 6 dietary concentrations ranging from 0 to 21 mg kg^{-1} feed for a total of 20 wk, which became the parent generation. Eggs were collected daily, set weekly and incubated for 18 d. Hatchlings were fed uncontaminated feed for 2 wk, euthanized to collect blood and liver. After 10 wk of egg collection, adults were euthanized to collect blood, liver and kidneys. All samples were processed by standard paraffin block embedding and H and E staining. Immunohistochemistry for smooth muscle actin (SMA) (Cell Marque) was performed using an automated stainer (Bond III) for a subsample of livers and kidneys across the dosage range to confirm presence of SMA expression in mesenchymal cell populations. Significantly increased SMA differentiation occurred in hepatic stellate cells with myofibroblastic activation and glomerular mesangial cells undergoing sclerosis. PFOS or AFFF PFOS did not significantly affect egg production but hatchability was decreased at the greatest PFOS dose. The no observed adverse effect levels for chick survivability, considered the critical effect, were 4.1 mg PFOS kg $feed^{-1}$ (0.55 mg kg body $weight^{-1}d^{-1}$) and 5.0 mg AFFF PFOS kg $feed^{-1}$ (0.66 mg kg body $weight^{-1}d^{-1}$) resulting in calculated average toxicity reference values of 0.25 mg kg $feed^{-1}$ and 0.034 mg kg $bw^{-1}d^{-1}$.

EVALUATION OF GENE TARGETED EDITING TO DISABLE THE ONCOGENIC RETROVIRUS HTLV-1 USING IN VITRO CELLULAR SCREENING AND IN VIVO NOG MICE MODELS

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Background: Human T-cell leukemia virus type 1 (HTLV-1) is the retroviral etiologic agent of both adult T-cell leukemia/lymphoma (ATL) and a progressive chronic neurodegenerative disease. HTLV-1 encodes two genes, *Tax* and *Hbz*, that are

essential for transformation, proliferation, and disease pathogenesis. Given that HTLV-1 persists in infected hosts through mitotic host cell division and the viral genome is highly conserved, genomic editing has strong potential as a treatment option for HTLV-1-mediated diseases.

Objective: This study examines the effectiveness of clustered regularly interspersed short palindromic repeat (CRISPR)/Cas9 genome editing for disabling HTLV-1 and will further inform genome editing strategies for HTLV-1 treatment.

Methods: We constructed a library of 163 gRNAs covering the Tax, HBZ, and viral long terminal repeats (LTRs) coding regions. These gRNAs were sub-cloned into a CRISPR lentiviral vector which expressed the *Cas9* gene and a puromycin resistance gene. VSV-G pseudotyped lentivirus was produced and transduced into HTLV-1-infected T-cell lines. Following puromycin selection, the cellular proliferation rate was analyzed by MTS assay. *Tax*, *hbz*, and *gag* gene expression was measured in each CRISPR-edited cell line.

Results: Our results suggest numerous gRNAs targeting *Tax*, LTR, and/or *Hbz* significantly decreased proliferation of HTLV-1-infected cells. Of these gRNAs, a total of 37 and 17 affected *tax*, *hbz,* or *gag* gene expression in HTLV-1 transformed and ATL-derived cells, respectively.

Conclusion: After off-target analysis, the top five gRNA candidates per viral gene/LTR will be selected for NSG sequencing and applied in our *in vivo* transplantation NOG mouse model.

COLOR CORRECTION IN WHOLE SLIDE IMAGING

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Introduction: Accurate color reproduction from histological slides by whole slide imaging scanners is essential for digital histopathological analysis. However, inconsistent optical and hardware characteristics of the scanners may result in distorted color measurement and inter-scanner color variation in the images. To address these issues, we developed a color correction method for color calibration and normalization of the images from different scanners.

Methods: Our color correction method includes two modules: deriving color correction models and monitoring the color variability of the scanners for updating the models over time.

First, a color calibration slide with standardized color patches and target specification was scanned by three in-house Hamamatsu scanners. Mathematical models were derived to minimize the distances between the measured and target color in the XYZ color space. The models were then applied to the scanned images to generate color-

corrected images, and the calibration performance was evaluated using the CIEDE2000 formula.

Second, to monitor the color variability, a color calibration slide and two H&E stained slides have been scanned once a week for nine months. The variation of chromaticity and luminance were analyzed over time.

Results: Our color correction method improves the color accuracy by 64.3% on average. The variation of chromaticity is 0.1% and luminance is 5% on average, showing negligible color variation over the period evaluated.

Conclusion: We developed a color correction method generating whole-slide images with more accurate color and improved contrast, which enhanced the pathologist viewing experience and the reliability of tissue features in histopathological images.

COMPARISON OF ACUTE DEHYDROPYRROLIZIDINE ALKALOID TOXICOSIS IN C57BL MICE GAVAGED WITH RIDDELLIINE, RIDDELLIINE N-OXIDE, SENECIONINE, SENECIONINE N-OXIDE, SENECIPHYLLINE, LASIOCARPINE OR HELIOTRINE.

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Background: Dehydro-pyrrolizidine alkaloids (DHPAs) are plant-derived toxins that frequently affect livestock and humans. At least 600 individual compounds are documented. They have similar chemical structures but vary in toxic potential.

Objective: To compare microscopic lesions and pyrrole concentrations in acute pyrrolizidine alkaloid poisoning cause by 5 DHPAs and 2 DHPA N-oxides in mice.

Methods: Male C57BL/6J mice were exposed by oral gavage to five DHPAs and two DHPA N-oxides for ten days. Three mice per dose and 24 mice per compound were used. Hepatic necrosis severity scores; serum ALT, ALP, and AST concentrations; and hepatic pyrrole concentrations were compared.

Results: Riddelliine and riddelliine N-oxide caused hepatocellular enlargement and necrosis in centrilobular areas at low doses progressing to panlobular hepatocellular enlargement with multifocal necrosis as dose increased. Senecionine and senecionine N-oxide exposure resulted in similar lesion but initially affected midzonal and periportal areas. Seneciphylline exposure also resulted in similar lesions but in midzonal areas at lower doses. Lasiocarpine caused periportal hepatocyte enlargement with individual hepatocellular necrosis at the highest dose. Heliotrine cause centrilobular hepatocyte enlargement without necrosis at the highest dose.

Conclusions: Liver enzymes and tissue bound pyrroles concentrations, and hepatic necrosis scores had strong correlations with dose for all compounds except heliotrine

and lasiocarpine. Contrary to previous reports, lesion severity was similar between the DHPA N-oxides and their parent compounds at comparable doses. Multiple sources state that acute pyrrolizidine alkaloid poisoning causes centrilobular hepatic necrosis, however in this study we demonstrate that the lobular area initially affected varies between compounds.

USE OF TRANSFER LEARNING IN DEVELOPMENT OF AN AI-BASED ALGORITHM FOR LESION DETECTION IN MOUSE LUNGS

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Background: The evaluation of lung tissue by toxicologic pathologists is an important component of xenobiotic safety assessment but can be time-intensive and subject to interobserver variability. The use of deep learning artificial intelligence (DLAI) has potential to improve workflow efficiency and quantitative and objective assessment of tissues by providing diagnostic decision support. However, supervised DLAI methods are labor intensive. Transfer learning allows DLAI to apply prior knowledge to a new but similar task to accelerate model development.

Objective: The development of a highly sensitive and specific DLAI mouse lung lesion decision support algorithm from a similar, successfully trained rat algorithm.

Methods: Whole slide images containing lung from control and treated mice were scanned at 40x magnification and uploaded to a Patholytix Study Browser platform. A successfully trained DLAI rat algorithm that detected four normal and three abnormal classes was applied to mouse slides. Four additional training runs with approximately 200 new annotations each were performed. The classifier was qualified at the pixel level using confusion matrices and F1 scores on 15% of the annotated data that was reserved for blinded validation.

Results: The DLAI algorithm identified normal pulmonary structures (alveolus, bronchiole/bronchus, BALT, interstitium) with 95% to 99% accuracy. The model accurately identified the three abnormal classes (atelectasis, alveolar macrophage aggregates, perivascular/alveolar mixed cell infiltrates) with a 98-99% accuracy. All class F1 scores exceeded the minimal acceptable performance level of 0.70.

Conclusion: Transfer-learning was successfully used to develop a highly accurate DLAI algorithm for detection of common pulmonary lesions in mice.

DEVELOPMENT OF A NOVEL AI-BASED ALGORITHM FOR VIRTUAL HEMATOXYLIN AND EOSIN STAINING OF RAT BRAIN

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Background: Conventional staining procedures are prone to pre-analytical variability, have workplace safety and environmental liabilities, and are not conducive to single section-labeling for multiple biomarkers.

Objective: The development of a deep learning AI algorithm that will produce a virtual HE stain on rat brain tissue that is concordant with conventional HE findings and supports improvements in sustainability and quality.

Methods: Whole slide autofluorescence images of unstained cover-slipped brain from control and rats administered test item were scanned at 40x equivalent magnification. The images were uploaded to a cloud-based virtual staining platform. The platform's core is a supervised image translation network, based on generative adversarial network. This network inferred a virtual HE stain on the unlabeled slide image. The results were viewed on the Canvas slide viewer. In a single pathologist verification study, a cohort of virtual slides were first presented for diagnostic review and quality assessment. Following this review the conventional H&E results were unblinded for the pathologist to compare side-by-side.

Results: The results were 100% diagnostically concordant between virtual and conventional H&E. In the matched slide review, the pathologist noted five distinctions which were not diagnostically significant; including a shift of color balance of the pineal gland. At the same time, the overall color balance of the virtual stains was preferred over the conventional.

Conclusion: Virtual HE staining is concordant with conventional HE staining in the rat brain and drives consistency and sustainability.

Impact Statement: This work highlights the potential for virtual staining in the digital toxicologic pathologist's workflow.

Industrial and Toxicologic Pathology Posters

T1: A DEEP LEARNING CONVOLUTIONAL NEURAL NETWORK METHOD TO DETECT SKIN PATHOLOGY ASSOCIATED WITH DELAYED-TYPE HYPERSENSITIVITY REACTION IN MONKEY SKIN

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Background: The monkey is an important animal model in assessing skin injury after local administration of xenobiotics. Histologic scoring methods have been used in the evaluation of skin in both standard repeat dose toxicology studies and specialized models evaluating reactions such as delayed type hypersensitivity (DTH). These

scoring methods are qualitative, subjective, and prone to inter- and intra-study variability.

Objective: We hypothesized that a deep learning method using a convolutional neural network (CNN) would facilitate the detection and scoring of skin for toxicologic pathologists.

Methods: A CNN was trained to identify normal structures and four classes of lesions in monkey skin: epidermal hyperkeratosis/hyperplasia, epidermal erosion/ulceration, dermal/subcutaneous inflammation/infiltrate, and panniculus/muscle myodegeneration/necrosis. Hematoxylin and eosin-stained sections of skin biopsies from monkeys were digitally scanned at 20x and uploaded into a cloud server. Training annotations were completed based on a strict ground truth established by two trained scientists. Model performance was first assessed by comparing model masks with the original training annotations (verification step) using both visual confirmation and confusion matrix calculations.

Results: When the CNN performed at a F-value of at least 0.7, additional qualification was done using new samples from the original study (testing set) and samples from studies not used for training. The performance of the algorithm exceeded precision of 0.85, sensitivity of 0.8, and F-values of 0.87 on unseen slides from unseen studies.

Conclusion: Qualification data suggest that the CNN model will be an effective decision support tool for the pathologist.

T2: DETERMINING OPTIMAL EXPOSURE INTERVALS FOR NAPTHALENE-INDUCED AIRWAY FIBROSIS

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Some organic compound exposures are implicated in causing fixed airways disease and bronchiolar fibrosis in people and laboratory animals. Naphthalene is a semi-volatile organic compound that causes acute necrosis of airway epithelial cells. After repeated weekly intraperitoneal injections, exposed mice develop bronchiolar fibrosis. However, naphthalene-induced airway epithelial necrosis is largely eliminated with daily exposure, a phenomenon known as tolerance. This tolerance reportedly gradually decreases with time. Based on the potential for some persistent tolerance, we hypothesized that fewer naphthalene exposures and longer intervals between exposures would produce airway fibrosis comparable to the fibrosis produced with weekly injections. Naphthalene (200 mg/kg) in corn oil was injected intraperitoneally into male and female C57BL6/J mice under three different exposure patterns: 1) weekly for 15 total injections, 2) every other week for 8 total injections, 3) weekly for 2 injections and then every 3 weeks for a total of 6 injections. Mice were sacrificed 5 days after the final injection and airway fibrosis was evaluated in trichrome-stained sections of lung and semi-quantitatively scored by a

veterinary pathologist. Airway fibrosis developed in all naphthalene-exposed mice but not in unexposed controls. Fibrosis was comparable in males and females and between the different exposure patterns. Thus, under the conditions of this study, the number of naphthalene exposures needed to produce airway fibrosis can be reduced by increasing the interval between exposures.

T3: ADVERSE REPRODUCTIVE OUTCOMES IN PREGNANT AND LACTATING BITCHES EXPOSED TO AFLATOXINS

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Background: In December 2020, the FDA was alerted to the illness and death of dogs consuming aflatoxin-contaminated pet food products manufactured by Midwestern Pet Foods, Inc., resulting in a voluntary recall of those products. The hepatotoxicity and hepatic carcinogenicity of these particular mycotoxin contaminants, produced primarily by Aspergillus flavus and parasiticus, is well-known.

Objective: To describe the reproductive outcomes of pregnant and lactating bitches exposed to aflatoxins.

Subjects: A commercial dog breeding facility consisting of approximately 75 adult dogs was the site of most of the canine mortality and morbidity initially reported to FDA.

Results: Approximately 50% of the adult dogs at this facility died from aflatoxin-associated liver disease. In this clinical setting, the adverse reproductive effects of aflatoxins, including teratogenesis previously reported in other species, were evident. Fetal and neonatal survival and growth were dramatically impacted in the litters of pregnant and lactating bitches exposed to high concentrations of dietary aflatoxins. Abortion and/or neonatal deaths were noted in all of the approximately 20 litters delivered during the timeframe of exposure. Litters of puppies born to aflatoxin-exposed dams that survived to term expired within several days from birth. At 8 weeks of age, puppies nursing on aflatoxin-exposed dams were smaller than puppies nursing on unexposed dams.

Conclusions: Maternal and fetal malnutrition and other stressors possibly involving transfer of aflatoxins across the placenta and in mammary secretions are likely explanations for these observations.

T4: DEVELOPMENT OF A DEEP LEARNING ALGORITHM FOR PRECLINICAL HISTOPATHOLOGICAL EVALUATION OF GRAFT-VS-HOST DISEASE IN A HUMANIZED MOUSE MODEL

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Artificial intelligence (AI) enabled “Deep Learning” for image analysis is a more recent addition to the pathologists’ portfolio. Deep learning is an unsupervised information generation method that attempts to mimic the human brain by using large amounts of training data to cluster data and make predictions. Here, we developed a deep learning algorithm for the evaluation of H&E-stained mouse liver sections (as digitally scanned whole slide images) to evaluate microscopic features of graft-vs-host disease (GvHD). Based on a limited training set, and pathologist’s input for iterative refinement, the AI algorithm was able to detect livers with GvHD with excellent sensitivity and acceptable F1 scores as well as good correlation to the pathologist’s semi-quantitative pathology scores. Further refinement and credentialing across different studies is ongoing. As such, this algorithm will be an excellent screening aid to our research histopathology workflow. We demonstrate that by using AI many of the tasks that are manual and subjective can become more automated and standardized with increased workflow efficiency, accuracy, and reproducibility.

T5: CELLULAR DENSITY MAP OVERLAYS DERIVED FROM AI BASED NUCLEAR SEGMENTATION CAN BE USED TO IDENTIFY TOXICOLOGIC PATHOLOGY OUTCOMES SUCH AS LIVER HYPERTROPHY.

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Background: Reliable identification of liver hypertrophy within toxicologic pathology workflows, informed by subtle differences in individual hepatocyte size and extent, is challenging. Application of digital pathology tools can help with reliable identification of such lesions, enabling detection of their presence in an unsupervised fashion.

Methods: Whole slide H&E images containing representative lesions were reviewed within the Patholytix Study Browser. StarDist, a well established cell segmentation AI algorithm was applied to the images, resulting in nucleus detection. Features including area, shape, and coordinates of each cell were calculated at the point of detection, permitting derivation of cellular density metrics.

Results & Conclusions: Following validation of nuclear segmentation performance against manual counts, we developed a “Cell Density” overlay permitting visualization of changes in cellular density across the tissue. We then applied this cell density overview to example regions of either hypertrophy (i.e. a decrease in nuclear density within an area), or immune infiltrate (revealed by an increase in nuclear density within an area).

Impact Statement: Here we present a methodology for the visualization of changes in cellular density within tissues, which can provide a pathologist reviewer with a heatmap visualizable at low resolution that captures changes happening at the cellular level,

which would normally require higher magnification views to observe directly. This view can enable the toxicologic pathologist to more rapidly screen the tissues of interest, to highlight the presence of tissue abnormalities in such a way as to increase the speed of screening of tissues for the presence of such lesions.

T6: HISTOPATHOLOGY AS A RISK ASSESSMENT TOOL FOR GONADOTOXICITY IN HONEY BEE DRONES

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Background: The widespread use of pesticides in cropping has increased the contact between honeybees and agrochemicals. Some pesticides have been shown to have negative effects on the reproductive system of honeybee. Considering the potential for negative effects in the reproductive development of honeybee drones, improved methodology for pesticide risk assessment is necessary. In other animals, histopathology is the gold standard method used to evaluate safety of new compounds. Currently, there has been limited investigation of the reproductive histopathology of the honeybee drone.

Objective: The objective of this study is to develop histopathology of honeybee drones as a potential risk assessment tool.

Methods: We are developing an *in vitro* method of rearing drones and evaluating morphological development of their testes *in vitro*, in comparison to testes of drones reared *in vivo*. We are also investigating potential gonadotoxic positive control compounds that induce dose-responsive histopathologic effects on the developing drone testes.

Results: Our preliminary results suggest excellent larval survival of honeybee drones reared *in vitro*, with 92% of drones surviving to pupation. Both *in vitro*-reared drones and *in vivo* will be used to develop an atlas of testicular histology. We have also successfully developed a method for *in vitro* pupal exposure of honeybee drones to potential gonadotoxic positive control compounds and histopathology of the testes of treatment and control pupae is in progress.

Conclusions: In conclusion, this research will enable us to use histopathology for evaluation of gonadotoxicity in honeybee drones which may enhance pesticide risk assessment for honeybees in the future.

T7: EVALUATION OF GENE TARGETED EDITING TO DISABLE THE ONCOGENIC RETROVIRUS HTLV-1 USING IN VITRO CELLULAR SCREENING AND IN VIVO NOG MICE MODELS

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Background: Human T-cell leukemia virus type 1 (HTLV-1) is the retroviral etiologic agent of both adult T-cell leukemia/lymphoma (ATL) and a progressive chronic neurodegenerative disease. HTLV-1 encodes two genes, *Tax* and *Hbz*, that are essential for transformation, proliferation, and disease pathogenesis. Given that HTLV-1 persists in infected hosts through mitotic host cell division and the viral genome is highly conserved, genomic editing has strong potential as a treatment option for HTLV-1-mediated diseases.

Objective: This study examines the effectiveness of clustered regularly interspersed short palindromic repeat (CRISPR)/Cas9 genome editing for disabling HTLV-1 and will further inform genome editing strategies for HTLV-1 treatment.

Methods: We constructed a library of 163 gRNAs covering the Tax, HBZ, and viral long terminal repeats (LTRs) coding regions. These gRNAs were sub-cloned into a CRISPR lentiviral vector which expressed the *Cas9* gene and a puromycin resistance gene. VSV-G pseudotyped lentivirus was produced and transduced into HTLV-1-infected T-cell lines. Following puromycin selection, the cellular proliferation rate was analyzed by MTS assay. *Tax*, *hbz*, and *gag* gene expression was measured in each CRISPR-edited cell line.

Results: Our results suggest numerous gRNAs targeting *Tax*, LTR, and/or *Hbz* significantly decreased proliferation of HTLV-1-infected cells. Of these gRNAs, a total of 37 and 17 affected *tax*, *hbz,* or *gag* gene expression in HTLV-1 transformed and ATL-derived cells, respectively.

Conclusion: After off-target analysis, the top five gRNA candidates per viral gene/LTR will be selected for NSG sequencing and applied in our *in vivo* transplantation NOG mouse model.

Natural Disease Focused Scientific Sessions

MICRORNA PROFILING OF PRIMARY APPENDICULAR AND PULMONARY METASTATIC OSTEOSARCOMA TISSUE IN DOGS

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Background: Appendicular osteosarcoma is a common bone tumor of dogs with a poor outcome despite aggressive therapy. Although few dogs have clinically detectable metastases at diagnosis, 90% of them are euthanized due to progressive metastatic disease. Novel molecules to predict prognosis and/or act as therapeutic targets are necessary as current grading schemes and biomarkers are inadequate at determining prognosis. MicroRNAs are non-coding RNA molecules that regulate translation of messenger RNA and are dysregulated in cancer. Our lab previously found that microRNAs in plasma from canine osteosarcoma patients are predictive of clinical outcomes, including disease-free interval.

Objective: We hypothesize that primary appendicular osteosarcoma (priOSA), pulmonary metastases (metOSA), and normal lung tissue from dogs will have different microRNA profiles.

Methods: MicroRNA was isolated from 41 priOSA, 12 metOSA, and 5 normal lungs. Fifty-nine microRNAs were evaluated for each sample using QIAGEN MIRCURY LNA custom PCR arrays. The Ct values were compared using $2^{-DeltaDetItaCt}$.

Results: Seven microRNAs were upregulated and 4 were downregulated in priOSAs compared to metOSAs. miR-200c was expressed in 10/12 metOSAs but had no expression in 39/41 priOSAs. Nineteen microRNAs were upregulated and 8 were downregulated in metOSAs compared to normal lung. All metOSAs had a Ct < 32 for miR-9, while all lung tissues had a Ct >34, with 4/5 having no expression.

Conclusions: These findings demonstrate differential expression of microRNAs between primary and metastatic osteosarcoma. miR-200c and miR-9 should be further investigated for their role in disease progression and as potential therapeutic targets for metastatic disease.

A SYSTEMATIC REVIEW OF METHODS AND PROGNOSTIC VALUE OF MITOTIC ACTIVITY IN CANINE TUMORS

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Background: Mitotic activity is considered a relevant prognostic marker for many canine tumors.

Objective: To review methods and prognostic value of mitotic activity in canine tumors.

Methods: A systematic literature search of two databases (Pubmed and Scopus) was conducted in April 2022. Of 412 potential papers identified, 87 were eligible for the review. Additional papers (N=50) were identified through manual search.

Results: The 137 eligible papers examined 23 tumor types/groups, with single studies on 8 tumor types/groups. The mitotic count (MC, number of mitotic figures per area) was conducted in 125/137 papers (91%) and the mitotic index (mitotic figures per number of tumor cells) in 5 papers. The methods were not specified in 7 papers. MA was predominantly evaluated in areas of high density (N=35/137, 27%), and less commonly in random (N=15/137), peripheral (N=3/137), tumor-representative (N=2/137) or highly cellular areas (N=1/137; not reported in 81 papers). For the MC, the evaluated area size (in mm^2) was reported in 27/125 papers (22%), while most of the remaining studies enumerated mitotic figures in 10 HPFs (N=82/98, 84%). The authors of 88/137 studies (64%) indicated the MA correlated with outcome of their study population, while most tumor types/groups with multiple studies (N=11/15) had conflicting conclusions.

Conclusion: Several studies evaluate the prognostic value of mitotic activity in canine tumors. However, the descriptions of the MC methods are often incomplete and different outcome metrics and statistical tests used, which impedes reproducibility and comparability.

AMDOPARVOVIRUS IN FREE-RANGING SKUNKS (MEPHITIS MEPHITIS)

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Background: Skunks are ubiquitous in North America and poised to play an important role as reservoirs and spreaders of viral infections among wildlife and domestic animals. Skunk Amdoparvovirus (SKAV) has a high prevalence in North America and is recognized as a potential host-jumping pathogen. The basic natural history of the virus, while assumed to be similar to its closest phylogenetic relative Aleutian Mink Disease Virus (AMDV), is unknown. Compared to intensively housed, genetically-bottlenecked mink, skunk are ubiquitous and free ranging, and the potential impact of SKAV infections is unknown. We have analyzed potential routes of SKAV transmission, tissue tropism, and disease association.

Objectives: We characterized the tissue/cell target spectrum for SKAV and its disease(s) association in a cohort of 28 animals.

Methods: In a retrospective analysis, we used a combination of PCR and sequencing that was designed to distinguish SKAV from closely related amdoparvoviruses and confirm SKAV infection. Viral distribution in SKAV tissues and lesions was further analyzed by in situ hybridization (ISH).

Results: In a cohort of animals primarily submitted by California rehabilitation facilities, SKAV was detected in FFPE tissue in 24/28 animals tested. SKAV was associated with meningitis, chronic kidney disease, and myocarditis.

Conclusions: SKAV was prevalent among submitted, mostly neurologic skunks. Both transmission and persistence of infection are likely to occur via epitheliotropism (kidney, intestine, skin). Other cellular targets (endothelium, e.g.) were present in SKAV associated diseases, including kidney, heart, and brain disease, and were unique in the skunk compared to the well characterized diseases of AMDV.

AMDOPARVOVIRUS IN ZOO-HOUSED RED PANDAS (AILURUS SPP.)

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Background: Persistent Amdoparvovirus (APV) infections occur in many small carnivores, but except for the prototypical Aleutian Mink Disease virus (AMDV), APV-associated diseases are not well studied. We discovered an APV infecting ~50% of red pandas in US zoos, including healthy and morbid animals. There is a critical need to understand the consequences of Red Panda Amdoparvovirus (RPAV) infection in this endangered species, and a unique opportunity to study APV infections in a population under careful long-term management.

Objectives: Our aims are 1) to establish persistence of infection and quantification of viral load using a 6-year prospective evaluation of fecal shedding, and 2) to investigate whether RPAV causes disease through a retrospective analysis of viral detection and tissue distribution in necropsied red pandas.

Methods: We designed and validated a qPCR assay to establish viral load in 77 fecal samples collected from two animals over a 6-year span. In 43 necropsied, infected pandas from 4 zoos, we correlated viral distribution with lesions using PCR and in situ hybridization (ISH).

Results: RPAV was persistently shed (76/77 fecal samples) in two animals over a 6-year span. Among necropsied pandas, RPAV was associated with significant lesions including important causes of morbidity and mortality in zoo red pandas: myocarditis (2/2 cases), nephritis (14/21), and interstitial pneumonia (2/4). Additional manifestations include multisystemic pyogranulomatous lesions, oral/pharyngeal mucosal inflammation, and dermatitis.

Conclusions: RPAV is a persistent infection in red pandas that that can be carried asymptomatically, but in a subset of infected red pandas is associated with significant disease.

NATURALLY ACQUIRED EQUINE PARVOVIRUS-HEPATITIS IS ASSOCIATED WITH A WIDE RANGE OF HEPATIC LESIONS IN HORSES

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Background: Recent studies have provided strong evidence that equine parvovirus-hepatitis (EqPV-H) is the causative agent of Theiler's disease. Mild to moderate hepatitis has been described following experimental inoculations with EqPV-H. However, it is poorly understood whether naturally acquired EqPV-H is associated with other clinical cases of liver disease in horses.

Objective: The objective of this study was to examine the prevalence and severity of EqPV-H infections in diagnostic liver samples.

Methods: Banked liver samples (n=98) from 2007 to 2022 were obtained from Cornell University and University of California Davis. Samples were evaluated for 15 individual histologic features and *in situ* hybridization was performed to visualize EqPV-H nucleic acid (NA).

Results: EqPV-H NA was detected in 48% (n=47) of samples. The most common histologic features of EqPV-H-positive samples included individual hepatocyte necrosis (n=40, 85%), lobular infiltrates (n=38, 80%), portal infiltrates (n=35, 74.4%), and ductular reaction (n=33, 70%). While centrilobular necrosis was less common, and mainly associated with Theiler's disease cases, it was significantly associated with EqPV-H hybridization. EqPV-H NA was also found in cases with megalocytosis, karyomegaly, bridging fibrosis, and multinucleated cells. Interestingly, EqPV-H was observed in two cases of hepatitis in foals and one case of lymphoma in the liver. To the best of our knowledge, this is the first report of EqPV-H-associated hepatitis in foals.

Conclusions: This study demonstrates that naturally acquired EqPV-H is common in a variety of liver diseases and should be considered as a differential diagnosis in cases of hepatitis other than Theiler's disease.

DOCUMENTATION AND MODELING OF PATHOLOGICAL ASYMMETRIES AND LONGITUDINAL CERVICAL COMPRESSION IN CASES OF EQUINE CERVICAL OSTEOARTHRITIS (ECOA)

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Equine cervical osteoarthritis (ECOA) is a degenerative condition that can cause spinal cord compression. While the individual vertebral lesions have been described, specific causes of ECOA have not been documented. The purpose of this study was to confirm a recent increase in ECOA and determine whether the chronic mechanical forces that created the lesions could be identified. A review of 24 ECOA cases submitted to the

Athens Veterinary Diagnostic Laboratory between 2002-2018 confirmed an increase in the number of cases in the last 9 vs the first 8 years (p=0.006). Most were middle aged (12.5 + SD 7.6 years old) Warmblood (15/24) geldings (22/24). Degenerative lesions were most common in the caudal cervical vertebrae between C6-7, C5-6 and C7-T1. The increase in cases and Warmblood predominance is associated with flexed head positions becoming more common in dressage. To characterize the mechanical forces associated, cervical vertebrae from 3 affected horses (2 Warmbloods, 1 Quarter Horse) were boiled and articulated. When examined in context the vertebral lesions revealed marked asymmetry and deformation of facets and severe compression characterized by one vertebra embedded in another. An animated 3D equine skeletal computer model was used to model the neck postures indicated by the chronic lesions and identified extreme head positions and persistent transversal vertebral rotations as likely causes. Photos of one of the horses working were available and confirmed the predicted cervical hyperflexion. Our findings indicate veterinary pathologists can make significant contributions to the understanding of ECOA by putting lesions into a mechanical context.

NOVEL SIMPLEXVIRUS (Dolichotine alphaherpesvirus 1) OUTBREAK IN FOUR PATAGONIA MARA (Dolichotis patagonum) RESULTING IN NECROTIZING RHOMBOENCEPHALOMYELITIS AND ACUTE DEATH

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Background: A viral outbreak affecting 4 of seven Patagonian mara (*Dolichotis patagonium*) at a zoological institution in Phoenix, Arizona resulted in acute neurologic signs with progression to tetraparesis and death (4/4) over eleven days. All affected individuals were young adult, intact females (10 months to 5 years old). Clinical signs were rapidly progressive which resulted in two natural deaths and two euthanasias. Postmortem gross findings included multifocal pinpoint white-tan foci distributed randomly throughout the myocardium (3/4) and widespread hemorrhage affecting the skeletal muscle, spinal cord, pericardium, urinary bladder, and skin. Histologically, there was a necrohemorrhagic rhombencephalomyelitis (4/4) with intranuclear viral inclusions in neurons of the brainstem and spinal cord (3/4). Viral inclusions were large (20 µm) and basophilic. Histologic findings within the heart included varying degrees of myocardial necrosis with mineralization (4/4).

Objective: To isolate and characterize the suspected herpesvirus responsible for infection and death.

Methods: Electron microscopy (EM) and consensus polymerase chain reaction (PCR) with sequencing was performed on cerebellum, brainstem, and spinal cord.

Results: EM findings confirmed herpesviral replication and assembly complex in neurons and oligodendrocytes, however virus was not associated with myocardial necrosis. PCR isolated a novel *Simplexvirus,* named *Dolichotine alphaherpesvius-1 (DAHV-1).* The same tissues were negative for adenovirus and polyomavirus. PCR on myocardium and oronasal swabs were negative for DAHV-1. DAHV-1 had 84-86.8% homology to *Leporid alphaherpesvirus 4 (LHV-4).*

Conclusions: Further research is needed to discern whether this virus is endemic in Patagonian mara or whether they represent an aberrant host.

HISTOPATHOLOGIC FINDINGS FROM FOUR MANATEE MORTALITY EVENTS IN THE INDIAN RIVER LAGOON, FLORIDA

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Since 2010, four Unusual Mortality Events (UME) from environmental factors have occurred in Florida manatees (*Trichechus manatus latirostris*) from the northern Indian River Lagoon (IRL). The 2010 and 2011 UMEs were from unusually cold weather. Observations included acute hypothermia and chronic cold stress with skin lesions and emaciation. Histopathologic findings included epidermal necrosis with dermal vasculitis and heterophilic dermatitis, pancreatic acinar atrophy, lymphoid depletion, enterocolitis, and myocardial degeneration. The 2013 UME resulted from clostridiosis caused by dysbiosis when a dietary shift to macroalgae occurred after phytoplankton blooms led to seagrass loss. Manatees were in good nutritional state with multi-organ congestion; macroalgae were present in the gastrointestinal tract. Intestines were thickened and had mucosal blebs. Histopathologic findings in the intestine included necrosis, edema, hemorrhage, superficial and intramucosal Gram positive bacterial rods, and mucosa-associated lymphoid depletion. Immunohistochemistry was immunoreactive for *Paeniclostriium sordelli* and *Clostridium perfringens* as well as *Clostridioides difficile toxin A*. Seagrass and macroalgae declines continued leading to the next UME in late 2020 which is ongoing. The hallmark feature of this UME is starvation. Manatees have markedly decreased body condition, serous atrophy of fat and muscle, small livers, and occasional lung bullae and torsions. Histopathologic findings include pancreatic atrophy, serous atrophy of fat, moderate to marked hepatic atrophy with hepatocellular lipofuscinosis, and muscle (heart and skeletal) atrophy. While gross and histopathologic findings differ among these UMEs, the findings demonstrate the likely initial and then mounting health impacts observed over a decade of environmental decline, stressing the importance of habitat restoration

CLINICAL, PATHOLOGICAL, AND IMMUNOHISTOCHEMICAL FEATURES OF ECTOPIC ODONTOGENIC-LIKE TUMORS IN THE RABBIT CHEEK

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Background: Odontogenic tumors are thought to arise from the germinal tissues of the developing tooth germ, effectively restricting their anatomic origin to the jaw and soft tissues of the oral cavity.

Methods: Tumors with histologic features reminiscent of odontogenic neoplasia were identified in the cheek of six, pet rabbits ranging from 6-8 years old. Immunohistochemistry for pancytokeratin, CK5/6, CK14, and vimentin was performed on these cases, and on control rabbit tissues: skin, oral mucosa, cutaneous trichoblastoma, and jaw-associated ameloblastoma.

Results: None of the animals had clinical signs referable to the tumors or radiographic evidence of tooth or bone association. Histologically, the tumors were composed of odontogenic-like epithelium with peripheral ameloblast-like cells and central stellate reticulum-like cells and a small to moderate amount of associated pulp-like ectomesenchyme. Neoplastic epithelial cells had variable keratinization, intra-epithelial mineralization, and subepithelial Vickers-Gorlin effect. One case had severe anisokaryosis, numerous invasive islands, and bizarre mitotic figures; the remaining cases lacked microscopic features of malignancy. Neoplastic epithelial cells had a unique immunohistochemical profile that was not identified in the other neoplasms or control tissues: ameloblasts and stellate reticulum had diffuse, strong immunoreactivity to pancytokeratin (6/6 cases) and CK14 (5/5 cases); peripheral neoplastic cells (ameloblast-like) often lacked immunoreactivity to CK5/6 (4/5 cases) while centrally located neoplastic cells (stellate reticulum-like) had diffuse strong immunoreactivity to CK5/6 (5/5 cases). Findings are consistent with ectopic ameloblastoma (n=3), ameloblastic fibroma (n=2), and ameloblastic carcinoma (n=1).

Conclusions: Tumors with histological features of ectopic ameloblastoma can occur in the cheek tissue of rabbits.

TROPICAL KERATOPATHY IS A DISORDER OF THE ANTERIOR CORNEAL STROMA IN THE AFRICAN GREEN MONKEY

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Background: Corneal opacities (tropical keratopathy) were frequently observed in African green monkeys (AGM) from a research facility on the island of St. Kitts with unknown etiology.

Objective: Describe the epidemiology, gross, histology and ultrastructural abnormalities associated with tropical keratopathy in AGM's.

Methods: 370 AGM's underwent slit-lamp examination. The location and number of lesions, age, and sex of each monkey were recorded. Ordinal logistic regression was performed to predict outcomes. Additionally, corneas were collected during autopsies of monkey from terminal studies and fixed in 10% formaldehyde for histology and modified Karnovsky's fixative for TEM and SAXS analysis.

Results: There was a statistically higher frequency of lesions located in the inferior quadrant of the cornea. The probability of having more than five lesions increases as monkeys age. Grossly, lesions consisted of small, white, dot-like cornea lesions. Histologically, the lesions were subtle consisting of thickened and disorganized collagen fibrils of the anterior stroma, sometimes associated with corneal epithelial hyperplasia, and sometimes with vacuolation of epithelial basal cells, Bowman's layer or collagen fibrils, with no inflammation. On TEM, the lesion was confined to the anterior stroma and consisted of disorganized collagen bundles and fibrils, increased elastic fibers, and proliferation of fibroblasts, some containing fat droplets. SAXS showed collagen fibril diameters and variation in size were greater in stroma containing the lesions compared to normal.

Conclusion: Tropical keratopathy in primates is a disorder of the anterior corneal stroma, more prevalent in the inferior quadrant of the eye, suggestive of exposure to a non-immunogenic environmental factor.

EXPRESSION OF RECEPTOR TYROSINE KINASES IN CANINE APPENDICULAR OSTEOSARCOMA

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Background: Osteosarcoma (OSA), the most common primary bone tumor of dogs, is an aggressive and often lethal disease. Receptor tyrosine kinases (RTKs) regulate cellular processes relevant to tumorigenesis and are targetable by treatment with tyrosine kinase inhibitors (TKIs). Published studies investigating RTKs and TKIs in canine OSA provide limited data detailing RTK expression patterns and their prognostic significance.

Objective: Determine expression and co-expression patterns of a set of RTKs and downstream signalling molecules in primary appendicular canine OSA to better inform potential clinical use of TKIs in OSA management.

Methods: Immunohistochemistry for c-KIT, EGFR, IGFR, PDGFR-B, VEGFR-2, and p-AKT was performed on a canine appendicular OSA tissue microarray incorporating 126 cases.

Results: Median survival time of the population was 218 days (90%-CI=152-284 days, range:0-1889 days). Standard of care therapy was received in 96/126 patients. Proportion scoring revealed PDGFR-B, IGFR, and p-AKT have immunolabelling in at least 20% of neoplastic cells in all cases, and at least 50% of neoplastic cells in 50% of cases. For c-KIT, EGFR, and VEGFR-2 more than 70% of cases lacked immunolabelling. Frequency of co-expression of at least 3 RTKs (about 30%) or 4 RTKs (less than 10%) was uncommon.

Conclusions: Information regarding the immunohistochemical expression patterns of a panel of multiple RTKs and their co-expression profiles was evaluated and compared within a large canine OSA population. These novel results can guide future research investigating the roles of RTKs as predictive biomarkers for TKI therapy, survival outcomes, and RTK profiles of metastases.

EXPRESSION OF IMMUNOINHIBITORY CHECKPOINT MOLECULES IN CANINE SOFT TISSUE SARCOMA

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Background: Canine Soft Tissue Sarcomas (STS) are common neoplasms in dogs and are considered immune deserts. Tumor infiltrating lymphocytes are sparse in STS, when present tend to organize around blood vessels or at the periphery of the neoplasm. This pattern has been associated with an immunosuppressive tumor microenvironment linked to overexpression of molecules of the PD-axis. PD-1, PD-L1 and PD-L2 expression correlates with malignancy and poor prognosis in other neoplasms in humans and dogs, but little is known about their role in canine STS, different grades, and how different therapies affect their expression.

Objective: Evaluate the expression of checkpoint molecules across STS tumor grades and subsequent to tumor ablation treatment.

Methods: Gene expression analysis of checkpoint molecules was performed by qRT-PCR from soft tissue sarcomas that undergo tumor ablation therapy (histotripsy) and from FFPE specimens of STS of different grades from the Virginia Tech Animal Laboratory Services archives.

Results: The expression of PD-1, PD-L1 and PD-L2 was detected in untreated STS tissue representing grades 1, 2, and 3. Trends of decreased expression of all markers were observed in tissue sampled from the treatment interface relative to untreated areas of the tumor.

Conclusions: The relatively lower expression of these checkpoint molecules at the periphery of the treated area may be related to liquefactive necrosis induced by the histotripsy treatment and would potentially allow TILs to infiltrate the tumor. The relative trend of increase of these checkpoint molecules in tumors of a higher-grade support previous reports that associate their expression with malignancy.

CARDIOVASCULAR PATHOLOGY IN CAPTIVE NORTHERN BALD IBIS (GERONTICUS EREMITA), AFRICAN SACRED IBIS (THRESKIORNIS AETHIOPICUS), AND SCARLET IBIS (EUDOCIMUS RUBER)

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Background: Published studies have demonstrated that captive ibises suffer from high rates of cardiovascular disease, particularly atherosclerosis and myocardial injury.

Objective: We describe the cardiovascular lesions diagnosed at post-mortem examination in captive northern bald ibis (*Geronticus eremita*), African sacred ibis (*Threskiornis aethiopicus*) and scarlet ibis (*Eudocimus ruber*) held at ZSL London Zoo between 2000-2020.

Methods: Retrospective analysis of postmortem records (144 birds) with subsequent histologic examination of stored tissues (64 birds) was performed to diagnose and characterize cardiovascular lesions. Differences in the frequency of disease manifestations with respect to species, sex, and age were evaluated.

Results: Cardiovascular lesions were diagnosed in 41 individuals. Vegetative valvular endocarditis (14/41) and bacterial myocarditis (5/41) were the most common infectious causes of death, with *Staphylococcus aureus*, *Erysipelothrix rhusiopathiae*, *Escherichia coli, Salmonella enteritidis* most frequently cultured. Fungal vasculitis was seen in three cases with systemic aspergillosis. Atherosclerosis was present in 10 cases though was only considered clinically significant in one where there was congestive heart failure secondary to marked aortic stenosis. There were two cases of cardiac trauma, one of which was iatrogenic due to misplacement of a microchip. Only one congenital lesion, a ventricular septal defect, was diagnosed. Epicardial gout was the only metabolic cause of death.

Conclusions: Cardiovascular lesions are common postmortem findings in ibis, with incidental atherosclerosis and fatal vegetative valvular endocarditis occurring most commonly in adult males and adult females, respectively. No significant interspecies differences in cardiovascular lesions were found. There was no link between bacterial cardiovascular disease and pododermatitis.

A PUTATIVELY NOVEL PAPILLOMAVIRUS ASSOCIATED WITH CUTANEOUS PLAQUES AND CUTANEOUS SQUAMOUS CELL CARCINOMA IN CAPTIVE NORTH AMERICAN SNOW LEOPARDS (PANTHERA UNCIA)

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Squamous cell carcinoma (SCC) is a common cause of mortality in captive North American (NA) snow leopards (*Panthera uncia*), accounting for over 33% of all reported cancers. In many species, including domestic cats, papillomaviruses are associated with precursor viral plaques that can progress to cancer. Viral plaques are also common in captive NA snow leopards affecting 12% of the population. Our objective was to determine if papillomaviruses are associated with cutaneous plaques and SCC in captive NA snow leopards. Samples of four cutaneous plaques from three animals and three cutaneous SCCs were collected from archived biopsy and necropsy samples. PCR was performed using degenerate primers that identified a putatively novel papillomavirus within cutaneous plaques. This putatively novel papillomavirus shares 76% DNA sequence identity to *Felis catus papillomavirus 2,* a PV associated with cutaneous viral plaques that can transition to SCC in domestic cats. PCR primers specific for this putatively novel papillomavirus were designed. These specific primers amplified DNA in 3/4 cutaneous plaques. In situ hybridization was performed using RNA scope technology and specific hybridization probes. Strong hybridization signals were present within epithelial cells forming cutaneous plaques (3/4) and neoplastic cells of cutaneous SCC (3/3) including metastatic disease. These findings suggest an association between this putatively novel papillomavirus and the development of cutaneous plaques and SCC in captive NA snow leopards. Ultimately, identification of a causal viral agent in the development of SCC in these animals will help guide therapeutic intervention and lay the foundation for the development of prophylactic vaccines.

REEMERGENCE OF CHLAMYDIA MURIDARUM WITH INFECTIONS IN THE LUNGS AND INTESTINES OF LABORATORY MICE

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Chlamydia muridarum (*Cm*), an intracellular pathogen that naturally infects mice, is used as a model organism for the study of human *Chlamydia trachomatis* urogenital tract infections. Despite its experimental use, natural infection by *Cm* has not been documented in laboratory mice since the 1940s and is not currently an excluded agent in mouse colony health surveillance programs. We recently reported a significant

prevalence of *Cm* infection among academic institutional mouse colonies based on testing of incoming shipments received by our institution (33% positive), and diagnostic (16%) and fecal microbiota (14%) samples. Herein, we evaluated whether *Cm* was present in tissues of selected immunocompetent and immunocompromised mouse strains from diagnostic necropsies and characterized the pathology of *Cm* infection in NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ (NSG) mice after cohousing with *Cm*-shedding mice. *Cm* was detected by immunohistochemistry, in-situ hybridization and/or qPCR in 25% (5/20 mice; positive / total # tested) of the lungs and 52.6% (10/19) of the intestines in immunocompetent mice. *Cm* antigen was detected in bronchiolar epithelium with or without peribronchiolar lymphoplasmacytic infiltrates and normal cecocolonic epithelium. NSG mice developed clinical disease frequently associated with a histiocytic and neutrophilic bronchointerstitial pneumonia and neutrophilia (89%, 17/19) and rarely endometritis/salpingitis (11.1%, 1/9), with intralesional *Chlamydia* inclusions. *Cm* antigen and/or nucleic acids were detected in the small and large intestine (100%, 8/8) of all mice examined without lesions. Given that experimental *Cm* infection elicits both myeloid and T-cell responses, these findings suggest that natural *Cm* infections may modulate immunological responses and cause morbidity in immunocompromised mouse strains.

GROSS, HISTOPATHOLOGIC, MICROBIOLOGIC AND RADIOLOGIC CHARACTERIZATION OF LESIONS ASSOCIATED WITH CLINICAL LAMENESS IN A COHORT OF GROUP-HOUSED SOWS EUTHANIZED FOR LAMENESS

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Background: Lameness in sows is reported as the most frequent cause of early culling from commercial farms, and results in reduced productivity, economic losses, and a negative impact on animal welfare. Osteochondrosis was reported as the leading cause of lameness in North American sows and, although more recent European studies report infectious arthritis as the leading cause, lameness in United States production facilities using group housing for gestating sows has not yet been evaluated.

Objective: To characterize lesions associated with lameness in the appendicular musculoskeletal system of 26 sows euthanized for lameness using pathologic, radiologic, and microbiologic analyses.

Results: Of 178 total lesions, infectious lesions were most common (54%), predominated in distal limb segments (*i.e*., at or distal to carpi and tarsi) and more often correlated with the clinically lame limb, while osteochondrosis and degenerative osteoarthritis predominated in proximal limb segments (*i.e*., at or proximal to cubital and stifle joints) and rarely correlated with the clinically lame limb. The location and characteristics of infectious lesions, including mixed bacterial growth isolated from 22/22 orthopedic sites representing 19 sows with *Trueperella pyogenes* isolated in 16/22 (73%) of samples, suggests an etiologic component involving trauma. Radiography had

a 70.6% sensitivity and 93.9% specificity for detecting infectious lesions affecting tarsocrural, antebrachiocarpal, and digital (*i.e*., claw) regions combined.

Conclusions: The frequency, type and location of infectious lesions identified in this cohort of sows euthanized for lameness differ from previous reports, indicating the need for further investigation of the etiopathogenesis, earlier detection methods and prevention.

Natural Disease Posters

N1: URINARY TRACT LESIONS IN HARVESTED BERING-CHUCKCHI BEAUFORT SEA BOWHEAD WHALES (BALAENA MYSTICETUS)

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Background: The Western Arctic Population of the bowhead whale (*Balaena mysticetus*) an ice -associated baleen whale inhabits Arctic Alaskan waters including the central Bering Sea to the Canadian Beaufort Sea. Bowhead whales are legally harvested by an aboriginal subsistence hunt and there is gross assessment and sampling for histopathologic evaluation to aid in assessing population health.

Objective: The urinary system, primarily the kidney, was examined from 288 animals between 2000 and 2020.

Methods: Samples of kidney were collected in formalin, sectioned routinely, and stained with hematoxylin and eosin.

Results: Histopathologic findings in the kidney were typically incidental and included interstitial fibrosis (n = 14), intraductal mineral (n = 14), interstitial nephritis (n = 3), and congestion (n = 9). However, in 2016, a spirurid nematode, *Crassicauda* sp., which has been reported in only four baleen whale species) was found in the kidney and ureter resulting in fibrosis, lymphoplasmacytic and eosinophilic nephritis, arterial medial hypertrophy and arteritis, corticomedullary effacement, and hydronephrosis. *Crassicauda* sp. has been observed in toothed cetaceans (odontocetes) in the mammary gland, male reproductive glands, subcutaneous tissues, cervical gill slit of *Kogia breviceps*, and kidney.

Conclusions: While not determined, climatic factors may have led to overlap with mysticete species known to carry kidney worms (humpback whales, blue whales, and fin whales) as well as prey range shifts (paratenic/intermediate parasite hosts). Continued monitoring and sampling for prevalence and population shifts are needed for this stock of bowhead whales.

N2: MOLECULAR FEATURES OF CANINE MEIBOMIAN GLAND ADENOMAS AND CARCINOMAS.

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Background: Human ocular adnexal sebaceous carcinomas commonly exhibit aggressive phenotypic features including pagetoid spread, mutations in tumor suppressor genes, and upregulation of protooncogenes such as MYC, and these tumors represent approximately 5% of all epithelial eyelid malignancies. Sebaceous carcinomas of the periocular region are uncommon in other species, and while the dog routinely develops Meibomian gland (MG) neoplasms, the majority are benign. The molecular drivers of MG tumors in dogs have not been elucidated.

Objective: The objective of the current study was to characterize the molecular features of canine MG adenomas and carcinomas.

Methods: A retrospective review of the COPLOW archives identified only two MG carcinomas in a total of 3,697 canine MG tumors. Immunohistochemistry was performed to characterize expression of adipophilin and MYC as markers of sebaceous differentiation and epithelial protooncogenic activity, respectively, and tumor expression was compared to normal tissue using H-scoring and evaluated using an ANOVA with a Dunnett's post hoc test for multiple comparisons ($\alpha = 0.05$).

Results: MYC expression was significantly greater in MG neoplasms (adenoma: 102.8 ± 29.4, $p \leq 0.0001$; carcinoma: 201.3 ± 45.9, $p \leq 0.0001$) compared to normal glands (39.3 ± 10.4), while ADRP expression was significantly lower in MG neoplasms (adenoma: 27.3 ± 26.6, $p \leq 0.0001$; carcinoma: 1.9 ± 0.9, $p = 0.006$) compared to the normal MG (122.9 ± 68.2).

Conclusions: Our findings suggest that MG oncogenesis in the dog may mirror tumor progression in the human condition.

N3: HOST EPIDERMAL GROWTH FACTOR RECEPTOR (EGF-R) EXPRESSION IS ASSOCIATED WITH ORF VIRUS INFECTION IN SHEEP AND GOATS

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Background: ORF virus (ORFV) is a large double-stranded DNA virus of the genus Parapoxvirus (PPVs) that causes contagious ecthyma (CE), which is characterized by crusted pustular lesions and multifocal coalescing verrucous nodules on the skin and mucosa.

We investigated the expression of host Epidermal Growth Factor Receptor (EGF-R) as 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 study oral and cutaneous proliferative lesions in neonatal lambs and kids from sheep flocks and goat herds with ongoing ORFV infections.

Results: The ORFV-affected skin and mucosae we examined were microscopically characterized by significant pseudo-papillomatous proliferation of the epithelium, while the derma was expanded by the presence of a proliferating fibro-vascular component representing a new-formed vascular spaces network. Immunofluorescence, confocal microscopy, and mRNA-ISH found expression of EGF-R associated with infiltrating CD163+ macrophages and endothelial cells of the new-formed vessel. In the epithelium, EGF-R was particularly over-expressed in the cells of the stratum basale.

Conclusions: The results of the study reveal that ORFV, in sheep and goats activates an inflammation reaction characterized by CD-163 positive macrophages expressing EGF-R. We suppose that in this way EGF-R might play an oncogenic-promoting role through synergistic action with viral Vascular Endothelial Grow Factor (VEGF), thus leading to the tumor-like changes sporadically observed during CE. This indicates that ORFV inflammation activates a mechanism stimulating cellular proliferative changes like what happens in numerous non-viral associated tumors.

N4: MORPHOLOGICAL STUDY OF APOCRINE GLAND TUMORS IN RICHARDSON'S GROUND SQUIRRELS (UROCITELLUS RICHARDSONII).

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Background and Objective: Richardson's ground squirrels (*Urocitellus richardsonii*) have peculiar scent glands located from the perioral region to the cheek, and from cervical skin to dorsal skin and the anal area, which are composed of modified sudoriferous and sebaceous glands. These glands have been considered to be related to ethology, such as greeting behavior and scent marking. Pathological information about these tumors originating from these glands is limited. The goal of this study was to clarify the morphological features and prognostic factors of these tumors.

Methods: A total of 57 surgically removed tumors from 30 cases and 1 necropsy case were histopathologically evaluated and classified into histological subtypes according to the WHO classification (epithelial and mammary tumors).

Results: Of the 58 tumors included in this study, 36 (62%) occurred at the cervical skin to dorsal skin, and 17 (29%) were at the perioral region to the cheek, with the location unrecorded for 5 tumors (9%). Histopathologically, 49 (84%) tumors were apocrine adenocarcinomas, followed by 6 apocrine adenomas (10%) and 3 apocrine cysts (5%).

Apocrine adenocarcinomas were subclassified into: cystic-papillary type (16; 33%), tubulopapillary type (16; 33%), solid type (12; 24%), and micropapillary invasive type (5; 10%). There were correlations between tumor subtypes and tumor diameters, nuclear atypia, tumor margins, vascular invasion, and PAS positivity.

Conclusions: The present study showed that apocrine adenocarcinomas predominated, and various histologic subtypes were identified. In addition, subclassification of these tumors was useful to predict the biological behavior of apocrine gland tumors in Richardson's ground squirrels.

N5: EMERGING OOMYCETE INFECTION IN HONG KONG

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Hong Kong is a city and special administrative region of China, with a humid, subtropical climate. Despite being densely populated, 75% of Hong Kong's land mass is categorized as conserved countryside including woodlands and mountain ranges with many natural water resources. Water molds or oomycetes such *Pythium insidiosum* and *Lagendium* spp, which are phylogenetically distinct from fungi, are known to cause disease of increasing significance in animals and humans in many parts of the world. The lifecycle involves release of the infectious, motile zoospores when spores contact water, which swim and encyst on various matter including plant matter and skin, develop a germ tube (hypha) which penetrates into host tissue. Whilst endemic in some South East Asian countries, oomycete infection has never been previously reported in Hong Kong, SAR, China. Five cats and six dogs presented with either cutaneous or intestinal disease manifesting as chronic pyogranulomatous to eosinophil rich, necrotizing inflammation centered around branching, colorless hyphae which stain poorly by periodic acid-Schiff but are well visualized with silver stains. The clinical manifestation of cutaneous infection in two cats in this cohort produced visually striking perianal swelling. Histopathology, fungal culture, ELISA and PCR diagnostic testing were required to reach a diagnosis. Infected animals had long term clinical follow up to monitor response to treatment, which revealed that oomycete infection is not necessarily a death sentence for cats, as good therapeutic response was achieved.

N6: A SYSTEMATIC REVIEW OF METHODS AND PROGNOSTIC VALUE OF MITOTIC ACTIVITY IN FELINE TUMORS

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Background: Mitotic activity has been evaluated as a prognostic marker in several studies on feline tumors.

Objective: To summarize the current literature by evaluating methods and prognostic value of mitotic activity in feline tumors.

Methods: A systematic literature search of two databases (Pubmed and Scopus) was conducted in June 2022. Of 167 papers screened, 25 were eligible and 17 additional papers were added through a manual literature search.

Results: The 42 included papers examined 17 tumor types, with multiple studies for 6 tumor types. The mitotic count (MC; number of mitotic figures per area) was performed in 36 instances (86%) and the mitotic index (MI; mitotic figures per number of tumor cells) in 3 instances (7%). The method was not reported in three papers. The tumor area selected for counting was not indicated in most papers (N=24), while 9 used hotspot locations (21%). For MCs, mitotic figures were counted mostly in 10 HPF (N=26, 72%) that were consecutive/contiguous in 6 instances (17%). However, the actual area size (in mm^2) was only available in 12 papers (33%). The authors of 28 papers (67%) concluded that the MC or MI was prognostically relevant in their studies on 10 tumor types (59%), with conflicting results for 4/6 tumor types.

Conclusion: Systematic review of the current literature indicates that the MC has prognostic value for multiple types of feline neoplasia. However, the MC methods are often incompletely reported and different outcome metrics are used. Standardization is required for better reproducibility and comparability between studies.

N7: REVIEW OF SPONTANEOUS LESIONS IN THE EXOCRINE PANCREAS OF FERRETS (MUSTELA FURO)

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Background: Reports of naturally occurring lesions in the exocrine pancreas of ferrets are rare and include individual case reports of exocrine tumors and pancreatitis.

Objective: Determine common lesions in the exocrine pancreas of ferrets, and identify any correlations to signalment, clinical signs, and comorbidities.

Methods: The Northwest ZooPath database was searched for biopsy and necropsy reports from domestic ferrets with exocrine pancreatic lesions.

Results: Lesions in the exocrine pancreas were described in 73/4412 reports and included acinar cell hyperplasia (n=32), chronic pancreatitis (n=16), acute pancreatitis (n=13), acinar cell adenoma (n=5), lymphoma (n=5), acinar cell carcinoma (n=4), atrophy (n=3), and hypoplasia (n=2). No trends in clinical signs were identified for animals with acinar cell hyperplasia. Animals with chronic pancreatitis also had inflammatory bowel disease (n=8), islet cell neoplasia (n=8), adrenocortical neoplasia (n=7), and diabetes mellitus (n=3). Clinical history was available in 9 cases with acute

pancreatitis, 4 of which had abdominal surgery 8-72 hours prior to death; clinical signs included lethargy (n=4) and diarrhea (n=3). There were no reported clinical signs referable to either acinar cell adenoma or carcinoma. The only non-pancreatic neoplasm identified in the pancreas was lymphoma, which was also present in lymph nodes (n=4) and ileum (n=1). All animals with atrophy had a history of weight loss. Both animals with hypoplasia had a history of hypoglycemia and had islet cell carcinomas (insulinomas).

Conclusions: Exocrine pancreatic lesions occur occasionally in domestic ferrets. Chronic pancreatitis was often associated with other chronic diseases. Acute pancreatitis was often associated with recent abdominal surgery.

N8: SPECTRUM OF LESIONS ASSOCIATED WITH WEST NILE INFECTION IN 7 BIRDS AND ONE ALPACA FROM CONNECTICUT, RHODE ISLAND AND MASSACHUSETTS

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West Nile Virus (WNV), a vector-borne flavivirus, has been the cause of significant neurologic disease across a multitude of avian species as their primary and/or reservoir hosts with mammals and humans as dead-end hosts. Across different animal species, particularly with avian, a variety of gross and histologic presentations occur that result in disease. From August 2021 to November 2021, seven birds and an alpaca (*Huacaya alpaca*) were submitted to the Connecticut Veterinary Medical Diagnostic Laboratory (CVMDL) at necropsy with a history ranging from sudden death to neurologic and/or ocular clinical signs prior to death. Of the avian species, there was a Northern cardinal (*Cardinalis cardinalis*), three American crows (*Corvus brachyrhynchos*), one red-tailed hawk (*Buteo jamaicensis*), one red-shouldered hawk (*Buteo lineatus*), and one Timneh grey parrot (*Psittacidae timneh*). All eight of these cases tested positive via RT-qPCR via submitted samples from necropsy. Grossly, these animals did not have lesions except for the Northern cardinal, which had cerebral hemorrhage. Microscopically, there was a variety of lesions to include perivascular mononuclear encephalitis (alpaca, red-tailed hawk), periocular mononuclear uveitis and ciliary body inflammation (red-tailed hawk), cerebral hemorrhage and necrosis (Timneh grey parrot), necrotic to necroulcerative enteritis (two of the three crows). The significance of these cases highlights the variety of lesions that can be seen in multiple avian and mammalian species but also that the typical season for vector borne diseases are extending.

N9: HISTOLOGIC FINDINGS IN CAPTIVE MADAGASCAR HISSING COCKROACHES (GROMPHADORHINA PORTENTOSA)

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Background: Madagascar hissing cockroaches (MHC) are members of the Blaberidae (giant cockroaches) family of the Insecta class. They are native to the African island of Madagascar where they live within leaf litter on the rain forest floor. Due to their large size, relative tameness, and general easy keeping, they have become popular in classrooms, zoological collections, museums, research laboratories, and as private exotic pets, but descriptions of diseases of MHC in the literature are rare.

Objective: The objective of this study is to describe and characterize postmortem histologic findings in 18 captive MHC from a single zoological collection.

Methods: In this retrospective study, eighteen (5 female, 13 males) adult MHC necropsies were submitted to Northwest ZooPath between 2016-2020 for evaluation. Bodies were formalin-fixed whole. After fixation, the viscera were removed and processed routinely for histology. Sections of the body wall were decalcified and dechitinized prior to processing. Inflammatory lesions were stained with histochemical stains to assess for infectious agents.

Results and Conclusions: The main organs with histologic lesions were chitinous gut (foregut and/or hindgut; n=13), body wall (n=11), tracheae (n=9), midgut (n=7), and Malpighian tubules (n=5). All animals had inflammatory lesions affecting various organs. Inflammatory lesions typically consisted of aggregates of hemocytes with variable amounts of melaninization and/or encapsulation. In some cases, bacterial, fungal, and parasitic organisms were observed within the inflammatory infiltrates. This study greatly expands our knowledge of pathologic findings and disease processes of MHC.

N10: PLEURAL MESOTHELIOMA IN A GOAT

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An eight-year-old female Saanen goat presented for several days of inappetence, as well as intermittent cyanosis of the oral mucous membranes. On physical exam, the goat exhibited reduced rumen contractility and a sunken abdominal contour, mild tachypnea and tachycardia, and muffled cardiac sounds on the left side of the chest during auscultation. Complete blood count and serum chemistry panel revealed hemoconcentration and increased globulin concentration. Euthanasia was elected. Necropsy revealed an infiltrative, multinodular, tan mass within the left thoracic cavity, with diffuse atelectasis of the left lung lobes. The mass contained pockets of viscous,

brown-green fluid. Innumerable small, tan nodules were disseminated across the pleura of the lungs, thoracic walls, and diaphragm. In one focus, the mass extended through the mediastinum into the right thoracic cavity. On histopathology, the mass was composed of streams, bundles, and nests of pleomorphic spindloid to polygonal cells. Neoplastic cells exhibited positive immunoreactivity for both cytokeratin and vimentin, consistent with a diagnosis of biphasic mesothelioma. Mesothelioma has rarely been described in the goat but should be considered as a differential diagnosis for thoracic masses in small ruminants, along with thymoma, metastatic carcinoma, carcinomatosis, and granulomatous lesions caused by parasites and bacteria.

N11: FATAL HERPESVIRUS INFECTION OUTBREAK IN A LITTER OF FRENCH BULLDOG PUPPIES

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Background: Canine herpesvirus (CaHV-1) is an enveloped virus belonging to the Herpesviridae family. CaHV-1 in adult dogs causes asymptomatic to mild respiratory or reproductive diseases with widespread diffusion mainly in breeding kennels, as confirmed by serological investigations. However, systemic fatal hemorrhagic CaHV-1 neonatal infections have been sporadically described.

Objective: We describe pathological and virological findings in an outbreak of CaHV-1 infection in a litter of French Bulldog puppies.

Methods: Three of five suspected CaHV-1-infected puppies, which died from 2 to 3 weeks after the onset of clinical signs, were submitted for necroscopy. Several organs were taken to histopathology, CaHV-1 Real Time-PCR, and viral isolation.

Results: Grossly, all puppies showed serohemorrhagic fluid in the abdominal and thoracic cavity, hepatomegaly, splenomegaly, and bilateral renomegaly with diffuse petechial and ecchymotic hemorrhages. Histologically, multifocal perivascular necrosis and hemorrhages with scarce mononuclear cell infiltration were determined in several organs, especially in the kidneys, lungs, and liver. Moreover, we observed severe thymic medullary atrophy and non-suppurative meningoencephalitis with glial nodules.

Real-time PCR technique determined CaHV-1 DNA presence in the brain, lungs, liver, thymus, kidneys, heart, and spleen collected from all the examined animals, and in the ocular swabs of the bitch. CaHV-1 was isolated from MDCK cells in 18 out of the 20 specimens examined.

Conclusions: Clinical and postmortem examination coupled with the increased sensibility of new molecular diagnostic tools help to make a differential diagnosis of

neonatal pathologies in puppies, and to gain insights into CaHV-1 spreading to improve biosecurity measures in the breeding facilities.

N12: THE GENE EXPRESSION PROFILE OF CANINE CD4+ PERIPHERAL T-CELL LYMPHOMA

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Background: Peripheral T-cell lymphoma (PTCL) refers to a poorly understood heterogeneous group of T-cell neoplasms, with 25-30% categorized as PTCL, not otherwise specified (PTCL-NOS). Gene expression profiling (GEP) has characterized human PTCL-NOS into clinically significant molecular subtypes. Canine PTCL clinically and immunophenotypically resembles human PTCL-NOS, and PTCL is more common in dogs than humans, leading to their interest as a naturally occurring model.

Objectives: 1) Determine whether the GEP of canine PTCL resembles human PTCL-NOS, and 2) investigate the cell of origin of canine CD4+ PTCL.

Methods: Differential gene expression analyses were conducted with DESeq2 on RNA-sequencing data from 26 dogs with CD4+ PTCL. The significantly differentially expressed genes were compared to human PTCL-NOS and various stages of T-cell development via gene set enrichment analyses (GSEA).

Results: Differential gene expression in canine CD4+ PTCL was analogous to human PTCL-NOS (ES=0.59, p<0.001). Canine CD4+ PTCL had increased expression of GATA3 (log2fc=1.9, p=4.44e-08) and was enriched for gene signatures associated with downregulation of PTEN (ES=0.52, p=0.001) and upregulation of the PI3K/AKT/mTOR pathway (ES=0.45, p=0.001), resembling the more aggressive human GATA3-PTCL subtype. Canine CD4+ PTCL cells were enriched for gene signatures associated with early thymocyte progenitor cells (ES=0.55, p=0.04) and had increased expression of genes of immaturity, such as CD34 (log2fc=7.04, p=4.31e-12) and KIT (log2fc=3.54, p=2.88e-06), even though CD34 is not detected by flow cytometry.

Conclusions: The GEP of canine CD4+ PTCL resembles that of human PTCL-NOS and suggests a possible thymic precursor cell of origin.

N13: ANALYSIS OF COLLAGEN AND ITS CROSSLINK CONTENT IN 2-YEAR-OLD DAIRY COWS WITH OSTEOPOROSIS FROM NEW ZEALAND

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Background: Outbreaks of spontaneous fractures of the humerus due to osteoporosis occur in dairy cows in New Zealand. Copper (Cu) deficiency (diagnosed from liver or serum analysis) is a common finding in these cows and thought to contribute to reduced bone strength leading to fractures.

Objective: Quantify and describe collagen and collagen crosslink content in bones from cows with humeral fracture and control cases and relate findings to liver Cu concentration (LiCuC) and bone Cu concentration (BoCuC).

Methods: Twenty-six slabs of cortical bone from cows with humeral fracture (affected) and 14 from control cows were processed. Cases were age matched. Crosslinks (immature: dihydroxylysinonorleucine (DHLNL) and mature: pyridinoline (PYR)) were quantified using liquid chromatography–mass spectrometry. Collagen content was then estimated using a colorimetric hydroxyproline assay. LiCuC and BoCuC were measured using inductively coupled plasma mass spectrometry.

Results: Control heifers had significantly higher total collagen content. Affected heifers had significantly lower LiCuC, significantly higher BoCuC, total crosslink content, DHLNL and PYR crosslink. BoCuC was positively correlated with DHLNL content. Increased BoCuC (OR 56.1), low LiCuC (OR 3.2), increased DHLNL and PYR were significantly associated with a high probability of humeral fracture.

Conclusions: Low LiCuC in affected cows appears, in part, to be due to mobilization of Cu from liver to bone, a response to mechanical stress aimed to increase the rate of collagen crosslink formation in bone. Low total collagen content suggests inadequate protein/calorie undernutrition, rather than Cu deficiency, could be a more important factor in the pathogenesis of these fractures.

N14: CHARACTERIZATION OF SOX10 EXPRESSION IN CANINE NEOPLASMS

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Background: Several members of the SOX genes family are implicated in tumorigenesis, metastasis, and regulation of the tumor microenvironment. SOX10, which is involved in neural crest cell migration and differentiation, has long been recognized a sensitive and specific marker in the diagnosis of malignant melanoma in humans. However, expression of SOX10 in other tumor types has infrequently been evaluated in humans and has not been investigated in the dog.

Objective: To characterize the expression of SOX10 in canine neoplasms.

Methods: Baseline labeling of SOX10 in healthy canine tissues was evaluated in a tissue microarray. Immunohistochemistry for SOX10 was performed on multiple batches of ten archived formalin-fixed paraffin-embedded tissues from representative canine

neoplasms of endodermal, mesodermal, and ectodermal origin. Oral and cutaneous canine melanocytic tumors were used as positive controls.

Results: Strong SOX10 immunolabeling was appreciated in most tumors of ectodermal origin, consistently expressed in glandular tumors (mammary, nasal, and salivary adenocarcinoma) and gliomas. Embryonal and follicular neoplasms inconsistently exhibited strong nuclear immunolabeling. Oral fibrosarcomas and oral sarcomas of undetermined embryonic origin both inconsistently exhibited moderate to strong nuclear immunolabeling. Neoplasms of mesodermal, endodermal, and of undetermined embryologic origin broadly lacked immunolabeling.

Conclusions: SOX10 expression, far from being limited to melanomas, is widely expressed in tumors arising in the peripheral and central nervous systems and originating from glands and cutaneous adnexa. Whether SOX10 constitutes a reliable prognostic factor or promising therapeutic target in canine neoplasia requires further investigation.

N15: TUBULO-PAPILLARY RENAL CELL CARCINOMA IN A CAPTIVE OCELOT (LEOPARDUS PARDALIS).

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A 12-year-old, male, captive ocelot (*Leopardus pardalis*) was diagnosed with a 2.6x2.2x1.6 cms mass on the cranial pole of the right kidney during an ultrasound exam. The mass was processed by standard Hematoxilin & Eosin staining. Periodic Acid-Schiff (PAS) staining was also performed. Under microscopic examination, the mass was surrounded by a fibrous pseudocapsule and compressed the adjacent normal renal tissue. The tumoral cells are small cuboidal epithelial cells arranged in a single layer. The cells have lightly eosinophilic cytoplasm, a rounded nucleus, and no mitoses. The epithelial cells grow in a papillary pattern which contains pseudo-rosettes in their lumen. Some of these pseudo-rosettes contain a PAS-positive material, most likely Tamm-Horsfall protein. The stroma was also PAS-positive. The number of pseudorotes varies from one to several in each tubule. The tissue was analyzed for the following markers by immunohistochemistry: WT1, CK Ae1/A3, CK19, CK7, Vimentin , Melan-1, and HMB45. Only Vimentin had a positive stain. Based on this finding the tumor was classified as a tubulo-papillary renal cell carcinoma. Although this neoplasia is well described in domestic cats it is rarely reported in wild felids. To our best knowledge, this is the first renal carcinoma reported in the *Leopardus* genera, which includes 13 new world felids. Its occurrence in an Ocelot is valuable since neoplasias in this specie are rarely reported in Latin America.

N16: AVIAN MALARIA DUE TO PLASMODIUM ELONGATUM (GRW6) AND PLASMODIUM MATUTINUM (LINN1) IN NEW ZEALAND KIWI (APTERYX SPP.)

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Aims: To describe the gross and histological lesions of avian malaria in kiwi (*Apteryx* spp.) in New Zealand, and to characterize the *Plasmodium* spp. involved.

Materials and Methods: Postmortem reports from kiwi (*Apteryx* spp.) were obtained from the Massey University/Te Kunenga ki Pūrehuroa School of Veterinary Science Pathology Register from August 2010 - August 2020. Reports in which a diagnosis of avian malaria was made or suspected on the basis of gross and histopathological findings were included in the study. Gross lesions were described from postmortem reports and archived H.E.-stained slides used for histopathological assessment. Nested PCR testing was performed on formalin-fixed paraffin-embedded tissues to assess the presence of *Plasmodium* spp DNA. Cases with a positive PCR result were sequenced to determine the *Plasmodium* spp. involved.

Results: Of 1,005 postmortem reports, 23 cases of confirmed or suspected avian malaria were included in this study. The most consistent gross lesions included splenomegaly, hepatomegaly, and interstitial pneumonia with edema. Histological lesions were characterized by severe interstitial pneumonia, pulmonary edema, myocarditis, hepatic sinusoidal hypercellularity and congestion, and splenic histiocytosis with numerous hemosiderophages and congestion. Cytoplasmic meronts were consistently found within endothelial cells of a variety of tissues, and within tissue macrophages of the liver, lung, and spleen. A diagnosis of avian malaria was confirmed via PCR testing in 13 cases, with sequencing revealing *P. matutinum* (LINN1) and *P. elongatum* (GRW6) as the species involved.

Conclusions: This report expands the knowledge of avian malaria in arguably the most iconic of New Zealand's avian species.

N17: COMPARATIVE IMMUNOHISTOCHEMICAL STUDY ON CANINE AND FELINE MENINGIOMAS

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Canine meningiomas exhibit various morphological patterns, while feline meningiomas show definite pattern. In this study, we focused on the morphological difference between canine and feline meningiomas, and examined the expression of E-cadherin, cytokeratin AE1/AE3, which are widely used in human and canine meningiomas, and the molecules (calretinin and cytokeratin 5/6) expressed in mesotheliomas. In 73 cases of canine meningioma examined, the tumor cells of 68 cases were immunopositive for E-cadherin, those of 52 cases were immunopositive for cytokeratin AE1/AE3, those of 43 cases were immunopositive for calretinin, and those of only one case was

immunopositive for cytokeratin 5/6. In 39 cases of feline meningiomas, the tumor cells of 36 cases were immunopositive for E-cadherin, those of 5 cases were immunopositive for cytokeratin AE1/AE3, those of only one case was immunopositive for calretinin, and all cases were negative for cytokeratin 5/6. Immunoreactivity for each antibody was not associated with histopathological grade and subtypes of canine and feline meningiomas. E-cadherin expression was widely observed in canine and feline meningiomas, which suggested utility of this antibody in diagnosis of meningiomas. Expression of cytokeratin AE1/AE3 is also commonly detected in dogs, but not in cats, suggesting the different morphological variation between dogs and cats. Interestingly, canine meningiomas frequently exhibited calretinin (43/73 cases), although the expression in feline meningiomas was rare (1/39 case). The finding may suggest that canine meningiomas have similar immunohistochemical natures to those of mesotheliomas which also exhibit biphasic (epithelial and mesenchymal) histological features.

N18: LIFE-THREATENING COMPLICATIONS OF ENDOMETRIOSIS IN RHESUS MACAQUES

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Endometriosis is a common reproductive disease affecting women and Old-World primates. It is characterized by the presence of endometrial tissue outside of the uterus, and affects up to 15% of reproductive aged women, and up to 30% of menstruating rhesus and cynomolgus macaques. The disease is considered benign and commonly causes clinical signs such as pain and dysmenorrhea, but more serious complications are rarely reported. Here, we present a series of life-threatening complications of endometriosis in three rhesus macaques.

Case 1 is an 18-year-old female rhesus macaque with a six-month history of waxing and waning abdominal distention, and constipation. Gross and histopathological findings showed severe fibrous adhesions secondary to endometriosis causing tight plication of the jejunum and marked gas distention and congestion of the orad segments. This case demonstrates intestinal obstruction secondary to invasive endometriosis.

Case 2 is a 20-year-old female who presented to necropsy after sudden death. Gross exam revealed turbid yellow abdominal effusion with fibrinous and fibrous adhesions throughout the abdominal viscera. Culture grew *Salmonella sp*. Histopathology showed widespread endometriosis of many abdominal organs with suppurative exudate and rod-shaped bacteria. This case demonstrates septic peritonitis and bacterial sepsis secondary to abdominal endometriosis.

Case 3 is a 17-year-old female with an acute clinical history of pleural effusion. Gross findings revealed left sided hemothorax with atelectasis, and hemoabdomen with fibrous adhesions between pelvic organs. Histopathology revealed widespread endometriosis throughout abdominal viscera and diaphragm, and suspected endometrial stroma on

the pleura. This case demonstrates the potential for intrathoracic complications of endometriosis.

N19: SPECIES -GROUP VARIATIONS WITH HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS INFECTION IN WILD BIRDS

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Background: Highly pathogenic influenza A virus (HPIAV) was detected in hunter-harvested waterfowl in the US in January 2022. Since then, HPIAV mortalities have been reported in numerous wild bird species in 42 states.

Objective: To characterize lesion patterns and tissue tropism in naturally HPIAV infected, wild birds submitted to the Southeastern Cooperative Wildlife Disease Study from February-June 2022.

Methods: Bird carcasses from the Eastern and Midwestern US were examined. Real-time reverse transcriptase polymerase chain reaction was performed on extracted RNA from pooled cloacal/choanal swabs and/or select tissues; primers were designed to clade 2.3.4.4B HP H5 IAV. Results were confirmed at the National Veterinary Services Laboratory. Select tissues were routinely processed for histopathology and immunohistochemistry.

Results: Seventy-four birds representing 22 species were diagnosed with HP IAV infection, these included: raptors (47%; 35/74), waterfowl (34%; 25/74), gulls and terns (12%; 9/74), seabirds (3%; 2/74), and others (4%; 3/74). Lytic necrosis was the most common microscopic finding in all cases, most frequently affecting the spleen in raptors (94%; 32/34) and seabirds (100%; 2/2) and the liver in waterfowl (90%; 18/20). Lesions in the brain were common in all groups (64%; 42/66), with neuronal necrosis (60%; 25/42) and lymphocytic perivascular cuffs (55%; 23/42) as the most frequently observed lesions. Distribution and severity of lesions and co-morbidities varied by species-group. Described lesions labeled immunohistochemically for HPIAV.

Conclusions: Species-group variation in lesions may represent differences in virus or host biology. Continued investigation into species susceptibility and pathogenesis to optimize sample collection for HPIAV diagnostics is necessary.

N20: NATURAL CHLAMYDIA MURIDARUM INFECTION CAUSES BRONCHOINTERSTITIAL PNEUMONIA IN NOD.CG-PRKDCSCIDIL2RGTM1WJL/SZJ (NSG) MICE

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The murine bacterial pathogen *Chlamydia muridarum* (Cm) has been used to study human *Chlamydia* infections in various mouse models. CD4 T-cells, NK cells, and *IFN-γ* mediated immunity are important to control experimentally induced Cm infections. While natural Cm infection has not been documented in laboratory mice since the 1940s, we recently reported a significant prevalence of natural Cm infections (33% positive) in academic mouse colonies. To investigate the impact of natural Cm infections in immunocompromised mice, nineteen NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ (NSG) mice were cohoused with Cm shedding naturally infected mice and/or their soiled bedding for four weeks and sacrificed. All mice developed clinical disease and had moderate neutrophilia (16 positive/18 total tested) and mild monocytosis (11/18). Gross examination revealed multifocal pale-pink-to-white regions of discoloration in the lung. The most consistent microscopic finding was mild to severe histiocytic and neutrophilic bronchointerstitial pneumonia (17/19) with intraepithelial chlamydial inclusions. Cm inclusions were also observed in the tracheal and nasopharyngeal epithelium with or without associated inflammation and throughout the small and large intestinal epithelium without lesions. Neutrophilic endometritis, vaginitis, and salpingitis with intraepithelial chlamydial inclusions was identified in one mouse. Cm was detected by IHC and/or ISH in the lungs (19/19), trachea (19/19), small and large intestine (16/16), and vagina/uterus/oviduct (1/9). Lung tissue from three NSG mice were qPCR-positive for Cm. These findings demonstrate that Cm infection from co-housing or exposure to soiled bedding causes significant pulmonary pathology in NSG mice, which may be due to the lack of functional T-cells and NK cells.

N21: MIR-361-5P, MIR-101, AND MIR-29C-3P ARE POTENTIAL ENDOGENOUS CONTROL IN RT-QPCR IN CANINE DLBCL

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Background: MicroRNAs(miRNAs) are short non-coding RNAs of 22 nucleotides that affect gene expression. The use of miRNAs as a biomarker is challenged by pre- and post-analytic variations, which affect the result's biological interpretation. Real-time PCR is a quantification method used to quantify the expression levels of microRNAs. Accurate data normalization is crucial for reproducible momentous results. According to recent studies, an endogenous miRNA control that is stably and moderately expressed

in the tested samples is a reliable normalization method for quantifying miRNA relative expression.

Objective: This study aimed to find the endogenous miRNAs control that corrects for any discrepancies in RNA extraction or reverse transcription to RT-qPCR data normalization.

Methods: We manually selected a set of miRNA candidates based on the coefficient of variation (CV%); the lowest CV% are included, followed by using RefFinder, a comprehensive web-based tool to screen and determine reference genes in a specific dataset. This user-friendly tool incorporates the most extensively used computational algorithms (geNorm, NormFinder, the comparative Delta-Cq method, and BestKeeper) to assess and order the pre-defined reference miRNAs according to their stable expression. Each algorithmic program ranks and ascribes weight to the tested miRNAs then the geometric mean of those weights is calculated for a complete picture expression evaluation.

Results: We found that miR-361-5p, miR-101, and miR-29c-3p with geomean ranking values (1.32, 1.86, and 2.06) are the most stably expressed miRNAs.

Conclusions: We emphasize the necessity for adopting a reliable normalization method suited to each experiment to avoid misleading data analysis.

N22: AUTOMATED NUCLEAR MORPHOMETRY USING A DEEP LEARNING-BASED ALGORITHM IS A STRONG PREDICTOR OF SURVIVAL IN CANINE CUTANEOUS MAST CELL TUMORS

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Background: Histological evaluation of canine cutaneous mast cell tumors (ccMCT) includes the degree of nuclear pleomorphism (variation in size and shape). Whereas nuclear pleomorphism is traditionally estimated by pathologists, accurate and time-efficient measurements can be done by automated morphometry.

Objective: To investigate the prognostic value of automated nuclear morphometry using deep learning.

Methods: An algorithm based on a UNet was trained (N=61), validated (N=11) and tested (segmentation performance, N=13) on H&E images (0.1185 mm²) from ccMCT. The algorithm measures different features of nuclear size and shape. Prognostic value of the algorithm was determined on 96 ccMCT with known tumor-specific survival by

two-fold cross validation. These cases were split in two parts, which were used to determine thresholds by a XGBoost model or to test the prognostic value.

Results: The segmentation performance of the algorithm compared to the ground truth was IoU=0.79. Tumor-related death could be predicted with an AUC of 0.82 and 0.86 for the two dataset parts. Based on the determined thresholds, the first dataset part had an accuracy, sensitivity and specificity of 79.2%, 66.7%, 81.0% and the second part had 93.8%, 71.4%, 97.6%, respectively. For comparison, the mitotic count at a cut-off of >5 had an accuracy, sensitivity and specificity of 85.4%, 50.0% and 90.5% and 93.8%, 85.7% and 95.1%, respectively, for the same dataset split.

Conclusion: Algorithmic morphometry has high prognostic value in ccMCT. Future studies should extract the relevant features and thresholds of automated morphometry and evaluate their application for pathologist estimation in a diagnostic setting.

N23: MYCOTOXINS IN PET FOOD: APROPOS OF RECENT CLINICAL-PATHOLOGICAL CASES IN CARACAS-VENEZUELA POST-SARS-COV-2 PANDEMIC

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Background: An aflatoxicosis outbreak affected 25 dogs and 10 cats in Caracas-Venezuela, after they were different fed commercial quality premium imported as a common food between January-June 2022. **Methods**: A physical examination was performed, the dogs and cats showing anorexia, polydipsia, icteric mucous membranes, hematemesis, hematochezia, or melena, and bleeding of the skin, eye, ear, and mouth. Liver samples taken by FNA, guided by ultrasound and all presented alterations of echogenicity of the liver in subjective homogeneous form of fatty infiltration and fibrosis. **Results:** The cytological findings reveal in all cases a degenerative process suggestive of mild to moderate fatty hepatocellular degeneration, severe cytoplasmic vacuolization, eccentric displacement of the nucleus of hepatocytes, fibroblasts, Kupffer cells loaded with hemosiderin, bilirubin, and abundant mature adipocytes, with dysplastic changes of the hepatocytes, in some more severe cases a degenerative process defined as postnecrotic nodular hyperplasia was diagnosed, consistent with the icterohemorrhagic syndrome. The main histological findings were hepatocyte fatty degeneration, bile duct hyperplasia, cholestasis, and postnecrotic nodular hyperplasia. On high performance liquid chromatography of food, Aflatoxin B_1 levels were 1091-1940ppb. **Conclusion**: Gross, histologic appearance and toxicology, were consistent with a diagnosis of aflatoxin poisoning in dogs associated with afalatoxin B_1- contaminated commercial food premium imported in Caracas-Venezuela.

N24: PORCINE PARVOVIRUS-2 VIRAL DISTRIBUTION IN CLINCAL CASES OF SYSTEMIC SWINE ILLNESS USING IN SITU HYBRIDIZATION

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Background: Porcine parvovirus-2 (PPV2) has been detected in swine tracing back to 1998 in Europe. Although recent studies linked PPV2 with the porcine respiratory disease complex, little is known about its role as a primary pathogen. Direct detection using in situ hybridization (ISH) has solely been reported on lung tissue and synovium. However, the cell target and PPV2 replication in other organs are still unexplored.

Objective: To determine PPV2 tissue distribution using ISH on 11 clinical cases of swine with systemic disease.

Method: PPV2 was detected by real-time PCR (Ct 10.1-30) in either serum, spleen, joint fluid, or pleural fibrin. Paraffin-embedded sections were prepared in microarray blocks, and ISH targeting PPV2 was performed.

Results: There was a PPV2 positive signal in 7/9 synovium, 5/5 spleen, 2/2 lymph nodes, 1/1 tonsil, 2/4 hearts, 5/6 kidneys, 3/4 lungs, 1/1 ileum, 0/1 spinal cord, and 0/4 livers. ISH demonstrated a marked PPV2 signal in synoviocytes, endothelial cells, and germinal centers of lymphoid organs. PPV2 mRNA was also detected in pneumocytes, pulmonary macrophages, glomerular tufts, mesothelial cells, and endothelial cells of multiple organs. Concurrent bacterial arthritis or systemic bacterial infection, and PRRSV were diagnosed in 5/11 and 4/11 animals, respectively.

Conclusion: The distribution of the virus in various organs indicates a multisystemic distribution, with the lymphoid organs and endothelial cells being the most prevalent sites of viral detection. Further characterization of viral replication mechanism, host immune response, and association with systemic disease should be conducted to elucidate the clinical relevance of PPV2.

N25: SPONTANEOUS EARLY-ONSET NEURODEGENERATION IN THE BRAINSTEM AND SPINAL CORD OF NSG MICE.

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Background: The NOD.Cg- *Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mouse is the model of choice in several research settings. While the spectrum of age-related lesions affecting NSG retired breeders has been thoroughly characterized, comprehensive studies focusing on the spontaneous neuropathological changes in younger animals used in preclinical studies are lacking.

Objective: In this work, we describe the development of spontaneous early-onset neurodegenerative changes in young adult NSG mice.

Methods: The study cohort consisted of about 300 animals (median age 20 weeks, interquartile range 14-23 weeks) primarily used for preclinical CAR T cell testing and generation of chimeric animals with humanized immune system. Histopathology was conducted on brain and spinal cord. Immunohistochemistry for AIF1, glial fibrillary acidic protein (GFAP), CD34, and CD45 was also performed.

Results: Neurodegenerative changes were observed in about 40% of the NSG mice without a clear correlation between lesion frequency and either sex, age, or type of experimental manipulation/treatment, including naïve control animals. No clinical signs or neurotropic infections were reported in association with neuropathological changes. The lesions were characterized by multiple foci of neuroparenchymal vacuolation associated with gliosis and neuronal loss. These changes were multifocally distributed throughout the brainstem and spinal cord. Immunohistochemical analysis confirmed the development of microgliosis and astrogliosis as well as the infiltration of peripheral macrophages.

Conclusions: These findings suggest that NSG mice are predisposed to the development of early neurodegenerative changes associated with a neuroinflammatory component. While the exact cause of these lesions is currently unclear, potential associations with the NSG genetic background are considered.

N26: RESVERATROL AND VISCUM ALBUM CH200 REDUCED IN VITRO CANINE OSTEOSARCOMA CELL PROLIFERATION AND MIGRATION

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Osteosarcoma (OS) is a locally aggressive and metastatic neoplasm whose current therapeutic options remain ineffective in high-grade/metastatic tumors both in humans and animals. Mistletoe and resveratrol, natural antitumor agents, showed dose-dependent cytotoxicity and reduced the proliferation and motility of human OS cells (*in vitro*), but they have not been tested yet in canine OS cells. This study aimed to describe the antineoplastic action of homeopathic mistletoe and resveratrol compared to doxorubicin. For this, canine osteosarcoma cell lines D17 (CCL-183, ATCC, USA) and UNESP-OSA 8-4 (FMVZ, Botucatu, SP, Brazil), were cultured *in vitro*, subdivided into treated and control groups, followed by metabolic evaluation by the cell viability assay (MTT - 24 to 72h), and of migration by the Trans well test. Homeopathic *Viscum album* CH200 (InjectCenter, SP, BRA), trans-resveratrol (Sigma Aldrich, SP, BRA) and doxorubicin (Cayman Chemical, MI, USA) exhibited selective (dose-dependent) inhibition of cell viability as well as inhibition of cell migration in the Trans well test, with best results achieved by Resveratrol ($p<0.001$). These preliminary results open new perspective for further studies in canine OS treatment, focusing migration of neoplastic

cells and combination of the chemotherapeutic protocol with these natural compounds.
Finnantioal Support FAPESP(2020/01639-9, 2019/00766-0), CNPq (309161/2020-7) and CAPES

N27: CARYOSPORA-LIKE COCCIDIOSIS IN A CLUTCH OF RED-EARED SLIDER TURTLES (TRACHEMYS SCRIPTA ELEGANS)

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Background: *Caryospora*-like organisms (CLOs) form a clade of at least 11 genotypes of related coccidia causing epizootic mortality in sea turtles.

Objective: The goal of this study is to describe an outbreak of systemic CLO in red-eared sliders (*Trachemys scripta elegans*), a species of freshwater turtles.

Methods: Gross and histologic evaluation were performed. Ancillary testing included aerobic and *Salmonella*-specific cultures, transmission electron microscopy, immunohistochemistry, and PCR targeting pan-apicomplexan organisms, *Monocercomanadidae* flagellates, ranaviruses, and reoviruses.

Results: Within a clutch of captive-raised red-eared slider hatchlings, unexpected deaths were recorded within a three-week period (n=4). Deceased animals had severe segmental to diffuse, transmural fibrinonecrotic enterocolitis and hepatic necrosis with numerous intralesional coccidia. Immunohistochemistry highlighted various coccidian developmental stages in numerous tissues, which were further characterized by transmission electron microscopy. Among the different developmental stages, merozoites were ultrastructurally characterized by an apical complex. The apicomplexan PCR targeting the rRNA gene locus yielded a 347 bp-amplicon matching the *Eimeriidae, Schellackia/Caryospora*-like clade with 99.1 percent homology to the US3 strain from green sea turtles and 99.1 percent homology to *Schellackia* sp. isolate OC116. PCR testing for other agents was negative. Surviving hatchlings were treated with ponazuril but were subsequently euthanized due to the risk of spread to other chelonids in the collection. The ponazuril-treated hatchlings (n=4) had mild proliferative anterior enteritis, with several intraepithelial coccidia in one hatchling, which was confirmed as CLO by PCR.

Conclusions: This report highlights the relevance of *Caryospora*-like coccidiosis as a potentially fatal systemic disease of turtles with cross species infectivity.

N28: EMERGING REOVIRUS INFECTIONS IN TURKEYS IN QUÉBEC, CANADA

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Since 2011, new reoviruses strains emerged in commercial turkey, tentatively named turkey arthritis reovirus (TARV) and turkey hepatitis virus (THRV). TARV is known to cause tenosynovitis of the gastrocnemius and/or digital flexor tendons in 12- to 17-week-old turkey and THRV is associated with necrotizing hepatitis in young turkey poults around 2 weeks of age. In 2020, the first infections to these viruses were diagnosed in Québec, Canada, leading to important economical loss due to death of young birds, and culling or sudden death of near slaughter age lame turkeys. Different flocks were affected by the hepatitis and tenosynovitis lesions and young birds that survived the hepatitis did not developed tenosynovitis when older. The tenosynovitis lesions were characterized by lymphoplasmacytic inflammation, hyperplasia of synoviocytes and variable fibrosis of the subsynovium. In poults suffering from hepatitis, randomly distributed necrotic foci were visible in the liver parenchyma, in which typical syncytial degenerated hepatocytes were found. Also, a reovirus-associated non-suppurative encephalitis, never described before, was detected in some flocks, leading to ataxia and tremors in 31- and 69-day-old poults, with no concurrent hepatitis or tenosynovitis. The cycle threshold of qPCR was especially low in hepatitis cases (average 10,88) compared to tenosynovitis cases (average 23,85). Viral culture and sequencing of the sigma-C gene were performed to determine the homology of these reoviruses with known strains and preliminary results identified two clusters, that were not correlated with the type of lesion (hepatitis or tenosynovitis).

N29: AN EPIDEMIOLOGICAL REVIEW OF "GOOD SAMARITAN" ANIMALS IN THE OHIO STATE UNIVERSITY VETERINARY MEDICAL CENTER FROM 2014 TO 2022

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Introduction: Good Samaritan cases are unique to veterinary medicine and are situations in which the clinician provides immediate medical care to animals without an owner of record. The anatomic pathology service at the Ohio State University College of Veterinary Medicine provides postmortem examination of Good Samaritan animals that die under hospitalization in the Veterinary Medical Center (OSU VMC). Although these animals provide an indirect epidemiological survey of unattended animals, there has been a gap in knowledge on the cause of major mortality and morbidity of Good Samaritan animals.

Objectives: The goal of this study is to perform epidemiological review of the major mortality and morbidity in all Good Samaritan animals admitted to OSU VMC and subsequently submitted for autopsy.

Methods: By cross-referencing the OSU VMC medical records and OSU Anatomic pathology archive from 2014 to 2022, 77 Good Samaritan cases and their medical records were retrieved. The gross autopsy results, histology slides and medical records were reviewed.

Results: The cohort of 77 Good Samaritan cases consists of 45 felines, 31 canines and 1 opossum. In 3 species combined, trauma accounted for 38% of main mortality/morbidity, followed by infectious disease (17%), unknown (13%), euthanasia for rabies test (9%) and other etiologies (23%). Interestingly, infectious disease was the second most frequent morbidity (29%) in dogs, but lower in cats (9%).

Conclusions: This is the first retrospective review of autopsy results of Good Samaritan animals. Further studies will help raise awareness on the cause of mortality and morbidity in stray and neglected animals.

N30: HISTOPATHOLOGY OF PROLIFERATIVE ENDOCRINE LESIONS IN AMPHIBIANS

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Background: The endocrine system of amphibians is similar to other vertebrates and consists of glands that create and release polypeptides. This system includes thyroid glands, parathyroid glands, ultimobranchial glands, paraphysis, pituitary gland, pancreatic islets, and adrenal glands. Despite multiple large scale studies on amphibian neoplasia, endocrine neoplasia is rarely reported.

Objective: Describe proliferative endocrine lesions in amphibians.

Methods: This retrospective study searched the Northwest ZooPath archives for proliferative lesions in the endocrine system. Additional cases were identified from one of the co-author's personal archives (APP).

Results: Thyroid neoplasms were identified in four amphibians from families Hylidae, Ambystomatidae, Salamandridae, and Ranidae, respectively. All thyroid neoplasms caused grossly visible cervical swellings ranging from 1-5 cm in greatest dimension. Three thyroid neoplasms were follicular cystadenomas and one was a follicular carcinoma with widespread metastasis; all four neoplasms were composed of well-differentiated cells that formed colloid-filled follicles and lacked mitoses. Pancreatic islet neoplasms were identified histologically in three salamanders from family Ambystomatidae; all were non-invasive and composed of cords of well-differentiated islet cells. Pheochromocytomas were diagnosed in two amphibians from families

Bufonidae and Phyllomedusidae, respectively, and were composed of packets of neoplastic cells with marked cellular atypia. Proliferations of presumed parathyroid tissue were identified in seven amphibians, including three *Atelopus* sp., two *Strabomantis budoniformis*, one from family Ranidae, and one from family Leptodactylidae. In all cases, sheets of polygonal cells formed large, cystic masses around the great vessels.

Conclusions: Immunohistochemistry will be performed in attempt to confirm cell of origin for these lesions.

N31: CHARACTERIZATION OF FUNGAL CONIDIAL ADHERENCE TO EXTRACELLULAR MATRIX: ASSOCIATION WITH FUNGAL SECRETED PROTEASES AND IDENTIFICATION OF EXTRACELLULAR MATRIX BINDING PROTEINS IN BAT WHITE-NOSE SYNDROME

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Background: *Pseudogymnoascus destructans* (Pd) is the fungal pathogen of bat white-nose syndrome. The mechanisms and virulence factors of Pd infection is still minimally known. Typically, fungal adherence to host cells and extracellular matrix (ECM) is the critical first step of the infection, leading to colonization and invasion. Studies of Pd transcriptomes and secretomes suggested fungal secreted proteases could be involved in these processes.

Objective: The goals of this study were to characterize Pd conidial adherence to ECM, investigate the roles of secreted proteases during this process, and identify specific fungal cell-surface proteins that can bind to ECM.

Methods: *In vitro* ECM adherence assays were performed on laminin or fibronectin-coated plates. Series of experiments varying the concentration of ECM, incubation time and pretreatments with inhibitors were performed. Identification of ECM binding proteins from fungal extracts was achieved by using the affinity ligand assay coupled with liquid chromatography-tandem mass spectrometry.

Results: Pd conidia adhered to laminin and fibronectin in a dose-dependent, time-dependent and saturable manner. However, the interaction between fungal conidia and ECM was not specific, nor was it facilitated by enzymatic activity of secreted proteases. Moreover, this study additionally proposed glyceraldehyde-3-phosphate dehydrogenase and elongation factor 1-alpha as potential fibronectin binding proteins.

Conclusions: Pd may use conidial surface proteins to recognize laminin and fibronectin, facilitating adhesion to ECM. Other non-specific interactions may also contribute to the conidial adherence. The ECM binding protein candidates identified in this study additionally highlighted potential Pd virulence factors worth investigating in future studies.

N32: HISTOPATHOLOGICAL SUBTYPING OF CANINE OVARIAN CARCINOMA AND ITS EXPRESSION OF 14-3-3 SIGMA PROTEIN AND CYCLOOXYGENASE-2

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Background: Subclassification of ovarian carcinomas (OC) as papillary or cystic unlikely represents the entire spectrum. Unequivocal histopathological distinction between OC and sex cord-stromal tumors (SCST) remains challenging and 14-3-3 sigma protein expression may aid in their differentiation. Cyclooxygenase-2 (COX-2) is a therapeutic target in canine neoplasms but its expression in OC has not been properly investigated.

Objective: To histopathologically subtype canine OC and investigate its 14-3-3 sigma and COX-2 expression.

Methods: Archived H&E sections from 24 OC were histopathologically examined and additional sections were subjected to immunohistochemistry for 14-3-3 sigma and COX-2.

Results: 75% of OC exhibited mixed histopathological patterns. Solid sheets of neoplastic cells with tubule formation predominated in 13 cases (solid-tubular subtype; 54.2%) and papillae in nine (papillary subtype; 37.5%). Two cases consisted of sessile polypoid structures with thin neoplastic cords supported by abundant hyalinized stroma (hyalinized subtype; 8.3%). Twelve cases (50%) included cystic areas. Thirteen cases (54.2%) exhibited cytoplasmic labelling for 14-3-3 sigma. COX-2 was expressed in two OC (8.3%), labelling neoplastic cells that blended with areas of coagulative necrosis.

Conclusions: The solid-tubular subtype of canine OC was most frequent. A previously undescribed subtype was termed hyalinized based on its histological features. 54.2% of cases overexpressed 14-3-3 sigma, in comparison to normal ovarian epithelium, suggesting its applicability in OC immunohistochemical panels. The overall low expression of COX-2 in canine OC differs from its overexpression in highly aggressive OC in women. Due to its apparent low expression in canine OC, COX-2 is presumed unsuitable as a therapeutic target.

N33: FIRST DESCRIPTION OF VESSEL CO-OPTION AS A MECHANISM OF TUMOR NUTRITION IN METASTATIC CANINE TUMORS.

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Background: Angiogenesis is essential for tumor progression and metastasis. The alternate and poorly known forms of tumor nutrition named vasculogenic mimicry and

vessel co-option (VCO) are related to poorer prognosis and anti-angiogenic treatments resistance in human oncology. In VCO highly malignant tumor cells can survive by hijacking pre-existing blood vessels of the invaded tissue. Despite its importance, VCO has never been described in Veterinary Medicine

Methods: Sixty necropsies of dogs with metastatic malignant tumors (metastases in lung, 33; liver, 7; brain, 2), were retrospectively retrieved. VCO histopathological characteristics were assessed following criteria described previously. Hematoxylin-eosin, PAS, Masson's trichrome and reticulin stains, and CD31 immunohistochemistry were used to evaluate VCO in the primary tumor or metastases.

Results: Eleven out of 60 cases (18.3%) presented VCO in pulmonary (9/33, 27.3%), hepatic (1/7, 14.3%) and brain (1/2, 50.0%) metastases, but not in primary tumors. Three patterns of VCO were found in lung: Alveolar pattern (7/9), cancer cells filled the alveolar spaces in a "honeycomb" pattern with an intact reticulin framework; Lepidic pattern (1/9), cancer cells grow lining the alveolar septa, adhering to the underlying basement membrane without completely filling the airspace; and Interstitial pattern (1/9), neoplastic cells expanded the alveolar interstitium by co-opting the alveolar capillaries. VCO in liver and brain metastasis showed expansion of perivascular spaces by neoplastic cells (perivascular pattern).

Conclusions: This study describes for the first time in Veterinary Medicine, the presence of VCO. Although the significance of VCO remains obscure, its observation should be considered in the pathology reports.

N34: IMMUNOHISTOCHEMICAL EVALUATION OF TUMOR MICROENVIRONMENT CHANGES INDUCED BY ECPMV NEOADJUVANT IMMUNOTHERAPY IN CANINE INFLAMMATORY AND NON-INFLAMMATORY MAMMARY CANCER.

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Background: Intratumoral vaccination with highly immunogenic empty nanoparticles of Cowpea Mosaic Virus (eCPMV) is a novel immunotherapy that improves survival and quality of life in dogs with inflammatory mammary cancer (IMC), the most aggressive and lethal type of mammary cancer, and canine malignant oral melanoma. This study evaluates cell diversity variations in the tumor microenvironment caused by eCPMV in IMC and non-IMC cases.

Methods: Tumor samples from 15 treated dogs (4 IMC and 11 non-IMC) were evaluated before (incisional biopsy) and after (excisional biopsy/necropsy) intratumoral treatment with eCPMV. A comprehensive immunohistochemical panel with

myeloperoxidase (MPO), CD3, CD20, FOXP3, MUM-1, Iba-1 and cKIT markers was performed on all samples (n=30). Positive cells/mm^2 for each marker were calculated.

Results: Immunotherapy with eCPMV significantly increased intratumoral neutrophils (mean 86.51 *vs* 574.44 MPO+ cells/mm^2), T lymphocytes (mean 101.11 vs 374.17 CD3+ cells/mm^2) and macrophages (212.19 vs 424.02 Iba-1+ cells/mm^2) in post-treatment tissues compared to pre-treatment biopsies (p<0.03 for all comparisons). In addition, the ratio of regulatory T cells (FOXP3+)/T lymphocytes (CD3+) was significantly reduced (p=0.006) after treatment.

Conclusions: Vaccination with eCPMV induces a potent immunostimulatory effect on IMC and non-IMC cases through recruitment of neutrophils as the main drivers of the inflammatory response, with a decrease in the FOXP3+/CD3+ ratio reducing the immunosuppressive action of regulatory T lymphocytes and producing a breakdown of tumor immunotolerance. The increase in macrophages and T lymphocytes may suggest an increase in antigen presentation with an activation of the adaptative immune response.

N35: CHARACTERIZING GROSS, HISTOLOGIC, AND IN SITU HYBRIDIZATION FEATURES OF EMERGING SKUNK ADENOVIRUS 1 IN NORTH AMERICAN PORCUPINES (ERETHIZON DORSATUM)

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Background: Skunk adenovirus 1 (SkAdV-1) is an emerging pathogen in eastern North America with multiple known outbreaks. This virus has been identified in a variety of taxonomically unrelated mammals, raising concern for its potential impacts on wildlife health. However, information on pathologic presentations is limited.

Objective: As clinical SkAdV-1 disease is most commonly observed in North American porcupines (*Erethizon dorsatum*), this study aimed to document gross and microscopic lesions in this species by standardized postmortem pathologic examinations, RNA in situ hybridization (ISH), and molecular analyses of retrospective and prospective samples.

Methods: 65 cases from 2010 to 2022 were examined grossly and histologically. Of these, 36 had changes in the respiratory tract and were further evaluated using ISH. SkAdV-1-specific PCR assays were conducted on nasal swabs and/or lung samples on 22 recent cases and additionally on ocular swabs and feces of SkAdV-1-positive cases.

Results: Hybridization was confirmed in 9/65 cases (13.9%) with 7/9 nine cases having classic respiratory lesions consisting of rhinotracheitis and bronchointerstitial pneumonia. The other two cases only had hepatitis and peritonitis, separately. Novel ocular and middle ear lesions were confirmed in five and three cases, respectively,

expanding the known range of SkAdV-1-associated disease. SkAdV-1 DNA was detected from the nasal passage and/or lung in 10/22 cases and in both ocular swabs and feces.

Conclusions: SkAdV-1 is a prevalent, primary respiratory pathogen in porcupines with various transmission routes, which may impact a wider wildlife population.

N36: IDIOPATHIC ILEAL HYPERTROPHY IN A CAPTIVE ONE-HORNED RHINOCEROS (RHINOCEROS UNICORNIS)

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A 17-year-old, one-horned rhinoceros presented unspecific signs of decreased appetite and fecal output, and lethargy lasting for approximately three months. Gastroscopy and abdominal ultrasonography revealed the presence of a large quantity of food in the stomach despite a 48-hour fast, and diffuse thickening (up to 2 cm) of the small intestinal wall. An exploratory laparotomy was attempted. However, marked distension of the large intestine by gas and fluid restricted access to the abdominal cavity. Given the poor prognosis, the rhinoceros was euthanized and submitted for necropsy.

Necropsy revealed that the terminal 1.26 m of the ileum was affected by marked thickening of the intestinal wall, up to 1 cm in width. This segment showed irregular, dark-brown discoloration of the serosa and presented one, 1 cm diameter diverticulum. Histology revealed that widening of the intestine was caused by thickening of the tunica muscularis, which was otherwise unremarkable. The width of the affected intestine was compared to historical controls, constituting of histological sections of the small intestines from 4 rhinoceros (one conspecific and 3 white rhinoceros). The tunica muscularis was 2.5- to 6.4-fold thicker compared to controls. Given the lack of other underlying diseases, a diagnosis of intestinal idiopathic muscular hypertrophy was made.

The gross and histological features of the intestinal smooth muscle hypertrophy of this rhinoceros were similar to what described in the horse, another perissodactyl. This is the first report of this condition in a rhinoceros, and it should be included in the list of differential diagnoses for this species.

N37: THE EXPRESSION OF YKL-40 IN CANINE CUTANEOUS MAST CELL TUMORS AND ITS ASSOCIATION WITH CLINICAL AND PATHOLOGICAL FEATURES

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Background: YKL-40, a novel glycoprotein, involves in tumor proliferation, metastasis, and angiogenesis in human cancer diseases. Overexpression of YKL-40 has correlated with poor prognosis in many human cancers. In veterinary medicine, overexpression of YKL-40 in the serum of canine cutaneous mast cell tumors (MCTs) has been found. However, the expression pattern of YKL-40 in MCT tissues associated with clinical and pathological features is still unknown.

Objective: The study aims to investigate the expression level of YKL-40 in canine MCTs and correlate it to clinical features, pathological features, and pathological grade.

Methods: Formalin-fixed paraffin-embedded tissues were collected from different individuals, including 15 high-grade and 17 low-grade MCTs. An anti-canine YKL-40 single-chain fragment variable (scFv) was used to detect YKL-40 by the immunohistochemistry. The expression levels of YKL-40 were semi-quantified by immunoreactivity score, then statistically compared to clinical and pathological features.

Results: Cytoplasmic immunoreactivity of YKL-40 was detected by scFv in most MCT cases (93%). YKL-40 appears to be relatively weaker expressed in high-grade than in low-grade. Additionally, the expression of YKL-40 is more prominent in mast cells infiltrating between collagens than those aggregating. The expression level was not significantly associated with clinical stage, lymph node metastasis, mass diameter, vessel density, mitotic counts, and histological grades.

Conclusions: Mast cell tumors moderately to strongly express YKL-40. Moreover, the expression distribution suggests YKL-40 may participate in tumor invasion of MCTs. *In vitro* study is needed to investigate the mechanism of YKL-40 in mast cell tumors.

N38: USING A CANINE SINGLE CELL TRANSCRIPTOMIC ATAS TO AID IN THE INVESTIGATION OF CANINE LYMPHOMA/LEUKEMIA

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Background: Hematopoietic malignancies are classified by multiple features, including identification of their normal counterpart, or cell of origin. Single cell transcriptomics offers the ability to characterize multiple leukocyte subsets simultaneously.

Objective: Generate a canine single cell transcriptomic reference of hematopoietic tissue to aid in the classification of canine lymphoma and leukemia.

Methods: Canine bone marrow (BM), thymus (Th), peripheral lymph node (PLN), and spleen (Sp) single cell suspensions were processed using 10x Genomics' technology. Libraries were sequenced using an Illumina NovaSeq 6000 and aligned to the Ros_cfam_1.0 canine genome. Data was analyzed with the Seurat R package. Cell type identification was cross-referenced with gene sets from publicly available databases. Bulk RNA-sequencing data from purified healthy CD4+ T cells and CD4+ T

cell lymphomas were deconvoluted using Cibersortx to impute cell fractions using the Th/PLN reference data sets.

Results: 12,990, 12,159, 25,330, 15,548 cells were sequenced from BM, Th, PLN, and Sp, respectively. Dimensionality reduction and clustering identified 26, 23, 17, and 28 predicted cell clusters globally within BM, Th, PLN, and Sp, respectively. Deconvolution of bulk RNA-seq CD4 T cell lymphoma revealed that these tumors had higher proportions of cell types classified as T cell precursors compared to sorted healthy CD4 T cells (p.adj. <0.05).

Conclusions: The atlas robustly identified major cell types within each hematopoietic tissue, including transcriptomically unique subtypes not identifiable with traditional methodologies, which can aid in identifying the cell of origin in canine lymphoma/leukemia.

N39: RETROSPECTIVE STUDY OF NEOPLASIA IN CAPTIVE AND FREE-RANGING WATER BIRDS, 2014-2022: PRELIMINARY FINDINGS

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Little is reported on neoplasia in water birds. This retrospective study reviewed submissions (n=1587) to the Infectious Diseases Laboratory, Athens Veterinary Diagnostic Laboratory, Department of Pathology, and the Southeastern Cooperative Wildlife Disease Study, The University of Georgia, between 2014-2022, for the diagnosis of neoplasia. This included submissions from five orders of water birds: Anseriformes, Pelecaniformes, Charadriiformes, Ciconiiformes, and Gruiformes. Twenty-nine birds were diagnosed with neoplasia. The majority of birds (n=19) were captive (pets and zoological collections). The hepatic (n=9) and urinary (n=8) systems were most commonly affected. The most common types of neoplasia were lymphoma (n=5), followed by tumors of the kidney and liver (n=4), and tumors of the reproductive tract (n=3). Affected animals ranged from 6 months to 40 years of age, but age was often unknown for free-ranging birds. Neoplasms were more commonly diagnosed in female birds and various species of ducks. Overall, the incidence of neoplasia in these five orders of water birds was low. Understanding patterns of neoplasia in water birds is important for captive and wild water bird disease surveillance and health management.

N40: EVALUATION OF SYSTEMIC AND RESPIRATORY INFLAMMATORY RESPONSES IN AUCTION CALVES AT HIGH RISK FOR BOVINE RESPIRATORY DISEASE AND LOW-RISK IN-HOUSE CALVES

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Background: Auction calves are considered at risk of BRD development due to the high stress of transportation, comingling, and pathogen transmission. Previously, an immunosuppressive pathogenesis has been suggested due to the latter predisposing factors but a more complex dysregulated pro-inflammatory response is suspected based on our recent findings.

Objective: Characterize systemic and local respiratory inflammatory responses in high-risk auction calves or lower-risk ones from the cow-calf production system, to determine if auction calves are at risk of developing excessive inflammatory responses after their arrival.

Methods: Two groups of calves were considered: Calves from Ontario Beef Research Center (low-stress) and calves purchased from an auction barn in Ontario. Blood and bronchoalveolar lavage fluid (BALF) samples were collected on days 1 and 4 post-arrival. Differential cell counts were performed on blood. Cell counts and expression of macrophage polarization markers (CD163, CD 206, and CD86) were evaluated in BALF.

Results: Compared to the low-risk group, there were a significantly lower number of blood neutrophils and monocytes ($P<0.05$) in auction calf groups on day 4. Moreover, the plateletcrit was significantly elevated in the auction calves on day 4. Calves in the auction groups had significantly higher expression of CD86 as well as a tendency for higher concentrations of neutrophils in the BALF on day 4.

Conclusions: Our preliminary results suggest that auction calves are more likely to have a pro-inflammatory response after arrival. These findings may suggest a novel mechanism by which auction calves are predisposed to BRD.

N41: PRIMARY LENS LUXATION AND ZONULAR LIGAMENT DYSPLASIA IN CHIHUAHUAS AND OTHER NON-TERRIER DOG BREEDS

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Background: Zonular ligament dysplasia (ZLD) is a well-known cause of primary lens luxation (PLL) in terrier dog breeds, but is poorly-documented in non-terrier breeds. In terriers, ZLD is caused by a single nucleotide polymorphism (SNP) in the ADAMTS17 gene, but the presence of this mutation has not been described in many non-terrier breeds with ZLD.

Objectives: Our objectives were to describe the non-terrier breed distribution of ZLD in the COPLOW database and to investigate the presence of the ADAMTS17 mutation in Chihuahuas, a previously unreported breed.

Methods: The COPLOW database was mined using the search terms “zonular ligament dysplasia” and “dysplastic zonular ligament protein” from the years 1989-2022. Chihuahuas with histologic evidence of ZLD were tested via a target-based assay to detect a SNP in the ADAMTS17 gene.

Results: Individuals from 39 unique pure breeds and 25 mixed breeds were diagnosed with ZLD in the COPLOW database. Notable non-terrier pure breeds included Australian cattle dogs (n=66, 16.26%), beagles (n=35, 8.62%), shar-peis (n=28, 6.9%), Chihuahuas (n=9, 2.22%), and Australian shepherds (n=3, 0.74%). When compared to the total number of individuals from these breeds in the database, Australian cattle dogs with ZLD=18.28%, beagles=3.83%, shar-peis=14.97%, Chihuahuas=0.85%, and Australian shepherds=0.51%. 81.25% of Chihuahuas with ZLD (n=13/16) were homozygous for the SNP in ADAMTS17.

Conclusions: ZLD is present in many dog breeds, some of which (Chihuahua, beagle, and Australian shepherd) are not reported in the literature. The ADAMTS17 SNP mutation is present in some ZLD-positive Chihuahuas, a breed not previously recognized as predisposed to ZLD.

N42: TRICHINELLOSIS IN A DOMESTIC CAT FROM OKLAHOMA WITH CONCURRENT FUNGAL CELLULITIS

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Tissue biopsy of a focal subcutaneous elbow lesion from an adult, castrated male, domestic shorthair cat in Payne County, Oklahoma was submitted to OADDL for histopathology. Microscopic findings included extensive pyogranulomatous cellulitis, and few, incidental, intramyocytic encysted nematode larvae. Morphologic features of the parasitized myocytes were consistent with *Trichinella* spp. Within the cellulitis, Grocott’s methenamine silver staining revealed scattered, 2-3 um, oval yeasts, most consistent with either *Histoplasma capsulatum* or *Sporothrix schenckii*. Giemsa and Ziehl-Neelsen acid fast stains were negative. Ancillary testing performed thus far includes fecal analysis (negative), Histoplasma urine antigen EIA (negative), panfungal PCR (pending) , and *Trichinella* spp. PCR (pending). To the authors’ knowledge, this is only the second report of trichinellosis in a domestic cat in the United States.

N43: FUNGAL PNEUMONIA AS A CAUSE OF MORTALITY IN 6 PET PIGS IN ARIZONA (2017-2022)

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Background: Between the years 2017-2022, Midwestern University received 6 pet pigs with severe eosinophilic-granulomatous pneumonia with intralesional hyphal fungal organisms. Individuals were 7 months – 8 years (median 1.46 years) with 4 females (2 intact, 2 spayed) and 2 males (1 intact, 1 castrated). Breeds listed were miniature and Vietnamese potbellied pig. Clinical signs prior to death included coughing, dyspnea, and respiratory distress. Two pigs had an eosinophilic leukocytosis on CBC. Due to disease progression, five pigs were euthanized, and one died naturally. On postmortem exam, common findings included a large space occupying, fibrous mass that replaced 70-100% of a lung field, with the right hemithorax most often affected. In two cases, there was direct extension into the diaphragm and abdomen. Microscopically, the fibrous masses were coalescing eosinophilic granulomas that contained large numbers of fungal hyphae. Hyphal structures were 4-7 um with parallel walls and irregular branching. In one case, hyphae were 21-30 um and ribbon-like with dichotomous branching. Hyphae were regularly PAS positive and GMS negative. Fungal culture (5/6) isolated Aspergillus spp. in 2 cases (2/5). Pan fungal PCR (6/6) either failed or did not match morphology of fungus. Given histologic morphology of hyphal structures, aerogenous infection with Conidiobolus spp. or Mucor spp. is suspected. This series demonstrates that fungal pneumonia is a cause of mortality within young pet pigs in Arizona as well as the difficulties of fungal isolation via culture and PCR for the suspected species of fungus.

N44: NEUROSENSORY LESIONS IN FIELD CASES OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) INFECTION

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Background: Field strains of PRRSV only sporadically induce neurological signs; however, inflammatory lesions are documented in experimentally infected animals that do not exhibit dramatic CNS aberrations. In commercial production, clinical recognition of neurologic disease is typically limited to overt deficits in locomotion or loss of central awareness. The SARS-CoV-2 pandemic has drawn scientific and laypublic attention to the myriad manifestations of viral neurotropism, including adverse impacts on quality of life associated with sensory neuropathies, such as anosmia and ageusia. Recently, recognition of unexpectedly widespread neurotropism in field cases of PRRSV received at Iowa State University Veterinary Diagnostic Laboratory (ISUVDL, internal data) has raised concerns regarding impacts of viral encephalitis on pig health and welfare due to damage of sensory pathways unamenable to routine clinical evaluation.

Objective and Methods: Our objective was to evaluate neurosensory pathology in pigs with PRRSV encephalitis. Animal selection was limited to pigs with CNS disease (ataxia, paddling) received in 2022, wherein intact heads/carcasses were retained for processing (n=10 pigs). Once PRRSV encephalitis was confirmed, sections of cribriform plate, eyes, midbrain, and tongue, when available, were retrospectively trimmed and stained with H&E.

Results: Viral lesions, including mononuclear perivasculitis, malacia, glial nodules, neuroglial and leukocytic degeneration were present in olfactory and optic tracts of pigs with PRRSV encephalitis (9/10). Olfactory nerves and rhinencephalon were most frequently affected (9/10), followed by iridoretinitis and optic neuritis (3/10).

Conclusions: PRRSV infection can damage olfactory and optic tracts, and may adversely impact productivity and welfare in survivors lacking overt CNS signs.

N45: PATHOLOGY OF RUMINAL ACIDOSIS IN CATTLE

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Background: Rumen acidosis (RA) is one of the most prevalent alimentary diseases of ruminants and is caused by excessive fermentation of feedstuff in the reticulorumen.

Objective: The aim of this study was to characterize histomorphometrically the microscopic lesions of acute and subacute RA in cattle.

Methods: Fifty-six RA cases submitted to CAHFS were selected for this study. RA was defined as ruminal pH < 5.5. A detailed autopsy was performed in each case and samples were collected for histopathology. Cases were classified as acute (n=29) or subacute (n=27) RA based on clinical history. In addition, 7 animals which had died of causes other than RA and had normal rumen content pH were included as controls. On rumen sections, number, length, and width of ruminal papillae, and thickness of the epithelium and stratum corneum were measured. Univariable lineal mixed models were used to compare groups.

Results: Acute cases had shorter papillae than control animals ($p<0.05$), and absent or few, less than <150 µm microabcesses within the ruminal epithelium. Sub-acute RA had shorter and wider rumen papillae than control animals ($p<0.05$), epithelial hyperplasia ($p<0.05$), parakeratotic hyperkeratosis ($p<0.05$) and intraepithelial microabcesses ranging from 150 µm to 500 µm.

Conclusions: Ruminal fluid pH measurement and pathologic findings are important to diagnose both forms RA. Histomorphometrically, subacute RA cases had wider rumen

papillae and more prominent epithelial hyperplasia than acute RA cases. Acute cases differed from control cases only by shorter ruminal papillae, which highlights the scant morphologic changes in this group.

N46: A NOVEL CHUVIRUS CAUSING MENINGOENCHEPATLITIS IN ALLIGATOR SNAPPING TURTLE, THE FIRST IN-SITU EVIDENCE OF CHUVIRAL DISEASE IN VETERBRATES

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Chuviruses, which are in the recently discovered family *Chuviridae*, were first identified in arthropods in 2015 and have been observed through metagenomics in other phyla including platyhelminths, cnidaria, nematodes, mollusks, echinoderms, and chordates. Several chuviruses have been found in vertebrates, e.g., long-spine snipefish (wenling fish chuvirus), hardyhead fish (hardyhead chuvirus), red-banded snake (Guangdong red-banded snake chuvirus), and boa constrictor (Herr Frank virus), but their pathological significance is unknown. This study identified a novel chuvirus in a wild alligator snapping turtle (*Macrochelys temminckii*) with non-suppurative meningoencephomyelitis. The animal was severely lethargic on the shore of a Newnans Lake (Alachua County, Florida) and later euthanized in a moribund state. The principal necropsy finding was moderate to severe, diffuse, lymphoplasmacytic meningoencephalomyelitis with numerous perivascular cuffs. Transmission electron microscopy of the brain demonstrated 85-nm diameter spherical, enveloped viral particles. The application of random, deep MinION sequencing successfully assembled a complete viral genome that best aligned to chuviruses. The genome sequence was used to create a custom RNAscope^{Ò} probe for in-situ hybridization, which confirmed the presence of chuviral RNA within the cerebrum and spinal cord, co-localizing with multiple areas of necrosis and non-suppurative inflammation. All other tissues lacked viral staining. Phylogenetic analysis illustrated that this virus clustered with other vertebrate chuviruses. Based on pairwise amino acid identities, this chuvirus belongs to a novel species, putatively named *Piscichuvirus macrochelydis*. Moreover, this study demonstrated the first in-situ evidence of chuvirus pathogenicity in animals. Additional studies of these viruses are needed to elucidate their role in animal disease.

N47: MICRORNA PROFILING OF PRIMARY APPENDICULAR AND PULMONARY METASTATIC OSTEOSARCOMA TISSUE IN DOGS

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Background: Appendicular osteosarcoma is a common bone tumor of dogs with a poor outcome despite aggressive therapy. Although few dogs have clinically detectable metastases at diagnosis, 90% of them are euthanized due to progressive metastatic

disease. Novel molecules to predict prognosis and/or act as therapeutic targets are necessary as current grading schemes and biomarkers are inadequate at determining prognosis. MicroRNAs are non-coding RNA molecules that regulate translation of messenger RNA and are dysregulated in cancer. Our lab previously found that microRNAs in plasma from canine osteosarcoma patients are predictive of clinical outcomes, including disease-free interval.

Objective: We hypothesize that primary appendicular osteosarcoma (priOSA), pulmonary metastases (metOSA), and normal lung tissue from dogs will have different microRNA profiles.

Methods: MicroRNA was isolated from 41 priOSA, 12 metOSA, and 5 normal lungs. Fifty-nine microRNAs were evaluated for each sample using QIAGEN MIRCURY LNA custom PCR arrays. The Ct values were compared using $2^{-DeltaDetItaCt}$.

Results: Seven microRNAs were upregulated and 4 were downregulated in priOSAs compared to metOSAs. miR-200c was expressed in 10/12 metOSAs but had no expression in 39/41 priOSAs. Nineteen microRNAs were upregulated and 8 were downregulated in metOSAs compared to normal lung. All metOSAs had a Ct < 32 for miR-9, while all lung tissues had a Ct >34, with 4/5 having no expression.

Conclusions: These findings demonstrate differential expression of microRNAs between primary and metastatic osteosarcoma. miR-200c and miR-9 should be further investigated for their role in disease progression and as potential therapeutic targets for metastatic disease.

N48: EXPRESSION OF IMMUNOINHIBITORY CHECKPOINT MOLECULES IN CANINE SOFT TISSUE SARCOMA

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Background: Canine Soft Tissue Sarcomas (STS) are common neoplasms in dogs and are considered immune deserts. Tumor infiltrating lymphocytes are sparse in STS, when present tend to organize around blood vessels or at the periphery of the neoplasm. This pattern has been associated with an immunosuppressive tumor microenvironment linked to overexpression of molecules of the PD-axis. PD-1, PD-L1 and PD-L2 expression correlates with malignancy and poor prognosis in other neoplasms in humans and dogs, but little is known about their role in canine STS, different grades, and how different therapies affect their expression.

Objective: Evaluate the expression of checkpoint molecules across STS tumor grades and subsequent to tumor ablation treatment.

Methods: Gene expression analysis of checkpoint molecules was performed by qRT-PCR from soft tissue sarcomas that undergo tumor ablation therapy (histotripsy) and

from FFPE specimens of STS of different grades from the Virginia Tech Animal Laboratory Services archives.

Results: The expression of PD-1, PD-L1 and PD-L2 was detected in untreated STS tissue representing grades 1, 2, and 3. Trends of decreased expression of all markers were observed in tissue sampled from the treatment interface relative to untreated areas of the tumor.

Conclusions: The relatively lower expression of these checkpoint molecules at the periphery of the treated area may be related to liquefactive necrosis induced by the histotripsy treatment and would potentially allow TILs to infiltrate the tumor. The relative trend of increase of these checkpoint molecules in tumors of a higher-grade support previous reports that associate their expression with malignancy.

Student Platform Presentations

MODELING AGE-RELATED CHANGES IN THE TUMOR MICROENVIRONMENT IN AN ORTHOTOPIC IMMUNOCOMPETENT MURINE PANCREATIC CANCER MODEL—A NOVEL APPROACH

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Pancreatic cancer is the third leading cause of cancer-related deaths in the U.S. and is most frequently diagnosed between the ages of 65-74 years. Only 11.5% of patients are expected to survive 5 years after diagnosis. There is extensive evidence that tumor microenvironment (TME) plays a critical role in cancer progression and metastasis. Despite the fact that most patients are diagnosed over the age of 60, most preclinical murine tumor models utilize young, healthy, adolescent mice, failing to account for structural and/or functional changes in stroma and immune cells that occur in tissues over time and contribute to tumor progression. Marked differences in lifespan between mice and humans preclude natural development of many age-related physiologic changes in mice that occur in humans over the course of decades. We performed a pilot study to test our hypothesis that KPC pancreatic tumors grown orthotopically in the pancreas of genetically engineered, LMNA-/- mice with features of accelerated aging would have altered tumor growth in comparison to tumors in WT controls. We found that tumor growth in LMNA-/- mice outpaced that of WT mice. Quantitative digital image analysis showed significantly reduced intratumoral infiltration of CD45+ leukocytes in LMNA-/- mice in comparison to WT. There was a significant reduction in tumor-infiltrating CD3+ T cells and F4/80+ macrophages. Our data supports the hypothesis that functional, age-related, immune and/or stromal TME changes impact pancreatic tumor growth and progression. Innovative strategies that better model age-related changes may better recapitulate the aggressive course of pancreatic cancer in aged patients.

USE OF A SUPEROXIDE DISMUTASE MIMETIC TO PREVENT SPACEFLIGHT-INDUCED BONE AND JOINT DEGRADATION IN MICE

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The damaging effects of microgravity on bone during spaceflight are well known; however effects on joint soft tissues remain poorly understood. The Rodent Research 9 mission identified cartilage and meniscal degradation in mice after ~35 days on the International Space Station (ISS). Damage occurred coincident with reduced antioxidant (Aox) defenses (e.g. superoxide dismutase (SOD)) and increased oxidative stress. As oxidative stress is associated with arthritis, we measured the efficacy of MnTnBuOE-2-PyP5+ (BuOE, 1 mg/kg IP, q.wk.), an Aox SOD mimetic, at preventing damage. Ten-week-old male C57BL/6 mice (n=10) in the Rodent Research 18 mission (Dec-Feb, 2022) spent 35 days on ISS with treatment (5 BuOE, 5 saline controls). Tissues were collected upon return, along with corresponding ground controls (n=10). Knee joints from right hindlimbs were isolated for histologic assessment of bone and cartilage using TRAP, MMP13, ADAMTS5, and Safranin O. Our microCT data indicates that the Aox is protective against articular cartilage and bone degradation during spaceflight. TRAP staining indicated Aox lowered osteoclast number overall, though by day 35 osteoclast number from FLIGHT mice was lower than GROUND control, indicating bone loss with microgravity occurred early in the mission. Aox may also reduce aggrecan degradation and promote formation of sulfated glycosaminoglycans (GAGs) in the cartilage and menisci during spaceflight, but as thinning occurred despite no loss of GAGs in flight, degradation of collagen may contribute to soft tissue damage during spaceflight. Taken together, our data indicate treatment with MnTnBuOE-2-PyP5 may protect against degradation of joint soft tissue and bone.

DERMATOPATHY IN A STRANDED MALE YEARLING NORTHERN ELEPHANT SEAL (MIROUNGA ANGUSTIROSTRIS)

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Background: In May 2021, a male yearling northern elephant seal presented to The Marine Mammal Center (TMMC) for lethargy and marked swelling of the right axilla, shoulder, and head. Approximately 80-90% of the skin was affected by alopecia, lichenification, dry black irregular cutaneous depressions, irregular tan moist ulcerations, and dry crusting ulcerations with raised thickened and puckering peripheral skin.

Objectives: To report a possible case of Northern Elephant Seal Skin Disease

(NESSD), which has not been reported for more than two decades.

Methods/Results: Complete blood cell count and biochemistry changes were consistent with inflammation, dehydration, and malnutrition. Due to poor prognosis, euthanasia was elected. Urine culture, fungal culture of the hair, and anaerobic and aerobic bacterial cultures of the subcutis and blubber were performed. Mixed bacteria were isolated from all sites with no fungal organisms isolated. On gross necropsy, large cavitations between the dermis and underlying subcutis were present. Dorsally there was emphysema emitting a putrid odor overlying approximately one liter of straw-colored oily non-viscous semi-translucent fluid in the deeper tissues. Histologic findings of the skin included subcutaneous and superficial dermal vasculitis, thrombosis, coagulative necrosis, sebaceous adenitis, and sebaceous gland metaplasia.

Conclusions: These skin lesions may be indicative of NESSD, a potentially fatal condition characterized by generalized, ulcerative dermatitis. From 1992 to 2001, it was the third most common presentation (9.8%) of northern elephant seals stranding at TMMC. The etiology is unknown and determining the pathophysiology may elucidate critical stressors to marine mammal and ocean health.

PERICARDIAL TREMATODIASIS IN JOHNNY DARTERS (ETHEOSTOMA NIGRUM) IN THE CACHE LA POUDRE RIVER

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Fish can be used as sentinels of environmental health. Granulomatous pericarditis and pericardial trematodes were incidentally recognized during a health survey of wild-caught Colorado Johnny Darters (Etheostoma nigrum) conducted in 2020-2021. The objective of this study was to determine the prevalence of pericarditis and pericardial trematodiasis in Johnny Darters at a collection site on the Cache la Poudre River. Fish and morphometric data were collected by the U.S. Geological Survey, Colorado Cooperative Fish and Wildlife Research Unit. One to two H&E sections from each fish were reviewed for the presence or absence of pericarditis and pericardial trematodes. Gram, GMS, and Acid-Fast preparations were applied to serial sections to investigate for bacteria and fungal agents. The sample included 53 female, 27 male, and 20 unknown sex fish with average total length 49.8mm (33-69mm) and average weight 1.1g (0.2-2.9g). Ninety-two percent of fish had pericarditis and 45% of those individuals had pericardial metacercariae. Gram, GMS, and Acid-Fast stains were negative in all serial preparations that included pericardium (72, 71, and 71 fish, respectively). The true prevalence of trematodiasis may be higher given the two-dimensional nature of histologic evaluation. Future work may include next generation sequencing to identify the trematode genus and species and identify any additional organisms, elucidation of parasite life cycle, and comparison of prevalence at other river locations in relation to environmental parameters.

DEEP LEARNING-BASED ASSESSMENT OF CORNEAL DAMAGE IN ISOLATED CHICKEN EYES

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Background: The isolated chicken eye test (ICET) is a validated method of identifying and assessing potential eye irritants. This *ex vivo* model utilizes a descriptive, semi-quantitative evaluation process to categorize substances for product safety (no category, mild, category 1). This procedure is time-consuming, ambiguous, and prone to bias and interobserver variability. Machine-learning-based artificial intelligence (AI) classifiers could facilitate a more consistent and quantitative assessment.

Objective: We developed a deep learning-based AI classifier for whole slide images (WSI) of cornea to support pathologists analyzing the ICET.

Methods: Routinely prepared hematoxylin and eosin-stained slides of chicken corneas were digitized at 40x and uploaded into Aiforia® Create, a commercial deep learning image analysis platform. Normal microanatomy and lesions were annotated by trained individuals based on a published ground truth. This supervised training approach produced a classifier using a convolutional neural network.

Results: AI-generated inference masks visually demonstrated erosion and loss of epithelial layers and the presence of vacuoles. Semantic segmentation of the cornea and corneal epithelial layers (superficial, middle, deep) produced area measurements to quantify epithelial erosion. Instance segmentation generated exact vacuole counts and sizes. Quantitative differences ($P<0.05$) in some of these measurements correlated with ICET categories and were consistent with the pathologists' original interpretation.

Conclusions: Deep learning AI-generated inference masks may provide decision support for pathologists during routine ICET slide review by reporting objective and quantitative data, reducing bias and variability.

PROGNOSTIC POTENTIAL OF MICRORNAS IN CANINE SPLENIC HEMANGIOSARCOMA

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Background: Canine hemangiosarcoma (HSA) commonly manifests as a visceral tumor that constitutes approximately 5% of cancers in dogs. The prognosis for visceral HSA is poor due to the aggressive nature of the tumor and lack of specific clinical signs until significant infiltration has occurred. Hence, most dogs present with metastatic disease that responds poorly to standard surgical and chemotherapeutic intervention. Grading systems for HSA have poor prognostic significance. Thus, improved markers are imperative to guide a patient's course of treatment. Non-coding microRNAs regulate gene expression and may serve as predictive biomarkers for HSA.

Objective: To investigate the potential of microRNAs in the prognostic assessment of canine splenic HSA.

Methods: Retrospective study using archived splenic biopsies from 18 cases of canine splenic HSA divided into three groups based on survival times (G1: <90 days, G2: 90-180 days, and G3: >180 days). Expression of four microRNAs (miR-126, miR-150, miR-214, miR-456) with documented roles in canine hemangiosarcoma was assessed by quantitative PCR. Quantification cycle (Cq) values were normalized using the exogenous control UniSp6 and results expressed as fold change. Differences in expression were determined via one-way ANOVA, followed by Tukey's test.

Results: From the four microRNAs analyzed, miR-214 was significantly upregulated in G1 and G2 compared to G3 ($p<0.05$).

Conclusions: Our findings suggest miR-214 may serve as a prognostic marker for canine HSA as upregulation was associated with decreased survival times. MiR-214 has been associated with regulating pro-tumorigenic processes in canine HSA and other cancers. Confirmatory studies are needed to evaluate this marker.

DIRECT DETECTION OF PORCINE HEMAGGLUTINATING ENCEPHALOMYELITIS VIRUS (PHEV) USING IN-SITU HYBRIDIZATION

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Porcine hemagglutinating encephalomyelitis virus (PHEV) is the only described betacoronavirus affecting pigs. Commonly, PHEV causes encephalitis and ganglioneuritis of the myenteric plexus resulting in tremors, muscle fasciculations, and vomiting. In 2015, multiple outbreaks of influenza-like respiratory cases were reported where PHEV was the only pathogen confirmed by PCR. Besides PHEV detection by PCR, *in-situ* detection in pulmonary lesions has not been reported. The objectives of this report are 1) to validate an *in-situ* hybridization protocol for direct detection of PHEV using primary porcine kidney cells experimentally infected as known status samples and 2) to confirm the presence of PHEV *in-situ* in clinical cases of bronchointerstitial pneumonia of unknown etiology. A retrospective selection of cases from 2019-21 (ISU VDL) was based on the presence of bronchointerstitial pneumonia of unknown etiology. The presence of PHEV was confirmed by PCR on paraffin-embed tissues. The direct detection of PHEV in cell culture and tissues from clinical cases, was carried out by small molecule inexpensive fluorescent *in situ* hybridization (smiFISH) and *in situ* chromogenic hybridization using RNAscope®, both targeting the S gene mRNA. In experimentally infected cells, both hybridization techniques showed a large proportion of cells with strong and localized perinuclear signal restricted to the cytoplasm. In positive lungs, the fluorescent signal was observed in the respiratory epithelium and interstitial macrophages. Our results show that both hybridization techniques can be used to

detect PHEV mRNA. Direct detection also confirmed that PHEV could be associated with cases of bronchointerstitial pneumonia.

HISTOPATHOLOGIC FINDINGS ASSOCIATED WITH NATURAL INFECTION WITH HIGHLY PATHOGENIC INFLUENZA VIRUS A (H5N1) IN THREE JUVENILE RACCOONS (Procyon lotor)

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Highly Pathogenic Avian Influenza (HPAI) H5N1 is a viral disease with a broad host range encompassing multiple species of birds and mammals, including humans. This study discusses the gross, histopathologic, and immunohistochemical findings associated with HPAI H5N1 in three juvenile raccoons. Each raccoon was found in regions with known HPAI infected avian species during an ongoing outbreak during the summer of 2022 in Washington state. All raccoons presented with neurologic signs, such as ataxia and circling, prior to mortality. Two of the three raccoon oropharyngeal swabs were positive for avian influenza virus (AIV), via real-time reverse transcriptase polymerase chain reaction (rRT-PCR) specific for the AIV matrix gene. Oropharyngeal swab and fresh brain of one animal had a confirmatory diagnosis of HPAI H5N1 via rRT-PCR. At necropsy, gross lesions attributable to HPAI infection were not present. One raccoon was shown to have pulmonary nodules attributable to lungworm parasitism. Histologically, all animals had a lymphocytic meningoencephalitis with neuronal necrosis and gliosis. Other histopathologic lesions attributable to HPAI infection included lymphoid depletion in the spleen in two raccoons, myocarditis in one raccoon and multifocal and random hepatic necrosis in another raccoon. Immunohistochemistry confirmed the presence of AIV antigen in sections of brain and heart. Many cases of HPAI H5N1 occur in avian species and pose an infection risk to other species, including humans. The objective of this study is to document pathologic changes associated with natural infection with HPAI H5N1 in raccoons to understand the virally induced changes in a novel species.

EVALUATION OF HISTOPATHOLOGIC SECTIONING OF CANINE SOFT TISSUE SARCOMAS

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Background: Soft tissue sarcomas (STS) are a group of mesenchymal neoplasms with the potential for metastasis and recurrence. The current method for determining excision status of canine STS is histological assessment of radial sections. Tangential, or en face, sectioning provides a complete margin evaluation and is a more sensitive method in some tumors.

Objective: The goal of this study was to compare standard radial sectioning to tangential sectioning in a retrospective study of 21 canine STS.

Methods: Canine STS were evaluated by radial and tangential sectioning. The total percent “dirty” surface areas from tangential margins were measured and calculated using image analysis. The measured histologic tumor-free margins (HTFM) of the radial sections were compared against the “gold standard” tangential results using statistical analysis and a receiver operating characteristic curve.

Results: Out of a total of 14 negative radial margins, 8 (57.1%) were positive on tangential margin analysis. Radial margin analysis had a low sensitivity (46.7%; 7/15) when compared to tangential sectioning when positive margins were defined as HTFM = 0 mm. Radial margin analysis reached 100% sensitivity when positive margins included HTFMs ≤ 4 mm.

Conclusions: Radial sections with HTFMs > 0 mm should not always be considered completely excised. For canine STS with HTFMs between 0 mm and 4 mm, tangential margin analysis would be the more sensitive method. Future studies should use methods to increase the accuracy of image analysis and consider case follow-up to determine the prognostic significance of positive margins detected by tangential sectioning

CYTOCHEMICAL CHARACTERIZATION OF HAMSTER NEUTROPHILS

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While the hamster is a widely utilized animal model for numerous diseases, little work has been done to characterize hamster neutrophils. There has been controversy over the name of the hamster primary polymorphonuclear cell (PMN) between neutrophil, heterophil, or pseudo-heterophil based on the faint eosin staining of the primary granules which is similar to human neutrophil granules. Often neutrophils and true heterophils differ in cytochemical staining which correlates to the biochemical content of granules. Hamsters have been assumed, as rodents, to have the same neutrophil granule cytochemical contents as mice and rats; however, those two species differ in alkaline phosphatase and defensin content. The neutrophil granule morphology and content are important to: 1) identify neutrophils from other granulocytes, 2) determine if cell staining morphology correlates to granule contents seen in neutrophils or heterophils (MPO often decreased to absent), and 3) determine if hamster neutrophil granule content differs from other species. Hamster blood smears were made with no anticoagulant or EDTA, with dog EDTA blood smears as quality controls. Neutrophils were positive for myeloperoxidase (MPO), Sudan black B, naphthol AS-D chloroacetate esterase, and periodic acid-Schiff (PAS); positive for acid phosphatase and tartrate sensitive (negative staining); and negative for alpha-naphthyl acetate esterase and Luna's eosinophil granule stain. Alkaline phosphatase reagents were not available. EDTA and the age of the blood smear, even within acceptable recommendations, diminished staining for some reactions. Hamster neutrophil cytochemical staining is consistent with mammalian neutrophil reactions.

Veterinary Student Posters

S1: MODELING AGE-RELATED CHANGES IN THE TUMOR MICROENVIRONMENT IN AN ORTHOTOPIC IMMUNOCOMPETENT MURINE PANCREATIC CANCER MODEL—A NOVEL APPROACH

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Pancreatic cancer is the third leading cause of cancer-related deaths in the U.S. and is most frequently diagnosed between the ages of 65-74 years. Only 11.5% of patients are expected to survive 5 years after diagnosis. There is extensive evidence that tumor microenvironment (TME) plays a critical role in cancer progression and metastasis. Despite the fact that most patients are diagnosed over the age of 60, most preclinical murine tumor models utilize young, healthy, adolescent mice, failing to account for structural and/or functional changes in stroma and immune cells that occur in tissues over time and contribute to tumor progression. Marked differences in lifespan between mice and humans preclude natural development of many age-related physiologic changes in mice that occur in humans over the course of decades. We performed a pilot study to test our hypothesis that KPC pancreatic tumors grown orthotopically in the pancreas of genetically engineered, LMNA-/- mice with features of accelerated aging would have altered tumor growth in comparison to tumors in WT controls. We found that tumor growth in LMNA-/- mice outpaced that of WT mice. Quantitative digital image analysis showed significantly reduced intratumoral infiltration of CD45+ leukocytes in LMNA-/- mice in comparison to WT. There was a significant reduction in tumor-infiltrating CD3+ T cells and F4/80+ macrophages. Our data supports the hypothesis that functional, age-related, immune and/or stromal TME changes impact pancreatic tumor growth and progression. Innovative strategies that better model age-related changes may better recapitulate the aggressive course of pancreatic cancer in aged patients.

S2: CHARACTERIZATION OF AUTOLYSIS OVER 24 HOURS IN CD1 MICE

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When unscheduled deaths occur within research mouse colonies, it is often impossible to determine the postmortem interval (PMI), with the closest estimate being the time between colony inspections. Autolytic changes can severely hinder macroscopic and microscopic evaluation. In toxicity studies, evaluating subtle lesions and differentiation of autolysis from test article-related lesions is critical. This project aims to characterize autolysis to determine utility of organ evaluation after extended PMIs.

Forty CD-1 male mice assigned PMIs of 0, 2, 4, 6, 8, 12, 18, and 24hr were euthanized and placed into cages. Autolytic changes were recorded at necropsy. Macroscopically, the brain, stomach, intestines, kidneys, and liver were most affected. Rigor mortis was present between 2 and 12hr. Friability became evident at 4hr, most affecting the brain. Discoloration became present in the lungs at 4hr and the liver at 6hr. Livor mortis and bile imbibition of subcutaneous tissue became apparent at 8hr.

Microscopically, tissue architecture remained at 24hr, except in intestine and spleen. Nuclear and cytoplasmic changes became apparent around 4hr in most organs. Liver, kidneys, and lungs were heavily congested at 12hr. Small intestines had moderate villus loss by 4hr, while sections of colon had moderate levels of mucosal sloughing by 12hr. Heart and testes were least affected by autolysis.

For most organs, autolytic changes were mild to moderate until 12hr. After 12hr, histopathology may have limited use in some organs, especially intestines. At this point, detection of subtle lesions or differentiating some findings from autolysis may be challenging.

S3: INTRATUMORAL INJECTION WITH STIMULATOR OF INTERFERON GENES (STING) AGONIST INCREASES B-CELL INFILTRATES IN CANINE OSTEOSARCOMA

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Background: Osteosarcoma is the most commonly diagnosed primary bone tumor in humans and in dogs. Even with standard of care treatment (chemotherapy and surgery), metastasis to the lungs is relatively common and incurable. Our group has an ongoing clinical trial treating canine osteosarcoma with neoadjuvant intratumoral delivery of a Stimulator of Interferon Genes (STING) agonist. Previous analysis of the STING agonist-treated tumors showed increased T-cell and myeloid cell immune infiltrates. Recent evidence indicates the importance of B-cells for the generation of an effective anti-tumor immune response. We hypothesized that STING agonist injection would increase B-cell infiltrates in the osteosarcoma tumor as well.

Objective: To assess B-cell infiltration of canine osteosarcoma tumors treated with the novel STING agonist, R229.

Methods: Six dogs with osteosarcoma received an intratumoral injection with R229 STING agonist on days 1 and 8, followed by amputation on day 15. Immunohistochemistry of the B-cell marker CD20 was performed on sections of the tumor obtained both at and away from the STING injection site. Density of $CD20^+$ cells was determined using HALO Image Analysis Software.

Results: Density of $CD20^+$ cells at the STING injection site was increased by an average of 10.4-fold (range 1.8-44-fold) as compared to tumor sections distant from the injection site.

Conclusions: Injection of STING agonist R229 into canine osteosarcoma tumors results in recruitment of B-cells to the injection site. These findings provide further evidence that this STING agonist may play a beneficial role in immunotherapy for osteosarcoma by increasing immune infiltrates within the tumor microenvironment.

S4: ASSESSING SEX-BASED DIFFERENCES IN THE CENTRAL NERVOUS SYSTEM OF SIV INFECTED ART-SUPPRESSED MACAQUES

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Background: There are known sex differences in HIV infection. Women with HIV (WWH) have increased immune activation, lower acute viral loads, and progress more rapidly to AIDS compared to men. Additionally, WWH are more prone to neurocognitive impairment (NCI) suggesting a sex-difference in the effect HIV has on the central nervous system (CNS). However, there have been no studies that have examined sex differences in immune cells within the CNS in people living with HIV.

Objective: To investigate the role biological sex plays in the innate immune response to HIV in the CNS utilizing the SIV-infected ART-suppressed macaque model of HIV.

Methods: Basal ganglia and spleen (control) tissues were compared between six female and six male SIV-infected ART-suppressed rhesus macaques. All animals were inoculated with SIVmac251, and began daily anti-retroviral therapy (ART: DTG/PMPA/TDF) 14 days post inoculation. Terminal time-points were completed at 300dpi after 8 months of suppression. At euthanasia, macaques were perfused with saline. Tissue samples were fixed, paraffin-embedded and sectioned onto positively charged slides. Immunohistochemistry for Iba-1 was completed to assess the number of brain macrophages present in each group. All samples were analyzed using digital image analysis software, Qupath.

Results: Female SIVmac251-infected ART suppressed macaques had higher numbers of brain macrophages in basal ganglia compared to male counterparts. No differences were observed in spleen.

Conclusion: Our findings suggest that SIV-infected ART-suppressed female macaques may have more robust innate immune responses in the CNS compared to males. These data may explain the observation of greater NCI in WWH.

S5: EFFECT OF PRO-INFLAMMATORY CYTOKINES TNF-ALPHA AND CXCL10 ON MIGRATION OF CANINE OSTEOSARCOMA CELLS

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Background: Osteosarcoma (OS) is a malignant mesenchymal neoplasm that targets long bones of children and young adults and middle-aged large breed dogs. Despite decades of research, prognosis remains poor in both species primarily due to progression and development of pulmonary metastases. New therapeutic approaches that inhibit or halt metastatic disease are needed. The tumor microenvironment, which includes neoplastic cells, host cells, extracellular matrix, and biochemical signals such cytokines, has been implicated in the progression, invasion, and metastasis of tumors. Our lab previously found that canine Abrams OS cells promote macrophage secretion of the pro-inflammatory cytokines, TNFa and CXCL10, by macrophages.

Objective: Our objective was to investigate whether pro-inflammatory cytokines the TNF-alpha and CXCL10 promote canine osteosarcoma cell migration in vitro.

Methods: Using a scratch assay, we quantified cell migration of Abrams osteosarcoma cells treated with or without recombinant canine TNF-alpha and CXCL10 at multiple concentrations.

Results: TNF-alpha had no effect on osteosarcoma migration. In contrast, we observed a dose-dependent inverse effect of CXCL10 on osteosarcoma cell migration. At low concentrations, CXCL10 promoted osteosarcoma migration; however, effect was attenuated with increasing concentrations of CXCL10.

Conclusion: We found that CXCL10, but not TNF-alpha, promoted osteosarcoma cell migration in vitro. Moreover, the effect of CXCL10 was dose-dependent. These findings give insight into the complex role that cytokines in the tumor microenvironment may play in regulating invasion and metastasis of osteosarcoma.

S6: TRANSGENIC L2-IL-1β MICE ARE PROTECTED AGAINST LIVER INFLAMMATION AND STEATOSIS CAUSED BY LIEBER-DECARLI CONTROL AND ETHANOL LIQUID DIETS

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Background: Alcoholic liver disease (ALD) is a major cause of mortality worldwide. The L2-interleukin (IL)-1β transgenic mouse, a model of Barret's esophagus, shows potential as an ALD model due to its persistent pro-inflammatory state. The Lieber-DeCarli (LDC) liquid diets are widely used for experimental models of ALD in rodents. More research is

needed to elucidate how the liver naturally responds when L2-IL-1β mice are fed LDC diets.

Objective: Our objective was to compare the histological features of livers from male and female transgenic L2-IL-1β and littermate control (wildtype, WT) mice maintained on either standard chow or LDC diets.

Methods: Livers were collected from nine-month-old, male and female L2-IL-1β and WT mice that were exclusively fed either standard chow, or LDC diet (0.0% or 2.5% ethanol v/v) for 20 weeks. The samples were scored according to the severity of steatosis and inflammation.

Results: WT animals fed 0.0% LDC diet had higher liver inflammation and mixed steatosis scores than those maintained on standard chow ($P<0.01$). For both sexes fed either LDC diet, WT mice had higher mixed steatosis scores than L2-IL-1β mice ($P<0.05$). In WT animals, female mice fed 0.0% LDC diet and male mice fed 2.5% LDC diet had greater hepatic inflammation scores compared to their L2-IL-1β counterparts ($P<0.05$).

Conclusions: LDC diets cause liver inflammation and steatosis in WT animals. L2-IL-1β mice, but not WT animals, are protected against LDC diet-induced hepatocellular injury. Our findings contribute to further characterization of the L2-IL-1β transgenic mouse as a model for ALD research.

S7: TNF-ALPHA AND CXCL10 DO NOT AFFECT CANINE OSTEOSARCOMA CELL PROLIFERATION

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Background: Osteosarcoma (OSA), an aggressive highly metastatic disease, is the most common bone tumor in dogs. Tumors survive by reprogramming the host immune response to evade destruction; however, precisely how tumors including OSA exert control over the immune system is not well understood. Macrophages, one of the most abundant immune cells in the tumor microenvironment, likely play a role. Tumor-associated macrophages facilitate angiogenesis, extravascular invasion, and immune suppression, thereby promoting tumor progression and metastasis. Our lab has shown that OSA secrete signals that upregulate expression of the classic pro-inflammatory cytokines TNF-alpha and CXCL10. In humans, TNF-alpha has been shown to promote OSA progression and tumorigenesis, while high concentrations of CXCL10 in circulation predicted worse survival rates.

Objective: We hypothesized that TNF-alpha and CXCL10 promote the proliferation of canine OSA cells.

Methods: We cultured Abrams OSA cells with or without recombinant canine TNF-alpha, CXCL10, or vehicle control at multiple concentrations. Cell viability and proliferation were measured with a colorimetric cell counting assay at 24, 48, and 72 hrs.

Results: Exogenous TNF-alpha and CXCL10 had no effect on OSA cell proliferation.

Conclusions: Exogenous TNF-alpha and CXCL10 do not directly promote OSA cell proliferation. Ongoing studies will determine whether TNF-alpha and/or CXCL10 affect other OSA cell functions, and/or play indirect roles on tumor cell survival.

S8: USE OF A SUPEROXIDE DISMUTASE MIMETIC TO PREVENT SPACEFLIGHT-INDUCED BONE AND JOINT DEGRADATION IN MICE

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The damaging effects of microgravity on bone during spaceflight are well known; however, effects on joint soft tissues remain poorly understood. The Rodent Research 9 mission identified cartilage and meniscal degradation in mice after ~35 days on the International Space Station (ISS). Damage occurred coincident with reduced antioxidant (Aox) defenses (e.g. superoxide dismutase (SOD)) and increased oxidative stress. As oxidative stress is associated with arthritis, we measured the efficacy of MnTnBuOE-2-PyP5+ (BuOE, 1 mg/kg IP, q.wk.), an Aox SOD mimetic, at preventing damage. Ten-week-old male C57BL/6 mice (n=10) in the Rodent Research 18 mission (Dec-Feb, 2022) spent 35 days on ISS with treatment (5 BuOE, 5 saline controls). Tissues were collected upon return, along with corresponding ground controls (n=10). Knee joints from right hindlimbs were isolated for histologic assessment of bone and cartilage using TRAP, MMP13, ADAMTS5, and Safranin O. Our microCT data indicates that the Aox is protective against articular cartilage and bone degradation during spaceflight. TRAP staining indicated Aox lowered osteoclast number overall, though by day 35 osteoclast number from FLIGHT mice was lower than GROUND control, indicating bone loss with microgravity occurred early in the mission. Aox may also reduce aggrecan degradation and promote formation of sulfated glycosaminoglycans (GAGs) in the cartilage and menisci during spaceflight, but as thinning occurred despite no loss of GAGs in flight, degradation of collagen may contribute to soft tissue damage during spaceflight. Taken together, our data indicate treatment with MnTnBuOE-2-PyP5 may protect against degradation of joint soft tissue and bone.

S9: LARYNGEAL HISTOPATHOLOGY OF THE GONADECTOMIZED AND DEHYDRATED RAT

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Background: Estradiol has a role in the body's response to systemic dehydration. Vocal folds are negatively affected by systemic dehydration. Additionally, they are a known target organ for estradiol. This research sets the groundwork for future studies investigating the role of sex hormones in influencing the adverse effects of dehydration and the voice.

Objective: This study investigates the histopathology of the larynx in gonadectomized rats undergoing systemic dehydration.

Methods: Twelve female and 12 male Sprague Dawley rats were divided into dehydrated (n=12) and euhydrated groups (n=12). Each hydration group had intact (n=6) and gonadectomized (n=6) rats. Blood was collected at beginning and end of experiment to measure and compare serum estradiol levels (plus androgen for males), packed cell volume, and total protein. Body weight and water intake were measured daily. The dehydrated rat groups received 4 ml water/100 g of baseline body weight (approximately 35% less than baseline average intake) compared with *ad lib* water in the euhydrated group. Sections of larynx were stained with hematoxylin and eosin and immunohistochemically for β-estrogen receptors.

Results: On HE staining, no difference in the vocal fold morphology was identified between euhydrated and dehydrated groups, intact or gonadectomized. β-estrogen receptor labeling was variable between individuals; however, no significant differences between groups were identified.

Conclusion: Standard histopathology did not reveal morphologic changes between groups. Variability in IHC requires further molecular investigation. This work provides important groundwork for future studies unraveling the molecular mechanisms contributing to changes in voice due to varying biological and environmental factors.

S10: INFECTIOUS BRONCHITIS DMV/1639 AND FALSE LAYER SYNDROME

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Infectious Bronchitis Virus (IBV), a gammacoronavirus of chickens, is considered a respiratory disease virus. Some strains of IBV cause reproductive disease and have been associated with False Layer Syndrome (characterized by large, fluid-filled cysts in the oviduct of laying hens that cannot lay eggs). Recently, IBV variant DMV/1639 has been isolated from field cases of FLS. Our research aimed to characterize the role of DMV/1639 infection in the development of FLS. Groups of SPF pullets were challenged with DMV/1639 or M41 at 3-, 7-, or 14-days of age and monitored for gross and histopathological reproductive lesions. Our results indicated that IBV DMV/1639 can cause cystic oviducts that will lead to FLS and younger birds are more affected. Our second study evaluated the role of vaccination on DMV/1639-associated reproductive disease. Based on lesions in vaccinated SPF birds challenged at 7DOA with DMV/1639, there didn't appear to be a relationship between vaccination timing and the development

of cystic oviduct. Because SPF pullets don't have maternal antibodies against IBV, we also investigated the role of maternal antibodies in the development of cystic oviducts. In this study, there appeared to be a pattern between maternal antibody-positive birds and lower incidence of cystic oviduct. Overall, our results indicate that the development of False Layer Syndrome caused by IBV DMV/1639 is multifactorial: influenced by age at infection, vaccination status, and maternal antibody status. Prevention of loss due to False Layer Syndrome should include a combination of biosecurity, efficient layer-breeder vaccination, and early vaccination for pullets.

S11: OSTEOSARCOMA EXOSOME PRIMING OF ALVEOLAR MACROPHAGES PROMOTES FORMATION OF A PRE-METASTATIC NICHE

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Background. The malignant bone tumor osteosarcoma (OS) frequently progresses to a highly fatal metastatic disease, most often in the lungs. Metastatic OS portends an unacceptable 20% 5-year survival rate, as chemotherapy dose-intensification, molecular targeted drugs, and immune checkpoint inhibitors have all failed to improve outcomes. Therapeutic development for relapsed OS is limited by a lack of understanding of the basic mechanisms driving lung metastasis. Primary tumors can remotely "prime" distant metastatic sites by secreting nano-sized extracellular vesicles called exosomes. Exosome targeting of resident cells in future metastatic sites is known to initiate a tumor-permissive pre-metastatic niche in other cancer types.

Objective. We aim to elucidate the biodistribution and role of exosomes in modulating resident lung cells during OS metastasis. *We hypothesize* that alveolar macrophages (AMs) are a primary target of osteosarcoma exosomes, and that exosome-primed AMs orchestrate metastasis-promoting immunological changes in the lungs.

Methods. Intravital serial imaging, multi-parameter flow cytometry, confocal microscopy, RNA sequencing and multiplex cytokine analysis was performed in mice and primary human donor AMs to assess the biodistribution and immunological effects of human OS exosomes on the lung.

Results. Osteosarcoma exosomes display a tropism for the lung, where they are selectively taken up by CD11c+ cells AMs. Osteosarcoma exosome uptake results in increased secretion of distinct tumor-promoting cytokines in the mouse lung and in donor-derived human AMs.

Conclusions. These data suggest exosomes play a role in OS lung pre-metastatic niche formation and identify AMs as a primary cellular target mediating exosome-induced secretion of pro-metastatic cytokines and chemokines.

S12: HISTOLOGIC ANALYSIS OF NANO-PLASTIC PARTICLES IN FRESHWATER MUSSELS

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Studies show that microscopic plastics can be found in detectable levels in both the environment and in tissues of organisms near populated areas; however, their tissue-level effects in animals remain relatively unknown. This study serves to inform our understanding of the pathologic effects of plastic particles on freshwater bivalves, keystone species in North Carolina waterways. The species of mussels used in this study were Elliptio complanata and Lampsilis radiata. These individuals were captured from the wild and after an acclimation period, were exposed to the plastic suspension, which consisted of 2.5mg/mL polyethylene terephthalate (PET) particles with a size of approximately 208nm per particle, suspended in bovine serum albumin (BSA). The experiment focused on three treatments, two with nanoparticles and one BSA control: 1) Three E. complanata and two L. radiata in 495mL water containing 5mL PET suspension; 2) three E. complanata and two L. radiata in 499.5mL water containing 0.5mL PET suspension; and 3) one E. complanata and two L. radiata, in 499.5mL water containing 0.5mL BSA as a control. After six hours of exposure, all individuals were euthanized in 5g/mL buffered MS-222 and placed in Davidson's fixative. Each individual was trimmed in cross-section and processed onto slides stained with hematoxylin and eosin. Histologic examination of gills revealed no lesions or loss of cilia in any of the groups. Histologic examination of the digestive tubules revealed marked sloughing and necrosis in all groups, including the control, suggesting artifact from the euthanasia process.

S13: DISSEMINATED MESOTHELIOMA WITH ABUNDANT INTRAVASCULAR INVASION IN A DOG

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A 6-year-old, female spayed Chihuahua presented as an emergency to the Texas A&M University Veterinary Medical Teaching Hospital for bicavitary effusion, circling to the right, tetraparesis, inappetence, and lethargy. The patient had a history of recurrent pericardial effusion and a subtotal pericardectomy was performed two years prior to presentation. Evaluation of pericardial and peritoneal fluids revealed a majority population of atypical cells that were round and typically individualized, with a moderate to high N:C ratio, and a small to moderate amount of blue-pink cytoplasm that often had a small peripheral pink fringe. Criteria of malignancy were more pronounced and the number of inflammatory cells was higher in the peritoneal effusion, providing suspicion for a neoplastic mesothelial population, although the cells were overall relatively uniform in appearance. Treatment included intravenous fluids, multiple antibiotics, steroids, and clopidogrel. The patient became hypercoagulable and whole body imaging revealed a probable multifocal thromboembolic event involving the brain, kidney, and spleen. Euthanasia was elected. Autopsy revealed a presumed infarct in the right frontal lobe of the brain and significant pleural and peritoneal effusion. Histopathology revealed

multisystemic neoplastic emboli and perivascular aggregates, with cells having a more prominent peripheral pink fringe compared with cytology. Neoplastic cells were positive for pancytokeratin and vimentin, and negative for uroplakin, most consistent with mesothelioma. Canine mesothelioma is a rare neoplasm with no definitive diagnostic criteria. The present case is unusual in its relatively uniform cytologic appearance and its wide-spread dissemination with numerous intravascular neoplastic emboli and resulting multisystemic infarcts.

S14: PUTATIVE, SEASONAL, WIDESPREAD, SYMMETRIC, NON-INFLAMMATORY ALOPECIA ASSOCIATED WITH FOLLICULAR DYSPLASIA IN THE AMERICAN RED SQUIRREL (TAMIASCIURUS HUDSONICUS)

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We describe a unique pattern of alopecia in 7 American red squirrels (Tamiasciurus hudsonicus) in New York State, USA from 2013 to 2021. All animals were juveniles, 5 female and 2 male, presenting between September and November. All squirrels had widespread, bilaterally symmetric, non-inflammatory, well-demarcated hypotrichosis to complete alopecia involving the entire dorsal and ventral trunk and neck and extending down the legs to variable degrees. Some of the animals had small to moderate numbers of guard hairs remaining in the affected regions. The fur on the head, face, pinnae, and tail was variably absent. All animals had normal fur on their muzzle and dorsal surfaces of all paws. Six months later, a normal fur coat regrew on 2 of the animals which were litter mates. To varying degrees, the following histopathologic changes were noted in the skin; abnormally oriented hair follicles, perforating folliculitis, kinked, bent and malacic hair shafts, and small cellular crusts. Complete necropsies were performed on 5 animals and no other gross nor histologic abnormalities were noted. In addition to the 7 animals reported here, we are aware of numerous anecdotal reports of a similar phenomenon in this species. Based on features of follicular dysplasia and apparent seasonality, this condition has some similarities to canine seasonal flank alopecia. Although we suspect a genetic etiology, the cause is unknown and other factors such as nutrition, hormones, stress and in utero exposure to toxins or infections may play a role.

S15: DISSEMINATED CRYPTOCOCCOSIS INCLUDING BONE MARROW INVOLVEMENT IN A DOMESTIC SHORTHAIR CAT

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A 6-year-old female spayed Domestic Shorthair presented to the University of Missouri Veterinary Health Center's internal medicine service for evaluation of chronic cough of two years duration and persistent neutropenia. The referring veterinarian previously diagnosed the patient with feline immunodeficiency virus (FIV), herpesvirus, and presumptive asthma. A complete blood count confirmed neutropenia and a biochemical

profile yielded hyperglobulinemia. On CT, the nasal passages were unremarkable. Thoracic CT scan revealed a right caudal pulmonary mass with associated pulmonary bronchial compression, pulmonary nodules, perihilar/cranial mediastinal lymphadenopathy and peribronchovascular thickening. Cytologic evaluation of bronchoalveolar lavage fluid and pulmonary mass aspirate slides demonstrated pyogranulomatous inflammation and the presence of *Cryptococcus* sp. yeast both extracellularly and phagocytosed in macrophages. A latex cryptococcus antigen test yielded a positive titer of 1:1024. A bone marrow aspirate was obtained to evaluate for a cause of persistent neutropenia, and cytology revealed ineffective granulocytic hyperplasia, evidence of mild granulocytic dysplasia, and low numbers of *Cryptococcus* sp. yeast. To our knowledge, this is the first documented case of disseminated cryptococcosis including marrow involvement in a cat. This phenomenon has been reported in humans, predominantly in HIV patients. We speculate that the respiratory tract was the first site of infection in this patient, and hematogenous spread led to secondary marrow involvement. Atypical dissemination in this case was likely facilitated by the patient's FIV infection and associated immunocompromised state. While cryptococcosis is not more common in cats with retroviral infections, it may negatively impact treatment and prolong recovery.

S16: MALIGNANT CATARRHAL FEVER IN A POTBELLIED PIG

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Malignant catarrhal fever (MCF) is a sporadic and fatal lymphoproliferative disease affecting a wide range of ungulates such as cattle, bison, and deer, and can rarely affect pigs. MCF is caused by the ruminant γ-herpesviruses alcelaphine herpesvirus 1 (AlHV-1) and ovine herpesvirus 2 (OvHV-2). These viruses are endemic in their reservoir host and cause inapparent infection in these species (wildebeest for AlHV-1 and sheep for OvHV-2). A 10-month-old female intact potbellied pig presented to the Atlantic Veterinary College at the University of Prince Edward Island teaching hospital for a history of lethargy, pyrexia, anorexia, erythema of the ventrum and legs, and generalized weakness. Despite supportive care, the patient deteriorated with signs of sepsis and papular and ulcerative dermatitis. The pig was euthanized, and postmortem examination revealed widespread multifocal arteritis ranging from fibrinonecrotizing to lymphoplasmacytic and granulomatous, in various sites including skin, kidneys, gastrointestinal tract, heart, lungs and meninges. Arteritis and thrombosis was interpreted as the underlying cause of small intestinal mural necrosis and subsequent septicemia with fibrinous peritonitis and pericarditis. Polymerase chain reaction (PCR) for detection of PCV-2, PCV-3, PRRSV and ASFV was negative; however, PCR for MCF was positive. Follow-up case history revealed the pig was housed with sheep. MCF has been sporadically diagnosed in cattle in Prince Edward Island, but to our knowledge, this is the first documented porcine case in Canada. This case serves as a reminder to consider the possibility of MCF in pigs with vasculitis and septicemia, especially when housed in close proximity to sheep.

S17: EPIDEMIOLOGY OF CANINE LEUKEMIA AND LYMPHOMA

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Background: Lymphoproliferative disorders affect many dogs across the U.S., but the frequency, clinical signs, and breeds associated with various subtypes are poorly characterized.
Objective: To identify breed predispositions and other traits associated with various B- and T-cell neoplasms in blood and lymph nodes.
Methods: Data on 34,888 neoplasms were obtained from Colorado State University's Clinical Hematopathology database from 1/1/2015-5/1/2022. Odds ratios (OR) comparing specific breeds to mixed breed dogs for each subtype were determined using population data from Banfield Pet Hospital (n=9,928,122 unique dogs). Physical exam and hematologic abnormalities were summarized by subtype.
Results: Subtypes investigated included nodal large cell (n=16,857) and small cell B-cell lymphoma (n=1,959), nodal CD4 T-cell lymphoma (n=3,530), B-cell chronic lymphocytic leukemia (BCLL) (n=3,410), CD8 T-cell leukemia (n=2,092) and T zone lymphoma/leukemia (n=5,934). Large breed dogs have greater odds of developing nodal large B-cell lymphoma and many small breed dogs have decreased odds. Conversely, many small breed dogs have greater odds of developing BCLL while large breed dogs have decreased odds. Breeds with markedly increased odds for specific subtypes included Scottish terriers for nodal small cell B-cell lymphoma (OR=36.0, 95% CI=26.9-47.5), Boxers for CD4 T-cell lymphoma (OR=24.7, 95% CI=22.4-27.2), Bull mastiffs and Golden retrievers for T zone lymphoma/leukemia (OR=34.1, 95% CI=25.5-45.6; OR=27.9, 95% CI=25.9-30), and Greyhounds for CD8 T-cell leukemia (OR=30.7, 95% CI=22.7-41.6).
Conclusions: Researchers and practitioners can use this information to better recognize and diagnose these disorders in their canine patients, and to identify breed-specific genetic risk factors for different B- and T-cell neoplasia subtypes.

S18: INVESTIGATION OF RETROVIRAL-INDUCED LYMPHOID PROLIFERATION IN WILD TURKEYS USING RNASCOPE

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Background: Recent population declines in wild turkeys (*Meleagris gallopavo*) in the eastern and midwestern US coincide with frequent detection of lymphoproliferative disease virus (LPDV), which manifests as subclinical infection to disseminated lymphoma. Reticuloendotheliosis virus (REV), a similar oncogenic retrovirus, often is detected concurrently with LPDV.

Objective: To determine: if tissues with lymphoid proliferation (including neoplasia) contain REV and/or LPDV RNA; tissue tropism for each virus; and whether individual lymphocytes exhibit co-infections in naturally infected wild turkeys.

Methods: RNAscope *in-situ* hybridization was performed on formalin-fixed paraffin-embedded tissues from seven naturally-infected wild turkeys to visualize LPDV and REV RNA within foci of abnormal lymphocyte infiltration.

Results: Lymphocytic proliferation with REV and/or LPDV RNA staining was most commonly observed in skin, heart, liver and lung. Up to ~60% of those lymphocytes exhibited intracytoplasmic and/or intranuclear, punctate to diffuse staining for REV RNA. LPDV RNA was observed in up to ~40% of those lymphocytes, as shown by intracytoplasmic, punctate to diffuse staining. Further, intravascular lymphocytes with REV and/or LPDV RNA staining was commonly observed in multiple tissues. Lymphocytes in splenic periarteriolar lymphoid sheaths primarily exhibited LPDV RNA staining, whereas lymphocytes in red pulp primarily exhibited REV RNA staining. Rare co-infection of lymphocytes was observed in tissues from multiple turkeys.

Conclusions: These results advance our understanding the pathogenesis of LPDV and REV and co-infections in wild turkeys. With continued evaluations of wild turkeys with varied infection and disease manifestations, refined diagnostic strategies can be applied to field-collected samples to better understand potential health threats.

S19: SPINAL DISEASE IN A CAPTIVE POPULATION OF PANTHERA SPP.: REVIEW OF 86 CASES (2003-2021)

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Objective: This retrospective study aimed to characterize and determine the prevalence of spinal disease in non-domestic felids within a sanctuary population.

Methods & Results: A review of 304 postmortem examination reports in *Panthera* species from 2003 to 2021 revealed that 86/304 (28%) were diagnosed with spinal disease. Spinal lesions were categorized according to pathologic process: degenerative (78/86, 91%), developmental (9/86, 10%), inflammatory (6/86, 7.0%), and neoplastic (8/86, 9.3%). Degenerative lesions included intervertebral disc disease (IVDD; 66/78, 85%), spondylosis without concurrent IVDD (4/78, 5.1%), and idiopathic (non-compressive) degenerative myelopathies (8/78, 10%). Developmental cases were vertebral (4/9) or spinal cord (3/9) malformations or both (2/9). Inflammatory lesions included meningitis (4/6) and meningomyelitis (2/6). Neoplasia included vertebral multiple myeloma (4/8) and others (4/8). Intervertebral disc disease often involved multiple disc spaces but primarily affected the cervical spine. A multivariate binary logistical model predicted the diagnosis of IVDD at postmortem examination (χ^2 [5, N = 303] = 37.237, $p < 0.001$), where odds of being affected (odds ratio [95% confidence interval]) were highest for males (2.64 [1.43-4.86]), lions (3.46 [1.09-10.99]), and geriatric age group (> 14 y; 8.19 [1.03-65.36]).

Conclusions: The spinal pathology documented in this study provides insight into high-risk signalment categories and predominant associated lesions affecting captive *Panthera* populations. Specifically, spinal disease, especially cervical IVDD, is common among *Panthera* species, and lions, males, and older cats are at increased risk.

S20: AN UNUSUAL INFECTION OF EXSEROHILUM ROSTRATUM IN AN AFRICAN LION

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A 14-year-old female African lion with a history of hemorrhagic nasal discharge and weight loss presented to Texas A&M Veterinary Medical Diagnostic Laboratory for necropsy. Pyogranulomatous lesions with fungal hyphae were evident in the nasal cavity, kidney, and lung. A qPCR assay of fresh nasal tissue revealed the presence of Feline Herpesvirus Type 1. The fungal hyphae were identified as *Exserohilum rostratum* on sequence analysis of the internal transcribed spacer (ITS) region. This heat tolerant fungal agent is a common plant pathogen found in soil in subtropical and tropical areas and is known to cause leaf spots, crown rot, and root rot in grasses and rare infections in humans. In veterinary medicine, *E. rostratum* was the causative agent in a single case of rhinitis in a goat. However, it has not been described as the causative agent of multifocal disease.

S21: PULMONARY ADENOCARCINOMA WITH PERICARDIAL, ESOPHAGEAL, AND RENAL METASTASIS IN A DOG

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An eight-year-old male neutered Pitbull-mix presented with dyspnea and non-productive cough. Radiographs revealed pleural effusion and thoracic masses. The dog deteriorated and was humanely euthanized.

On autopsy, the left caudal lung lobe was infiltrated by a poorly-demarcated irregularly-shaped mass, 10 cm in largest diameter, exuding copious mucus on cross-section. The distal esophagus contained a 7-cm-diameter cyst. Multifocal nodular lesions were documented in lungs, pericardium, and left renal cortex. Pleura contained fibrotic plaques on parietal surface and adhesions to the pericardium. Hypertrophic osteopathy was noted and interpreted as paraneoplastic syndrome. Cytology of post-mortem imprint of pulmonary lesions suggested epithelial proliferation exhibiting malignant criteria and glandular features.

On histology, the pulmonary neoplasm exhibited acinar and papillary patterns, with intra-airway neoplastic cell clusters present. Morphologically, these cells appeared predominantly columnar with basal ovoid nuclei, frequently exhibiting pleomorphism, multinucleation, and atypical mitotic figures. Immunohistochemistry revealed

cytokeratin, thyroid transcription factor-1 (TTF-1), and napsin A reactivity. The pink amorphous matrices were PAS-positive. Neoplastic invasions were confirmed in pericardium and esophagus. Fibrous pleuritis with rare neoplastic cells was found in parietal pleura. Left renal cortex contained suspected metastasis, showing similar histo-anatomic features to pulmonary neoplasms, and lymphovascular invasion, and had areas of end-stage kidney disease.

The final diagnosis was primary pulmonary adenocarcinoma, papillary and acinar type, with metastasis: pericardial, esophageal, and renal. Fibrous pleuritis was suspected to be caused by both direct extension and rubbing effects from the pulmonary neoplasm. TTF-1 and napsin A, combined with PAS, can be useful in differentiating primary from metastatic pulmonary tumors.

S22: CYTOLOGICAL AND MOLECULAR EVALUATION OF PROTOZOAL HEMOPARASITES IN ARIZONA AVIAN SPECIES

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Background: Protozoal hemoparasites are a diverse group of etiologic agents that result in significant morbidity and mortality in birds worldwide. Within the genus Haematoproteus the commonly known reservoirs are columbiformes and high grade infections can occur in non-reservoir species. Leucocytozoon is more prevalent in waterfowl and can cause high mortality in domestic poults. Plasmodium persists in endemic species like the North American Song Bird but can cause high mortality in captive penguins and commercial poultry. Unfortunately, information is limited regarding anthropomorphic activity in relation to the distribution of Haemosporidia in Arizona. With the control of infections limited to reducing and eliminating vector populations, full characterization of hemoparasites is necessary for proactive avian wildlife conservation and commercial population control.

Objective: Characterize the prevalence and diversity of hemoparasites in Arizona avian species using opportunistic surveying.

Methods: Recently deceased birds were collected across the Arizona MWU campus from 2020-2022. Necropsy, tissue impression cytology and PCR were performed on all birds.

Results:
- White Winged Dove were the most common bird collected (37%) and demonstrated the highest prevalence of hemoparasite infection (64%).
- Coinfections 20.67% (74/358 total birds collected)
- Nested PCR and cytologic presence of trophozoites support reclassification of H. multipigmentatus to Plasmodium.

- Babesia identified in two birds.

Conclusions: Haemosporidia are endemic in Arizona with columbiformes acting as a vital hosts within the hemoparasite life cycle. The presence of Babesia spp. and H. multipigmentatus in native birds, suggests the presence of a novel vector.

S23: OPOSSUMS AS SENTINELS FOR CHAGAS DISEASE IN NORTH CAROLINA

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Chagas disease is caused by the protozoan *Trypanosoma cruzi*, which can infect a wide range of mammals including humans. The parasite is transmitted by the kissing bugs native to Central and South America, Mexico, and the southern US. Kissing bugs have been identified as far north as Delaware and reported in North Carolina since 2015. Acutely infected individuals may be asymptomatic or develop potentially fatal myocarditis. Dilated cardiomyopathy can occur in the chronic disease phase. Opossums, common to the eastern US, can be infected with *T. cruzi* and may develop associated pathology, thus could serve as sentinel animals for Chagas disease in this region.

FFPE blocks from 2016-2021 and recently collected samples of opossum necropsy cases in North Carolina were assessed for the presence of *T. cruzi* and characteristic histopathology of Chagas disease. To detect *T. cruzi*, DNA was extracted from scrolls or ethanol-fixed tissue and subjected to qPCR. Histopathologic examination of heart was also performed.

No *T. cruzi* DNA was detected via qPCR (n = 7). Of the six complete necropsies performed, four had a gross diagnosis of cardiomegaly. Of these four, three had histologic evidence of cardiac dilation and one of injury and inflammation. No amastigotes were seen.

Although opossums in North Carolina can have heart disease, the current study suggests Chagas disease is not affecting opossums in this state. This is ongoing work of a consortium of wildlife veterinarians, veterinary pathologists, parasitologists, entomologists, and researchers dedicated to the advancement of human, animal, and environmental health.

S24: CLINICAL AND PATHOLOGIC FEATURES OF FELINE INFECTIOUS PERITONITIS (FIP) IN SENIOR CATS

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Background: Feline infectious peritonitis (FIP) has a reported bimodal age distribution with a second peak in senior cats. However, variability in disease manifestation, comorbidities, and higher-ranked differentials complicates diagnosis in seniors. Currently, reported clinical, clinicopathologic, gross, and histological features of FIP+ senior cats is lacking.

Objectives: Our aim was to characterize features of FIP+ senior cats compared to FIP+ younger cats. We hypothesized that FIP+ seniors would have unique characteristics.

Methods: Cases were identified via retrospective search of the OSU-CVM Applied Pathology archives. Cats were considered FIP+ if pathognomonic lesions were noted in reports; confirmatory histologic review was performed on a sub-set, resulting in 237 FIP+ cases. Patient signalment, presence/location(s) of effusion, tissues affected, and gross/histologic features were analyzed.

Results: Kittens were more often affected (n=117), than young adults (n=80), mature adults (n=21), or seniors (n=14). Trends in senior cats were similar to the other age groups, including over-representation of males and mixed-breed cats with enrichment in the population by purebred cats. The majority of senior (n=10/14) and younger cats (n=148/223) had effusions, with unicavitary and peritoneal effusion predominating. Grossly the liver was most frequently affected tissue in both senior (n=7/14) and younger cats (n=101/223). Gross renal involvement was more common in younger cats (n=94/223) than seniors (n=4/14). Histologically, hepatitis was common in seniors (n=10/14), and across all tissues pyogranulomatous inflammation and vasculitis were consistently present.

Conclusions: No unique features have been identified in FIP+ seniors. Further investigation into FIP immunohistochemistry and PCR status for senior cats is ongoing.

S25: DERMATOPATHY IN A STRANDED MALE YEARLING NORTHERN ELEPHANT SEAL (MIROUNGA ANGUSTIROSTRIS)

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Background: In May 2021, a male yearling northern elephant seal presented to The Marine Mammal Center (TMMC) for lethargy and marked swelling of the right axilla, shoulder, and head. Approximately 80-90% of the skin was affected by alopecia, lichenification, dry black irregular cutaneous depressions, irregular tan moist ulcerations, and dry crusting ulcerations with raised thickened and puckering peripheral skin.

Objectives: To report a possible case of Northern Elephant Seal Skin Disease (NESSD), which has not been reported for more than two decades.

Methods/Results: Complete blood cell count and biochemistry changes were consistent with inflammation, dehydration, and malnutrition. Due to poor prognosis, euthanasia was elected. Urine culture, fungal culture of the hair, and anaerobic and aerobic bacterial cultures of the subcutis and blubber were performed. Mixed bacteria were isolated from all sites with no fungal organisms isolated. On gross necropsy, large cavitations between the dermis and underlying subcutis were present. Dorsally there was emphysema emitting a putrid odor overlying approximately one liter of straw-colored oily non-viscous semi-translucent fluid in the deeper tissues. Histologic findings of the skin included subcutaneous and superficial dermal vasculitis, thrombosis, coagulative necrosis, sebaceous adenitis, and sebaceous gland metaplasia.

Conclusions: These skin lesions may be indicative of NESSD, a potentially fatal condition characterized by generalized, ulcerative dermatitis. From 1992 to 2001, it was the third most common presentation (9.8%) of northern elephant seals stranding at TMMC. The etiology is unknown and determining the pathophysiology may elucidate critical stressors to marine mammal and ocean health.

S26: SPLENIC DISEASE IN THE DOG: A RETROSPECTIVE STUDY (2007-2020)

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Background: Retrospective studies of the incidence of canine splenic disease are few. Clinicians are frequently taught that two-thirds of splenic masses are malignant, and of those malignancies, two-thirds are hemangiosarcoma. Additional data suggests that over 2/3 of splenic masses associated with hemoabdomen are hemangiosarcoma. However, this data is based on outdated retrospective studies that do not incorporate the wide breadth of splenic disease categorizations that are utilized today.

Objective: To characterize the incidence of canine splenic disease via retrospective analysis.

Methods: Archived cases submitted to the New York State Animal Health Diagnostic Center from January 1, 2007 to May 29, 2020 were searched for all splenic biopsies from dogs. Necropsy cases were omitted from analysis as well as cases that did not have the entire spleen submitted for examination. The cases were re-reviewed and categorized based on the diagnosis.

Results: 1035 cases were identified with 1418 diagnoses. The most common diagnoses were: hemangiosarcoma 21% (300/1418), simple nodular hyperplasia 21% (295/1418), hematoma 13% (190/1418), extramedullary hematopoiesis 8% (109/1418), and congestion 6% (84/1418). Of the diseases causing masses in the spleen, only 34% (449/1319) were malignant. Of these malignancies, 67% (300/449) were hemangiosarcoma, 12% (55/449) were histiocytic sarcoma, 7% (32/449) were stromal

sarcoma, and 6% (25/449) were lymphoma. Of cases that had reported hemoabdomen, 72% (137/190) had malignant neoplasia, with 93% (127/137) being hemangiosarcoma.

Conclusions: The splenic masses rule of two-thirds over-estimates the incidence of malignant causes of splenic masses, but accurately estimates the incidence of hemangiosarcoma as splenic malignancies.

S27: PERICARDIAL TREMATODIASIS IN JOHNNY DARTERS (ETHEOSTOMA NIGRUM) IN THE CACHE LA POUDRE RIVER

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Fish can be used as sentinels of environmental health. Granulomatous pericarditis and pericardial trematodes were incidentally recognized during a health survey of wild-caught Colorado Johnny Darters (Etheostoma nigrum) conducted in 2020-2021. The objective of this study was to determine the prevalence of pericarditis and pericardial trematodiasis in Johnny Darters at a collection site on the Cache la Poudre River. Fish and morphometric data were collected by the U.S. Geological Survey, Colorado Cooperative Fish and Wildlife Research Unit. One to two H&E sections from each fish were reviewed for the presence or absence of pericarditis and pericardial trematodes. Gram, GMS, and Acid-Fast preparations were applied to serial sections to investigate for bacteria and fungal agents. The sample included 53 female, 27 male, and 20 unknown sex fish with average total length 49.8mm (33-69mm) and average weight 1.1g (0.2-2.9g). Ninety-two percent of fish had pericarditis and 45% of those individuals had pericardial metacercariae. Gram, GMS, and Acid-Fast stains were negative in all serial preparations that included pericardium (72, 71, and 71 fish, respectively). The true prevalence of trematodiasis may be higher given the two-dimensional nature of histologic evaluation. Future work may include next generation sequencing to identify the trematode genus and species and identify any additional organisms, elucidation of parasite life cycle, and comparison of prevalence at other river locations in relation to environmental parameters.

S28: MICRORNA EXPRESSION PROFILING OF HEMANGIOSARCOMA AND OTHER SPLENIC MASSES IN DOGS

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Neoplastic and non-neoplastic splenic masses are common in dogs. Hemangiosarcoma is the most common malignancy of the spleen and can form large, blood-filled masses. Following splenectomy, an accurate diagnosis of hemangiosarcoma can be challenging, since many of these masses are poorly cellular and contain large regions of hemorrhage (hematomas). Thus, an inappropriate sample site could result in a diagnosis of a hematoma rather than hemangiosarcoma, leading to inappropriate treatment and reduced survival. MicroRNAs are non-coding RNA molecules that

regulate mRNA translation and are dysregulated in cancer. We hypothesize that microRNA expression can be used to differentiate between splenic masses in dogs. MicroRNAs were isolated from tissue of dogs with splenic hemangiosarcoma (n=10), lymphoma (n=8), undifferentiated sarcoma (n=4), histiocytic sarcoma (n=2), nodular hyperplasia (n=8), hematomas (n=8) and histologically normal spleen (n=3). Fifty-nine microRNAs were evaluated per tissue using custom miRCURY PCR arrays (QIAGEN) and relative expression was determined using the $2^{-DeltaDeltaCt}$ method. Compared to healthy spleens, 2 microRNAs were significantly downregulated ($p<0.05$), and 4 were significantly upregulated in hemangiosarcoma. Compared to hematomas and nodular hyperplasia, 2 microRNAs were significantly downregulated, and 4 were significantly upregulated in hemangiosarcoma. MiR-214 was downregulated in hemangiosarcoma compared to all other splenic masses. Overall, 4 microRNAs had significant upregulation in neoplastic compared to non-neoplastic splenic masses. These results demonstrate differential expression of microRNAs between splenic masses in dogs. Further investigation of miR-214 is required as low expression in splenic masses may indicate hemangiosarcoma and could prompt pathologists to review additional tissue sections to reduce diagnostic error.

S29: INVESTIGATION OF NEORICKETTSIA RISTICII AND NEORICKETTSIA FINDLAYENSIS INFECTION AND CO-INFECTION IN CLINICAL CASES OF POTOMAC HORSE FEVER IN 3 CANADIAN PROVINCES IN 2021

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Potomac Horse Fever (PHF) is an acute colitis that affects horses seasonally. It causes fever, diarrhea, colic, laminitis, abortion, and death in 5-30 % of cases. Previously, *Neorickettsia risticii* was identified and widely accepted as the causative agent for PHF. A novel *Neorickettsia* spp*., Neorickettsia findlayensis*, with an 11.6 % genomic divergence from *N. risticii*, was shown to also be a causative agent for PHF in 2020. Molecular detection using PCR was performed on blood and fecal samples to identify the specific *Neorickettsia* spp. in 18 clinical cases from Eastern Canada (Ontario and Quebec), and 13 from Western Canada (Alberta). The goal was to identify the frequency of co-infection with *N. risticii* and *N. findlayensis*, and compare the causative agent by location. qPCR was performed on DNA isolated from 31 blood samples and on DNA isolated from 26 fecal samples using a general Neorickettsia primer. If positive, additional PCRs were performed using primers specific to *N. risticii* and *N. findlayensis*. 12/31 blood samples were positive on qPCR using a general Neorickettsia primer; with 10/12 positive for *N. risticii,* and 5/12 for *N. findlayensis,* with 1 sample testing positive for both *N. risticii* and *N. findlayensis.* 8/26 fecal samples were positive using the general Neorickettsia primer. All 8 fecal samples were positive for both *N. risticii* and *N. findlayensis.* This study has established that co-infection with *N. risticii* and *N. findlayensis* is possible, and found in cases across Canada.

S30: ACUTE TUBULAR INJURY FROM NERIUM OLEADER TOXICOSIS IN AN ALPACA

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Oleander (*Nerium oleander*) is a hearty evergreen shrub of the Apocynaceae family native to the Mediterranean and southern Asia. Due to its drought tolerance, oleander has been globally cultivated as an ornamental plant. Oleander is a well-known toxic plant affecting humans and animals, mainly ruminants, camelids, and horses. A 2-year-old male alpaca was presented to the Texas A&M Large Animal Hospital with bradycardia, arrhythmia, dehydration, severe azotemia, hyperphosphatemia, hypermagnesemia, mild hypokalemia, leukocytosis with neutrophilia, acidemia, and low body condition score. The patient was placed on supportive treatment, but due to poor prognosis, the owner elected for euthanasia. At necropsy, both kidneys were mottled pale tan to red and the cortices were pale and contained linear white areas. The peritoneal cavity contained 500 mL of thin, clear to yellow effusion. Serous atrophy of fat was appreciated in the epicardial adipose tissue and bone marrow. Histologically, the kidneys displayed marked, acute, multifocal, tubular simplification, degeneration, and necrosis with intratubular granular casts, tubular proteinosis, and mild tubular mineralization. Microscopic analysis of C1 contents revealed fragments of oleander. Typical signs of oleander toxicosis include a triad of simultaneous gastrointestinal tract, cardiac, and renal problems. Myocardial necrosis occurs due to inhibition of Na/K-ATPase by cardenolides that may also directly alter ATPase-dependent transport in renal tubules. Gastrointestinal lesions associated with the irritant effects of triterpenoids on the mucosa. This report highlights a case of oleander toxicosis without typical cardiac lesions. Oleander toxicosis should be considered in the differential diagnosis of acute tubular injury.

S31: DIRECT DETECTION OF PORCINE HEMAGGLUTINATING ENCEPHALOMYELITIS VIRUS (PHEV) USING IN-SITU HYBRIDIZATION

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Porcine hemagglutinating encephalomyelitis virus (PHEV) is the only described betacoronavirus affecting pigs. Commonly, PHEV causes encephalitis and ganglioneuritis of the myenteric plexus resulting in tremors, muscle fasciculations, and vomiting. In 2015, multiple outbreaks of influenza-like respiratory cases were reported where PHEV was the only pathogen confirmed by PCR. Besides PHEV detection by PCR, *in-situ* detection in pulmonary lesions has not been reported. The objectives of this report are 1) to validate an *in-situ* hybridization protocol for direct detection of PHEV using primary porcine kidney cells experimentally infected as known status samples and 2) to confirm the presence of PHEV *in-situ* in clinical cases of bronchointerstitial

pneumonia of unknown etiology. A retrospective selection of cases from 2019-21 (ISU VDL) was based on the presence of bronchointerstitial pneumonia of unknown etiology. The presence of PHEV was confirmed by PCR on paraffin-embed tissues. The direct detection of PHEV in cell culture and tissues from clinical cases, was carried out by small molecule inexpensive fluorescent *in situ* hybridization (smiFISH) and *in situ* chromogenic hybridization using RNAscope®, both targeting the S gene mRNA. In experimentally infected cells, both hybridization techniques showed a large proportion of cells with strong and localized perinuclear signal restricted to the cytoplasm. In positive lungs, the fluorescent signal was observed in the respiratory epithelium and interstitial macrophages. Our results show that both hybridization techniques can be used to detect PHEV mRNA. Direct detection also confirmed that PHEV could be associated with cases of bronchointerstitial pneumonia.

S32: INVESTIGATION OF PLATELET MEASURANDS IN DOGS WITH HEMATOLOGIC NEOPLASIA

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Introduction: Thrombocytopenia has been associated with some neoplastic processes, including hematologic neoplasia. There is no information regarding specific changes in platelet measurands in dogs with hematologic neoplasia compared with healthy dogs. The objectives of our study were to establish RIs, evaluate platelet measurands in dogs with hematologic neoplasia, and compare these measurands in patients with hematologic malignancies with or without thrombocytopenia.

Methods: This was a retrospective study. Platelet measurands were determined using the ADVIA 120 Hematology analyzer when a CBC was performed and included the platelet count, MPV, platelet distribution width (PDW), plateletcrit (PCT), mean platelet component (MPC), platelet component distribution width (PCDW), mean platelet mass (MPM), platelet mass distribution width (PMDW), and number of large platelets. Reference intervals were determined retrospectively using data from 129 healthy dogs. Patients with hematologic neoplasia (n = 50) were identified through retrospective evaluation of medical records from the Auburn University Veterinary Teaching Hospital and separated into thrombocytopenic (n = 20) and nonthrombocytopenic groups (n = 30).

Results: Platelet count and PCT were significantly higher in older healthy dogs compared with younger dogs. Significant differences were identified when comparing healthy dogs with those with hematologic neoplasia without thrombocytopenia for PDW, PCDW, PMDW, and the number of large platelets, indicating the presence of more heterogeneous platelets. Thrombocytopenic dogs with hematologic neoplasia had significantly decreased MPCs and increased MPVs, MPMs, and PCDWs compared with nonthrombocytopenic dogs with neoplasia.

Conclusions: Dogs with hematologic neoplasia had more heterogeneous platelets, whereas thrombocytopenic patients with neoplasia had more activated platelets.

S33: AN IRONIC CASE OF PIMA

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Routine blood work for an apparently healthy 8-year-old spayed female dog revealed a macrocytic, normochromic, nonregenerative anemia. The nonregenerative anemia persisted and progressed over months, without an apparent cause, so bone marrow core and aspirate samples were collected. The marrow samples showed ineffective erythroid hyperplasia characterized by increased hematopoietic cellularity, a decreased M:E ratio, and rubriphagocytosis of late-stage erythroid precursors and polychromatophils (aspirate). This was accompanied by marked hemosiderosis and regionally severe collagen myelofibrosis (core). Aspirate smears also revealed low to moderate numbers of coarse green granular inclusions in the cytoplasm of plasma cells, thought to be iron, and what appeared to be erythroid precursors of various maturation stages undergoing cell death with irregularly shaped homogeneous nuclei. One week later, the dog's condition suddenly worsened, and euthanasia was elected. On gross necropsy 4 days later, there was splenomegaly and the full thickness of the walls of the duodenum and proximal jejunum were dark red. Histopathology showed severe fibrinonecrotizing vasculitis in the affected small intestine, potentially contributing to the worsening clinical status; the cause was unclear.

Precursor-targeted immune mediated anemia (PIMA) is the most common diagnosis for dogs undergoing marrow evaluation for nonregenerative anemia at our institution. This was an atypical case of PIMA with respect to the large amount of hemosiderin in the bone marrow, the death of individual erythroid precursors prior to phagocytosis, and the accumulation of iron in plasma cells. We hypothesize that excessive accumulation of iron in the bone marrow caused ferroptosis of erythroid precursors.

S34: HISTOPATHOLOGIC FINDINGS ASSOCIATED WITH NATURAL INFECTION WITH HIGHLY PATHOGENIC INFLUENZA VIRUS A (H5N1) IN THREE JUVENILE RACCOONS (Procyon lotor)

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Highly Pathogenic Avian Influenza (HPAI) H5N1 is a viral disease with a broad host range encompassing multiple species of birds and mammals, including humans. This study discusses the gross, histopathologic, and immunohistochemical findings

associated with HPAI H5N1 in three juvenile raccoons. Each raccoon was found in regions with known HPAI infected avian species during an ongoing outbreak during the summer of 2022 in Washington state. All raccoons presented with neurologic signs, such as ataxia and circling, prior to mortality. Two of the three raccoon oropharyngeal swabs were positive for avian influenza virus (AIV), via real-time reverse transcriptase polymerase chain reaction (rRT-PCR) specific for the AIV matrix gene. Oropharyngeal swab and fresh brain of one animal had a confirmatory diagnosis of HPAI H5N1 via rRT-PCR. At necropsy, gross lesions attributable to HPAI infection were not present. One raccoon was shown to have pulmonary nodules attributable to lungworm parasitism. Histologically, all animals had a lymphocytic meningoencephalitis with neuronal necrosis and gliosis. Other histopathologic lesions attributable to HPAI infection included lymphoid depletion in the spleen in two raccoons, myocarditis in one raccoon and multifocal and random hepatic necrosis in another raccoon. Immunohistochemistry confirmed the presence of AIV antigen in sections of brain and heart. Many cases of HPAI H5N1 occur in avian species and pose an infection risk to other species, including humans. The objective of this study is to document pathologic changes associated with natural infection with HPAI H5N1 in raccoons to understand the virally induced changes in a novel species.

S35: DETERMINATION OF THE OPTIMAL PACKING TIME FOR MICROHEMATOCRIT IN A UNIVERSITY NETWORK OF TWO IN-CLINIC AND THE CLINICAL PATHOLOGY LABORATORIES

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Objective: To determine the optimal packing time (OPT) for 3 microhematocrit centrifuges from the Royal (Dick) School of Veterinary Studies (R(D)SVS) Clinical Pathology, Farm Animal Hospital and Equine Hospital laboratories for doing spun packed cell volume (PCV) on ovine, bovine, and equine blood samples.

Materials and Methods: Anticoagulated venous whole blood samples from the 3 species were collected in either EDTA or heparin coated tubes. Microhematocrit tubes were prepared following standard WHO protocol. The tubes were spun at 12,000 rpm starting at 2 minutes. PCV of each sample was measured with the Hawksley microhematocrit reader. The spinning time was increased by an increment of 1 minute for the subsequent sets of tubes. The OPT was determined when the PCV measurement showed no further reduction in subsequent spin times.

Results: The OPT for ovine, bovine and equine samples using the Clinical Pathology laboratory centrifuge are 5 minutes; for ovine and bovine samples using the Farm Animal Hospital laboratory centrifuge are 5 minutes and 6 minutes, respectively; and for equine blood using the Equine Hospital laboratory centrifuge is 5 minutes.

Clinical Significance: The standard operating procedures (SOP) followed in the Clinical Pathology and Equine Hospital laboratories are acceptable for doing spun PCV on ovine and equine samples. The packing time in the Farm Animal Hospital laboratory is acceptable for ovine samples, but an update in the packing time stated in the SOP for bovine samples is necessary.

S36: DEEP LEARNING-BASED ASSESSMENT OF CORNEAL DAMAGE IN ISOLATED CHICKEN EYES

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Background: The isolated chicken eye test (ICET) is a validated method of identifying and assessing potential eye irritants. This *ex vivo* model utilizes a descriptive, semi-quantitative evaluation process to categorize substances for product safety (no category, mild, category 1). This procedure is time-consuming, ambiguous, and prone to bias and interobserver variability. Machine-learning-based artificial intelligence (AI) classifiers could facilitate a more consistent and quantitative assessment.

Objective: We developed a deep learning-based AI classifier for whole slide images (WSI) of cornea to support pathologists analyzing the ICET.

Methods: Routinely prepared hematoxylin and eosin-stained slides of chicken corneas were digitized at 40x and uploaded into Aiforia® Create, a commercial deep learning image analysis platform. Normal microanatomy and lesions were annotated by trained individuals based on a published ground truth. This supervised training approach produced a classifier using a convolutional neural network.

Results: AI-generated inference masks visually demonstrated erosion and loss of epithelial layers and the presence of vacuoles. Semantic segmentation of the cornea and corneal epithelial layers (superficial, middle, deep) produced area measurements to quantify epithelial erosion. Instance segmentation generated exact vacuole counts and sizes. Quantitative differences ($P<0.05$) in some of these measurements correlated with ICET categories and were consistent with the pathologists' original interpretation.

Conclusions: Deep learning AI-generated inference masks may provide decision support for pathologists during routine ICET slide review by reporting objective and quantitative data, reducing bias and variability.

S37: LETHAL BLUNT FORCE TRAUMA IN A KITTEN AND A HAMSTER THROWN AGAINST FIRM SURFACES

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A hamster and a kitten were submitted as unrelated cases under criminal investigation to the Louisiana Animal Disease Diagnostic Laboratory (LSU) for forensic necropsy. The hamster had forcefully been thrown to the floor by a teenager during an episode of anger. Gross findings were severe subdural hemorrhages; complete, non-displaced, mildly comminuted fractures of both nasal bones; fractures of both lower incisors; left eye exopthalmos and hyphema; and a focal area of abdominal wall hemorrhage. The animal likely impacted the ground with nose and head, where it sustained the lethal subdural hemorrhage. The abdominal wall hemorrhage likely resulted from the grip of the offender.

The kitten was thrown against a wall by a man with a history of domestic violence during an episode of abuse of his female companion. Gross findings were acute full thickness fracture of the spine at the T2- T3 junction, severe hemorrhages over the head, neck, and scapulae. The spinal fracture represents the deadly impact point, and likely caused a neurogenic shock.

Death of both animals resulted from the direct action of a human and the manner of death was non-accidental killing, which is the animal equivalent to “homicide”. These two cases confirm again, that acts of animal cruelty result from deviant behaviors of humans, also known to be linked to domestic abuse. Here, the victims were small enough to be held in one hand, even by a young individual, and propelled in a single movement with enough force towards a hard surface and cause death.

S38: ASSESSMENT OF PRECLINICAL DEVELOPMENTAL NEUROPATHOLOGY USING IMAGE ANALYSIS

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Exposure to various chemicals and compounds can lead to neurotoxic effects in the developing nervous system. Pharmaceutical and agrochemical industries are required by the EPA to assess for toxicologic effects of their products in the brain. Morphometric measurements of various areas of the brain are analyzed as part of these Developmental Neurotoxicity (DNT) studies. The current process involves taking manual linear measurements, which are subject to variability and bias. Our goal was to see if artificial intelligence (AI) could complete this process with greater precision and higher throughput. Digitally scanned PND 21/22 and PND 71/73 rat brain samples were imported into a Good Laboratory Practices (GLP)-validated artificial intelligence (AI)-assisted image analysis platform (Visiopharm®) at Charles River Laboratories - Durham. The rat brains were sectioned according to Garman 2016 and levels 1, 2, and 4 were accessed. We developed a highly reproducible and precise APP (Analysis Protocol Package) that automatically generates linear measurements of the neocortex, striatum, corpus callosum, hippocampus, and cerebellum. These measurements

correlate highly with the traditional, manual measurements. Further, demonstration of the ability of a trained neural network to automatically segment brain regions may make more accurate, but traditionally more time consuming, stereological methods of volume analysis more practical.

S39: SEVERE BRONCHIECTASIS WITH CHRONIC BACTERIAL BRONCHITIS AND BRONCHOPNEUMONIA IN JUNGLE CAT (FELIS CHAUS)

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Background: Bronchiectasis is irreversible bronchus dilation that can be congenital or acquired secondary to chronic airway obstruction. Feline bronchiectasis is rare and has not been previously reported in a non-domestic felid. An approximately 10-year-old female jungle cat (*Felis chaus*) was presented for evaluation of an abdominal mass and suspected pulmonary metastasis. The animal arrested during exploratory laparotomy and was submitted for a cosmetic postmortem examination.

Objectives: Our aim was to characterize gross and histologic lesions causing this animal's death.

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

Results: Gross examination revealed consolidation of the left caudal lung lobe and hilus of cranial lung lobes, which were dark red and heavy and had several pale yellow, flat, pleural foci (endogenous lipid pneumonia). On cut section there was severe distension of bronchi that contained abundant clear-yellow, mucoid to turbid fluid. Remaining lung lobes were multifocally expanded by air-trapping that was most severe marginally (emphysema). Histologically, ectactic bronchi, bronchioles, and few alveoli contained numerous degenerate neutrophils, fibrin, and mucin (suppurative bronchitis/bronchopneumonia) with rare gram-negative bacteria. Aerobic culture yielded low growth of *Proteus mirabilis* and *Escherichia coli*. Surrounding affected bronchi there was moderate bronchial gland hyperplasia, lymphoplasmacytic inflammation, and lymphoid hyperplasia. Additionally, an exophytic mass was identified in lumen of the distal right uterine horn that was histologically consistent with an endometrial polyp with squamous metaplasia.

Conclusions: Chronic bronchitis should be considered as a cause of bronchiectasis and a differential diagnosis for respiratory disease in non-domestic felids.

S40: CO-OCCURRENCE OF TRICHOMONIASIS AND ORAL ADENOCARCINOMA IN A BACKYARD, FREE-RANGE CHICKEN

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Background: An approximately one-year-old hen was submitted for necropsy to the Michigan State University (MSU) Veterinary Diagnostic Laboratory (VDL). This bird was part of a backyard, free-range flock in which three others (out of a flock of 32) had died within the past two weeks. There were no reported clinical signs prior to death.

Methods: A full necropsy was performed by a board-certified anatomic pathologist (MT) and representative samples of all major organs were collected for histopathologic examination using hematoxylin and eosin, and Giemsa stains.

Results: Gross examination revealed abundant tan to black caseonecrotic material that filled the oropharynx and partially adhered to the mucosa. Microscopically, the oral mucosal epithelium was extensively ulcerated and lined by a thick membrane of necrotic cell debris containing low numbers of degenerate 5-7 µm round to pear-shaped protozoa that were highlighted using Giemsa stain. The stroma also contained a neoplastic population of epithelial cells that formed nests and tubules, invaded into skeletal muscle bundles and surrounded nerve fascicles, surrounded by a dense fibrous stroma.

Conclusions: This hen was diagnosed with a severe necrotizing pharyngitis, which was caused by both infection with Trichomonas gallinae and an oral adenocarcinoma. Poultry are thought to become infected with trichomonas by contaminated water or by direct contact with pigeons, the natural host of this protozoan. This case report highlights the importance of biosecurity in backyard flocks, notably segregation from wild birds, and postulates the role of chronic inflammation in tumorigenesis.

S41: BIPHASIC PLEURAL MESOTHELIOMA WITH METASTASES AND CONCURRENT RIGHT MIDDLE LUNG LOBE TORSION IN A DOG

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A nine-year-old male neutered mixed breed dog presented with exercise-induced coughing over a four-week period. Radiographs revealed pleural effusion in addition to a mediastinal mass, and euthanasia was elected due to the progression of clinical signs. On necropsy, the pleural cavity contained 200 ml of serosanguineous fluid. The right middle lung lobe was diffusely dark red, swollen, and firm with a 360-degree torsion. Throughout the lung lobes (primarily subpleural), parietal pleura, and pericardial sac were multifocal, off-white, firm nodules up to 0.5 cm in diameter. A multinodular, mottled dark brown and red, firm, 4.0x3.0x2.5 cm mass was within the cranial mediastinum. Two dark brown, firm, 2.0 cm in diameter nodules surrounded part of the abdominal aorta. On histopathology, the visceral and parietal pleural masses were characterized by an unencapsulated, infiltrative, densely cellular neoplasm composed of two cell populations; an epithelial-like population of polygonal cells arranged in papillary

projections and packets supported by fibrovascular stroma, and a mesenchymal-like cell population composed of spindle cells arranged in streams on a fibrovascular stroma. Both tumor cell populations had marked anisocytosis and anisokaryosis. Anaplasia, multinucleation, and vacuolization were common. The cranial mediastinal and abdominal periaortic lymph nodes were effaced by neoplastic cells with epithelial-like morphology. On immunohistochemistry, neoplastic cells were positive for cytokeratin and vimentin and negative for TTF-1. These findings and the lack of a primary neoplasm are consistent with the diagnosis of mesothelioma, a rare tumor in dogs. Pleural effusion and concomitant intrathoracic neoplasia likely contributed to the lung lobe torsion.

S42: EVALUATION OF HISTOPATHOLOGIC SECTIONING OF CANINE SOFT TISSUE SARCOMAS

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Background: Soft tissue sarcomas (STS) are a group of mesenchymal neoplasms with the potential for metastasis and recurrence. The current method for determining excision status of canine STS is histological assessment of radial sections. Tangential, or en face, sectioning provides a complete margin evaluation and is a more sensitive method in some tumors.

Objective: The goal of this study was to compare standard radial sectioning to tangential sectioning in a retrospective study of 21 canine STS.

Methods: Canine STS were evaluated by radial and tangential sectioning. The total percent “dirty” surface areas from tangential margins were measured and calculated using image analysis. The measured histologic tumor-free margins (HTFM) of the radial sections were compared against the “gold standard” tangential results using statistical analysis and a receiver operating characteristic curve.

Results: Out of a total of 14 negative radial margins, 8 (57.1%) were positive on tangential margin analysis. Radial margin analysis had a low sensitivity (46.7%; 7/15) when compared to tangential sectioning when positive margins were defined as HTFM = 0 mm. Radial margin analysis reached 100% sensitivity when positive margins included HTFMs ≤ 4 mm.

Conclusions: Radial sections with HTFMs > 0 mm should not always be considered completely excised. For canine STS with HTFMs between 0 mm and 4 mm, tangential margin analysis would be the more sensitive method. Future studies should use methods to increase the accuracy of image analysis and consider case follow-up to determine the prognostic significance of positive margins detected by tangential sectioning

S43: INTRAOPERATIVE CYTOLOGY OF A PARAGANGLIOMA IN THE LUMBAR VERTEBRAL CANAL OF A DOG

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Background: Paragangliomas are neuroendocrine neoplasms derived from paraganglia associated with the parasympathetic and sympathetic nervous systems. Due to their wide distribution throughout the body and varied morphologic features, diagnosis of paragangliomas can be challenging. Few cases of extra-adrenal paragangliomas have been described in veterinary literature, with little focus on cytomorphology.

Objective: Describe the clinical, cytologic, and histopathologic features of a paraganglioma in the lumbar spine of a dog.

Methods: A 5-year-old female Airedale Terrier presented for lumbosacral pain and ambulatory paraparesis. MRI revealed a L5-6 extradural mass causing severe compressive myelopathy. The mass was removed and submitted for histopathologic evaluation. Intraoperative touch impressions were submitted for cytologic evaluation.

Results: Cytologic impression smears were highly cellular. Cells were present individually and in clusters with rosette-like arrangements surrounding pink fibrillar material. Cells were pleomorphic with prominent anisokaryosis, multinucleation, and intranuclear pseudoinclusions. Nuclei were round and often lacking surrounding cytoplasm, with fine chromatin and 0-2 prominent nucleoli. Mitotic figures were occasionally seen. When present, cytoplasm was abundant and finely granular. Histologically, cells were arranged in packets surrounded by fibrovascular stroma. Cells were positive for neuron-specific enolase, chromogranin A, vimentin, myelin basic protein, and S-100, and negative for synaptophysin, pan-cytokeratin, glial fibrillary acid protein, PNL2, MelanA, and CD18. A diagnosis of paraganglioma was made.

Conclusions: Paragangliomas are pleomorphic on cytology and can stray from typical descriptions of neuroendocrine neoplasms. Pathologists should be aware of cytologic and histologic features of extra-adrenal spinal paragangliomas. Paraganglioma should be included in the differential diagnosis of extramedullary spinal tumors.

S44: KI-67 EXPRESSION IN FELINE THYROID CARCINOMA, THYROID ADENOMA, AND NORMAL THYROID: A PILOT STUDY

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Background: Thyroid carcinoma is an uncommon, often aggressive tumor of the thyroid gland in felines. Ki-67 is a nuclear biomarker used as a prognostic indicator in various tumor types, but has not been evaluated in feline thyroid carcinomas.

Objective: To compare expression of Ki-67 in feline thyroid carcinoma, thyroid adenoma, and normal thyroid.

Methods: Seven cases of histologically diagnosed thyroid carcinoma and five cases of histologically diagnosed thyroid adenoma with normal tissue were identified retrospectively in the Colorado State University Veterinary Diagnostic Lab database. Medical records, H&E, and KI-67 immunohistochemical preparations were evaluated.

Results: Thyroid carcinomas had a mean mitotic index of 5/2.37mm2 (range 2-7), KI-67 index of 7.8/mm2 (range 4.2-10.2), and KI-67% of 4% (range 2-7). Metastatic foci in regional lymph nodes of 2 cases had KI-67 indices of 19/mm2 and 4.2/mm2, respectively, and KI-67% of 2% and 7%, respectively. Among thyroid adenomas, one case had 1/2.37 mm2 mitotic figures, a KI-67 index of 1.6/mm2, and KI-67% of 3%. Otherwise, no mitotic figures or KI-67 expression was seen in adenomas or normal thyroid tissue.

Conclusions: Thyroid carcinomas appear to express higher rates of KI-67 than thyroid adenomas and healthy tissue. More work needs to be done in order to determine whether KI-67 expression could be useful as a prognostic indicator or diagnostic tool to differentiate adenoma from carcinoma.

S45: ETHYLENE GLYCOL TOXICITY IN A PREGNANT CANINE

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A five-year-old intact female Border Collie presented with vomiting, lethargy, and collapse following an exposure to antifreeze. The patient was subsequently admitted in the hospital's intensive care unit. She became oliguric and was euthanized. At autopsy, the patient was found to be pregnant. The kidneys of the dam and the puppies contained pale green to clear crystals obstructing the tubules with the cortex and medulla. No crystals were present in the placenta. This case illustrates the classic presentation of ethylene glycol toxicity in an adult canine, with the additional unique finding of crystals within the kidney of the fetuses. This finding is important for understanding the potential for impaired renal function in puppies exposed to ethylene glycol *in utero.*

S46: HISTIOCYTIC ULCERATIVE COLITIS IN A FRENCH BULLDOG PUPPY

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A 3-month-old female French bulldog presented to Mississippi State University College of Veterinary Medicine on March 3, 2022 for a 4-week history of unsuccessful management of a rectal stricture. Unfortunately, due to the lack of clinical response, humane euthanasia was elected. Postmortem examination revealed severe thickening

of the colonic mucosa and submucosa with multifocal, mucosal ulcerations. Microscopically, there was marked expansion and disruption of the colonic mucosa and submucosa by dense sheets of macrophages containing intracytoplasmic PAS positive, granular material. Aerobic culture of an adjacent markedly enlarged mesenteric lymph node resulted in moderate growth of *Escherichia coli*. In-situ hybridization on a section of colon demonstrated had strongly positive intracytoplasmic macrophage staining for an *E. coli* probe. Based on the clinical history and postmortem findings, the lesions are diagnostic for histiocytic ulcerative colitis (HUC) also known as “Boxer colitis”. HUC is best described in young dogs with Boxers and French Bulldogs being overrepresented. Current research supports that *E. coli* has a causative role in this disease due to its clinical response to enrofloxacin.

S47: PRESUMED CUTANEOUS LYMPHANGIOSARCOMA/LYMPHANGIOMATOSIS IN YOUNG DOG

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A 13-week-old, female, German Shepherd dog presented to a referral clinic with a mass effect present in her right inguinal region suspected to be an inguinal hernia or seroma-like formation. Grossly, there were red-purple macules in the left caudal mammary area that extended towards the stifle as well as swelling in the cranial stifle. Fine needle aspirate performed in-house was equivocal. The rDVM began a course of Clavamox; however, over the next week, the skin of the medial and lateral stifle progressively reddened and progressed to ulceration both laterally and medially. The seroma-like lesion had reappeared with exuberant clear exudative fluid from the inguinal area to the stifle. Skin punch biopsy samples from the affected area were submitted to The Ohio State University Veterinary Medical Center Dermatopathology service. Histologically, the dermis and subcutis contained plump spindle cells that lined irregular, empty spaces (endothelium). There was mild, regionally extensive, acute to subacute hemorrhage and mild perivascular lymphohistiocytic and neutrophilic infiltration. One mitotic figure was present in ten high-power fields. The differential diagnoses included hamartomatous vascular anomalies, progressive angiomatosis, and well-differentiated angiosarcoma. The neoplastic endothelial cells were immunopositive for lymphatic vessel endothelial hyaluronic acid receptor-1 (LYVE-1) and CD31 antigens, supporting a possible lymphatic endothelial origin. The affected limb was amputated, and as of six-months post-surgery, there are no signs of reoccurrence, and the dog remains clinically healthy. The primary differential diagnosis is lymphangiosarcoma based on histological features, yet lymphangiomatosis cannot be ruled out because of clinical features.

S48: MAST CELL TUMOR OF DISTAL METACARPUS WITH INTEROSSEOUS LIGAMENT INVOLVEMENT IN A HORSE

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Cutaneous mast cell tumors are uncommon in horses and usually benign. Studies indicate that Arabians are overrepresented in mast cell tumor diagnoses. A 2-year-old Arabian gelding presented for a small mass on the right forelimb (RF) at the level of the distal metacarpus that enlarged over several months and progressed to lameness and eventual recumbency. Radiographs revealed smooth periosteal proliferation and marked cortical thickening of the lateral distal metacarpus. MRI and CT confirmed a heterogenous RF palmar metacarpal soft tissue mass with focal bone lysis and periosteal proliferation of metacarpal bones III and IV (MCIII & MCIV). Biopsy of the mass confirmed an incompletely excised mast cell tumor, and the owners elected euthanasia. On necropsy, a 6x4x2 cm, tan, firm, granular mass was on the lateral distal aspect of MCIII surrounding the interosseous ligament and distal MCIV. The caudal aspect of MCIII in the area of the mass was irregular and expanded by an 8 mm band of new bone growth. Histologically, the soft tissue and interosseous ligament showed a mast cell tumor with severe eosinophilic and granulomatous inflammation. MCIII showed mild, multifocal, chronic, eosinophilic and granulomatous osteomyelitis with periosteal new bone formation. Neoplastic cells infiltrated the interosseous ligament but did not enter the bone in examined sections. To the best of the authors' knowledge, this is the first report of an equine cutaneous mast cell tumor with interosseous ligament involvement.

S49: COX-2 EXPRESSION IN FELINE AND CANINE CHOLANGIOCARCINOMA

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Background: Cholangiocarcinoma (CCA) is a common malignant neoplasm of the liver in dogs and cats with a poor prognosis and short overall survival time after diagnosis. Currently, surgical resection is the only palliative treatment option. COX-2 enzyme is neo-expressed and involved in the tumorigenesis of several neoplasms including canine and feline mammary tumors, canine mast cell tumors and canine renal cell carcinomas. Tumors overexpressing this enzyme are candidates for COX-2 selective-inhibitor treatments.

Objective: The objective of this research was to investigate, for the first time, the expression of COX-2 in a cohort of canine and feline cholangiocarcinomas.

Results: COX-2 is not expressed in normal canine and feline liver parenchyma. COX-2 was neo-expressed in 85% and 60% of canine and feline CCA respectively. COX-2 neo-expression was not significantly associated with histological subtype, mitotic count, necrosis, inflammation, desmoplasia, or lipid change ($p>0.05$). Intravascular neoplastic

emboli and metastatic lesions also neo-expressed COX-2 with similar immunolabeling as the corresponding primary neoplasm.

Conclusion: To the authors knowledge, this is the first study analyzing the expression of COX-2 in canine and feline CCA. Our results may suggest that COX-2 selective-inhibitor treatments could be of therapeutic value in the medical treatment of canine and feline CCA.

S50: ULCERATIVE DERMATITIS DUE TO PYTHIUM INSIDIOSUM IN AN ANGUS COW

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A 3-year-old Angus cow presented to Mississippi State University College of Veterinary Medicine for a seven-day progressive history of anorexia and severe ulcerative cutaneous lesions on the rostral mandible, ventral neck, shoulder, mid-thoracic, and flank areas that were refractory to treatment. On presentation, she was tachypneic and pyretic with ptyalism and enlarged prescapular and prefemoral lymph nodes. Multifocal circular, well-demarcated, flat, firm, malodorous, ulcerated skin lesions were present on the rostral mandible and extended multifocally along the ventrum, lateral thorax, and flank bilaterally. Bovine viral diarrhea virus SNAP test was negative. No immunosuppressive disease was identified. Culture of the skin lesions yielded light growth of *Staphylococcus hyicus* and *Clostridium perfringens*. Biopsy revealed severe multifocal pyogranulomatous dermatitis with intralesional fungal hyphae, vasculitis, and epidermal ulceration. Fungal hyphae were poorly staining, 5 µm in diameter, and pauciseptate with nonparallel walls and bulbous ends. Initial differentials included mucormycosis (previously zygomycosis) and pythiosis. Panfungal PCR and DNA sequencing performed on paraffin embedded tissue identified the fungus as *Alternaria* sp., thought to be a contaminant. Additional PCR and sequencing was performed on frozen samples, with 100% similarity to *Pythium insidiosum*. *P. insidiosum* is an opportunistic oomycete enzootic to tropical and subtropical climates, including the southeastern United States. Pythiosis commonly causes cutaneous lesions in dogs and horses but is rarely reported in cattle. Some reports suggest that bovine pythiosis may be self-limiting. In this case, the cow was treated with sodium iodide and at the time of follow-up (7 weeks), the cutaneous lesions were healing.

S51: CLINICAL STAGING, HISTOPATHOLOGY AND PROGNOSTIC FACTOR EVALUATION OF 24 MAMMARY TUMORS IN DOGS FROM PANAMA CITY, PANAMA

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Mammary gland tumors are heterogeneous, both in their morphological aspect and clinical behavior, creating a need to find clinical and pathological parameters with prognostic and therapeutic value. The purpose of this study was to stage canine

mammary tumors and to correlate this with the histopathological diagnosis and possible prognostic indicators. Twenty-four mammary tumors from four veterinary clinics located in Panama City were collected. The tumors were clinically staged using the TNM system: tumor sizing [T1 (<3cm); T2 (> 3 cm, up to 5 cm); T3 (> 5cm)], lymph node evaluation for metastasis [N, (0=no metastasis)], and radiographic examination for distant metastasis [M, (0=no metastasis)]. After surgical excision, tumors were histologically assessed and categorized. Some risks factors such as age, breed, spay status, and prognostic indicators like tumor size and presence of distant metastasis were taken into account.

Our findings: age of presentation was between five and fourteen years old, most affected dogs (79%) were purebreds, and the majority (88%) were intact. Most tumors (14/24) were categorized as T3; 22/24 patients had normal lymph nodes (N0) and 21/24 did not show clinical evidence of pulmonary metastases (M0).

We concluded that larger tumor size often correlates with a malignant phenotype, since in this study 10/14 tumors classified as T3 were categorized as malignant by histopathological evaluation. The most frequent histopathological diagnosis was mammary gland carcinoma (12/24), primarily the complex type. Clinically, mammary tumor size appears to be a useful prognostic indicator when assessing mammary neoplasia in dogs.

S52: GRANULOSA CELL PROLIFERATION IN THE ORANGUTAN OVARY

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Orangutans *(Pongo pygmaeus*) are critically endangered and conservation efforts are important for their survival. However, there is little research on orangutan reproduction, including the normal changes associated with aging. From an archive of 27 female orangutan reproductive tracts, three had lesions composed of discrete granulosa cell aggregates in the ovarian stroma, often associated with atretic follicles, and lacking the invasive or atypical nature of neoplastic granulosa cells. The affected females were nulliparous and aged 14, 25, and 34 years; the mean age for archived population was 30 years of age and parity was 3.5. Similar lesions have been described in the human literature as granulosa cell proliferations of pregnancy and may be related to the progression of menopause. In humans, these lesions have been described as incidental findings and are similarly associated with atretic follicles. This case study describes lesions not previously identified in orangutans and highlights the value of comparative pathology to better understand the reproductive pathophysiology of both humans and orangutan. In addition, these findings may help develop a better understanding of the regulation of menopause.

S53: EFFECT OF ANTICOAGULANTS (HEPARIN, ETHYLENEDIAMINETETRAACETIC ACID, ACID CITRATE DEXTROSE SOLUTION A) ON HEMATOLOGY OF BUFF ORPINGTON HENS

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Hematology is a crucial component of medicine. Stopping the coagulation pathway of the blood during its processing is a critical step leading up to the proper evaluation of blood and is easily obtained by use of commercially available anticoagulants. However, there are important gaps in knowledge regarding the effects of common anticoagulants (e.g., ethylenediaminetetraacetic acid [EDTA], lithium heparin (HEP), acid citrate dextrose solution A [ACD-A] and their species specific effects on hematology. Chickens have become increasingly popular as pets and therefore routine diagnostics have also increased in prevalence. The goal of this study was to determine what anticoagulant (EDTA, HEP, ACD-A) best resembles the hematological results of the fresh blood in Buff Orpington hens. The 3 parameters measured were packed cell volume (PCV), total solids (TS), and absolute white blood cell count (WBC). Blood smears and PCV tubes using fresh blood were made immediately after venipuncture, and remaining blood was transferred to EDTA, HEP, and ACD-A vials in randomized order. From those vials, PCV tubes and blood smears were also made. The PCV (%, mean ± standard deviation) from EDTA (29±2.6, $p=0.7$) and HEP (27±2.7, $p=0.54$) was not significantly different from fresh blood (28±2.4), however, ACD-A (25±2.5, $p<0.01$) caused significant differences. Fresh blood TS (5.5±1.3, mg/L) was significantly different from ACD-A (5.1±1.2, $p<0.01$) and HEP (5.3±1.1, $p<0.01$), but not from EDTA (5.6±1.3, $p=0.21$). No significant differences on WBC were identified with all 3 anticoagulants. The results of this study will provide valuable information for veterinarians working with chickens.

S54: CHRONIC PANCREATITIS LEADING TO MESENTERIC AND SUBCUTANEOUS NECROTIZING STEATITIS AND INTESTINAL LYMPHANGITIS/LYMPHANGIECTASIA IN A CAT

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A 10-year-old spayed female DSH cat was presented with a history of anorexia, progressive weight loss, polydipsia and polyuria. Physical examination revealed ascites and ill-defined subcutaneous nodules along the ventral abdomen. Bloodwork showed amylase and Precision PL elevations, suggesting pancreatitis. In addition, low RBC and hemoglobin values indicated anemia. Due to the poor prognosis the cat was euthanized.

Significant autopsy findings included ascites, hydrothorax, chronic nephritis with urolithiasis, chronic pancreatitis and widespread ventro-abdominal subcutaneous and mesenteric necrotizing and granulomatous steatitis. The inflammatory process within the mesenteric fat extended locally to lymphatics resulting in prominent mesenteric and intestinal lymphangiectasia. In addition to mycobacteriosis, pancreatitis and pancreatic

tumors, necrotizing pansteatitis in cats has been linked to vitamin E (a-tocopherol) deficiency. Vitamin E levels in the liver of this cat were adequate, there was no gross or microscopic evidence of a pancreatic neoplasm and no acid fast positive bacteria were detected within the affected adipose tissue. Fat necrosis and inflammation therefore was likely the result of chronic pancreatitis. To our knowledge intestinal lymphangiectasia, a common cause of protein losing enteropathy in dogs, is not reported in cats. In this particular case, secondary intestinal lymphangitis and lymphangiectasis (sequelae of pancreatitis and fat necrosis) led to protein-losing enteropathy and likely played a significant role in the reportedly severe and progressive weight loss.

S55: CYTOCHEMICAL CHARACTERIZATION OF HAMSTER NEUTROPHILS

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While the hamster is a widely utilized animal model for numerous diseases, little work has been done to characterize hamster neutrophils. There has been controversy over the name of the hamster primary polymorphonuclear cell (PMN) between neutrophil, heterophil, or pseudo-heterophil based on the faint eosin staining of the primary granules which is similar to human neutrophil granules. Often neutrophils and true heterophils differ in cytochemical staining which correlates to the biochemical content of granules. Hamsters have been assumed, as rodents, to have the same neutrophil granule cytochemical contents as mice and rats; however, those two species differ in alkaline phosphatase and defensin content. The neutrophil granule morphology and content are important to: 1) identify neutrophils from other granulocytes, 2) determine if cell staining morphology correlates to granule contents seen in neutrophils or heterophils (MPO often decreased to absent), and 3) determine if hamster neutrophil granule content differs from other species. Hamster blood smears were made with no anticoagulant or EDTA, with dog EDTA blood smears as quality controls. Neutrophils were positive for myeloperoxidase (MPO), Sudan black B, naphthol AS-D chloroacetate esterase, and periodic acid-Schiff (PAS); positive for acid phosphatase and tartrate sensitive (negative staining); and negative for alpha-naphthyl acetate esterase and Luna's eosinophil granule stain. Alkaline phosphatase reagents were not available. EDTA and the age of the blood smear, even within acceptable recommendations, diminished staining for some reactions. Hamster neutrophil cytochemical staining is consistent with mammalian neutrophil reactions.

S56: GALLBLADDER AGENESIS IN A FOUR-WEEK-OLD AMERICAN PITBULL TERRIER

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An approximately four-week-old, male intact American pitbull terrier dog was reported to have been abandoned in a trash dumpster for 20 hours. An in-clinic parvovirus test was negative and the dog was euthanized. A full necropsy was performed including gross and microscopic examination as well as fecal testing. Gross examination revealed that

the dog was severely emaciated. The liver weighed 60 grams (4.4% of body weight) and no gallbladder was identified. The common bile duct drained directly to the duodenum. Microscopically, the portal triads and central veins in the liver were closely apposed and the hepatic cell cords between these portal triads and central veins were mildly atrophied. Multifocally, the portal triads were expanded by small amounts of well-differentiated paucicellular collagenous connective tissue. Moderate *Toxocara canis* eggs were detected on fecal analysis. This dog was diagnosed with emaciation, endoparasitism, hepatic atrophy, and gallbladder agenesis. The etiology of gallbladder agenesis is not fully understood or well documented in either humans or veterinary species, but is assumed to arise from a disruption in embryologic development. In this case, the gallbladder agenesis may be correlated with the hepatic atrophy noted microscopically. This case report highlights the importance of further research as gallbladder agenesis has been rarely diagnosed in small breed dogs and has not been reported in American pitbull terriers.

S57: PERIANAL GLAND ADENOMA ON THE FACE OF A CANINE PATIENT

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An eight-year-old collie was presented to the hospital with a 9-mm mass on the left side of his face, just caudal to the lip commissure. No major abnormalities were noted on the physical exam, CBC, and biochemistry profile. Fine needle aspiration of the mass yielded moderately to highly cellular slides. Frequent epithelial cell clusters were observed, including cells with 3 distinct morphologies. Vacuolated epithelial cells, consistent with typical sebaceous epithelium, epithelial cells with high nuclear to cytoplasmic ratios consistent with basaloid epithelium, and epithelial cells with a “hepatoid” appearance were found in separate clusters and variably intermixed. Each respective epithelial population displayed minimal anisocytosis and anisokaryosis. The hepatoid epithelial cells were reminiscent of a perianal gland neoplasm, however the anatomical location of the mass was unusual. The mass was surgically excised and submitted for histopathologic evaluation. As observed cytologically, a mixture of hepatoid and classical sebaceous epithelial cells were evident, arranged in lobules and trabeculae, and surrounded by moderate numbers of basal cells. Despite the unusual location, the number of hepatoid epithelial cells present rendered a diagnosis of perianal gland adenoma. Perianal gland tumors have been well documented in aberrant locations in dogs. However, these locations have been limited to the tail, thighs, trunk, and neck. The facial location of this perianal gland adenoma makes this case of particular significance. Upon recheck, the patient recovered well with no post-operative complications.

S58: RHODOCOCCUS EQUI INFECTION OF A JUVENILE CAPRINE IN GRENADA, WEST INDIES

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Rhodococcus equi is a gram-positive bacterium frequently isolated from soil and feces of diseased and healthy animals. *Rhodococcus equi* infection is commonly reported in foals where it causes pyogranulomatous pneumonia. There are only a few reported cases of rhodococcosis in goats worldwide. The disease has been reported in goats with underlying clinical conditions. A juvenile female goat presented for necropsy at St. George's University School of Veterinary Medicine following euthanasia. The animal had a poor body condition. The liver was markedly enlarged up to three times the normal size with the hepatic parenchyma distorted by hundreds of multifocal to coalescing well-demarcated, raised, white nodules (0.5cm-2cm). These nodules contained a soft to firm center with yellow exudate. Approximately two dozen similar pyogranulomas were found in the lungs. The mesenteric lymph nodes were moderately enlarged with a soft cortex and a medullary parenchyma with multifocal areas of dark brown to black discoloration. A heavy *Haemonchus contortus* load was present in the abomasum. The nasal cavity and frontal sinuses revealed a moderate burden of *Oestrus ovis* larvae. There was straw colored and serosanguinous fluid within the abdominal (10ml), thoracic (50ml), and pericardial (30ml) cavities. Histologically, the liver and lung lesions revealed pyogranulomatous hepatitis and pyogranulomatous pneumonia, respectively. *Rhodococcus equi* was isolated from the liver and the lung on bacterial culture. Findings are consistent with systemic *Rhodococcus equi* infection in a goat with severe endoparasitism in Grenada, West Indies.

S59: HEMOCYTIC SARCOMA IN A CAPTIVE ASIAN JUNGLE SCORPION

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Background: Several tumor types are documented in invertebrates, including carapacial wall tumors, papillomas, carcinomas, adenocarcinomas, and the most described, disseminated hemic neoplasms. Neoplasia has not been reported in scorpions and only a single case is reported in arachnids.

Objective: Describe histologic features of hemocytic sarcoma in an arachnid to assist pathologists in discerning histologic lesions in these animals, including differentiating inflammation and hemocytic neoplasia.

Methods: A four-year-old, adult, male scorpion was found dead without antemortem clinical signs. The scorpion was fixed whole in 10% neutral buffered formalin for <24 hours and then placed in Davidson's solution for 72 hours. Whole body, transverse sections were made through the carcass, sections were placed in 70% ethanol for 24 hours, and were routinely processed for histologic examination. Slides were stained with H&E as well as select special stains.

Results: Multiple non-encapsulated, well-demarcated masses comprised of dense sheets of monomorphic hemocytes expanded the dorsal portion of the prosomal and

opisthosomal body cavity and extended into adjacent striated muscle. Hemocyte cellular atypia was moderate. Sheets of hemocytes had multifocal areas of lytic necrosis with loss of architecture and replacement by cellular and karyorrhectic debris.

Conclusions: Diagnosis of neoplasia was based on the large sheets of monomorphic hemocytes lacking features of arthropod inflammation, including melanization, encapsulation, and hemocyte elongation. Multifocal areas of necrosis suggest this neoplasm outgrew its blood supply. This is consistent with a hemocytic sarcoma, which is previously undescribed in arachnids. Differentiating inflammation and neoplasia can be a diagnostic challenge in invertebrate pathology.

S60: SYSTEMIC SALMONELLOSIS IN TWO JUVENILE OPOSSUMS AS PART OF A LARGER OUTBREAK

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Two juvenile female Virginia opossums were presented for postmortem examination at the University of Illinois Veterinary Diagnostic Lab after being found deceased. The animals had been clinically normal up until time of death. Primary gross examination findings include petechial hemorrhages in various organs of both animals and generalized icterus of subcutaneous tissues. Histopathologic examination revealed necrosuppurative inflammation with vasculitis in many organs and tissues, often with associated intralesional bacilli. The most severely affected organs included lung, liver, kidney, spleen and urinary bladder in both animals, heart and stomach in animal A, and the gastrointestinal tract in animal B. Heavy growth of *Salmonella* sp. Group B was cultured in the spleen and kidney of both animals with aerobic culture, which was further confirmed by positive PCR for *Salmonella* and growth with *Salmonella* isolation culture using liquid enrichment media. Salmonella isolates were confirmed to be Salmonella enterica serovar Typhimurium through testing at the National Veterinary Services Laboratory. These animals are part of a broader outbreak of Salmonellosis cases in Virginia opossums at a regional wildlife rehabilitation center, which has been ongoing despite biosecurity, hygiene, and diet changes. An investigation into the outbreak has been initiated and is in progress.

S61: STAINING FOR ALKALINE PHOSPHATASE ACTIVITY CAN BE USED TO IDENTIFY CARCINOMA IN ABDOMINAL AND THORACIC FLUID SAMPLES

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Background: Distinguishing reactive mesothelium from neoplastic cells within cavity fluid can be challenging by Wright-Giemsa-stained cytology. Cytokeratin and vimentin immunolabeling can aid this distinction but is of limited availability. Alkaline phosphatase (ALP) enzyme activity is documented in some mesenchymal and epithelial tissues but not in mesothelium. Evaluation of ALP activity could prove a quick and

inexpensive way to identify carcinoma and other neoplasia in body cavity fluids.

Objective: Evaluate the diagnostic utility of ALP staining to identify neoplastic cells in abdominal and thoracic fluid samples.

Methods: Records and samples from canine and feline abdominal or thoracic cavity fluids cases submitted between 1 Jan 2019 and 1 Sep 2022 which had been immunolabeled for cytokeratin and vimentin at the time of initial submission were included. Archived Wright-Giemsa stained slides were stained for ALP activity using the 1 hour protocol. Sensitivity and specificity were calculated.

Results: 42 samples were included from 33 dogs and 9 cats. In the whole group, for detection of carcinoma, sensitivity was 65% (95%CI=38-86%) and specificity was 88% (95%CI=69-97%). In dogs, sensitivity was 50% (95%CI=19-81%) and specificity was 86% (95%CI=66-97%). In cats, sensitivity was 86% (95%CI=42-100%) and specificity was 100% (95%CI=16-100%). One false positive dog case was an ALP+ mesenchymal neoplasia. Another false positive dog case had ALP+ atypical cells and a documented history of disseminated carcinoma but carcinoma was not confirmed by immunolabeling. ALP+ mesothelial cells were not identified.

Conclusions: ALP activity can aid identification of carcinoma and was not seen in mesothelium in cavity fluids

S62: NOVEL IGG LAMBDA CLONAL PLASMA CELL NEOPLASTIC PLEURAL EFFUSION IN A DOG

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A 6-year-old, neutered male, Australian Shepherd dog was presented to a veterinary practice with a four-day history of lethargy, nonproductive cough, and exercise intolerance. Physical examination revealed a pleural effusion, and a sample was collected for evaluation at the Clinical Pathology Section of the Veterinary Diagnostic Laboratory. The pleural fluid cell and total protein concentrations were 2,773/µL and 3.0 g/dL, respectively. Large, individualized cells with abundant, moderately blue cytoplasm that commonly contained Mott cell inclusions predominated on cytospin concentrated, modified Wright-stained slides. The large cell nuclei were round to pleomorphic with dispersed to condensed chromatin, and eccentrically located. Anisocytosis and anisokaryosis were moderate to marked with a small proportion of bi- to tri-nucleated cells. There were smaller numbers of nondegenerate neutrophils and few small lymphocytes, macrophages, and eosinophils. Immunocytochemically (ICC) stained, cytospin concentrated, fresh cell preparations documented that the large cells expressed CD20 (B cell marker) and lacked expression of CD3 (T cell marker). Additionally, immunohistochemical (IHC) staining of formalin-fixed, paraffin-embedded cell pellet sections revealed that the large cells exhibited strong expression of multiple

myeloma oncogene 1 (MUM1), IgG, and lambda light chains, while IgA and IgM expression appeared similar to the negative control. A B cell PARR (PCR for Antigen Receptor Rearrangements) clonality assay performed on formalin-fixed cell block shavings was positive. These diagnostic test results support an IgG-lambda plasma cell clonal neoplastic effusion. This is a rare diagnosis with a grave prognosis in humans that has not to our knowledge, previously been reported in animals.

S63: MENINGEAL AND OCULAR FELINE INFECTIOUS PERITONITIS IN A KITTEN

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Background: A 5-month-old intact male kitten was submitted for necropsy to the Michigan State University (MSU) Veterinary Diagnostic Laboratory (VDL). This kitten had a several-day history of pneumonia which was being treated prior to death.

Methods: A full necropsy was performed and representative sections of major organs stained with hematoxylin and eosin were histopathologically examined. Immunohistochemistry was performed on sections of the brain and an eye to evaluate for the presence of feline enteric coronavirus (FeCV) antigen.

Results: There were no obvious gross lesions. Microscopically, the leptomeninges overlying the cerebrum and cerebellum were expanded by amorphous to flocculent eosinophilic debris and regional perivascular aggregates to sheets of macrophages with interspersed scattered neutrophils, lymphocytes, and plasma cells. Similar infiltrates and debris were in the uveal stroma, vitreous, and aqueous of the eye.

Conclusions: This kitten was diagnosed with feline infectious peritonitis caused by a mutated FeCV. Many cats are commonly infected with innocuous strains of feline enteric coronavirus, however, in roughly 10% of cats, the virus mutates, altering its nature to infect macrophages, resulting in severe systemic inflammation. The molecular manifestation for this conversion is currently unknown.

S64: CYTOLOGIC DIAGNOSIS OF MYCOPLASMA FELIS ARTHRITIS IN A CAT

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A 10-year-old castrated male Domestic Shorthair cat presented on emergency to the University of Missouri Veterinary Health Center with a chronic history of intermittent right hind limb lameness and recent progression to non-weight bearing lameness. The right hock was swollen with a pressure sore present on the lateral aspect of the joint. Radiographs revealed mineral fragments in the right tarsal and stifle joints. The patient was discharged with pain medication, a recommendation for recheck at the primary care veterinarian within a few days, and a recommendation for full evaluation by the MU

orthopedic service if clinical signs failed to improve. The patient was febrile at presentation to the primary veterinarian 4 days later. Right tarsal synovial fluid was collected and submitted for cytology. Cytologic examination revealed markedly increased numbers of nucleated cells with the predominant cell type being neutrophils. Low numbers of pleomorphic bacteria were noted in the background as well as within neutrophils and macrophages. Bacteria were <1 micrometer, pale blue-grey to medium blue, and ranged from ring-shaped, to rod-shaped to coccoid. This morphology is consistent with *Mycoplasma* sp*.* Presumed ragocytes were also present in low numbers. PCR using primers specific to *Mycoplasma* spp, followed by sequencing of PCR products, confirmed the bacterial population to be *Mycoplasma felis.* Both *Mycoplasma felis* and *Mycoplasma gateae* have been implicated in cases of feline arthritis, however culture or PCR were required for diagnosis. To our knowledge, this is the first documentation of cytologic diagnosis of *Mycoplasma* arthritis in a cat.

S65: METASTATIC ATYPICAL CUTANEOUS MAST CELL TUMOR WITH CONCURRENT ERYTHROPHAGOCYTOSIS IN A CAT

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A two-year-old spayed female Domestic Shorthair cat presented to the emergency veterinarian for collapse, unusual behavior, and vomiting. She was being treated for an ulcerated mass on her dorsum. Upon presentation, the patient had seizure-like activity, arrested, and died. On necropsy, there was a 3.0 cm in diameter, ulcerated cutaneous mass on the dorsum. The right axillary lymph node was enlarged, measuring 3.5 x 1.0 x 0.5 cm, off-white, and soft with a loss of corticomedullary distinction. There was moderate hepatosplenomegaly. The lungs were wet with multifocal, irregular areas of congestion, atelectasis and emphysema. On histopathology, the cutaneous mass was characterized by an infiltrative, dermal to subcutaneous, unencapsulated, densely cellular neoplasm composed of large, round to polygonal cells arranged in sheets on a preexisting collagenous stroma. Neoplastic cells had distinct borders, abundant eosinophilic cytoplasm and round to bean-shaped, central to eccentric, slightly indented nuclei with finely stippled chromatin and a single prominent nucleolus. Bi- and multinucleated cells were common. Anisocytosis and anisokaryosis were moderate. The mitotic count (MC) was 7 per 2.37 mm^2. Similar neoplastic cells were within the right axillary lymph node, spleen, liver, lung and bone marrow. Neoplastic cell erythrophagocytosis was primarily seen in the spleen and liver. Neoplastic cells in all aforementioned organs were negative for c-kit and contained numerous intracytoplasmic, metachromatic granules on Giemsa, confirming the diagnosis of metastatic atypical (histiocyte-like) cutaneous mast cell tumor, which is rare in felines. Despite minimal documentation of their biological behavior, high MC suggests an unfavorable outcome.

S66: IRON OVERLOAD IN CAPTIVE AMARGOSA VOLES (MICROTUS CALIFORNICUS SCIRPENSIS)

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In 2014, a breeding colony of endangered Amargosa voles was established for population insurance. Recently, hemochromatosis was identified in 2 colony voles. This study was designed to investigate the extent of hepatic iron overload in the colony and identify risk factors such as time-in-captivity, sex, and lineage that could influence management decisions. In the 2 voles with hemochromatosis, hepatic iron content was quantified by mass spectroscopy and compared to unaffected vole and mouse controls. To identify risk factors, 44 voles from the pathology archives were randomly selected within 5 stratified age groups. Liver tissue from these voles was stained with Prussian blue and analyzed using automated image analysis of binary pixels per region of interest (ROI). Based on the group mean, a threshold of 20% ROI was set to identify voles with elevated hepatic iron content. Mean hepatic iron content in the 2 voles with hemochromatosis (20,050ppm) was 10 - 20x greater than unaffected voles (2,443 ppm) and mice (1,010ppm). Linear regression of Prussian blue-differential staining and time-in-captivity showed no significant correlation (R^2=0.0222) but allowed for the identification of 4 additional voles with elevated hepatic iron content. Females had significantly higher hepatic iron content (P=0.0046) and sex was a risk factor (P=0.021). Pedigree analysis revealed that 5 of 6 voles (83.3%) with elevated hepatic iron were related to a single male founder (tag 4585) and lineage was a definitive risk factor (P<0.0001). Iron overload in this Amargosa vole colony is thus confirmed and potentially due to a heritable cause.

S67: BILATERAL ADRENOCORTICAL NECROSIS AND HEMORRHAGE IN AN 11-YEAR-OLD DACHSHUND RECIVING TRILOSTANE THERAPY FOR THE MANAGEMENT OF PITUITARY-DEPENDANT HYPERADRENOCORTICISM.

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An 11-year-old, female spayed, miniature dachshund was presented with signs of polyuria, polydipsia, weight loss, alopecia, and fatigue. Ultrasound revealed degenerative mitral valve disease [ACVIM STAGE B2], a mildly enlarged, hyperechoic liver, thickened urinary bladder and enlarged adrenal glands. An ACTH stimulation test revealed hyperadrenocorticism, and the dog was started on Trilostane (Vetoryl ®), a short-acting reversible inhibitor of steroid synthesis, at 30mg once a day. Four weeks after the initiation of therapy a second ACTH stimulation test revealed improved control of cortisol levels, however clinical signs remained unchanged. The Trilostane dose was increased to 40mg once a day; a week later the dog become severely ill, listless, developed shaking, vomiting and mucoid diarrhea and died within a few hours. Postmortem findings included valvular endocardiosis, subacute cystitis and

pyelonephritis and gross lesions consistent with bilateral adrenocortical hyperplasia secondary to the presence of a functional pituitary chromophobe adenoma. The adrenal glands were diffusely enlarged and dark red. Sagittal sections revealed thickening and red discoloration of the cortex. Microscopically, multifocal to locally extensive areas of hemorrhage, coagulative necrosis and acute inflammation were present throughout the hyperplastic cortices. Trilostane is considered safe and effective for the treatment and management of hyperadrenocorticism in dogs. Rarely, however, adrenocortical necrosis (as seen in this case) leading to life-threatening hypoadrenocorticism may occur. Small animal clinicians should be aware of this rare but possible fatal adverse drug reaction when managing patients with Cushing's disease.

S68: MULTISYSTEMIC LISTERIOSIS IN A SUGAR GLIDER

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An adult female albino sugar glider was presented for necropsy with no clinical signs. This animal was housed with four other individuals with varying signs of lameness and anorexia. This animal was the second to die but the first in the group to have a necropsy. Gross necropsy examination revealed disseminated, tan to dark red, flat pinpoint discolorations on the liver that extended throughout the parenchyma. The craniodorsal lung lobes were mottled red, and the heart was enlarged.

Necrosuppurative hepatitis, interstitial pneumonia, and suppurative encephalomyelitis with microabscessation and intralesional bacteria were appreciated histologically. Bacteria were predominantly extracellular, rod-shaped, and formed small haphazardly arranged stacks. The bacteria stained positively with Steiner's silver stain. Aerobic culture of the liver and spleen yielded growth of *Listeria monocytogenes*. Multisystemic listeriosis was based on visceral and central nervous system lesions and culture results. *Listeria monocytogenes* is a zoonotic food-borne pathogen that can survive refrigeration and is most commonly transmitted via ingestion of contaminated produce. Listeriosis has rarely been reported in sugar gliders and emphasizes the zoonotic potential when owning exotic pets.

S69: PRIMARY PULMONARY MAST CELL TUMOR IN A DOG

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A 9.5-year-old male castrated Great Dane mix presented for intermittent vomiting and anorexia. A thoracic mass was noted incidentally on abdominal radiographs and the patient subsequently developed respiratory distress. CT scan with contrast revealed an 8.5x7.0x7.2cm mass in the left caudal lung lobe. The affected lung lobe was removed and submitted for histopathology. Lung parenchyma was effaced by broad, variably dense sheets of neoplastic round cells with discrete cell borders, moderate amounts of faintly to modestly well-granulated amphophilic cytoplasm, round to oval nuclei with

stippled chromatin, and one to two very large nucleoli. Anisocytosis and anisokaryosis were moderate to occasionally marked with 47 mitoses in 10 400X fields. There were low numbers of eosinophils multifocally associated with the neoplastic cells as well as aggregates of lymphocytes and plasma cells within the mass. Toluidine blue revealed sparse to abundant metachromatic granules in the neoplastic cells. Immunohistochemistry for c-kit showed predominantly membranous immunoreactivity. Ki-67 average was >486/mm^2. Ki-67 index was 29%. No mutations were detected in *c-kit* exons 8 or 11. Primary pulmonary mast cell tumor is a rare diagnosis. This is the first case reporting *c-kit* mutation analysis alongside c-kit immunohistochemistry and ki-67 expression.

S70: EVALUATION OF ALKALINE PHOSPHATASE ENZYMATIC ACTIVITY IN CD34+ CANINE ACUTE LEUKEMIA USING CYTOCHEMICAL STAINING AND FLOW CYTOMETRY

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Background: Alkaline phosphatase enzymatic activity (ALP) has been proposed to help in classifying acute leukemia (AL) lineage (myeloid vs. lymphoid). However, human pluripotent stem cells are ALP+ and ALP may not be indicative of the lineage in AL cases with high proportions of CD34+ cells.

Objective: Assess if ALP, determined via cytochemical staining and flow cytometry (FC), is indicative of AL subtype.

Methods: 37 CD34+ AL, 7 B-cell chronic lymphocytic leukemia (B-CLL) controls, and 3 clinically healthy patients were evaluated for ALP via cytochemical staining and FC. Based on published FC criteria, 18 cases were myeloid, 12 were unclassifiable, and 7 were lymphoid. We determined the proportion of positive neoplastic cells and degree of cytoplasmic reactivity via cytochemical staining. A positive case was defined as >3% neoplastic cells with ALP cytoplasmic stippling. ALP Live Stain was incubated for 30 minutes prior to performing FC. Samples were considered ALP+ by FC if there were >3% CD34+ALP+ cells.

Results: There was no statistical difference between cases determined to be ALP+ by FC or cytology (chi-square p-value=0.91) and 36/37 AL cases were considered ALP+ cytologically. Correlation between the proportion of ALP+ cells by cytology and ALP+CD34+ by FC was poor (Spearman's rho=0.25). Neither the proportion of ALP+ cells by cytology and FC (p-value >0.05) nor the strength of staining via cytology (chi-square p-value=0.54) was statistically different between AL subtypes. No B-CLL cases were ALP+.

Conclusions: ALP was unable to distinguish AL subtype via cytology or FC.

S71: ANGIOCENTRIC LYMPHOMA IN A CAT: CASE REPORT

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A 15-year-old male castrated domestic short hair cat was presented with a three-day history of anemia, multicavity effusions, lethargy and inappetence. Cytology of abdominal effusion revealed increased numbers of lymphocytes suggestive of large granular lymphocyte (LGL). Splenic and abdominal lymph node aspirates showed lymphoid hyperplasia and rare similar atypical LGL lymphocytes, some exhibiting cannibalism of erythrocytes. Lymphoid neoplasia was suspected but could not be confirmed with cytology. Due to poor prognosis, euthanasia was selected. At necropsy, there was abdominal and pleural effusion, lungs were mottled red and firm, the liver had an increased reticular pattern, and the spleen evidenced a focal infarct. Microscopically in sections of lungs, liver, spleen, kidneys, lymph node, small intestine and brain, numerous atypical round cells were discovered in the lumen of blood vessels and extending transmurally into the adventitia. In the lung, multifocal infiltrates caused alveolar thickening, with numerous foamy macrophages and edema in alveoli. Erythrophagocytosis was noted in the lumen of vessels and in the spleen. Immunohistochemistry revealed numerous CD-3 positive cells in pulmonary vessels. PCR for antigen receptor rearrangement (PARR) using lung tissue scrolls revealed a clonally rearranged T cell receptor gene. These findings confirm a T-cell neoplasia. Given the accumulation of neoplastic cells in the vasculature, a diagnosis of angiocentric T-cell lymphoma was reached. Angiocentric T-cell lymphoma is rare malignancy, so the clinical, pathological, and histological findings of this case will help define the characteristics of this entity and provide reference for future diagnosis.

S72: SARCOCYSTIS SPP. PERITONITIS IN A DOMESTIC CAT

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A 5-year-old, male castrated, domestic shorthair cat was presented to its primary veterinarian with a 5-day history of lethargy and vomiting. The patient had outdoor access and was being treated with cyclosporine. Free peritoneal fluid was collected via abdominocentesis and submitted to the University of Illinois VDL. The fluid was exudative and contained many crescent-shaped protozoal merozoites, measuring approximately 4-6 micrometers on the long axis. These organisms had a small amount of lightly basophilic cytoplasm with centrally located nuclei and were noted throughout the background of the sample and phagocytized within leukocytes. The sample was interpreted as marked suppurative inflammation with sarcocystid organisms. Samples were negative by qPCR for *Toxoplasma Gondii*. Fluid was submitted to the *MSU VDL for pan-Apicomplexan PCR and sequencing, which yielded a DNA nucleotide sequence with 100% homology for Sarcocystis neurona, felis, or speeri. Due to deteriorating condition, the owner elected humane euthanasia. Postmortem examination was*

declined, and no additional clinical or diagnostic data was collected. Sarcocystis spp. has been previously observed in peripheral blood, cerebrospinal fluid, and potentially skeletal muscle in cats, but to our knowledge has not been implicated as a cause of peritonitis. These cases typically involved immunosuppression (e.g. FIV, FeLV, or steroid administration). We suspect that cyclosporine administration predisposed this patient to clinical illness. Based on *Sarcocystis*' apparent tissue tropism in cats, potential explanations for the presence of the organism in peritoneal fluid include hematogenous spread and tissue-associated (organ or skeletal muscle) cyst rupture.

S73: PROGNOSTIC POTENTIAL OF MICRORNAS IN CANINE SPLENIC HEMANGIOSARCOMA

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Background: Canine hemangiosarcoma (HSA) commonly manifests as a visceral tumor that constitutes approximately 5% of cancers in dogs. The prognosis for visceral HSA is poor due to the aggressive nature of the tumor and lack of specific clinical signs until significant infiltration has occurred. Hence, most dogs present with metastatic disease that responds poorly to standard surgical and chemotherapeutic intervention. Grading systems for HSA have poor prognostic significance. Thus, improved markers are imperative to guide a patient's course of treatment. Non-coding microRNAs regulate gene expression and may serve as predictive biomarkers for HSA.

Objective: To investigate the potential of microRNAs in the prognostic assessment of canine splenic HSA.

Methods: Retrospective study using archived splenic biopsies from 18 cases of canine splenic HSA divided into three groups based on survival times (G1: <90 days, G2: 90-180 days, and G3: >180 days). Expression of four microRNAs (miR-126, miR-150, miR-214, miR-456) with documented roles in canine hemangiosarcoma was assessed by quantitative PCR. Quantification cycle (Cq) values were normalized using the exogenous control UniSp6 and results expressed as fold change. Differences in expression were determined via one-way ANOVA, followed by Tukey's test.

Results: From the four microRNAs analyzed, miR-214 was significantly upregulated in G1 and G2 compared to G3 ($p<0.05$).

Conclusions: Our findings suggest miR-214 may serve as a prognostic marker for canine HSA as upregulation was associated with decreased survival times. MiR-214 has been associated with regulating pro-tumorigenic processes in canine HSA and other cancers. Confirmatory studies are needed to evaluate this marker.

S74: POLYCYSTIC LIVER DISEASE WITH FEATURES OF BILIARY CYSTADENOMA IN A GERIATRIC SYRIAN HAMSTER (MESOCRICETUS AURATUS)

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A 3-year-old, intact male Syrian Hamster was presented for necropsy after six months of deteriorating health. At the time of necropsy, most of the liver was encompassed by soft, multilobular masses that were black to red, soft, fluid filled with dark red to brown serous fluid. On histopathology, the hepatic parenchyma was replaced by large cystic structures lined primarily by a single layer of cuboidal epithelial cells with occasionally up to 3 layers of cells. The cells form papillary projections and there is hepatic degeneration, necrosis, and leukocyte infiltration. A diagnosis of polycystic liver disease was made. Polycystic liver disease in hamsters is characterized by multiple hepatic cysts that eventually lead to the replacement of normal liver parenchyma. Syrian hamsters have a low prevalence of spontaneous tumors of the liver, however, hepatic cysts associated with PLD must be differentiated from cysts that might be seen in association with hepatic neoplasms in which cysts may develop such as cholangiocarcinoma or cystadenoma. The epithelial cell papillary projections, hepatic degeneration, necrosis, and leukocyte infiltration seen in this case is not typically seen in PLD but are common findings in neoplastic disease of the liver. True cysts are usually multiple and lined by a single layer of epithelium, whereas tumors can be single or multiple and may have a complex arboriform pattern. The lack of cellular pleomorphism, stromal invasion, and mitotic figures suggests that the lesions seen in this case are a non-malignant process

S75: EQUINE PLACENTAL TERATOCARCINOMA WITH METASTASIS TO A FETUS

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Reports of fetal metastatic neoplasia in domestic animals are rare. A 273-day gestated female fetus aborted from a 10-year-old Welsh Cob mare presented for postmortem examination. On gross examination, the placenta was markedly thickened with approximately 25-40 semi-discrete, variably sized, pale-tan to yellow nodules. Within the fetus, the umbilicus and umbilical artery were regionally thickened by similar tan-yellow masses. Expanding the liver were numerous variably sized, discrete red-brown, firm nodules with soft and gritty centers. Histopathologically, hepatic, umbilical, and placental nodules consisted primarily of sheets of poorly preserved and poorly differentiated neoplastic polygonal to round cells with heterochromatic nuclei and a moderate amount of cytoplasm, separated by extensive necrosis and mineralization. The mitotic count was high (58 in 10 HPF, 2.37mm2). Within the placenta were rare areas of chondroid differentiation, few epithelial islands, and areas resembling poorly differentiated neuropil. The neoplastic population was variably reactive to cytokeratin,

and negative for vimentin and Hep Par-1. Additional immunohistochemical markers including GFAP, were unrewarding, most likely due to marked autolysis. Despite extensive metastasis within fetal tissues, the mare remains in good health with no indication of neoplasia. Based on cellular morphology, differentials included a malignant germ cell tumor or teratocarcinoma. Due to the presence of differentiation into multiple germ cell lines in the placenta and lack of HepPar-1 labeling, a placental teratocarcinoma with metastasis to the fetus is suspected. Teratocarcinomas are uncommon in domestic animals, and to our knowledge, only one additional report exists of a similar case with metastasis to a foal.

Late Breaking Posters

LB2: G-RATIO DETERMINATION USING AN ARTIFICIAL INTELLIGENCE-BASED IMAGE ANALYSIS PLATFORM

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G-ratio (inner to outer diameter myelinated nerve fiber ratio) assessment in preclinical models is a relevant aspect of peripheral nerve assessment. It correlates with nerve conduction velocity data, and/or histopathological and clinical observations. Current methodology can be time consuming, yield low fiber counts, and be potentially biased. We present an automated solution using rat resin-embedded peripheral nerve samples stained with toluidine blue and digitally scanned for image analysis. A commercial artificial intelligence-based image analysis platform (Aiforia®) was used to train a convolutional neural network (CNN)-based supervised deep learning model. Training included: semantic segmentation of the nerve fascicle, instance segmentation of nerve fibers, and instance segmentation of axons. Measurements obtained were highly consistent and amenable to statistical analysis. Thus, deep learning image analysis is a valuable tool in acquiring pivotal quantitative and nonbiased myelinated fiber data used for G-ratio determination to aid in decision-making during preclinical development.

LB3: EVALUATION OF THE XN-31 ANALYZER FOR THE RAPID DIAGNOSIS OF EQUINE PIROPLASMOSIS

Akihiro Ochi[1], Yuji Toya[2], Mikako Sengoku[2], Seiichiro Tsuchiya[2], Takashi Yamanaka[3,4], Takanori Ueno[1], Daiki Kishi[1], Toshio Nukada[1], Yoshinari Katayama[1]
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Background and Objectives: Equine piroplasmosis (EP) is a protozoal disease of equids that is caused by *Theileria equi* and *Babesia caballi*. Diagnosis of EP is conventionally performed by microscopic, molecular, and serological methods. These

methods are time-consuming, so there is a need for faster testing methods. In this study, we evaluated the application to EP diagnosis of Sysmex's XN-31 automated hematology analyzer, a rapid tester for human malaria.

Methods: The cultured parasites were measured using an XN-31 that had been customized to detect EP. The following parameters were evaluated: limits of detection (LoD), quantitation (LoQ), linearity, carryover, precision, and correlation with microscopic examination. To evaluate the practicality of the XN-31, PCR and serological tests were also performed on 120 horses, including five EP-positive horses, at the 2020 Tokyo Olympics.

Results: The XN-31 detected infected red blood cells (RBCs) in approximately 1 minute. To investigate the XN-31's reliability, the ratio of identified infected RBCs was compared between the XN-31's results and microscopic examinations. The iRBC% correlation was high (R^2 > 0.98). LOB was 0.70 cells/µl, and the LoD and LoQ for *T. equi* were 4.5 cells/µl and 16.9 cells/µl, respectively, while the LoD and LoQ for *B. caballi* were 5.5 cells/µl and 15.1 cells/µl. Linearity was good (R^2 > 0.99). Carryover never exceeded 0.5%. The XN-31's coefficient of variation was under 5%.

The Olympics test results showed the XN-31 results to be consistent with the PCR results, indicating the XN-31 to be effective in detecting EP-infected horses.

LB4: ANTE-MORTEM DIAGNOSIS OF FELINE PULMONARY LANGERHANS CELL HISTIOCYTOSIS

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Feline pulmonary Langerhans cell histiocytosis (FPLCH) is a rare proliferative disease in middle-aged to older domestic cats. Pulmonary Langerhans cells in the terminal airways proliferate and infiltrate the interstitium and the airways to a lesser degree, widely effacing normal parenchyma. Historically, definitive diagnosis has required post-mortem evaluation where pulmonary lesions have a classic gross and histologic morphology. Here, we present the first documented ante-mortem diagnosis of FPLH utilizing bronchoalveolar lavage (BAL) cytology and immunocytochemistry in a 9-year-old British shorthair mix. The cat had a 3-month history of respiratory difficulty that was refractory to steroids and antimicrobials. Pulmonary radiographs had marked diffuse changes with a complex bronchointerstitial and micronodular pattern. BAL cytology revealed neutrophilic inflammation and markedly increased histiocytes with morphology distinct from typical pulmonary macrophages. Immunocytochemistry characterized histiocytes as CD1+/ E-Cadherin+/ CD11b-/ PanCK-, consistent with a Langerhans cell phenotype. The cat was humanely euthanized due to poor prognosis and presented for necropsy. Gross, histopathologic, immunophenotypic and ultrastructural findings confirmed a diagnosis of FPLH. On immunohistochemistry, proliferative cells were E-cad+/ Iba-1+/ CD18+/ CD1+/ CD5+/ MHCII+/ CD204-/ CD4-, and transmission electron

microscopy identified the presence of Birbeck's granules, consistent with previous reports of FPLCH.

LB5: MORTALITY IN JUVENILE HATCHERY-RAISED SALMON ASSOCIATED WITH HYPERINFECTION BY BLOOD FLUKES (PRESUMPTIVE SANGUINICOLA SP.)

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Background: Increased mortality occurred in a group of >100,000 hatchery-raised juvenile kokanee salmon (*Oncorhynchus nerka)*. Daily mortality rates reached 0.6%, with 25% morbidity and 4,000 mortalities in a 28-day period. Administration of medicated feeds containing broad-spectrum antibiotics did not reduce mortality. Gross lesions observed in affected fish include exophthalmia, petechiae on the ventral skin, and intracoelomic hemorrhage.

Objective: To determine a cause for increased mortality in juvenile kokanee salmon from a hatchery in northwestern Washington state.

Methods: Routine histology was performed on seven recently deceased fish. Standard aquatic bacterial and viral cultures were performed on pooled fresh tissues. DNA extracted from formalin-fixed, paraffin-embedded tissue was amplified with PCR using universal trematode primers.

Results: Histologically, all seven fish had disseminated intravascular trematodiasis. Large numbers of intravascular trematode eggs were observed in the gills, heart, kidney, and spleen. Blood vessels throughout the head, body, and tail also contained numerous adult flukes. Hemorrhage expanded the surrounding connective tissues, and blood vessels had secondary endothelial hypertrophy and myointimal proliferation. PCR and sequencing for definitive parasite identification are currently in progress. There was no histologic evidence of viral or bacterial disease, and bacterial and viral cultures were negative.

Conclusions: Blood flukes within the genus *Sanguinicola* infect the vascular system of fish following transmission from a gastropod or bivalve intermediate host. Although low numbers of these parasites are a common incidental finding in salmonid fish, vascular damage and tissue injury due to hyperinfections can cause significant mortality.

LB6: INTRAMURAL CORONARY ARTERY AND MYOCARDIAL PATHOLOGY IN CAPTIVE TIGERS (PANTHERA TIGRIS) AND AFRICAN LIONS (PANTHERA LEO)

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Cardiovascular disease is considered the leading cause of human mortality worldwide, with coronary heart disease being the most common. Though coronary heart disease is relatively uncommon in animals, other forms of spontaneous cardiac disease have been reported in several veterinary species. Currently, there is limited published data regarding cardiovascular disease in nondomestic felid populations. To address this knowledge gap, necropsy cases of tigers and lions with representative myocardial samples submitted from 2019-2022 to the diagnostic laboratory at Colorado State University were histologically examined. Tissue sections were assessed with H&E and Sirius red stains for arteriosclerosis, perivascular fibrosis, myocardial fibrosis, and cardiomyocyte degeneration and loss. A total of 32 submissions (15 tigers, 17 lions) were identified. All tigers and 88.2% of lions had some degree of coronary artery lesions in the left ventricle and/or interventricular septum. The most significant lesions included moderate to marked arteriosclerosis (53.3% tiger and 23.5% lion cases) and moderate to marked perivascular fibrosis (53.3% tiger and 35.3% lion cases). 60.0% of tigers and 47.1% of lions had coronary artery lesions with some degree of perivascular cardiomyocyte degeneration and/or loss. To our knowledge, this is the first report describing coronary artery pathology in captive tigers and lions. Unfortunately, a definitive cause of coronary artery pathology in this population was not determined and all cases were euthanized or died due to other clinical concerns. However, the severity of the lesions in some cases may suggest an emerging, clinically silent cardiomyopathy. Possible contributing/inciting factors include obesity, hypertension, genetics, and aging.

LB7: MULTIPLE EPICARDIAL SQUAMOUS CYSTS AND OTHER SPONTANEOUS FINDINGS IN THE HEART OF AN AGED CYNOMOLGUS MACAQUE.

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A 28-year-old male cynomolgus macaque of Mauritius origin was on a senescence study. The animal was euthanized due to numerous clinical problems (e.g., chronic diarrhea, infection surrounding a vascular access port, a testicular abscess, carpal joint osteoarthritis, and infections leading to necrosis necessitating amputation of the tail tip and several digits). At necropsy, macroscopic findings involving the heart included a 4.5 cm-diameter mass near the right atrium that exuded green-brown material on cut section; a firm 1.5 cm-diameter nodule in the pericardial sac; multifocal areas of white granular material on the epicardium; and irregular nodular thickening of mitral valve leaflets. The first three gross findings each correlated histologically with keratin-filled cysts lined by well-differentiated stratified squamous epithelium. The valvular thickening correlated histologically with myxomatous degeneration. In the myocardium, findings consistent with mild spontaneous cardiomyopathy of cynomolgus monkeys were present, and there was minimal adipocyte infiltration. Minimal intimal hyperplasia affected several coronary artery branches.

Based on the animal's clinical history, the heart masses noted at necropsy were suspected to be consistent with abscess formation. Surprisingly, however, they actually

correlated histologically with the spontaneous finding of squamous cysts. The remaining heart findings were common spontaneous age-related processes in cynomolgus monkeys.

LB8: EFFECT OF JANUS-KINASE INHIBITORS ON CELL GROWTH AND SURVIVAL IN CANINE B- AND T-CELL LYMPHOMA CELL LINES

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Background: Constitutive activation of Janus Kinase (JAK) / Signal Transducer and Activator of Transcription (STAT) pathways play important roles in the pathogenesis of human hematopoietic tumors and various JAK inhibitors are used to treat human lymphomas in clinical trials. Previous *in vitro* studies reported antitumor effects of JAK inhibitors in canine B-cell lymphoma. However, no studies have investigated the role of the JAK/STAT pathway in dogs with T-cell lymphoma.

Objective: Evaluate different JAK inhibitors to determine the survival dependency of canine B- and T-cell lymphoma cell lines on JAK/STAT signaling.

Methods: The effects of four JAK inhibitors (AZD1480, CP-690550, INCB39110, oclacitinib) on cell growth, viability, and apoptosis were evaluated in canine lymphoma cell lines (OSW, Ema, 17-71, CLBL-1) using a bioreductive spectrophotometric assay, trypan blue exclusion assay, light microscopic morphology, and flow cytometry.

Results: The JAK2 inhibitor (i) AZD1480 significantly inhibited proliferation, decreased viability, and induced apoptosis and necrosis in a dose-dependent manner in both B-cell and T-cell lymphoma cells. Oclacitinib (JAK1/2-i) significantly inhibited cell proliferation at a high dose and results suggested it induced cell cycle arrest rather than cell death. The effects of INCB39110 (JAK1-i) and CP-690550 (pan-JAK-i) were not significant in any cell line.

Conclusions: As previously demonstrated in human and canine B-cell lymphoma, aberrant JAK-2 signaling may also play a role in canine T-cell lymphomagenesis and JAK-2 inhibition may represent a promising new treatment approach in canine T-cell lymphoma. Future studies should focus on expression levels of the different JAK subsets in canine neoplastic T-cells.

LB9: OXIDATIVE STRESS AND NEURODEGENERATION DURING NATURAL AGING OF NONHUMAN PRIMATES

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Aging is a complex process that represents the change brought about by a lifetime of responses to stimuli, both endogenous and exogenous. Oxidative stress has long been

implicated in aging and is due to the accumulation of damage caused by reactive oxygen and nitrogen species created as byproducts of normal metabolism and inflammation. Chronic inflammation increases oxidative stress and has been shown to be associated with dementia and Alzheimer's disease. Small mammal models have been crucial in our understanding of aging and age-related conditions but are limited in their ability to model spontaneous neurodegeneration and dementia. NHPs provide a superior model to study neurodegeneration and dementias due to their similar neuroanatomy and higher cognitive function which allow for the development of tests, biomarkers, and therapies that are more translatable to people. Herein we investigated the role of oxidative damage on natural, age-related, neurodegeneration in rhesus macaques. We utilized archival formalin-fixed, paraffin-embedded (FFPE) brain tissue to measure the immunohistochemical (IHC) expression of markers of oxidative damage in different aged cohorts (young-adult, adult, and geriatric) of rhesus macaques. Markers of oxidative damage were correlated with histopathologic changes of neurodegeneration and an IHC marker of dementia (B- amyloid). The identification of IHC markers that correlate with aging, neurodegeneration, and dementia establishes tissue-based methods for detecting NHPs in future studies that exhibit accelerated aging or "inflammaging".

LB10: CALLITRICHINE HERPESVIRUS 3 (CALHV-3) ASSOCIATED B-CELL LYMPHOMA IN THE COMMON MARMOSET (CALLITHRIX JACCHUS)

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Background: Callitrichine herpesvirus 3 (CalHV-3), a gammaherpesvirus and Epstein-Barr (EBV) related lymphocryptovirus, was first isolated from a B-cell lymphoma in a common marmoset (*Callithrix jacchus)*. While initial studies showed CalHV-3 infection was prevalent in research colonies, associated clinical lymphoproliferative disease, such as lymphoma, was suspected to be rare.

Objective: The goal of this study was to further characterize the role of CalHV-3 in lymphoma in the common marmoset.

Methods: The archives of the Department of Veterinary Resources at the National Institutes of Health were searched for marmoset autopsies from 1992-2022, and specimens from nine cases of suspected lymphoma were reviewed. Immunohistochemistry (IHC) for CD3 and CD20 was performed to confirm neoplastic cell of origin. DNA was extracted from formalin-fixed paraffin-embedded tissues, and presence of CalHV-3 DNA was detected via droplet digital PCR (ddPCR).

Results: 15 (1.8%) of 834 marmoset autopsies were diagnosed with neoplasia, with lymphoma suspected in 67% (10/15) of these cases. Eight lymphomas were positive for

CD20, consistent with B-cell origin. All B-cell lymphomas (100%) were positive for CalHV-3 DNA, with high viral loads of millions of copies of virus per million cells.

Conclusions: All cases of B-cell lymphoma, the most common neoplasm in the marmoset, were associated with CalHV-3 infection, highlighting the important role of an oncogenic herpesvirus and a potentially useful model of virally induced hematopoietic neoplasia. As utilization of the common marmoset in biomedical research continues to grow, further investigation of CalHV-3 induced oncogenesis and other related pathology is warranted.

LB11: A NEW NON-TOXIC ACID-FREE GLYOXAL FIXATIVE FOR VETERINARY MOLECULAR PATHOLOGY

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Formaldehyde (NBF) fixation followed by paraffin-embedding is routinely used for histopathology and immunohistochemistry. However, toxicity and poor preservation of proteins and nucleic acids represent severe limitations of NBF. Alternative fixatives guaranteeing similar morphological quality and costs, but less toxicity and better preservation of proteins and nucleic acids would therefore be very appealing. This multi-institutional study aimed to compare the performances of a newly patented, non-toxic, acid- free Glyoxal (GAF) fixative with NBF.

GAF fixation was satisfactory for histology and immunohistochemistry from different animal species and organs (60 samples). Only mast cell tumors were suboptimally preserved at histology. Molecular analyses were performed on a subset of 15 samples, assessing RNA/DNA quality and quantity and genes amplification.

Interestingly, GAF fixed samples yielded a higher DNA concentration (microgram/microliter). The amplification of selected genes was either obtained from GAF samples only (e.g. TP53, exon 6) or with lower Ct values (e.g. COX1) for GAF vs NBF –fixed samples. Moreover, when amplification of a specific viral agent was obtained with both fixatives, sequencing was completely successful only from GAF samples. RNAScope was preliminarily tested in canine mast cells tumors. RNAScope allowed RNA amplification of CD117 and positive control CI-PPIB probes as cytoplasmic, red, small punctate dots. No signal was observed for negative control dapB probe.

Based on these results GAF appears as a highly encouraging fixative that could be used with less environmental and public health impact than NBF and with better results for molecular analyses.

LB12: ABUNDANCE OF BACTERIA ON THE SMALL INTESTINAL MUCOSA OF DOGS WITH CHRONIC INFLAMMATORY ENTEROPATHIES

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Background: Canine chronic inflammatory enteropathy (CIE) can be classified according to treatment response as food-responsive (FRE), steroid-responsive (SRE), or antibiotic-responsive (ARE).

Objective: We aimed to quantify bacteria on the small intestinal mucosa of dogs with different categories of CIE and healthy control (HC) dogs.

Methods: Formalin-fixed paraffin-embedded samples of duodenum and ileum from 53 dogs with CIE (FRE, SRE, and ARE) and eleven HC dogs were used. Bacteria were labeled using fluorescence in situ hybridization with a eubacterial probe (EUB338) and quantified with ImageJ software in ten random 400x fields on the mucosal surface for each case and intestinal segment. The median area of labeled bacteria and cumulative WSAVA histopathologic scores were compared among groups using Kruskal-Wallis tests, followed by Dunn's multiple comparisons test.

Results: In the ileum, the median area of bacteria was higher in dogs with ARE than in HC dogs (adjusted p=0.04), however, it did not differ from those of dogs with FRE or SRE. Ileal histopathologic scores were higher in dogs with ARE and SRE than in HC dogs (adjusted p=0.021 and 0.0004, respectively). In the duodenum, there was no significant difference in bacterial counts among groups. However, dogs with ARE, FRE, or SRE had higher duodenal histopathologic scores (adjusted p=0.048, <0.0001, and <0.0001, respectively) compared to HC dogs.

Conclusion: The higher mucosal bacterial abundance in the ileum of dogs with ARE compared with HC may represent evidence of ongoing dysbiosis in this group. Dogs with FRE and SRE showed more variability in mucosal bacterial abundance.

LB13: 3D IN-VITRO MODELS OF COMMON BOTTLENOSE DOLPHIN (TURSIOPS TRUNCATUS) CELL LINES ON NOVEL COLLAGEN-FREE SCAFFOLDS

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Marine mammals are important indicators of environmental conditions such as pollution and viral spread. The creation of in-vitro complex systems to assess their interaction with environmental conditions, is therefore of very considerable interest.
2D cell cultures are very minimally resembling the tissue of origin, whereas in 3D cultures, cells grow in complex interactions with the extracellular matrix (ECM), better mimicking the condition of cells in vivo. Hence, 3D systems are considered a potential bridge between 2D cultures and in vivo animal models, and in the past years different 3D models have been developed in human and veterinary medicine.
However, no study to date has accomplished the creation of marine mammals' 3D models.

Thus, in this study, two new 3D systems of common bottlenose dolphin (Tursiops truncatus) fibroblasts on novel scaffolds (hyaluronic acid and a ionic-complementary self-assembling peptide) and on Matrigel have been developed and assessed. Histological (Hematoxylin and Eosin and Masson Trichrome) and fluorescent staining with both nuclear, cytoplasmic, and membrane dyes (DAPI, Hoechst, and Red CellBrite), and viability assays (Presto blue, and CellTiter-Glo 3D Cell) have been performed on the models. RT-PCR has instead been used to detect ECM components. Results have shown that Matrigel induced cells to form complex spheroids while the scaffolds allowed single cells to grow surrounded by a collagenous ECM (collagen1a1, laminin B1 and elastin).

The development of this innovative approach is the first step towards the possibility to create 3D in-vitro models also for wild animals better resembling the in vivo systems.

LB14: INCIDENTAL HISTOCHEMICAL STAIN CONTAMINATION AS A COMPLICATION OF MITIGATION EFFORTS FOR SUPPLY CHAIN DISRUPTIONS DURING THE COVID-19 PANDEMIC

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The Coronavirus Disease 2019 (COVID-19) pandemic has caused significant morbidity and mortality, and it has received significant attention. However, indirect consequences of the pandemic such as supply chain disruptions, can also impact standard laboratory practices for research cores. During the pandemic, our core lab was faced with a short-term forecast of hematoxylin shortages that would require rationing the stain. This was accomplished by increasing the time interval between replenishing and discarding each batch of hematoxylin. We used HE sections of liver as a tissue control to assure that HE stains were of acceptable quality during the extended usage period. This approach proved successful for several months, but then during a routine screening of an immunohistochemistry slide with 3,3′-diaminobenzidine (DAB) as chromogen, brown to black pigmented cells were identified in known negative control tissues. Pathologist examination identified uncommon aggregates of yeast-like oval to round bodies ranging

~2-10 microns with most averaging ~4-6 microns in diameter. The fungal organisms had prominent cell wall and were readily detected by its pigment using HE stain or IHC with hematoxylin counterstain, suggesting the hematoxylin reagent was contaminated. Cleaning and replenishing the hematoxylin container yielded no further evidence of the organisms. Previous reports of contamination by a similar fungus (e.g. Exophiala jeanselmei) have led to medical misdiagnosis thus emphasizing the importance of quality controls in the laboratory. This report highlights how incidental stain contamination can occur under pandemic conditions and how routine quality controls were able to identify the contamination before it influenced research studies.

LB15: ASSOCIATION OF DEFICIENT COPPER WITH BLACKLEG (CLOSTRIDIUM CHAUVEOI) IN CATTLE

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Muscle trauma has a central role in the pathogenesis of clostridial myositis (blackleg) caused by intramuscular germination of *Clostridium chauvoei* spores; however, the occurrence of blackleg in muscles like the heart that are less easily traumatized suggests that other factors may contribute. We hypothesized that deficiencies in micronutrients important to muscle health, in particular copper and/or selenium, contribute to muscle damage and the pathogenesis of blackleg. We performed a retrospective analysis for bovine clostridial myositis/myonecrosis (blackleg) cases to determine if disease occurred in association with alterations in these micronutrients. Fifteen cases were identified via a database search for positive muscle *C. chauvoei* immunofluorescence from March 2017 through July 2022; liver copper and selenium levels available for blackleg cases (n=8) was compared to levels in control bovines (n=13) dying during the same period without evidence of blackleg disease. Liver copper levels in blackleg cases (median=21.6ppm, SD=35.4) were significantly lower (Mann-Whitney U test p=0.045) compared to controls (median=72.4 ppm, SD=65.9) while selenium levels were not different between groups. These data suggest a contributing role for copper deficiency in blackleg disease and provide a basis for increased monitoring of micronutrient levels in clinical cases and herds with history of blackleg.

LB16: CYTOLOGIC, HISTOLOGIC, AND RADIOGRAPHIC FINDINGS OF OSTEOCHONDROMA IN A DOMESTIC FERRET

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Background: Osteochondromas are cartilage capped bony proliferations that arise from the surface of endochondral bones. Lesions that occur in dogs and horses are generally present at or occur shortly after birth. They are thought to arise from displaced physeal cartilage. Bone tumors are rarely reported in ferrets; there are no published reports of osteochondroma.

Methods: A 4.5-year-old female spayed ferret presented for a hard, 1.5-cm-diameter swelling proximal to the right elbow. Radiographs were performed. The mass was aspirated and stained with Wright's giemsa and alkaline phosphatase (ALP). The limb was amputated, fixed whole in 10% neutral buffered formalin for >24 hours, decalcified, and processed routinely for histology.

Results: Radiographs of the limb showed a variably mineralized mass continuous with the distal medial aspect of the right humeral cortex with no evidence of bone lysis or periosteal reaction. Cytologic samples consisted of pleomorphic spindle cells with rare mitotic figures on a background of bright magenta matrix (chondroid). Cells demonstrated diffuse cytoplasmic ALP staining, and the lesion was interpreted as likely osteosarcoma. Histologically, the mass was multilobular and composed of outer caps of hyaline cartilage with normal endochondral ossification and regular trabeculae of woven bone. Trabeculae were lined by osteoblasts and fewer osteoclasts and the intertrabecular spaces contained bone marrow. It was continuous with underlying cortical bone of the humerus. This is typical for osteochondroma.

Conclusions: This case demonstrates the importance of a multimodal approach of diagnostic imaging, cytology, histochemistry, and histology, when diagnosing proliferative bony lesions.

LB17: PATHOLOGY, GENOMIC CHARACTERIZATION, AND IN SITU HYBRIDIZATION OF DENSOVIRUS-INFECTED SUPERWORMS (ZOPHOBAS MORIO)

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Superworms, known as giant mealworms *Zophobas morio*, are common captive-raised beetle larvae used as a food source for birds, reptiles, and amphibians due to the ease of mass-rearing and high protein content. In 2021, superworms imported into Washington experienced high morbidity and mortality. Clinical signs included lethargy, dehydration, and dark discoloration. Samples were submitted to the Washington Animal Disease Diagnostic Laboratory for investigation. Histopathology from affected animals revealed karyomegaly and basophilic intranuclear inclusion bodies throughout tissues, including cells of the epidermis, circulating hemocytes, foregut, myocytes, and fat body. DNA extracted from filtered tissue homogenate was used for Nanopore library construction. Reads which aligned to the only publicly available sequences of *Z. morio* densovirus (accession MN732869.1) were queried and matched with high concordance

to *Densovirinae* sp. isolate hwf061par2 (DNV, accession MT138252.1). Reads mapping to DNVsp. hwf061par2 were *de novo* assembled and polished. Our final 5791bp genome contains open reading frames (ORFs) for three non-structural proteins and two anti-sense ORFs encoding structural proteins, flanked by inverted terminal repeats. Our genome bears 97.98% nucleotide similarity to DNVsp. hwf061par2 and 99.66% to DNVsp. isolate T2002773. We developed a PCR capable of discriminating *Z.morio* DNV from other related densoviruses. An *in-situ* hybridization probe was developed targeting VP1 nucleotides of our sequenced genome. Localization of viral genome revealed the widespread presence of viral RNA throughout the DNV-infected superworm, and an absence of detection in uninfected controls. Further investigation into transmission dynamics, age susceptibility, and effective preventative measures are warranted to further safeguard this important food source.

LB18: ANALYTICAL VALIDATION OF A CANINE HBA1C POINT-OF-CARE ASSAY: PRELIMINARY FINDINGS

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Background: Hemoglobin A1c (glycated hemoglobin) is a measure of the proportion of glucose-bound hemoglobin in the blood. The measurement of HbA1c provides an estimation of plasma glucose levels over the approximate lifespan of an erythrocyte. This parameter is widely used in people to monitor glycemic control; however, HbA1c is infrequently measured in companion animals primarily due to the lack of available, validated bedside tests that can provide real-time information.

Objectives: To validate the Vet Chroma™ point-of-care test for the measurement of canine HbA1c. This analyzer uses immunofluorescent technology to measure HbA1c in whole blood samples. We hypothesize that the Vet Chroma™ is a valid assay for the measurement of HbA1c in dogs.

Methods: ASVCP quality control guidelines for instrument validation are being followed. Surplus whole blood samples submitted to the diagnostic lab are being used to assess intra- and inter-assay variability, linearity, effect of interfering substances, method comparison, and storage stability.

Results (preliminary): Intra-assay variability ranges from 3.5-10.0% and inter-assay variability ranges from 7.9-14.5% for samples representing the low and high ends of the analyzer's reportable range. Linearity testing shows that the assay is linear within the reported range and shows evidence of constant bias.

Conclusions: The preliminary results indicate promising potential for the Vet Chroma™ as a bedside test for the measurement of canine HbA1c.

LB19: ELEVATED SERUM GAMMA-GLUTAMYL TRANSFERASE IN TWO DOGS WITH RENAL CARCINOMA

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During a 3-year period, a 15-year-old male castrated Terrier mix (dog 1) and a 6-year-old female spayed Labrador Retriever (dog 2) presented to the NC State Veterinary Hospital with similar blood work abnormalities and no significant physical exam findings. A complete blood count (CBC), chemistry panel, and urinalysis performed on both dogs were relatively unremarkable other than a marked increase in serum gamma-glutamyl transferase (GGT). Imaging revealed a renal mass in both patients. Histopathology of both masses revealed a carcinoma. Immunohistochemical staining of the mass in dog 1 was intensely positive for GGT. Dog 1 had the affected kidney removed, which normalized the GGT value. Dog 2 was euthanized, and metastasis to the lung was noted upon autopsy. There have been limited case studies documenting an elevation in serum GGT in dogs diagnosed with renal carcinoma. However, while uncommon, it is an important differential to keep in mind when there is a marked increase in serum GGT without accompanying increases in other measured liver enzymes. In addition, serum GGT can serve as a helpful biomarker for disease resolution and recurrence, as surgical removal of the renal mass (dog 1) led to resolution of the elevated serum GGT.

LB20: ONCOCYTIC MYOEPITHELIAL CHANGE IN A CANINE MAMMARY COMPLEX ADENOMA

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Background: Oncocytic change is cytoplasmic accumulation of fine, eosinophilic granules due to abundant mitochondria. Diagnosis is based on histologic appearance with positive staining of granules for periodic acid-Schiff (PAS) with diastase resistance, and transmission electron microscopy revealing increased numbers of mitochondria. In dogs, oncocytomas rarely occur in the salivary gland, renal collecting ducts, and skeletal muscle. Oncocytic change has been reported in suspected neuroendocrine neoplasms in the canine mammary gland.

Objective: Describe oncocytic change in a canine mammary neoplasm and investigate the cell of origin.

Methods: A 10-year-old intact female mixed breed dog presented for excision of multiple mammary masses. Tissues were fixed in 10% neutral buffered formalin for >24 hours, processed routinely for histology, and stained with HE, PAS, and PAS-diastase stains. Immunohistochemistry was performed with antibodies to calponin, p63, pancytokeratin (AE1/AE3), and Iba-1.

Results/Conclusions: Multiple benign and malignant mammary neoplasms were present. The neoplasm of interest was a 2.95 x 1.75 mm, well-demarcated, moderately cellular neoplasm composed of two cell populations. Well-differentiated epithelial cells formed tubules immediately surrounded and separated by nests of polygonal cells. Polygonal cells had distinct borders, an eccentric oval nucleus with coarse chromatin, and abundant pale eosinophilic cytoplasm with abundant intensely eosinophilic granules. Anisocytosis and anisokaryosis were moderate and mitotic count was 1 per 2.37 sq. mm. Polygonal cells displayed immunoreactivity to calponin and p63, and were negative for pancytokeratin and Iba-1. Granules were PAS-positive and diastase resistant. These findings are consistent with a complex adenoma with oncocytic change of the myoepithelium.

LB21: PATHOLOGICAL FINDINGS IN SEA TURTLES STRANDED IN THE NORTH-WESTERN ADRIATIC SEA, ITALY

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In the Mediterranean Sea, the loggerhead turtle (*Caretta caretta*) is the most abundant sea turtle species representing a distinct population with specific demographic and genetic features. Due to its conservation interest, it is listed in different international conventions. Sea turtle strandings are considered a strategic indicator of at-sea mortalities for the assessment of the health status of a regional and seasonal population, for the estimation of trends and patterns of demographic parameters, for the establishment of index of mortality and human activities interaction. Between 2018 and 2022, 403 sea turtles were found dead along the north-western Adriatic Sea, especially during summer season, and were analyzed by applying systematic and standardized post-mortem investigations to monitor stranding trends and assess the causes of death. The 16,8% of the carcasses showed a decomposition code suitable for post-mortem investigation. Among these cases, a focally extensive/multifocal granulomatous inflammation detected in gastrointestinal tract (GIT) and/or at visceral level represents the major finding (20,5%). Signs of interaction with human activities were related to fishery (by-catch and presence of fishing gear in the GIT) (7,3%), ship-strike (8,8%), and marine litter ingestion (14,7%). These results underline the importance of performing comprehensive and standardized post-mortem analysis for the detection of pathological processes affecting these animals, to understand the anthropogenic impacts, and also the natural infectious diseases that threaten this species. In addition, this information is essential for the conservation and management of certain populations and habitats.

LB22: CHARACTERIZATION OF EXTRACELLULAR VESICLES PURIFIED WITH ULTRACENTRIFUGATION OR SIZE EXCLUSION CHROMATOGRAPHY FROM TWO CETACEAN CELL LINES

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Extracellular vesicles (EVs) are intercellular communicators which play a role in physiological and pathological processes and in host-to-environment interplay. EVs are released in body fluids, being mainly studied in humans as diagnostic biomarkers. Preliminary and scarce studies on aquatic animals have introduced the multiple biological modulating functions of EVs and mentioned their potential applications as markers.

The aim of this study was to characterize EVs isolated with two different techniques from the cell culture media of bottlenose dolphin (*Tursiops truncatus*) and of Cuvier's beaked whale (*Ziphius cavirostris*) fibroblast cell lines.

EVs were isolated by ultracentrifugation (UC) or size exclusion chromatography (SEC) and characterized for size and concentration with Nanoparticle Tracking Analysis (NTA), for protein expression by Western Blot (WB), and for morphology via Transmission Electron Microscopy (TEM).

NTA showed the typical size range of EVs (50-700 nm) and a higher concentration of particles in UC samples compared to SEC in both cell lines (10^{11} and 10^{9} particles/mL, respectively). WB evidenced the expression of membrane EV-markers (CD9, integrin-beta) in all EV-samples and the expression of the cytosolic marker TSG10 only in UC derived EVs from both cell lines.

To our knowledge, this is the first characterization of EVs derived from cetacean cell lines. Though further investigations are needed, this preliminary research offers a new prospective system for the *in vitro* study of these species and for the possible identification of new biomarkers to assess their health status.

LB23: BRONCHOALVEOLAR LAVAGE FLUID (BALF) CYTOLOGY PROFILES OF VACCINATED AND UNVACCINATED PIGS FOLLOWING EXPERIMENTAL INFLUENZA INFECTION

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Background: Influenza A virus (IAV) infections pose a significant threat to both human and animal health. Developing IAV vaccine strategies, able to elicit broad immunity against antigenically diverse influenza strains, is pivotal in controlling the disease.

Objective: The goal of this study was to evaluate the clinicopathological features of infection in vaccinated animals with either a homologous or a heterologous to the challenge virus vaccine, compared to non-vaccinated controls.

Methods: A total of 18 influenza-seronegative piglets were used in the study. They were divided into 3 groups of 6 animals per group and were prime-boost vaccinated with a 3-week interval with either a whole inactivated virus (WIV) A/swine/GA/27480/2019/(H1N2) vaccine (homologous group), a WIV A/swine/MN/02636116/2021/(H1N1) vaccine (heterologous group) or adjuvant only (mock-vaccinated group), respectively. Pigs were intranasally and intratracheally challenged four weeks post-boost with A/swine/GA/27480/2019/(H1N2), a swine IAV field isolate. Vaccine-induced protection was evaluated based on three parameters, (i) serological responses, (ii) virus titers in nasal swabs and respiratory tissue homogenates, and (iii) BALF cytology.

Results: While homologous vaccination was highly immunogenic, resulting in limited pulmonary virus replication and normal BALF differential cytology profile, heterologous vaccination failed to significantly reduce lung virus titers. Additionally, the BALF cytology of the heterologous vaccinated group was characterized by mild to moderate lymphocytosis. In contrast, mock-vaccinated controls demonstrated moderate to marked neutrophilic BALF infiltration.

Conclusions: This study demonstrates that heterologous influenza vaccination failed to confer protection against challenge and elicited a different BALF cytology profile after influenza infection compared to the homologous and mock-vaccinated groups.

LB24: NEONATAL INITIATION OF PENTOSAN POLYSULFATE THERAPY IN CANINE MUCOPOLYSACCHARIDOSIS IIIB IMPACTS OCULAR PATHOLOGY

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Background: Mucopolysaccharidosis (MPS) type IIIB is a lysosomal storage disease (LSD) caused by deficiency of a-N-acetylglucosaminidase. This enzyme deficiency results in accumulation of heparan sulfate in numerous tissues and organs, including retina and optic nerve. There are currently no approved therapies for MPS IIIB. Pentosan polysulfate (PPS) has been shown to decrease markers of disease in other LSDs but has not been evaluated as a therapeutic for MPS IIIB.

Objective: The objective of this study was to evaluate the efficacy of PPS in canine MPS IIIB ocular disease by evaluating retinal lysosomal compartment size and gliosis in treated dogs.

Methods: MPS IIIB dogs (n=3) were treated every two weeks following birth with 2.88 mg/kg subcutaneous PPS. Age-matched untreated MPS IIIB dogs (n=3) and unaffected dogs (n=3) were used as controls. Semiquantitative immunohistochemistry was performed on retina for lysosomal integral membrane protein 2 (LIMP-2), a marker of lysosomes, and vimentin, a marker of Müller glia and astrocytes. Four non-overlapping contiguous images of the retina were taken at 200x magnification, and images were analyzed for percent immunoreactivity.

Results: Mean LIMP-2 immunoreactivity was decreased in MPS IIIB treated dogs (2.314 +/- 0.367 SD), compared to untreated IIIB controls (2.999 +/- 0.591 SD), but this change was not statistically significant. No differences in vimentin immunoreactivity were detected between groups (IIIB control= 7.703 +/- 1.729 SD, IIIB treated= 8.068 +/- 0.117 SD).

Conclusions: Results of this study suggest PPS treatment decreases retinal lysosome volume in MPS IIIB, but does not impact glial vimentin expression.

LB25: SPATIAL TRANSCRIPTOMICS REVEALS NEURAL-LIKE PROGENITOR PROGRAMS ASSOCIATED WITH MALIGNANT TRANSFORMATION AND METASTASES IN MURINE PANCREATIC CANCER MODELS

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Spatially resolved transcriptomics and proteomics are emerging methodologies that enable discovery and validation of molecular events within the context of histopathologic features. Such approaches are well-suited for characterizing molecular events involved in tumorigenesis, particularly for tumors composed of heterogeneous neoplastic cell, immune cell, and stromal cell populations. Animal models provide unique opportunities for utilizing spatial methodologies; models with well-characterized, repeatable disease progression enable the evaluation of both early- and late-stage events during tumor progression and evaluation of treatment effects on cell populations with specific histologic features. The KPC model of pancreatic ductal adenocarcinoma (PDAC), driven by the Kras and Trp53 transgenes, follows a consistent, stepwise process of neoplastic transformation, separable into various stages based on well-described histopathologic features. Pancreatic lesions occurring in KPC mice closely recapitulate the biology of human PDAC in terms of histopathology and clinical features. Using the GeoMx-mouse whole transcriptome atlas, we identified differentially expressed genes in normal pancreas (acini, ducts, and islets), precursor lesions (PanIN and IPMN), PDAC, and metastases and integrated spatial transcriptomics with scRNA-seq datasets for cell-type deconvolution within each histologic category. We identify that malignant progression is associated with enrichment for neural-like progenitor and neuro-endocrine pathways including genes associated with axonogenesis, neurogenesis, and synapse organization.

LB26: HISTOLOGIC CHARACTERIZATION OF CANINE HISTIOCYTIC AND NEUTROPHILIC HEPATITIS: AN EMERGING INFLAMMATORY CONDITION IN DOGS

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Background: Most cases of chronic hepatitis (CH) in dogs remain idiopathic. As such, key histologic features communicate lesion severity and facilitate clinical decisions. We recognize an emerging form of canine CH characterized by histiocytic and neutrophilic infiltrates.

Objective: Characterize clinical and histologic features of canine histiocytic and neutrophilic hepatitis to assist veterinary pathologists discern this inflammatory condition.

Methods: Medical and pathology records were retrospectively reviewed. Archived liver histology slides were assessed including special stains for pigment, tissue architecture, and infectious agents. Cases with granuloma formation or significant copper accumulation were excluded. Immunohistochemistry with IBA-1 and MHCII was performed on 5 cases.

Results: 45 cases met inclusion criteria. All biopsies had periportal to nodular sheets of activated macrophages with admixed non-degenerative neutrophils, fewer lymphocytes, and scattered necrotic hepatocytes. Strong IBA1 and MHCII positive immunoreactivity highlighted activated macrophages and resident Kupfer cells. Special stains and antemortem bacterial cultures did not reveal involvement of infectious agents. The average age at diagnosis was 6.5 years (2-14 years); females were overrepresented (23/45). Post-biopsy survival ranged from 0 to 1563 days. Clinical improvement was reported for patients receiving at least one course of antimicrobial therapy combined with a lifelong immunomodulatory agent. PCR and eubacterial fluorescent in situ hybridization (FISH) on affected dogs and age-matched controls are pending.

Conclusions: This study characterizes canine histiocytic and neutrophilic hepatitis as a form of CH that may have a favorable outcome if identified and treated prior to protracted disease. An etiology has not been identified.

LB27: EFFECTS OF THERAPEUTIC AND LIFELONG AEROBIC TRAINING ON STRUCTURAL, INFLAMMATORY AND REDOX MUSCLE CHANGES IN AN ANIMAL MODEL OF SARCOPENIC OBESITY

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Background: Sustained inflammation and oxidative stress observed in aging and obesity contribute to muscle structure and function damage by interfering with protein turnover, leading to sarcopenia. Physical training is a non-pharmacological therapeutic alternative for muscle protection by adaptive response mechanisms. However, studies evaluating therapeutic or lifelong training on muscle caused by aging and high-fat diet (HFD)-induced obesity are lacking.

Objective: To evaluate the effects of therapeutic and lifelong training on muscle structure, inflammation, and oxidative stress in an animal model of sarcopenic obesity.

Methods: Thirty-two male Wistar rats were distributed into four aging groups: sedentary (ASed), Ased+HFD, therapeutically trained (ATT)+HFD, and lifelong trained (ALT)+HFD. Animals were trained on a treadmill at moderate intensity and alternate days. Quadriceps muscles were collected at age 14 months for fiber cross-sectional area (CSA) analysis, measurement of inflammatory cytokines (TNF-α, IL-6, and IL-10), and redox balance (lipidic peroxidation, carbonylated proteins, and GPx activity and expression). ASed and ASed+HFD were compared with Student's t-test; ASed+HFD and trained groups with One-Way ANOVA.

Results: The HFD reduced CSA with an increase in muscle TNF-α, IL-6, lipid peroxidation, and carbonyl proteins and reduced IL-10 and GPx activity and expression. Both trainings were effective in attenuating HFD effects, and lifelong training showed the best results among the trained groups for CSA, IL-10, lipid peroxidation, and GPx.

Conclusions: The deleterious outcomes of sarcopenic obesity are strongly associated with inflammation and oxidative stress, and training protocols attenuate these changes. Furthermore, regular exercise throughout life was the best alternative to protect skeletal muscle.

LB28: INTESTINAL HELMINTHS IN DOGS INFECTED WITH LEISHMANIA INFANTUM AND THEIR RELATIONSHIPS WITH CLINICAL SIGNS AND HISTOLOGICAL CHANGES

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Background: The association of intestinal helminths with canine leishmaniasis is unclear.

Objectives: To evaluate the occurrence of intestinal helminths in dogs infected with *Leishmania infantum* and to compare clinical signs and histological alterations between those coinfected and non-coinfected with intestinal helminths.

Methods: Forty-one dogs were euthanized, and sections of jejunum and cecum were fixed in 10% buffered formalin for histopathology and immunohistochemistry.

Results: A positive frequency of 54% was observed for helminths. *Ancylostoma* spp., *Dipylidium caninum, Trichuris vulpis* and *Toxocara canis* were identified. In the non-coinfected group, dogs with clinical signs were 53% and 31% had intestinal inflammation. In the jejunum, non-granulomatous inflammation was observed in 31%, villi atrophy in 21%, fibrosis in 21%, glandular hyperplasia in 16% and granulomatous inflammation in 5%. In the cecum, non-granulomatous inflammation was observed in 31%, fibrosis in 26%, lymphoid hyperplasia in 26%, and granulomatous inflammation in 5%. The positivity for amastigotes was 32% in the jejunum and 37% in the cecum. In the coinfected group, dogs with clinical signs was 77% and 69% had intestinal inflammation. In the jejunum, non-granulomatous inflammation was observed in 59%, villi atrophy in 36%, fibrosis in 27%, hyperplasia glandular in 23% and granulomatous inflammation in 14%. In the cecum, non-granulomatous inflammation was observed in 54%, fibrosis in 18%, lymphoid hyperplasia in 9%, and granulomatous inflammation in 9%. The positivity for amastigotes was 18% in the jejunum and 50% in the cecum.

Conclusion: Coinfection by intestinal helminths may contributes to the worsening of canine leishmaniasis.

LB29: SPONTANEOUS LYSOSOMAL STORAGE DISEASE IN TWO SENTINEL CD1 MICE

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Lysosomal storage disease (LSD) is an inherited metabolic disorder secondary to dysfunction or deficiency of lysosomal enzymes. This leads to an accumulation of intracellular substrates that can result in cell death and organ dysfunction. The current report documents two female CD1 mice that were examined for routine sentinel testing. Gross necropsy findings included hepatomegaly, splenomegaly, and diffuse dilation and thickening of the intestines. Viscera including the liver, pancreas, uterus, and intestines were diffusely pale. Microscopically there were clusters of large foamy round cells widely disseminated throughout intestinal villi, spleen, liver, uterus, ovaries, and lymph nodes. The central nervous system was unaffected. Wright-Giesma stain showed a characteristic “sea blue” staining of these cells in the liver, spleen, and lymph nodes which is consistent with a lysosomal storage disease. Due to the lack of CNS involvement and the appearance of the accumulated cytoplasmic material, cholesterol ester storage disease is suspected. Cholesterol ester storage disease is caused by a genetic mutation in the lysosomal acid lipase (LIPA) gene, which results in the accumulation of lipids and calcium in tissue macrophages. A similar condition had also been documented in an outbred mouse on a CD1 background in a previous case report. Further investigation is needed to characterize this condition with ultrastructural imaging and genotyping of the mouse strain. This may facilitate the development of additional murine models for LSD.

LB30: COMPARISON BETWEEN CYTOLOGY OF EAR SAMPLE SWABS COLLECTED FROM DOGS AND CATS WITHOUT OTITIS EXTERNA AND STORED IN DRY TUBES OR TUBES WITH AMIES SOLUTION

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Background: Effects of sample storage conditions and examination time delays on canine and feline ear cytologic results—yes/no and numbers of bacteria, yeast, mites, and skin cells—are unknown.

Objective: Objective was to compare diagnostic quality of day zero (D_0) ear swab samples stored at room temperature in dry, sterile tubes versus diagnostic quality of day zero (AD_0) and day three (AD_3) samples stored in sterile tubes with sponge moistened with Amies solution (preservative).

Methods: Three samples/ear of 10 dogs and 10 cats without ear infections were collected and randomized. One swab was stored in dry transport tube for microscopic examination on D_0 and one swab each was stored in tubes with Amies for microscopy on AD_0 and AD_3. The same laboratory technician who was blinded to sample storage conditions used standardized, semi-quantitative scales to score samples for bacteria, yeast, mites, and skin cells.

Results: Numerous cocci were common (10 samples; abnormal score, 3+ or 4+) at various timepoints and tube types. More variability was noted between D_0 and AD_0 but was less between D_0 and AD_3 and AD_0 and AD_3. Cocci scores for 17 samples were more variable than for yeast, with same yeast scores for all time points for 10. Changes for yeast or cocci were minimally variable for 17 or 12 samples (85% or 60% precision), respectively. Samples had minor changes for rods and skin cells; no samples had mites.

Conclusion: Amies sponge tubes may be acceptable for ear swab transport to the laboratory for diagnostic application.

LB31: GAMMA HERPESVIRUS ASSOCIATED INTERSTITIAL PNEUMONIA IN A BLACK-TAILED PRAIRIE DOG (CYNOMYS LUDOVICIANUS)

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Background: Formalin-fixed tissues from a ~3-year-old, female, zoo-housed, Black-tailed prairie dog (*Cynomys ludovicianus*) were submitted for histopathology at the New Hampshire Veterinary Diagnostic Laboratory. The primary lesion was severe, subacute, neutrophilic and histiocytic, fibrinonecrotizing and hemorrhagic interstitial pneumonia, highlighted by large intranuclear inclusion bodies in numerous bronchiolar epithelial cells and some histiocytes.

Objective: To characterize the virus in the observed intranuclear inclusions.

Methods: Scrolls of formalin-fixed paraffin-embedded (FFPE) lung were sent to the Veterinary Diagnostic Laboratory at Michigan State University for degenerate herpesvirus PCR. Nucleic acid extracted from the FFPE tissue and fecal samples from the prairie dog colony were sent to the Baker Institute at Cornell University for shotgun metagenomics sequencing.

Results: Degenerate herpesvirus (polymerase gene) PCR generated an amplicon with 98.12% sequence similarity to Cynomys herpesvirus (GenBank# EU863200), previously identified in a single prairie dog in 2011 not associated with illness or any lesions. Shotgun metagenomics sequencing of DNA extracted from FFPE recovered partial sequences of five genes of Cynomys herpesvirus. In addition to Cynomys herpesvirus, a large amount of endogenous Prairie dog retrovirus and Woodchuck hepatitis virus were recovered from the extracted genetic material. No Cynomys herpesvirus DNA was recovered from the fecal samples.

Conclusions: To the authors' knowledge, this is the first report of clinical disease or lesions associated with Cynomys herpesvirus, as well as the first report of pneumonia with intranuclear inclusion bodies in Black-tailed prairie dogs. Cynomys herpesvirus pneumonia should be considered a differential etiologic diagnosis for respiratory disease in this species.

LB32: ECTOPIC THYROID CARCINOMA IN THE HEART-BASE OF A DOG: A CASE REPORT

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Background: A 10-year-old male dog of an undefined breed presented with a history of severe ascites and apathy. Echocardiographic examination showed pericardial effusion with cardiac tamponade and neoformation in the heart base measuring approximately 3.58 x 4.51 cm. The animal was euthanized, and the samples of the cardiac mass were sent for histopathological analysis.

Objective: To report a case of ectopic thyroid carcinoma located in an adult dog's heart base of an undefined breed.

Methods: The mass fragments were sent in 10% buffered formalin solution, cleaved, and submitted to histological processing. The slides were stained with hematoxylin and eosin and evaluated under light microscopy. A paraffin block was sent to a veterinary laboratory for immunohistochemical analysis to determine the cell origin.

Results: Microscopically, there was a highly cellular neoplastic proliferation of epithelial cells, forming aggregates separated by delicate fibrous septa. Occasional foci of rudimentary tubular differentiation were observed, rarely containing amorphous and weakly eosinophilic material in the lumen. The cells are cuboidal to polyhedral, cytoplasm sparse and eosinophilic, central nucleus rounded and hypochromatic, coarse chromatin and nucleoli evident, sometimes multiple. There are foci of neoplastic emboli in lymphatic vessels. The neoplastic cells were positive for TTF1, Thyroglobulin, and the cell proliferation marker Ki67 in approximately 25% of the tumor.

Conclusions: Ectopic thyroid carcinoma is a rare tumor in animals. Discrete foci of glandular differentiation and immunohistochemical findings allow the diagnosis. It should be considered in the differential diagnosis of tumors at the base of the heart and aortic body.

LB33: RENAL CARCINOMA WITH SYSTEMIC METASTASIS IN A YOUNG DOG: CASE REPORT

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Background: A 3-year-old female Golden Retriever was referred for postmortem examination at the UNESP Veterinary Pathology Service, Jaboticabal, São Paulo State, Brazil. The animal presented a history of hematuria, generalized pain, and progressive paralysis of the four limbs. In the ultrasound, there was suspicion of renal neoplasm.

Objective: To report a case of renal cell carcinoma with multiple foci of metastasis in a three-year-old dog.

Methods: The animal was referred for necropsy. During the examination, the material was collected for histopathological analysis.

Results: Macroscopically, the right kidney measured 14 x 10 x 7 cm, with an irregular surface; on cut surface, it was heterogeneous, with a firm, yellowish-white mass occupying 80% of the parenchyma, extending from the cortex to the renal papilla. On microscopy, the direct kidney showed a neoplastic proliferation of epithelial origin with a

tubular pattern, moderate anisokaryosis, marked karyomegaly, and three figures of mitosis in 10 fields of higher magnification (2.37 mm). The diagnosis was tubular renal cell carcinoma with metastasis to the lung, adrenals, left kidney, lymph nodes, head muscles, and bone marrow. In addition, the brain and spinal cord exhibited spongiosis, most evident in the white matter (vasogenic edema) and marked reactive astrocytosis (Alzheimer type II astrocytes), indicative of renal encephalopathy.

Conclusions: Young animals can present aggressive neoplasms with systemic metastasis, demonstrating the importance of considering them in the differential diagnosis of renal diseases.

LB34: CO-TARGETING TBL1X AND AKT IN DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL)

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Diffuse large B-cell lymphoma (DLBCL) is characterized by poor prognosis in the relapsed and refractory setting, underscoring a need for novel therapies. Our published work shows that DLBCL cells overexpress transducin ß-like protein 1 (TBL1X), a scaffold protein that regulates the stability of critical oncoproteins, c-MYC and PLK1, via interaction with the Skp1/Cul1/F-Box (SCF) degradation complex. Genetic deletion of TBL1X and pharmacologic targeting with tegavivint (Iterion), a first-in class small molecule, induce significant DLBCL cell death *in vitro*, and while treatment with tegavivint significantly prolongs survival in mouse models of DLBCL, cure is not appreciated. Given that TBL1X regulates multiple oncogenic signaling pathways, we hypothesized that treatment with tegavivint may result in activation of compensatory pro-survival mechanisms, providing rationale for combination strategies. Our data show that TBL1X, via the SCF complex, modulates the stability of mTOR signaling regulatory protein, Rheb, an upstream activator of mTORC1. TBL1X genetic knockdown and treatment with tegavivint promote degradation of Rheb, followed by decreased mTORC1 activity. We hypothesize that tegavivint induces compensatory hyperphosphorylation of AKT as a consequence of selective mTORC1 signaling inhibition. The orally-bioavailable pan-AKT inhibitor AZD-5363 (capivasertib) has significant activity in preclinical models of DLBCL and is currently in several clinical trials. We show that the combination of tegavivint and AZD-5363 results in synergistic killing of DLBCL cells compared to monotherapy. These data suggest that co-targeting TBL1X and AKT may represent a promising novel combination strategy in AKT-addicted DLBCL.