

## Clinical Pathology Focused Scientific Session I

Sunday, October 31, 2021 | 2:30 p.m. – 2:45 p.m. CDT

Sunday, October 31, 2021

2:30 p.m. – 2:45 p.m. CDT

### **VALIDATION OF A POINT-OF-CARE BENCHTOP ANALYZER FOR QUANTITATIVE MEASUREMENT OF SERUM AMYLOID A IN FELINE SERUM AND PLASMA**

Samantha Lee, Sara Connolly, Arnon Gal, Meghan Fick, Anne Barger  
University of Illinois, Urbana, IL, USA

**Background:** Serum amyloid A (SAA) is a valuable biomarker for detection of inflammation and can provide important diagnostic and prognostic information in many disease processes. Routine measurement of feline SAA (fSAA) in clinical practice has been limited due to the lack of available assays. **Objectives:** To validate the Vet Chroma™ point-of-care test for the measurement of fSAA. The analyzer uses immunofluorescent technology to measure analytes in serum and plasma using analyte specific cartridges. We hypothesized that the Vet Chroma™ fSAA cartridge is a valid assay for measurement of fSAA within biologic variability.

**Methods:** Serum and plasma from previous diagnostic feline patient samples were used. Validation was performed according to the ASCVP general quality control guidelines and included intra- and inter-assay variation, linearity, spike recovery, effect of interfering substances, sample matrix comparison and method comparison.

**Results:** Intra-assay CVs ranged from 9.8-12.1%, and inter-assay CVs ranged from 3.8-10.5%. Linearity testing showed evidence of constant bias. Recovery of fSAA in feline serum was acceptable at concentrations  $\geq 36.8$  mg/L. Interference testing revealed significant differences in fSAA concentration with moderate to high levels of icterus, lipemia and hemolysis. The fSAA concentrations of paired plasma and serum samples were close to equivalent. Comparison of the Vet Chroma™ fSAA assay to an established SAA immunoturbidometric assay revealed significantly different results.

**Conclusions:** The Vet Chroma™ analyzer has acceptable test performance to measure serum or plasma fSAA concentration in a sample without evidence of interfering substances; however, results may not be interchangeable with other fSAA assays.

Sunday, October 31, 2021

2:45 p.m. – 3:00 p.m. CDT

### **IMPACT ON CANINE WHITE BLOOD CELL PRESERVATION WITH ADDITION OF BOVINE SERUM ALBUMIN TO K3-EDTA WHOLE BLOOD SAMPLES**

Brodie Reinhart, Cornelia Gilroy, Noel Clancey, Shelley Burton, Jason Stull, Nicole Bishop  
Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE,  
Canada

**Background:** Cellular deterioration occurs with blood sample aging and may impact white blood cell (WBC) identification and differential accuracy, potentially worsened in inflammatory states. Bovine serum albumin (BSA) has been used to improve cellular preservation in other studies, but effect on cell preservation in canine blood with or without inflammatory leukograms has not been assessed.

**Objectives:** This study evaluated effects on WBC preservation with 22% bovine serum albumin (BSA) added to potassium ethylenediaminetetraacetic acid (K<sub>3</sub>-EDTA)-anticoagulated canine blood prior to smear preparation in samples stored at 4°C and 20°C.

**Methods:** Ten individual canine K<sub>3</sub>-EDTA-anticoagulated blood samples stored at 4°C and 20°C were utilized, 5 with and 5 without inflammatory leukograms. Blood smears were prepared from aliquots with or without addition of 22% BSA at 0, 4, 8, 24, 48 and 72 hours post submission. Slides were digitally scanned, and nuclear area measured for 25 randomly selected neutrophils per slide. Kruskal-Wallis and Mann-Whitney tests were performed (significance  $p < 0.05$ ).

**Results:** Nuclear area increased over time with and without BSA (both  $p < 0.01$ ). Samples with BSA had median nuclear areas greater than those without at 0, 4 and 72 hours for inflammatory samples at 4°C; at 0, 4, 48 and 72 hours for those without inflammatory leukograms at 4°C; and at 0, 4, and 8 hours for inflammatory samples at 20°C (all  $p < 0.05$ ).

**Conclusions:** Addition of 22% BSA sporadically increased neutrophil nuclear area in 4°C and 20°C stored samples. Evaluation suggests BSA does not improve cellular preservation regardless of storage time or inflammatory leukogram status.

Sunday, October 31, 2021

3:00 p.m. – 3:15 p.m. CDT

## **CYTOCHEMICAL STAINING OF LEUKOCYTES AND PLATELETS IN FLORIDA MANATEES**

Laura Cagle<sup>1</sup>, Tracy Stokol<sup>2</sup>, John Harvey<sup>3</sup>, Laurie Adler<sup>4</sup>, Michael Walsh<sup>1</sup>, Martine deWit<sup>5</sup>, Robert Bonde<sup>6</sup>, Nicole Stacy<sup>1</sup>

<sup>1</sup>Department of Comparative, Diagnostic, and Population Medicine; College of Veterinary Medicine, University of Florida, Gainesville, FL, USA, <sup>2</sup>Department of Population Medicine and Diagnostic Sciences, Cornell University College of Veterinary Medicine, Ithaca, NY, USA, <sup>3</sup>Department of Physiological Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA, <sup>4</sup>Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA, <sup>5</sup>Florida Fish and Wildlife Conservation Commission, the Marine Mammal Pathobiology Laboratory, St. Petersburg, FL, USA, <sup>6</sup>Wetland and Aquatic Research Center, U.S. Geological Survey, Gainesville, FL, USA

**Background:** Florida manatees (*Trichechus manatus latirostris*) belong to the Order *Afrotheria*, along with elephants and rock hyraxes, both of which have a known unique cell type, the bilobed monocyte. **Objective:** To characterize leukocytes and platelets in

blood films of manatees (n=8) using one routine and eight cytochemical stains.

**Methods:** Blood films were stained with the Wright-Giemsa, alkaline phosphatase (ALP), a-naphthyl butyrate esterase (ANBE), chloroacetate esterase (CAE), Luna, myeloperoxidase (MPx), periodic acid-Schiff (PAS), Sudan black B (SBB), and toluidine blue (TB). **Results:** Leukocytes were identified as granulocytes (heterophils, eosinophils, basophils), and mononuclear cells (lymphocytes, monocytes), with rare numbers of bilobed mononuclear cells of undetermined lineage (1-3%). Manatee leukocytes had several similar staining reactions to elephants (e.g. heterophils: ALP<sup>+</sup>, MPx<sup>+</sup>, CAE<sup>+</sup>, ANBE<sup>-</sup>; eosinophils: MPx<sup>+</sup>, CAE<sup>-</sup>). Unlike elephant leukocytes, eosinophils were ANBE<sup>-</sup> and ALP<sup>+</sup>, most lymphocytes and platelets were ANBE<sup>+</sup> and monocytes were variably ALP<sup>+</sup> and ANBE<sup>+</sup>. Monocytes and bilobed mononuclear cells had similar staining reactions (variably positive for all stains, except Luna and TB), which is similar to the respective elephant cells (e.g. MPx<sup>+</sup>, ALP<sup>+</sup>, variable CAE<sup>+</sup>). All leukocytes, except lymphocytes, were PAS<sup>+</sup> and SBB<sup>+</sup>, to varying degrees. Platelets were PAS<sup>+</sup>.

**Conclusions:** Bilobed mononuclear cells were classified as monocytes based on their morphological and cytochemical features. Their proportions were similar to rock hyraxes (1-3%), but far less than elephants (approximate range 20-60%). This study provides new information on the morphologic features and cytochemical staining characteristics of Florida manatee leukocytes and platelets and will aid in obtaining accurate leukocyte differential counts.

Sunday, October 31, 2021

3:45 p.m. – 4:00 p.m. CDT

### **COMPARISON OF DIPSTICK URINE PROTEIN WITH QUANTITATIVE URINE PROTEIN AND URINE PROTEIN-TO-CREATININE RATIO**

Clark Broughton<sup>1</sup>, Nick Jeffery<sup>2</sup>, Julie Piccione<sup>3</sup>, Jessica Hokamp<sup>4</sup>, Mary Nability<sup>1</sup>

<sup>1</sup>Department of Veterinary Pathobiology, Texas A&M University College of Veterinary Medicine, College Station, TX, USA, <sup>2</sup>Department of Small Animal Clinical Sciences, Texas A&M University College of Veterinary Medicine, College Station, TX, USA,

<sup>3</sup>Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL), College Station, TX, USA, <sup>4</sup>Department of Veterinary Biosciences, The Ohio State University College of Veterinary Medicine, Columbus, OH, USA

**Background:** Dipstick urinalysis can suggest if urine protein-to-creatinine ratio (UPC) is warranted and may aid monitoring treatment of proteinuric kidney disease. There is uncertainty regarding interpretation of semi-quantitative urine dipstick protein results.

**Objectives:** To determine quantitative protein concentration associated with each dipstick urine protein value, effects of urine specific gravity (USG) and pH, and cutoff values to guide when to pursue UPC.

**Methods:** Retrospective analysis was performed by tabulation of odds, non-parametric ANOVA, and regression of USG and matching UPC on dogs presenting to the Texas A&M Veterinary Medical Teaching Hospital (n = 392). Siemens Multistix 10SG was run on Siemens Clinitek Status+ and UPC on Vitros 4600 Chemistry System.

**Results:** Quantitative microprotein concentration differed significantly between dipstick values ( $P < 0.001$ ), although considerable overlap was observed (median [interquartile range (IQR)] mg/dL: negative 9 [6-13]; trace 10.5 [8-23]; 1+ 18.5 [8-33]; 2+ 50.5 [15-94]; 3+ 177 [72.5-333]; 4+ 467.5 [272.3-829]). A pH  $\geq 8$  and USG  $\geq 1.030$  were associated with higher dipstick protein compared with quantitative measurement in urine with pH 6.5-7.9 or USG 1.015-1.029, respectively ( $P < 0.001$ ). Dogs with USG  $\geq 1.030$  and dipstick  $\leq 1+$  were unlikely (0/31) to have UPC  $> 0.5$ . Dogs with USG  $< 1.015$  and dipstick  $\geq 1+$  are highly likely (odds = 17 [95% CI: 4.1-70.8]) to have UPC  $> 0.5$ .

**Conclusions:** Each dipstick urine protein category corresponds with a wide range of quantitative microprotein values. Urine pH and USG impact the dipstick value, and should be considered in deciding the value of obtaining UPC.

Sunday, October 31, 2021

4:00 p.m. – 4:15 p.m. CDT

### **THE MIRNOME OF CANINE UROTHELIAL CARCINOMA**

Mara Varvil, Deepika Dhawan, Deborah Knapp, José Ramos-Vara, Andrea dos Santos

**Background:** Urothelial carcinoma (UC) is a prominent cancer in dogs, comprising up to 2% of all naturally occurring neoplasia. Currently, UC can be challenging to diagnose. Urine sediment exams lack sensitivity and specificity. Surgical biopsies are invasive. Cystoscopic biopsies require special equipment and can be limited by small sample size. BRAF mutation testing is available; however, not all tumors express this mutation, reducing the sensitivity of this test. The microRNAs (miRNAs) are regulatory RNAs that are stable in fluids and tissues and are promising diagnostic tools for disease. miRNA expression has been evaluated in human UC; however, there is limited information regarding the miRNA transcriptome of UC in dogs.

**Objectives:** To characterize the miRNA transcriptome of UC.

**Methods:** We performed Next-Generation RNA sequencing (RNA-Seq) in dogs with UC ( $n=29$ ) and normal canine urothelium ( $n=4$ ) to identify miRNA expression markers for canine UC. Findings were validated by RT-qPCR. IPA software (QIAGEN) and targetscan.org were used to identify targets of these miRNAs and predict their pathway interactions in UC.

**Results:** Twenty-eight differentially expressed miRNAs were identified. Six were validated by RT-qPCR: miR-32, miR-105a, miR-143, miR-181a, miR-214, and miR-374b, and confirmed to be downregulated in UC ( $n=5$ ) compared to normal urothelial samples ( $n=5$ ). Affected pathways include the RAS-MEK-ERK, ATM/TP53/CDK, and the AKT signaling pathway, similar to those found in human UC.

**Conclusions:** Several miRNA are differentially expressed with interactions in pathways known to be disrupted in canine UC. These markers should be further evaluated as potential diagnostic or therapeutic markers for UC.

Sunday, October 31, 2021  
4:15 p.m. – 4:30 p.m. CDT

**DISCOVERY OF POTENTIAL URINE BIOMARKERS OF CANINE MEMBRANOPROLIFERATIVE IMMUNE COMPLEX MEDIATED GLOMERULONEPHRITIS BY LC-MS/MS**

Jessica Hokamp, Miranda Gardner, Andrew Reed, Rachel Cianciolo  
The Ohio State University, Columbus, OH, USA

**Background:** Membranoproliferative glomerulonephritis (MPGN), a common glomerular disease in dogs that is often treated with immunosuppression, currently requires comprehensive renal biopsy for definitive diagnosis. The International Veterinary Renal Pathology Service found a unique protein banding pattern on Bis-Tris gel electrophoresis in urine from dogs with MPGN/mixed MPGN. Identification of proteins in the banding pattern could lead to discovery of a urine biomarker for MPGN.

**Objective:** Use liquid chromatography tandem mass spectrometry (LC-MS/MS) to discover protein(s) in urine that will distinguish dogs with MPGN/mixed MPGN vs. non-MPGN diseases.

**Methods:** Urine supernatant from dogs with MPGN/mixed MPGN (n=10), membranous glomerulonephritis (n=5), amyloidosis (n=5), and focal segmental glomerulosclerosis (n=5), as diagnosed by comprehensive renal biopsy, were prepared for tandem mass spectrometry by protein precipitation and tryptic digestion. MS/MS spectra were acquired by reverse phase liquid chromatography (nanoRPLC) coupled to a Fusion containing a FAIMS device. Data were separated into different compensation voltages and searched against a *Canis lupus* UniProt database containing reviewed and unreviewed proteins. Total normalized spectral counts were used to quantify relative protein abundance. Protein abundances from dogs with MPGN/mixed MPGN were compared with those from all non-MPGN diseases.

**Results:** Urine from dogs with MPGN/mixed MPGN contained 88 significantly differentially expressed proteins ( $p < 0.05$ ) including apolipoprotein B-100 (APOB), upregulated in 100% of MPGN/mixed MPGN samples by a log-fold change of 2.8.

**Conclusions:** LC-MS/MS identified candidate urine biomarkers for differentiation of canine MPGN/mixed MPGN from non-MPGN glomerular diseases. Validation of APOB as a quantifiable urine biomarker of MPGN is ongoing.

Sunday, October 31, 2021  
4:30 p.m. – 4:45 p.m. CDT

**CHARACTERIZATION OF CANINE CHRONIC MYELOID NEOPLASMS DIAGNOSED BY ANDROGEN RECEPTOR CLONALITY TESTING**

Marika Klosowski, Janna Yoshimoto, Robert Burnett, Anne Avery, Emily Rout  
Colorado State University, Fort Collins, CO, USA

**Background:** Chronic myeloid neoplasms are reported as rare causes for marked neutrophilia or monocytosis in dogs, but definitive diagnosis of these malignancies is

challenging and the clinicopathologic features and outcomes in these cases remain poorly characterized. An assay to test canine leukocyte clonality using the androgen receptor gene (CANARA) can support a diagnosis of chronic myeloid neoplasia in female dogs.

**Objectives:** Characterize the clinicopathologic findings and outcomes in 12 canine patients with suspected chronic myeloid neoplasia and a clonal CANARA result.

**Methods:** We identified cases of marked neutrophilic or monocytic leukocytosis in dogs that lacked a CD34+ cell population by flow cytometry and had a clonal CANARA result supportive of chronic myeloid neoplasia. Medical records were obtained and clinical presentation, laboratory data, treatment, and outcomes were summarized.

**Results:** Mean age at diagnosis was 6.1 years (range 1.4-11). Median total leukocyte count was 98,520/ul (range 50,160-203,100/ul) and median segmented neutrophil count was 58,500/ul (range 17,556-144,200/ul). 92% of cases had band neutrophils (median 11,600/ul; range 502-57,443/ul), 50% had metamyelocytes, and 33% had myelocytes. Four cases had 2-5% blasts described. Toxic change was reported in 67% of cases. All cases had monocytosis (median 7,550 monocytes/ul; range 1,003-21,100/ul) and 33% had eosinophilia (range 1,800-9,727 eosinophils/ul). 58% were anemic and 17% were thrombocytopenic. By flow cytometry, CD4+CD5-CD18+ cells correlated with mature neutrophil counts and CD4-CD14-CD18+ cells correlated with summed left-shifted granulocytes and eosinophils. Five cases died with variable outcomes.

**Conclusions:** Chronic myeloid neoplasia cases identified by CANARA have variable age, hematologic findings, and outcomes.

Sunday, October 31, 2021

4:45 p.m. – 5:00 p.m. CDT

### **IDENTIFICATION OF POTENTIAL PLASMA PROTEIN BIOMARKERS FOR FELINE PANCREATIC CARCINOMA BY LIQUID-CHROMATOGRAPHY TANDEM MASS SPECTROMETRY**

Rina Nabeta, George Katselis, Paulos Chumala, Ryan Dickinson, Nicole Fernandez, Melissa Meachem

University of Saskatchewan, Saskatoon, SK, Canada

**Background:** Feline pancreatic carcinoma (FePAC) is an aggressive cancer with a grave prognosis. Early detection while the disease is still localized is the key to improving prognosis. Proteomic techniques have been successfully used to investigate biomarkers of human pancreatic neoplasia and may identify plasma proteins of interest for the early diagnosis of FePAC.

**Objective:** Our aim was to compare immunodepleted plasma proteomes between cats with malignant and inflammatory pancreatic disease and healthy control cats in order to identify differentially expressed plasma proteins that may serve as potential biomarkers of FePAC.

**Methods:** Plasma samples from 9 cats with FePAC, 10 cats with pancreatitis, and 10 healthy control cats were immunodepleted before undergoing liquid-chromatography tandem mass spectrometry. Mass spectral data were searched against mammalian species in the UniProtKB Swiss-Prot database for protein identification. Signal intensity was utilized for relative quantification. Bioinformatics analysis was performed using specialized software. Differences between groups were evaluated by one-way ANOVA and fold-change analysis.

**Results:** Principal component analysis demonstrated overall proteomic profiles of each study group was distinct from each other. Differential analyses identified 37 differentially expressed proteins ( $p < 0.05$  in one-way ANOVA and  $\geq 2$ -fold change in fold-change analysis). Among these proteins, ETS variant transcription factor 4 (ETV4) ( $p = 0.007$ ) was overexpressed while gelsolin ( $p < 0.001$ ) and apolipoprotein A-IV ( $p < 0.001$ ) were down-regulated in cats with FePAC compared to cats with pancreatitis and healthy controls.

**Conclusions:** ETV4, gelsolin, and apolipoprotein A-IV may be novel plasma biomarkers for FePAC; further studies are needed to investigate their diagnostic potential.

## Diagnostic Pathology Focused Scientific Session I

Sunday, October 31, 2021 | 1:30 p.m. – 1:40 p.m. CDT

Sunday, October 31, 2021

1:30 p.m. – 1:40 p.m. CDT

### A DEEP LEARNING APPROACH TO TUMOR VS. NORMAL DETECTION IN DIGITIZED HISTOPATHOLOGY SLIDES OF HUMAN SOFT TISSUE LEIOMYOSARCOMA

James Cronin, Asmaa Aljuhani, Monika Karera, Xiaoyan Cui, Raghu Machiraju, David Liebner

The Ohio State University College of Medicine, Columbus, OH, USA

**Background:** Deep learning methods for digital histopathology analysis can achieve accurate and reproducible diagnoses, particularly in the domain of oncology.

**Objective:** We sought to implement a deep convolutional neural network on digitized histopathology images to discriminate tissue regions of soft tissue leiomyosarcoma from peri-tumoral non-neoplastic tissue.

**Methods:** 120 H&E stained whole slide images from 51 human soft tissue (non-uterine) leiomyosarcoma patients were collected from The Cancer Genome Atlas and Clinical Proteomic Tumor Analysis Consortium. A board-certified pathologist exhaustively annotated the slides for regions of leiomyosarcoma and peri-tumoral non-neoplastic tissue. The annotated images were then processed into non-overlapping patches, which were assigned labels based on the pixel level annotation masks. Oversampling was implemented to overcome class imbalance in our training dataset (192,549 leiomyosarcoma patches vs. 32,385 non-neoplastic patches). We manipulated

the following experimental conditions to maximize performance: patch size and magnification, normalization technique, Otsu's binarization threshold, oversampling strategy, network architecture, training epochs, and learning rate.

**Results:** Our best performing algorithm (a pre-trained ResNet18) achieved 94.5% accuracy, 96.5% F1-score, 94.9% precision, and 98.1% recall in the validation set for the classification of leiomyosarcoma vs. non-neoplastic tissue at the patch level.

**Conclusions:** Our network achieved good performance in classifying leiomyosarcoma vs. peri-tumoral non-neoplastic tissue. The most common source of error was in distinguishing well-differentiated leiomyosarcoma from surrounding normal smooth muscle, which can be a challenging distinction for pathologists. This work serves as an initial step in a computational pathology analysis pipeline for risk stratification in human soft tissue sarcoma.

Sunday, October 31, 2021

1:40 p.m. – 1:50 p.m. CDT

## **QUANTIFICATION OF MUCOSAL LYMPHOCYTES IN FELINE SMALL INTESTINAL BIOPSIES USING ARTIFICIAL INTELLIGENCE**

Judit Wulcan<sup>1</sup>, Jonatan Wulcan<sup>2</sup>, Peter Moore<sup>1</sup>, Kevin Keel<sup>1</sup>, Stefan Keller<sup>1</sup>

<sup>1</sup>University of California Davis, Davis, CA, USA, <sup>2</sup>Technogarden, Malmö, Sweden

**Background:** Semi-quantitative grading of mucosal inflammation in veterinary diagnostic pathology is time-consuming and poorly reproducible, which compromises confidence in the method. The use of artificial intelligence (AI) for whole slide analysis allows the identification and quantification of tissues and cells and has the potential to improve the speed and reproducibility of lymphocyte quantification in small intestinal biopsies. **Objectives:** To assess the performance of AI-based quantification of lymphocytes in small intestinal biopsies from cats. **Methods:** Whole slide images of randomly selected biopsy specimens from the small intestine of cats were used to train, validate and test a convolutional neural network. Separate models for the recognition of regions (epithelium and lamina propria) and objects (lymphocytes) were developed and results were merged post-analysis. **Results:** In comparing computer-generated area and object recognition, to manual test set annotations, best performance metrics were obtained for epithelium (88% recall, 86 % precision and 86% F1-score) followed by lamina propria (85% recall, 75% precision and 78% F1-score) and lymphocytes (77% recall, 84% precision and 77% F1-score). In addition, a reference dataset for features such as tissue area, lymphocyte density and distance was created based on the analysis of 348 slides. **Conclusion:** The generated algorithm performed well in object and region recognition and might provide a more reproducible alternative to manual quantification of mucosal inflammation. The generated dataset can be used as an objective reference for assessing the degree of abnormality for individual biopsies.



Sunday, October 31, 2021  
1:50 p.m. – 2:00 p.m. CDT

### **ATYPICAL MALIGNANT BEHAVIOR FOR CUTANEOUS HISTIOCYTOMA IN A YOUNG DOG**

Jenny Wyrick<sup>1</sup>, Heather Herd<sup>1</sup>, Mark Payton<sup>2</sup>, Jerry Ritchey<sup>1</sup>

<sup>1</sup>Oklahoma State University, Stillwater, OK, USA, <sup>2</sup>Rocky Vista University, Parker, CO, USA

A cutaneous histiocytoma was diagnosed in a 1.5-year-old, Scottish Terrier from a biopsy specimen removed from the lower lip margin. Five months after the original diagnosis, the patient re-presented with markedly enlarged submandibular and prescapular lymph nodes. Lymph node biopsies revealed effacement by histiocytic cells similar to that previously seen in the skin. Immunostains performed indicated a neoplastic cell phenotype of CD18+, CD3-, CD20- and CD204-, all consistent with benign cutaneous histiocytoma. Extension of cutaneous histiocytomas to local lymph nodes has been previously reported in very few canine patients, yet even in these patients, regression had resulted in favorable outcomes. In this case, there was unexpected tumor progression with extension into other lymph nodes of the head and neck, mediastinal lymph nodes and skin resulting in euthanasia of the patient 7 months after the original diagnosis. Benign cutaneous histiocytomas are histologically indistinguishable from histiocytic malignancies; young age is often used as a diagnostic criterion. A review of 2,699 cases of cutaneous histiocytoma revealed an average patient age of 4.4 years (median 3.9) with 50% of the cases diagnosed in patients < 4 years of age. The other cases were diagnosed in patients over 4-years of age with 18% occurring in patients >8-years-old. A blinded, retrospective review of the original biopsy from the young Scottish Terrier by other pathologists returned consensus agreement for a morphologic diagnosis of benign cutaneous histiocytoma; however, neoplastic cell infiltration into deep skeletal muscle may have been a clue to potential malignant behavior.

Sunday, October 31, 2021  
2:00 p.m. – 2:10 p.m. CDT

### **COMPARISON OF ANTI-MULLERIAN HORMONE AND INHIBIN IMMUNOLABELING IN CANINE AND EQUINE GRANULOSA CELL TUMORS**

Sophie Nelissen, Andrew Miller

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**Background:** Granulosa cell tumors (GCTs) are common ovarian neoplasms in the mare and bitch. While in mares, the classic honeycomb pattern imparted by the cystic histomorphology of those tumors usually allows for a straightforward diagnosis, canine GCTs often present in various histomorphologic patterns and can represent a diagnostic challenge. Inhibin has long been the standard immunohistochemical marker for granulosa cell tumors; however, anti-Mullerian hormone (AMH) has not been evaluated as a diagnostic IHC in the dog and horse.

**Objective:** This study aims to compare the efficacy of AMH and inhibin as

immunohistochemical markers in canine and equine GCTs.

**Methods:** We performed a retrospective search of equine and canine GCTs in the histologic database of the New York State Animal Health Diagnostic Center. A total of 18 equine and 15 canine cases were selected based on histomorphological criteria assessed on hematoxylin and eosin.

**Results:** Virtually all equine tumors were dominated by a cystic pattern, while canine tumors had a more solid, follicular pattern. Both inhibin and AMH had a cytoplasmic, granular pattern of immunolabeling. Labeling for AMH occurred in 12/15 canine cases (varying from 1-50% of cells) and 18/18 equine cases ( $\geq 75\%$  of cells). Labeling for inhibin occurred in 15/15 canine cases (from 50 - 75% of cells) and 18/18 equine cases (25 - 75% of cells). Distribution and intensity of the labeling were unrelated to histomorphologic pattern.

**Conclusions:** While inhibin and AMH performed comparably in dogs, AMH had more diffuse immunolabeling than inhibin in mares.

Sunday, October 31, 2021

2:10 p.m. – 2:20 p.m. CDT

### **CASE REPORT: MUCINOUS INTRAHEPATIC CHOLANGIOCARCINOMA IN A HORSE**

Daniel Felipe Barrantes Murillo<sup>1</sup>, Rachel Pfeifle<sup>1</sup>, Anne Wooldrige<sup>1</sup>, Russell Cattley<sup>1</sup>, John Cullen<sup>2</sup>, Rachel L.A.L.T. Neto<sup>1</sup>

<sup>1</sup>Auburn University, Auburn, AL, USA, <sup>2</sup>North Carolina State University, Raleigh, NC, USA

Cholangiocarcinomas are malignant tumors arising from the biliary epithelium and are reported in several domestic species including dogs, cats, ruminants, and horses. In humans, a mucinous variant of intrahepatic cholangiocarcinoma is infrequently diagnosed. By convention, mucinous carcinoma is defined when extracellular mucus lakes occupy at least 50% of the entire neoplasm. In humans, mucinous variants have been reported in tumors arising from the breast, pancreas, colon, and gallbladder. A 17-year-old, intact female, Arabian horse was presented to the Teaching Hospital for a long-term history of intermittent mild colic responsive to nonsteroidal anti-inflammatory drugs. The patient slightly improved with supportive treatment but, due to the chronic history, the owner elected euthanasia. On necropsy, the liver had multiple, white to tan, raised, 0.5-1 cm diameter parenchymal nodules on all lobes. In the right hepatic lobe, a larger 3 cm diameter, cavitated nodule was filled with a light brown, mucinous content. Histologically, the hepatic nodules consisted of a variably cystic, paucicellular cellular, infiltrative neoplasm forming dilated tubules and papillary projections lined by a single layer of columnar cells with goblet cell-like differentiation. The cysts were filled with abundant amphophilic, alcianophilic amorphous to laminated mucus, also highlighted with mucicarmine stain. Neoplastic cells had cytoplasmic reactivity against cytokeratin 19, similar to normal biliary epithelium, and lacked immunoreactivity against HepPar1. Based on these findings and the absence of other neoplastic processes, a diagnosis of

mucinous intrahepatic cholangiocarcinoma is concluded, which, to the best of the authors' knowledge, is the first described in an equid.

Sunday, October 31, 2021

2:20 p.m. – 2:30 p.m. CDT

**A TALE OF TWO BEARS: HISTOLOGIC AND IMMUNOHISTOCHEMICAL FEATURES OF TUMORS OF THE PERIPHERAL NERVOUS SYSTEM IN TWO FREE-RANGING AMERICAN BLACK BEARS (*URSUS AMERICANUS*)**

Deborah Chong<sup>1</sup>, Robert Bildfell<sup>1</sup>, Kurt Licence<sup>2</sup>, Julia Burco<sup>2</sup>, Colin Gillin<sup>2</sup>, Peregrine Wolff<sup>3</sup>, Christiane Löhner<sup>1</sup>

<sup>1</sup>Oregon State University, Corvallis, OR, USA, <sup>2</sup>Oregon Department of Fish and Wildlife, Corvallis, OR, USA, <sup>3</sup>Wildlife Disease Association, Moorpark, CA, USA

**Background:** Reports of tumors of the nervous system in ursids are rare. Here we present two cases of peripheral nervous system tumors in two free-ranging American black bears (*Ursus americanus*). Case 1: Adult male with a subcutaneous, right cheek mass. Histologic and immunohistochemical findings: An infiltrative mass composed of mildly anisokaryotic, fusiform to spindle cells, arranged in haphazard streams and cords to an Antoni type B pattern. No mitoses were observed. Extensive areas of necrosis were present. Neoplastic cells were variably immunopositive for Sox-10, S100 and vimentin, and immunonegative for laminin, neurofilament, neuron specific enolase and GFAP. Diagnosis: Malignant nerve sheath tumor. Case 2: Adult female with a mass associated with the left trigeminal ganglion. Histologic and immunohistochemical findings: A well-encapsulated mass composed of a few variably-differentiated ganglion cells (<5% of the neoplastic cells) and packets of mildly anisokaryotic, polygonal cells with wispy amphophilic cytoplasm and a round nucleus with finely stippled chromatin and prominent nucleolus. They are supported by a fine fibrovascular and pale basophilic stroma. Mitotic count was 1.5 per 400x field (2.37mm<sup>2</sup>). Extensive areas of necrosis were present. Neoplastic cells were variably immunopositive for Sox-10, S100, vimentin, neuron specific enolase and synaptophysin, and immunonegative for laminin, neurofilament, and GFAP.

Diagnosis: Neuroblastoma, poorly differentiated subtype

Normal tissues from adult American black bears used as tissue controls for immunohistochemistry performed as expected.

**Conclusion:** These findings help document the range of ursid neoplastic diseases and the utility of specific immunohistochemical markers in American black bears.

Sunday, October 31, 2021

2:30 p.m. – 2:40 p.m. CDT

**MIXED MAMMARY TUMORS IN FEMALE NORTHERN GREATER GALAGOS (*OTOLEMUR GARNETTI*)**

Michael McKinney, Katherine Shuster, Carissa Jones, Christopher Pinelli, Katherine Gibson-Corley

Vanderbilt University Medical Center, Nashville, TN, USA

Here we describe two cases of mixed mammary tumors in a northern greater galago colony at our institution. The first case was an 11-year-old, pregnant female presenting with a firm, lobulated subcutaneous mass of the right middle mammary gland. Euthanasia was elected following weaning of the infant. Grossly, the mass was ~2.5 cm in diameter and well circumscribed. Histologically, the mass was markedly cavitated with variable regions of mixed neoplastic cells. Some regions contained disorganized, densely cellular stroma with others well organized into acinar glandular tissue. Both regions contained malignant characteristics such as frequent mitotic figures and anisokaryosis. The second case was a 9-year-old, retired breeding female presenting with a firm, lobulated mammary mass of the left thoracic mammary gland. Given the age of the animal, euthanasia was elected. The mass appeared grossly and histologically similar except for fewer malignant characteristics. Based on these findings, both neoplasms resemble a rare, stromal-epithelial mammary tumor of women known as a phyllodes tumor. Although these tumors are mixed in origin, their rapid growth is more characteristic of a sarcoma. While 75% of these tumors are benign, malignant forms carry a poor prognosis due to extreme chemotherapy and radiation resistance. Historical reports of mammary tumors in galagos are non-descript, including fibrosarcomas of dwarf galagos and mixed spindle cell sarcomas in thick-tailed galagos. Therefore, these reports may be consistent with our institutional findings and altogether indicate a predisposition to phyllodes-like mammary tumors in galagos.

Sunday, October 31, 2021

2:40 p.m. – 2:50 p.m. CDT

## **MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERIZATION AND MOLECULAR CLASSIFICATION OF SPONTANEOUS MAMMARY GLAND TUMORS IN MACROPODS**

Tu Chun (Steven) Hsu<sup>1</sup>, Michael Garner<sup>2</sup>, Matti Kiupel<sup>3</sup>

<sup>1</sup>National Cancer Institute, Frederick, MD, USA, <sup>2</sup>Northwest ZooPath, Monroe, WA, USA, <sup>3</sup>Michigan State University, East Lansing, MI, USA

Mammary gland neoplasms in macropods are uncommonly reported, and the morphological and immunohistochemical characteristics are incompletely described. The goal of this study was to describe the morphologic features of macropod mammary neoplasms and to determine the molecular subtypes of mammary carcinomas using a panel of antibodies against estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (Her-2), p63, smooth muscle actin (SMA), and epidermal growth factor receptor (EGFR). Biopsy and necropsy specimens were examined from macropods with mammary tumors submitted to Northwest ZooPath from 1996 to 2019. In accordance with the histologic classification of canine mammary tumors proposed by Goldschmidt and colleagues, tubulopapillary (2), tubular (10), and comedo-carcinomas (2), adenoma (1), lobular hyperplasia (2), fibroadenomatous hyperplasia (1), and mastitis (2) were diagnosed. Red kangaroos (*Osphranter rufus*) were most commonly diagnosed with mammary carcinomas (78% of all carcinomas) compared to other macropods. Seven cases of carcinomas had lymphovascular invasion and 2 animals had pulmonary metastases. Six of these carcinomas were classified as grade 3. Immunohistochemistry for all antibodies was performed on 9/14 carcinomas, and partial immunohistochemistry was performed for 3 cases. All 12

carcinomas were immunoreactive for PR, 5 for ER, 9 for EGFR and none for Her-2. Five of the 9 mammary carcinomas with complete immunohistochemistry data were classified as luminal A subtype, and 4 were normal-like subtype. Accurate classification of mammary tumors in macropods based on morphology, immunohistological characteristics, and molecular subtype may be helpful in guiding clinical management, prognosis, and potential therapeutic targets.

Sunday, October 31, 2021

2:50 p.m. – 3:00 p.m. CDT

### **A PRESUMPTIVE CASE OF MUCOLIPIDOSIS IN A PIT BULL TERRIER**

Rebecca Bacon<sup>1</sup>, Brian Porter<sup>1</sup>, Margret Casal<sup>2</sup>, Petra Werner<sup>2</sup>, Carmen Lau<sup>1</sup>

<sup>1</sup>Texas A&M University College of Veterinary Medicine, College Station, TX, USA,

<sup>2</sup>University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA, USA

A 7-year-old, male castrated Pit Bull Terrier presented for respiratory distress with a history of difficulty breathing for 3 months, a one-year history of urinary incontinence, and a several year history of seizure-like activity following exercise. Following radiographs and bloodwork, the dog was diagnosed with megaesophagus and chronic aspiration pneumonia. Despite aggressive antimicrobial and oxygen therapy, the dog continued to decompensate and was humanely euthanized. Necropsy examination revealed a severe megaesophagus, aspiration pneumonia, and a markedly enlarged vagus nerve. Histopathology demonstrated variably sized accumulations of eosinophilic intracytoplasmic material within neurons of the brainstem, spinal cord, esophageal ganglia, and vagus nerve, as well as within hepatocytes and histiocytes throughout the liver, intestine, and lymph nodes. These accumulations stained strongly with Luxol fast blue, lightly with Periodic-acid Schiff, and did not stain with Alcian blue pH 2.5. On transmission electron microscopy, the accumulations were confirmed as intracytoplasmic, membrane-bound aggregates of storage material which were arranged in concentric lamellations or parallel arrays. Enzyme analysis showed greatly elevated levels of many enzymes typically associated with mucopolysaccharidoses, gangliosidoses, and mannosidoses, suggesting a form of mucolipidosis. Mucolipidoses have been reported only in cats, mice, and humans. While most forms of mucolipidosis in animals are severe, with affected animals dying at a young age, some forms in humans are less severe and patients may live well into adulthood. Genetic analysis is pending. This is the first report of a presumptive mucolipidosis in a dog.

Sunday, October 31, 2021

3:00 p.m. – 3:10 p.m. CDT

### **CASE REPORT: UNILATERAL PAMPINIFORM PLEXUS ARTERIOVENOUS HAMARTOMATOSIS OF THE VAGINAL TUNIC OF A DOG**

Daniel Felipe Barrantes Murillo<sup>1</sup>, Ellen Newsom<sup>2</sup>, John Edwards<sup>3</sup>, Kellye Joiner<sup>1</sup>

<sup>1</sup>Auburn University, Auburn, AL, USA, <sup>2</sup>Animal Medical Center, Tuscaloosa, AL, USA,

<sup>3</sup>Texas A & M University, College Station, TX, USA

Hamartoma is the term used to describe the focal, disorganized, overgrowth of a well-differentiated tissue in an organ, in which the tissue constitutes a normal component. Vascular hamartoma is the non-neoplastic proliferation of disorganized, well-

differentiated vasculature, forming a focal developmental abnormality. Since vascular cells are in almost all organs, vascular hamartomas can arise from any anatomical location. A 12-year-old, 12.44 kg, intact male, Dachshund dog was presented to the veterinary clinic for an elective orchiectomy. Routine castration was performed using a pre-scrotal incision. Many, discrete, 2- 3 mm diameter, slightly raised, flat to nodular, dark red nodules similar in appearance to blood clots, were noted along the pampiniform plexus, the gubernaculum towards the scrotum on the tunica vaginalis. The testicular parenchyma was normal. Histologically, within the fibrous stroma of the visceral tunica vaginalis, were multiple, disorganized, variable-sized, thin-walled, blood vessels, lined by a single layer of flattened, spindled, endothelial cells with and ovoid nuclei, coarse chromatin, and indistinct nucleolus. The endothelium is supported by a thin, layer of subendothelial pericytes. Endothelial cells have a cytoplasmic immunoreactivity against CD31, whereas subendothelial pericytes and smooth muscle fibers have a strong cytoplasmic immunoreactivity against  $\alpha$ -SMA. The blood vessels are filled by erythrocytes without thrombi formation. Thus unilateral pampiniform plexus arteriovenous hamartomatosis of the vaginal tunic diagnosis was made.

Sunday, October 31, 2021

3:10 p.m. – 3:20 p.m. CDT

### **A CASE OF INFECTIOUS NECROTIC HEPATITIS CAUSED BY CLOSTRIDIUM NOVYI TYPE B IN A CANINE PATIENT WITH NO PREDISPOSING LIVER LESIONS**

Briana Trusiano, Sarah Barrett, Virginia Corrigan, Katie Krebs, Stephanie Todd, Kevin Lahmers, Vanessa Oakes, Francisco Carvallo, Michael Ciepluch, Tessa LeCuyer  
Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, USA

An 8-year old female spayed mixed breed dog presented to the Virginia-Maryland College of Veterinary Medicine for acute onset of hyporexia and vomiting. Serum biochemistry confirmed hepatocellular injury and cholestasis. Ultrasound revealed free fluid within the abdomen and multiple hepatic masses. Cytologic examination of liver aspirates and peritoneal fluid revealed frequent 1 x 4  $\mu$ m bacilli with a terminal endospore. Aerobic culture yielded no growth and anaerobic bacterial growth isolated from the fluid sample could not be identified using typical laboratory identification techniques. Long-read, whole genome sequencing was performed and the organism was identified as *Clostridium novyi* type B, the causative agent of infectious necrotic hepatitis (INH). Treatment was initiated and a 30-day follow up anaerobic culture was negative. Bacteria were also not appreciated on fine needle aspiration of the liver. The patient presented five months later and a large hepatic mass with peritoneal fluid were again identified on abdominal ultrasound. Cytologic examination of the peritoneal fluid revealed bacilli similar to those identified on initial presentation. The patient was euthanized and the hepatic lesion was collected for histopathology. The most significant finding was necrotizing hepatitis with intralesional spore-forming bacilli compatible with recurrence of *Clostridium novyi* type B. INH is typically a disease of ruminants with growth of the causative agent supported by development of an anaerobic environment within the liver. In dogs, INH is rare and has only been reported secondary to metastatic pancreatic adenocarcinoma. The present case had no identifiable cause of an anaerobic insult to the liver.

Sunday, October 31, 2021

3:20 p.m. – 3:30 p.m. CDT

## **INFECTIOUS BRONCHITIS VIRUS PREVALENCE, CHARACTERIZATION, AND STRAIN IDENTIFICATION IN CALIFORNIA BACKYARD CHICKENS**

OMAR GONZALES VIERA<sup>1</sup>, Beate Crossley<sup>1</sup>, Francisco Carvallo-Chaigneau<sup>2</sup>, Emily Blair<sup>3</sup>, Daniel Rejmanek<sup>1</sup>, Özge Erdoğan-Bamac<sup>4</sup>, Karen Sverlow<sup>1</sup>, Alejandra Figueroa<sup>5</sup>, Rodrigo Gallardo<sup>5</sup>, Aslı Mete<sup>1</sup>

<sup>1</sup>California Animal Health and Food Safety (CAHFS), Davis Laboratory, UC Davis, Davis, CA, USA, <sup>2</sup>Department of Biomedical Sciences & Pathobiology, Virginia–Maryland, College of Veterinary Medicine, Virginia, VA, USA, <sup>3</sup>College of Agriculture and Environmental Sciences, University of California, Davis, Davis, CA, USA, <sup>4</sup>Istanbul University-Cerrahpasa, School of Veterinary Medicine, Istanbul, Turkey, <sup>5</sup>Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, Davis, CA, USA

**Background:** Infectious bronchitis virus (IBV) causes significant losses in poultry.

**Objective:** To characterize the lesions of infectious bronchitis (IB) and IBV prevalence and identify the circulating strains in small flocks in California.

**Methods:** Backyard chickens submitted to the Davis (Northern California; NorCal) and San Bernardino (Southern California; SoCal) branches of the California Animal Health and Food Safety Laboratory System from January-March 2019 were included in the study. Trachea, kidney, and cecal tonsils were collected for real-time reverse transcriptase (qRT)-PCR, histology, immunohistochemistry (IHC), and sequence analysis.

**Results:** 50 chickens out of 169 submissions tested positive for IBV by qRT-PCR. Of these, 16% (20/123) were from NorCal and 65% (30/46) from the SoCal laboratory. The cecal tonsil was the most frequently positive tissue by qRT-PCR and IHC. Lymphoplasmacytic tracheitis was the most frequent histopathologic finding in 24 of 39 birds, while the kidney showed interstitial nephritis, tubular necrosis, tubular dilation, and/or gout in 14 of 43 chickens. Infectious bronchitis virus played a primary role or a synergistic effect in the mortality of chickens that succumbed to other infections. Sequences of IBV detected in 22 birds were analyzed, and 14 strains were most similar to CA1737, an endemic California genotype. One strain each matched Conn46, Cal99, and ArkDPI, and the remaining five did not have a substantial match to any available reference strains.

**Conclusions:** The findings indicate that small flocks can be reservoirs of IBV and might facilitate the evolution of new variants as well as the reversion of attenuated strains to virulence.

Sunday, October 31, 2021

3:30 p.m. – 3:40 p.m. CDT

### **FOCAL INOCULATION OF HISTOPLASMA CAPSULATUM IN A BUDDING RESIDENT PATHOLOGIST—A CASE FOR ZONOTIC RISK AWARENESS**

Ariel Carlson, Kim Newkirk

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

*Histoplasma capsulatum* is a free-living, soil-borne, dimorphic fungus. Histoplasmosis is reported in captive exotic felids, primarily in tigers (*Panthera tigris*) and less commonly leopards (*Panthera pardus*) and snow leopards (*Panthera uncia*). In humans, it primarily causes severe pneumonia in immunocompromised hosts, and is referred to as “Darling’s disease;” immunocompetent individuals may also be affected. An immunocompetent veterinary anatomic pathology resident performing a tiger necropsy sustained an accidental needle stick to the tip of the left index finger while doing a fine-needle aspirate of lung nodules, to make a cytologic assessment of the lesions. A full microscopic examination of the tissues from the tiger revealed a disseminated infection with *H. capsulatum*. Approximately 3 weeks after the July 2020 necropsy, the resident developed swelling, hyperemia, blanching, and pain in the finger as well as a regional lymphadenopathy; there was no response to antibiotic therapy. Radiographs of the resident’s finger and thorax were normal. The resident’s finger was biopsied in September 2020 which identified a granulomatous dermatitis with yeasts that were consistent with *H. capsulatum*. An 8-month course of itraconazole therapy and surgical excision was curative in the resident. This incident underscores the importance of zoonotic disease awareness in veterinary medicine and importance for proper safety techniques during routine diagnostic procedures.

Sunday, October 31, 2021

3:40 p.m. – 3:50 p.m. CDT

### **FIRST REPORTED CASE OF COVID-19 INFECTION IN A CANINE IN CONNECTICUT WITH A 3-MONTH-OLD GERMAN SHEPHERD PUPPY**

Natalie Tocco, Kirklyn Kerr, Neha Mishra, Alizza Barbieri, Zeinab Helal, Dong-Hun Lee, Guillermo Risatti

Connecticut Veterinary Medical Diagnostic Laboratory, Department of Pathobiology and Veterinary Sciences, College of Agriculture, Health, and Natural Resources, University of Connecticut, Storrs, CT, USA

COVID-19 or SARS-CoV-2, a novel coronavirus, has been the source of a pandemic and has been a focal point of study in humans. The effects of the infection on other species are being investigated. Active disease has been reported in large felids, primates, and mink and in domestic animals including cats and dogs. Currently, there is limited information regarding the scope of pathologies affecting domestic dogs as a result the infection or if cases of sudden death are associated with COVID-19 exposure. In the winter of 2021, the Connecticut Veterinary Medical Diagnostic Laboratory (CVMDL) received a 3-month-old German Shepherd dog with a history of sudden death presented for postmortem examination. Grossly, notable lesions were pulmonary congestion, pulmonary edema, and multifocal areas of pallor in the visceral pericardium extending slightly into the myocardium. Microscopically, significant changes were



pulmonary congestion, pulmonary edema, minimal pulmonary hemorrhage, intra-alveolar fibrin accumulation, alveolar damage with hyaline membrane formation, epicardial edema, and pancardial congestion. The dog was included in the CVMDL's background surveillance testing of domestic cats and dogs. Nasal swabs, lungs, heart and kidney tested positive in RT-qPCR for COVID-19. This was the first reported case of a positive COVID-19 in a domestic dog in the state of Connecticut.

Sunday, October 31, 2021

3:50 p.m. – 4:00 p.m. CDT

### **SARS-COV-2 INFECTION IN TWO FARMED MINKS (NEOVISON VISON)**

Ryan Yanez, Dodd Sledge, Matti Kiupel

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

A facility with 17,000 minks (*Neovison vison*) had 12 mink die on 9/27/2020 and 7 die on 9/28/2020. Coughing, anorexia and bloody nasal discharge were reported prior to death. The facility owner reported that these clinical signs were present for several weeks in the facility. Two male minks were submitted for necropsy to Michigan State University Veterinary Diagnostic Laboratory. In general, the gross findings were unremarkable. Histologically, the nasal epithelium was eroded to ulcerated and had a lymphoplasmacytic to suppurative infiltrate. The turbinates were coated by mucus, hemorrhage and numerous degenerate neutrophils and foamy macrophages. In the lungs, large to small sized veins and arteries had prominent lymphoplasmacytic cuffing with vasculitis and fibrinoid necrosis. Extensive loss of terminal bronchiolar epithelium was a prominent feature. The alveolar interstitium had a lymphoplasmacytic to suppurative inflammation and fibrin thrombi within alveolar capillaries. The formation of hyaline membranes was not observed. PCR for SARS-CoV-2 was performed on nasal turbinate and lung samples from both minks and was found to be positive and confirmatory testing performed by the National Veterinary Services Laboratory (NVSL) in Ames, Iowa was also positive. Immunohistochemistry for the SARS coronavirus antigen was performed. Strong cytoplasmic labeling for the SARS coronavirus antigen was observed within mononuclear inflammatory cells, the nasal epithelium, tracheal epithelium and bronchial and terminal bronchiolar epithelium. Rarely, there was cytoplasmic labeling of endothelial cells in the subepithelial stroma of the turbinates. This report documents unique histologic lesions and positive endothelial immunohistochemical labeling in farmed minks diagnosed with SARS-Cov-2.

Sunday, October 31, 2021

4:00 p.m. – 4:10 p.m. CDT

### **SEVERE ENDOMETRITIS AND SEPTICEMIA IN A CAPTIVE STINGRAY**

Clare Brown<sup>1</sup>, Debra Moore<sup>1,2</sup>, Christa Barrett<sup>1,2</sup>, Tim Morgan<sup>1</sup>

<sup>1</sup>Mississippi State University College of Veterinary Medicine, Starkville, MS, USA,

<sup>2</sup>Institute for Marine Mammal Studies, Gulfport, MS, USA

A captive adult female Cownose stingray (*Rhinoptera bonasus*) was submitted for necropsy to the Mississippi State University College of Veterinary Medicine following a one-day history of anorexia and lethargy. Significant postmortem exam findings

included emaciation, severe reddening around the vent, subcutaneous edema, approximately 0.5 liters of flocculent serosanguinous coelomic fluid, a diffusely purple liver, and an enlarged uterus containing abundant histotroph and a macerated fetus. Additional findings included monogenetic trematode infestation and widely disseminated calcinosis circumscripta. Histologically, the liver had severe hepatocellular lipid depletion, although some small foci of hepatic lipidosis were present. Sinusoids contained large numbers of pigment-laden macrophages. Numerous bacilli occupied hepatic vasculature, and the serosa was covered with abundant heterophils with fibrin and bacilli. The epigonal gland contained increased numbers of lymphocytes and mature heterophils. The uterine lumen contained scattered aggregates of heterophils. Multifocal to coalescing areas of mineralization were present in the external muscularis of the intestine and the dermis. Culture of uterine fluid and coelomic fluid grew *Vibrio vulnificus*. The likely cause of death in this stingray was septicemia and coelomitis without a clearly identifiable site of origin. Mild uterine inflammation was present but was considered unlikely to be the source of the infection. Emaciation and hepatic lipid depletion probably occurred due to anorexia secondary to septicemia, although concurrent trematode infestation may also have contributed to overall morbidity in this individual. It is unclear whether early death of the fetus was a contributing factor to morbidity in this animal or if it was secondary to multifactorial debilitation.

Sunday, October 31, 2021

4:10 p.m. – 4:20 p.m. CDT

### **DISSEMINATED COCCIDIOIDOMYCOSIS IN A WHITE-FACED SAKI (PITHECIA PITHECIA)**

Jason Struthers<sup>1</sup>, Alyssa Palmer<sup>2</sup>, Gary West<sup>2</sup>, Kristen Phair<sup>2</sup>, Alexandra Goe<sup>1</sup>

<sup>1</sup>Animal Health Institute, Midwestern University, Glendale, AZ, USA, <sup>2</sup>Phoenix Zoo, Phoenix, AZ, USA

A 6-y-o intact male white-faced saki (*Pithecia pithecia*) developed generalized pruritic dermatopathy of undetermined etiology that initially responded to antihistamines. 1.5 years later, the patient's pruritus progressed to self-induced ulcerative dermatitis and alopecia, with weight loss. Anti-inflammatory doses of prednisolone, gabapentin, and antibiotics improved the dermatopathy, yet the patient subsequently developed weakness, mild ataxia, lethargy, neutrophilic leukocytosis, cholestasis, and cachexia. Abdominal ultrasound noted a mottled liver with peritoneal effusion. A CT scan revealed pulmonary nodules and hilar lymphadenomegaly. *Coccidioides* spp. titers were negative at the initial diagnostic exam and five weeks later; however, serum obtained three weeks later, 1 month after beginning the new skin treatment regimen and two days prior to euthanasia, returned with a positive IgG titer at 1:64. Postmortem examination confirmed emaciation and diagnosed disseminated coccidioidomycosis that involved the liver, spleen, kidney, heart, lung, adrenal gland, bone marrow, bicavitary lymph nodes, pleura, brain, and testes. Incidentally, the patient had epiglottal, pharyngeal, and esophageal gonglyonemiasis. The dermatitis was non-infectious inflammatory and atrophic, which favored environmental or immune-mediated causes not otherwise specified. The saki's disseminated coccidioidomycosis may have been predisposed by co-morbidities and stress-related immunosuppression, coupled with presumptive species-sensitivity, since some non-human primates are regarded as

particularly susceptible to *Coccidioides* spp. *Coccidioides* spp. is an endemic and opportunistic dimorphic fungus of the southwestern USA that more commonly infects immunocompromised hosts. Because of this regional health risk, clinicians should consider a cost-benefit analysis for empiric anti-fungal treatment in ailing non-native naïve mammals where immune status is a concern.

Sunday, October 31, 2021

4:20 p.m. – 4:30 p.m. CDT

### **ANGIOSTRONGYLUS CANTONENSIS IN A RED-RUFFED LEMUR**

Jessica Rizer<sup>1</sup>, Ryan Yanez<sup>1</sup>, Shannon Gonsoulin<sup>2</sup>, Matti Kiupel<sup>1</sup>

<sup>1</sup>Michigan State University Veterinary Diagnostic Laboratory and Department of Pathobiology and Diagnostic Investigation, East Lansing, MI, USA, <sup>2</sup>All Creatures Veterinary Hospital, New Iberia, LA, USA

*Angiostrongylus cantonensis* is a metastrongyloid nematode that has recently become endemic in the Southeastern United States. Rats are the definitive and gastropod species are the intermediate host. *A. cantonensis* is a common cause of eosinophilic meningitis in humans in Southeast Asia and the Pacific Islands and also affects nonhuman primates. A 9-year-old male Red-ruffed lemur (*Varecia rubra*), from Louisiana, was euthanized for worsening hind limb paresis. Sections of the brain and spinal cord were collected at necropsy for microscopic examination. Microscopically, within the subarachnoid space and neuropil of the cerebellum and brainstem, there were numerous transverse and longitudinal sections of adult nematodes. These nematodes were 50-70µm in diameter and had a 3-4µm thick smooth eosinophilic cuticle. The nematodes had a coelomyarian musculature, a pseudocoelom, a reproductive tract, and an intestine lined by uni-nucleated cells and intraluminal eosinophilic to brown flocculent material. The surrounding neuropil and affected cerebellar folia were effaced by hemorrhage and small numbers of eosinophils, neutrophils, macrophages, and glial cells. A single adult nematode was observed within the subdural space of the thoracic spinal cord. Another nematode had regionally effaced the dorsal horn in a section of the lumbar spinal cord causing regional axonal degeneration. The leptomeninges of the cerebellum and spinal cord were expanded by numerous eosinophils and fewer neutrophils and macrophages. The morphologic features of the nematodes were consistent with *Angiostrongylus* spec. Considering the affected species, the associated pathology, and the geographic origin of the lemur, these nematodes were most consistent with *A. cantonensis*.

Sunday, October 31, 2021

4:30 p.m. – 4:40 p.m. CDT

### **CHAGAS DISEASE IN A VIRGINIA OPOSSUM FROM NORTH CAROLINA**

Andeliene Croce<sup>1</sup>, Lisa Auckland<sup>2</sup>, Sarah Hamer<sup>2</sup>, Grace Mathis<sup>3</sup>, Bonnie Brenseke<sup>1,4</sup>

<sup>1</sup>North Carolina State University, Raleigh, NC, USA, <sup>2</sup>Texas A&M University, College Station, TX, USA, <sup>3</sup>Lincoln Memorial University, Harrogate, TN, USA, <sup>4</sup>Campbell University, Buies Creek, NC, USA

A 3-year-old male Virginia opossum from a local science museum was presented for a two-week history of fatigue and increased respiratory effort. The opossum came to the

museum from a rehabber based in the Research Triangle area of North Carolina. Physical exam and imaging findings indicated heart failure. Grossly, the animal had dilated cardiomyopathy, serosanguineous pleural effusion, and hepatomegaly. Microscopically, cardiac myofibers varied in size, the interstitium had multifocal cellular infiltrates (neutrophils and fewer mononuclear cells), and there was passive congestion in the liver. Additional findings included an organizing pneumonia with lipid accumulation, pleuritis, and pleural fibrosis. Scrolls from formalin-fixed paraffin-embedded heart tested positive for *Trypanosoma cruzi* via polymerase chain reaction. Chagas disease is caused by the protozoan parasite, *T. cruzi*, which can infect a wide range of mammals including opossums, dogs, and humans. The parasite is primarily transmitted by triatomine vectors native to Central and South America, Mexico, and the southern United States, with higher concentrations in Texas, Arizona, and New Mexico. Triatomines have been identified as far north as Delaware (confirmed by CDC in 2019) and have been reported in North Carolina since 2015. In the acute phase of the disease, infected individuals may be asymptomatic or develop potentially fatal myocarditis. In the chronic phase, patients may develop dilated cardiomyopathy. Opossums are common to the eastern United States, can be infected with *T. cruzi*, and may develop associated pathology, thus can serve as an important sentinel species for risk of Chagas disease for humans in this region.

Sunday, October 31, 2021

4:40 p.m. – 4:50 p.m. CDT

**TRYPANOSOMA CRUZI INFECTION IN A JUVENILE ASIAN-SMALL-CLAWED OTTER (AONYX CINEREUS) AND A JUVENILE TWO-TOED SLOTH (CHOLOEPUS SPP.)**

Samantha Hughes, Brittany Baughman

Mississippi State University College of Veterinary Medicine, Mississippi State, MS, USA

In April 2021, a 9-week-old Asian Small-Clawed Otter with limited clinical history was submitted to the Mississippi State University College of Veterinary Medicine for necropsy. No gross lesions were appreciated on gross examination. Histopathology revealed a multiorgan protozoal infection, and frozen tissue was PCR-positive for *Trypanosoma cruzi*. After the diagnosis, the owner mentioned that a 4-month-old Two-Toed Sloth died in December 2020 and was submitted to the Mississippi State Diagnostic Laboratory where it was diagnosed with a protozoal myocarditis. Formalin fixed paraffin embedded tissue scrolls from the sloth's heart revealed a PCR-positive result for *Trypanosoma cruzi*. Additional history indicated that the otter and sloth were housed in the same enclosure at an exotic animal park in southern Mississippi, but were purchased from facilities in different states months apart. This additional information was interesting as it suggests these animals may have become infected on site in Mississippi. *Trypanosoma cruzi* is a protozoal parasite that is transmitted by blood-sucking triatomine bugs, also known as “kissing bugs.” These insect vectors are known to inhabit Mississippi; however, natural *Trypanosoma cruzi* infections have not been documented in humans or domestic species. These cases are notable because they are the first documented cases of *Trypanosoma cruzi* infections in exotic animal species within the state of Mississippi.

## Natural Disease Focused Scientific Session I

Monday, November 1, 2021 | 2:30 p.m. – 2:45 p.m. CDT

Monday, November 1, 2021

2:30 p.m. – 2:45 p.m. CDT

### **DIAGNOSTIC IMMUNOHISTOCHEMISTRY OF CANINE GLIOMA**

Gregory Krane<sup>1,2,3</sup>, Carly O'Dea<sup>4</sup>, David Malarkey<sup>1,2</sup>, Andrew Miller<sup>5</sup>, C. Miller<sup>6</sup>, Debra Tokarz<sup>1,7</sup>, Heather Jensen<sup>2</sup>, Kyathanahalli Janardhan<sup>8,9</sup>, Keith Shockley<sup>2</sup>, Christopher Mariani<sup>1</sup>

<sup>1</sup>North Carolina State University - College of Veterinary Medicine, Raleigh, NC, USA, <sup>2</sup>National Institute of Environmental Health Sciences - National Toxicology Program, Research Triangle Park, NC, USA, <sup>3</sup>Charles River Laboratories, Shrewsbury, MA, USA, <sup>4</sup>Charles River Laboratories - PAI, Durham, NC, USA, <sup>5</sup>Cornell University - College of Veterinary Medicine, Ithaca, NY, USA, <sup>6</sup>University of Alabama at Birmingham - School of Medicine, Birmingham, AL, USA, <sup>7</sup>Experimental Pathology Laboratories, Research Triangle Park, NC, USA, <sup>8</sup>Integrated Laboratory Systems, Research Triangle Park, NC, USA, <sup>9</sup>Abbvie, North Chicago, IL, USA

Glioma is a devastating cancer with diverse histologic features that can create diagnostic difficulty and generate inter-pathologist diagnostic disagreement. Immunohistochemistry, which has been shown to increase inter-pathologist diagnostic agreement of canine brain tumors, can help diagnose canine gliomas. Though the literature describes qualitative canine glioma immunolabeling, quantitative assessment has not been reported. We report immunolabeling characteristics for 73 canine gliomas diagnosed with consensus by a five-pathologist panel utilizing NCI diagnostic recommendations with access to H&E, Olig2, GFAP, and CNPase slides. Cases were reported as positive or negative for immunolabeling based on manual examination by a single evaluator, and area fractions were measured by digital analysis. Astrocytoma had greater frequency of tumors positive for GFAP immunolabeling than oligodendroglioma (83 vs 17%;  $p < 0.01$ , Chi-squared test), and oligodendroglioma had greater frequency of tumors positive for CNPase immunolabeling than astrocytoma (55 vs 17%;  $p = 0.01$ , Chi-squared test). Olig2 median area fraction was higher in oligodendroglioma than in astrocytoma (16 vs 1.5%;  $p < 0.01$ , Mann-Whitney test). GFAP median area fraction was higher in pooled low-grade than in pooled high-grade tumors (45 vs 21%;  $p < 0.01$ , Mann-Whitney test), and GFAP median area fraction was higher in astrocytoma than in oligodendroglioma (52 vs 21%;  $p < 0.01$ , Mann-Whitney test). These data reinforce the utility of these markers in helping to differentiate oligodendroglioma from astrocytoma. Future studies are indicated to determine to what degree these immunohistochemical profiles predict prognosis and response to therapy for canine patients with glioma.

Monday, November 1, 2021

2:45 p.m. – 3:00 p.m. CDT

### **MOLECULAR AND MORPHOLOGIC CHARACTERIZATION OF FUNGAL ENCEPHALITIS IN DOGS SUBMITTED TO NEW YORK STATE DIAGNOSTIC LABORATORY FROM 1996-2020**

Cristy Rocío González Barrientos<sup>1</sup>, Andrew David Miller<sup>1</sup>, Courtney Meason-Smith<sup>2</sup>, Aline Rodrigues Hoffmann<sup>2</sup>, Eric Glass<sup>3</sup>, James Hammond<sup>4</sup>, Elena Alina Demeter<sup>1</sup>

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**Background:** Fungal infections of the CNS are under-characterized causes of neurologic disease in dogs. **Objective:** Characterize cases of fungal encephalitis in dogs (1996-2020) by histopathology and molecular identification. **Methods:** Formalin-fixed paraffin-embedded (FFPE) tissue from 19 dogs was evaluated applying H&E, GMS, Fontana-Masson stains and immunohistochemistry (IHC) for canine distemper virus (CDV). DNA was extracted from FFPE blocks. Panfungal PCR targeting the internal transcribed spacer (ITS) and large (28S) subunit (LSU) regions was followed by sequencing and comparison to NCBI database. **Results:** The age range of affected dogs was 1-10 years, with most cases being 2-4 years. German Shepherd (7/19, 36.8%) and mixed breed dogs (5/19, 26.3%) were primarily affected. Histopathologic changes included necrosis (18/19, 94.7%), encephalitis (17/19, 89.5%), meningitis (16/19, 84.2%), perivascular cuffing (14/19, 73.7%), and vasculitis (12/19, 63.2%). Nine (47.4%) of 19 cases had yeast morphologically compatible with *Cryptococcus neoformans* (6/19, 31.6%) and *Blastomyces dermatitidis* (3/19, 15.8%). Ten of 19 cases had pigmented (4/19, 21.1 %) and hyaline hyphae (6/19, 31.6%). Fungal identification was achieved in 9 cases: *Cladophialophora bantiana* (3/19, 15.8%), *Coniochaeta* sp. (2/19, 10.5%), *Cryptococcus neoformans* (2/19, 10.5%), *Blastomyces dermatitidis* (1/19, 5.3%) and *Phialemonium obovatum* (1/19, 5.3%). CDV IHC was positive in one case co-infected with *Cladophialophora bantiana*. **Conclusions:** Fungal agents are important causes of encephalitis in dogs younger than 4 years and German shepherds are overrepresented. Morphologically, *Cryptococcus neoformans* and hyaline septate fungi were most common, while *Cladophialophora bantiana*, a dematiaceous fungus, was the most commonly confirmed by molecular techniques. Fungal coinfection with CDV was infrequent.

## Clinical Pathology Focused Scientific Session II

Tuesday, November 2, 2021 | 9:00 a.m. – 9:15 a.m. CDT

Tuesday, November 2, 2021

9:00 a.m. – 9:15 a.m. CDT

### **PSEUDORETICULOCYTOSIS BY THE ADVIA 2120 HEMATOLOGY ANALYZER AND OTHER HEMATOLOGIC CHANGES IN A CYNOMOLGUS MONKEY (*MACACA FASCICULARIS*) WITH MALARIA**

Diya Sharma, Heather Priest, Angela Wilcox

Simian malaria is one of the most frequent parasitic diseases of nonhuman primates (NHPs) imported from endemic regions. This report discusses significant hematologic changes in a NHP with malaria, with emphasis on pseudoreticulocytosis as reported by a flow cytometry hematology analyzer. A 5-year-old male purpose-bred Cambodian cynomolgus monkey (*Macaca fascicularis*) was enrolled in a study investigating the effects of an anti-neoplastic test article and was found to have a high burden of *Plasmodium spp* on blood smear review three weeks after the first dose. Automated

hematologic measurements were performed by the ADVIA 2120 hematology analyzer, and increased monocytes ( $2.20 \times 10^3/\mu\text{L}$ , RI:  $0.17 - 0.76 \times 10^3/\mu\text{L}$ ) were observed at that time. Progressively higher parasite burdens (% of infected red blood cells) and persistent monocytosis (up to  $10.38 \times 10^3/\mu\text{L}$ ) were observed on all subsequent hematologic evaluations with eventual development of severe anemia and clinical malarial disease. The analyzer reported artifactual increases in reticulocyte counts (percent reticulocytes and absolute counts) when compared with manual counts performed on new methylene blue smear evaluations on study Days 43 ( $\Delta 6.7\%$ ,  $\Delta 266.2 \times 10^9/\text{L}$ ) and 50 ( $\Delta 18.9\%$ ,  $\Delta 409.8 \times 10^9/\text{L}$ ). The discrepancy between analyzer and manual counts was greater with higher parasite burdens. Abnormal reticulocyte scatter plot distributions were also observed at time points with high parasite burdens. Verification of automated reticulocyte counts and recognition of pseudoreticulocytosis are important to assess the appropriateness of the regenerative response. Marked monocytosis correlated with higher parasite burdens and may be an indicator of advanced disease.

Tuesday, November 2, 2021

9:15 a.m. – 9:30 a.m. CDT

## **ASSESSING AN IMMUNOTURBIDIMETRIC ASSAY FOR FELINE SERUM AMYLOID A**

Elsbeth Waugh, Hayley Haining, James Harvie, David Eckersall  
University of Glasgow, Glasgow, United Kingdom

**Background:** Serum Amyloid A (SAA) is a major acute phase protein in cats, increasing rapidly in response to various inflammatory diseases. An automated latex-enhanced immunoturbidimetric assay for human SAA has been adapted and improved for use in veterinary diagnostic laboratories but has yet to be validated in cats.

**Objectives:** To validate the VET-SAA assay (Eiken) for use with feline samples.

**Methods:** Assays were run on a Pentra analyser (Horiba).

**Results:** Intra-assay and inter-assay imprecision were  $<3.3\%$  and  $<6.7\%$ , respectively, for both low and high SAA concentrations. Linearity under dilution was acceptable with no prozone effect observed. Limit of detection was  $1.65 \text{ mg/L}$  and limit of quantification (LoQ) was  $6 \text{ mg/L}$ . Haemoglobin and triglyceride showed no adverse interference, but bilirubin produced positive bias in samples with low SAA. Comparison with the LZ-SAA assay (Eiken), previously validated in cats, showed significant correlation with slight proportional bias increasing as SAA concentration increased, possibly related to differing calibration standards. SAA was significantly higher in patients with moderate inflammatory disease compared with mild or non-inflammatory disease (medians  $58.5$ ,  $1.2$  and  $0 \text{ mg/L}$  respectively;  $p < 0.001$ ). A significant difference was observed between mild and non-inflammatory disease, however the median SAA levels in these cases were below the LoQ, suggesting improvement of the assay range at lower levels may be worthwhile.

**Conclusions:** The automated VET-SAA assay is a robust, precise and accurate method for measurement of feline SAA which can clearly identify patients with moderate inflammation. It should be a valuable biomarker for use in feline medicine.

Tuesday, November 2, 2021

9:30 a.m. – 9:45 a.m. CDT

**CLINICAL OUTCOME AND KI67 EVALUATION IN CANINE NODAL SMALL B-CELL LYMPHOMA DIAGNOSED BY FLOW CYTOMETRY**

Emily Rout, Monica Fernandez, Janna Yoshimoto, Kelly Hughes, Anne Avery, Jenna Burton

Colorado State University, Fort Collins, CO, USA

**Background:** Canine B-cell lymphoma subtypes comprised of small to intermediate-sized cells include marginal zone, follicular, mantle cell, small-cell lymphocytic and diffuse small B-cell lymphoma, which cannot be distinguished by flow cytometry. We hypothesized that Ki67 expression measured by flow cytometry may offer prognostic information, particularly for cases where histologic subtyping is not pursued.

**Objective:** Describe clinical outcome in canine nodal small B-cell lymphoma cases and correlate clinical, laboratory and Ki67 expression data with survival.

**Methods:** Small B-cell lymphoma cases were identified by flow cytometry by an expansion (>80%) of CD21+ B cells that were small-intermediate in size by forward light scatter. The percentage of Ki67-expressing B cells was measured in all cases by flow cytometry. Outcome data were extracted from medical records.

**Results:** 49 nodal small B-cell lymphoma cases were included. The median B-cell Ki67% was 41% (range 3-97%). The median overall survival time (MST) was 222 days across all cases and 267 days among cases treated with a CHOP-based chemotherapy protocol (n=32). Among CHOP-treated cases, those with very low proliferation (<11% Ki67) had longer survival (MST 542 days) than cases with >11% Ki67 (MST 242 days; p=0.014). However, this association was not significant when all treatment types were combined (p=0.077; <11% Ki67 MST 542 days; >11% Ki67 MST 176 days).

**Conclusions:** The majority (78%) of nodal small B-cell lymphoma cases had an aggressive course surviving <1 year from diagnosis. A small subset had an indolent clinical course. Low Ki67 expression may be useful in identifying cases with better prognosis.

Tuesday, November 2, 2021

9:45 a.m. – 10:00 a.m. CDT

**REPEAT PATIENT TESTING AS AN ALTERNATIVE TO COMMERCIAL QUALITY CONTROL MATERIAL FOR VETERINARY HEMATOLOGY ANALYZERS**

Susan Daly<sup>1,2</sup>, Kathleen Freeman<sup>3</sup>, Peter Graham<sup>2</sup>

<sup>1</sup>Synlab VPG Cork, Cork, Ireland, <sup>2</sup>University of Nottingham, Nottingham, United Kingdom, <sup>3</sup>Synlab VPG Exeter, Exeter, United Kingdom



**Background:** Repeat patient testing quality control (RPT-QC) utilizes retained patient samples as an alternative to commercial quality control material (QCM-QC). We elected to validate RPT- QC for RBC, HGB, HCT and WBC.

**Objectives:** (1) Validate RPT-QC across a network of harmonized hematology analyzers. (2) Determine the total error that can be controlled with RPT-QC. (3) Challenge RPT-QC to ensure acceptable sensitivity. (4) Educate technicians and clinical pathologists about application of RPT-QC.

**Methods:** Fresh adult canine EDTA samples with results within reference intervals were selected and re-run on day 2, 3 and 4. Limits were generated from the standard deviation (SD) of the duplicate measurement differences. The limits were challenged using interventions designed to promote unstable system performance. The total error detectable by RPT-QC was determined using EZRULES3 software. RPT-QC education was provided by seminars advertised to all employees.

**Results:** Twenty or 40 data points were needed for RPT-QC validation and confirmed using 20 additional data points. The total error which could be controlled was the same or better than QCM-QC for all measurands except haematocrit, which required a higher total error to achieve acceptable probability of error detection. The challenges designed to mimic unstable system performance were successful in provoking out-of-control QC. Feedback following educational sessions indicated increased understanding and acceptance of the statistical bases for RPT-QC.

**Conclusions:** RPT-QC successfully identified in-control system performance. The challenges for RPT-QC resulted in acceptable detection of potential unstable system performance. Personnel education regarding RPT-QC was crucial to gaining increased understanding and acceptance.

Tuesday, November 2, 2021  
10:30 a.m. – 10:45 a.m. CDT

### **SEMI-QUANTITATIVE AUTOMATED URINE SEDIMENT QUALITY ASSESSMENT**

Jessica Hokamp<sup>1</sup>, Kathleen Freeman<sup>2</sup>, Kendal Harr<sup>3</sup>

<sup>1</sup>The Ohio State University, Columbus, OH, USA, <sup>2</sup>Syn Labs VPG, Exeter, United Kingdom, <sup>3</sup>URIKA, LLC, Mukilteo, WA, USA

**Background:** The Zoetis VetScan SA and IDEXX SediVue Dx are marketed for in-clinic urine sediment analysis but have not been evaluated objectively using quality specifications.

**Objective:** Evaluate the performance of the VETSCAN SA and SediVue Dx using assayed, bilevel urine quality control material (QCM) to determine if instrument specifications are acceptable for possible clinical urine sediment evaluation.

**Methods:** Accuracy, precision, and clinical utility of the VETSCAN SA and SediVue Dx measurements were evaluated using a bilevel, assayed QCM in 23 veterinary practices.

**Results:** The VETSCAN SA and SediVue Dx instruments reported RBCs and WBCs within manufacturer specifications with good to excellent sensitivity (93-100%) and specificity (100%). The VETSCAN SA and SediVue Dx instruments under identified the presence of crystals with an 83% and 13% inaccuracy in the positive QCM, respectively. Neither instrument identified clinically important cystine crystals without human review. Both instruments over reported bacteria in the sterile QCM (confirmed with Romanowsky stain), with 27% and 13% inaccuracy reported from VETSCAN SA and SediVue Dx, respectively, for QCM Level 2.

**Conclusions:** The VETSCAN SA and SediVue Dx performed adequately as a semiquantitative screening tool in RBC and WBC identification in normal and abnormal samples. Additional improvement is needed to better classify crystal types and reduce false positives for bacteria prior to clinical use for these urine components; recommended use is as a tool for digital documentation including photomicrographs. Manual review of abnormal urine samples is required to ensure that clinically important urine components are correctly evaluated.

Tuesday, November 2, 2021  
10:45 a.m. – 11:00 a.m. CDT

### **PLATELET FUNCTION IN HEARTWORM-INFECTED DOGS: EVALUATION OF RESPONSES FROM PLATELET-RICH PLASMA AND WHOLE BLOOD SAMPLES**

Carisa Fraser, Benjamin Brainard, Bridget Garner, Andrew Moorhead, Jaime Tarigo, Mandy Wallace  
University of Georgia, Athens, GA, USA

**Background:** Platelet hyperreactivity has been demonstrated in heartworm-infected dogs through *in vitro* platelet aggregation, but the root cause and clinical implications of hyperreactivity have not been fully elucidated.

**Objective:** To more broadly evaluate platelet function in heartworm-infected (HWI) dogs using novel testing modalities.

**Methods:** Anticoagulated whole blood (citrate, EDTA and hirudin) samples collected from eight HWI and eight uninfected dogs were evaluated using optical platelet aggregometry, a platelet function analyzer (PFA-100), a total thrombus-formation analysis system (T-TAS), tissue factor-activated and tPA modified thromboelastography (TF- and tPA-TEG), a CBC including conventional platelet indices (MPV, PCT and MPC, using the ADVIA 2120i) and coagulation parameters (vWF and fibrinogen concentration).

**Results:** Platelets from HWI dogs demonstrated enhanced aggregation with area under the curve for higher concentrations of collagen (20 $\mu$ g/mL) compared to control dogs ( $P = 0.023$ ). Platelet aggregation in response to adenosine diphosphate (10 $\mu$ M) or lower concentrations of collagen (10 $\mu$ g/mL), PFA-100 closure times, and T-TAS occlusion times were not significantly different between groups. TEG values TF-R, tPA-R, TF-K, and TF-LY60 were decreased ( $P = 0.025$ ,  $P = 0.047$ ,  $P = 0.038$ ,  $P = 0.025$ ), and TF-MA, tPA-MA, TF-G, tPA-G, and TF-alpha angle were increased ( $P < 0.04$ ) in HWI dogs. HWI

dogs had significantly higher fibrinogen ( $P = 0.008$ ) and eosinophil ( $P = 0.003$ ) levels. There was no difference in hematocrit between groups ( $P = 0.383$ ) and all HWI dogs were microfilaremic.

**Conclusions:** Canine heartworm disease was associated with enhanced platelet response to high concentrations of collagen, hypercoagulability, and decreased fibrinolysis.

Tuesday, November 2, 2021  
11:00 a.m. – 11:15 a.m. CDT

### **CLINICAL FEATURES OF A RARE CD21-CD22+ B-CELL LYMPHOPROLIFERATIVE DISEASE BY FLOW CYTOMETRY IN CANINE PERIPHERAL BLOOD**

Kari Frankhouse, Emily Rout, Adam Harris, Janna Yoshimoto, Anne Avery

**Background:** Immunophenotypic analysis of canine blood samples revealed a rare B-cell lymphoproliferative disease with a unique CD21-CD22+ phenotype that most often presents in young dogs.

**Objectives:** Describe a rare, CD21-CD22+ lymphoma/leukemia in the peripheral blood of dogs, and evaluate the frequency, clinical presentation, and outcome of this subtype.

**Methods:** We identified cases with an expansion of CD21-CD22+ cells in the blood between 2018-2021. Hematology and biochemical data were reviewed. Blood smears were evaluated for cytomorphology when available, and clinical presentation was described.

**Results:** 21 cases with CD21-CD22+ B-cell lymphocytosis were identified. These cells often expressed low levels of the pan-leukocyte antigen CD45. Class II major histocompatibility complex expression was variable. The cells did not express CD34. Neoplastic cells were described as large-sized with moderate to deeply basophilic cytoplasm and a round nucleus with smooth chromatin that occasionally contained one or multiple nucleoli. Among cases with available clinical information, 95% were thrombocytopenic, 52% were anemic, and 53% were hypercalcemic. Only 29% of cases had peripheral lymphadenopathy; however, 56% had mesenteric lymphadenopathy. 46% of cases had splenomegaly and 23% had hepatomegaly. The median age of these dogs was 2.25 years and 71% were male.

**Conclusions:** This case series describes a rare B-cell neoplasm presenting primarily in young male dogs. The disorder is associated with hypercalcemia and is comprised of cells with immature cytologic features. Future studies are designed to determine if this is a disorder of immature B cells (acute B-cell leukemia) or an unusual disease of mature B cells.

Tuesday, November 2, 2021  
11:15 a.m. – 11:30 a.m. CDT

### **CYTOMORPHOMETRIC DIAGNOSIS OF FELINE LYMPHOMA**

Peter James O'Brien, Nicholas Hegarty, Lindsay Purvis  
University College Dublin, Dublin, Ireland

**Background:** We previously demonstrated that in dogs cytological diagnoses of benign and malignant lymphoid tissue concorded with a single cytomorphometric parameter,

percentage of lymphocytes  $>11\mu\text{m}$ . **Objective:** Test hypothesis that a similar concordance occurs for feline lymphoma. **Methods:** Lymphoid tissue smears with an unequivocal cytological diagnosis of benign tissue or malignancy were used. Lengths for 8-9, randomly-selected cells (CL) in 3 images of 50x-objective fields-of-view that were enriched in large cells from an area of the smear that was similarly enriched. The percentage of the 25 cells  $>11\mu\text{m}$  was determined. 12/9 benign and 22/11 lymphoma cat/dog cases were studied. **Results:** Feline and canine CL (in  $\mu\text{m}$ ) were similar,  $8.5\pm 0.8(7.2-10.1)$  and  $8.9\pm 0.6(8.2-10.0)$ , respectively, for benign tissue, but much larger for lymphoma with only slight overlap with benign tissue:  $2.1\pm 1.1(10.1-14.8)$  and  $11.9\pm 1.0(10.5-14.4)$ . For all benign tissues  $<25\%$  of cells were  $>11\mu\text{m}$ ; however with lymphoma, this percentage ranged from 40-100% for cats and 36-100% for dogs. **Conclusion:** There was 100% concordance between cytomorphometric measurement and diagnoses on cytology reports for both cat and dog. Test values in the grey-zone of 25-36% large cells, would require exam by a certified, clinical pathologist for definitive diagnosis. This cytomorphometric test could be a screen or alternative to full cytological examination. thereby being less time-consuming, not requiring a board-certified clinical pathologist and with potential for automation.

Tuesday, November 2, 2021  
11:30 a.m. – 11:45 a.m. CDT

### **ANALYTICAL VALIDATION OF A HUMAN PORTABLE GLUCOMETER IN HONEYBEE HEMOLYMPH**

Antoine Cournoyer<sup>1,2</sup>, Pascal Dubreuil<sup>1</sup>, Annie Deschamps<sup>1</sup>, Marie-Odile Benoit-Biancamano<sup>1,2</sup>

<sup>1</sup>Département de pathologie et microbiologie, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, QC, Canada, <sup>2</sup>Groupe de recherche sur les maladies infectieuses en production animale (GREMIP), Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, QC, Canada

#### Background

Glucose and trehalose are the main energy sources in honeybees (*Apis mellifera*) for daily activities. Portable glucometer can compensate for large volume samples and high costs required from standard methods.

#### Objective

The primary aim of this study was to perform an analytical validation of a portable human glucometer (Accu-Check®) in honeybees and to assess its agreement with the reference method (Beckman, GluCH).

#### Methods

Thirty pooled hemolymph samples collected from the antenna of anesthetized honeybees and diluted 1:5 0.9% were used. Detection limits, dilution linearity, spike-and-recover analysis and inter- and intra-assay imprecisions were evaluated. Glucose concentration was measured over time at different storage temperature ( $25^{\circ}\text{C}$ ,  $4^{\circ}\text{C}$ ,  $-20^{\circ}\text{C}$ ,  $-80^{\circ}\text{C}$ ). Trehalose concentration was indirectly measured after trehalase hydrolyzation with an enzymatic protocol.

## Results

Results showed a good correlation between values measured by both instruments (0,985,  $p < 0,0001$ ); a bias of 1.466 (95% CI 2.739-5.673), where a linear agreement between both methods is seen below 20 mmol/L. Accuracy of the glucometer decreases with higher glucose concentration. A recovery of 115% to 130% of diluted spikes indicated good specificity. Average inter- and intra-assay imprecisions were 2.50% and 2.21%, respectively. Because of endogenous trehalase activity, glucose concentration fluctuates in stored samples in a time- and temperature-dependent manner, where storage at  $-80^{\circ}\text{C}$  showed the most stable conservation, with relatively constant glucose concentrations over time (around 1.7 mmol/L).

## Conclusion

Accu-Check® glucometer is an adequate instrument to measure honeybee glucose concentration with good accuracy and precision below 20 mmol/L, and hemolymph storage at  $-80^{\circ}\text{C}$  is suitable for long-term conservation.

## Diagnostic Pathology Focused Scientific Session II

Tuesday, November 2, 2021 | 8:10 a.m. – 8:20 a.m. CDT

Tuesday, November 2, 2021

8:10 a.m. – 8:20 a.m. CDT

### **HISTOPATHOLOGIC CHANGES ASSOCIATED WITH CHRONIC SUPERFICIAL DIGITAL FLEXOR TENDINOPATHY IN AN AMERICAN SADDLEBRED**

Kerry Goldin<sup>1,2,3</sup>, Stephanie French<sup>1</sup>, Kurt Williams<sup>1</sup>

<sup>1</sup>Michigan State University Veterinary Diagnostic Laboratory, Lansing, MI, USA,

<sup>2</sup>National Institutes of Health Comparative Biomedical Scientist Training Program, Bethesda, MD, USA, <sup>3</sup>Laboratory of Virology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, USA

Superficial digital flexor tendon (SDFT) injuries are one of the most common tendon injuries in horses. The pathophysiology of the SDFT injuries in horses is complex and multifactorial. It is likely a combination of mechanical stressors, the biochemical environment within the tendon, and patient factors (such as age, breed, etc.). Histopathologic changes associated with chronic SDF tendinopathy in horses is not well described in the literature.

A 16-year-old American Saddlebred gelding was euthanized and submitted for necropsy after going down and being unable to rise, and a chronic history of suspected suspensory ligament issues. Grossly the left SDFT was markedly swollen, with multifocal red to black pinpoint foci observed on cross section. The right SDFT had similar, less severe changes. Sections of normal SDFT were collected from an unaffected horse for comparison. The left SDF, stained with H&E and Masson's trichrome revealed a spectrum of changes, including necrosis of individual fascicles, swelling of fascicles with loss of individual collagen fibers, loss of birefringence under polarized light, and replacement of fascicle bundles with abundant homogenous eosinophilic material. The peritenon was markedly expanded by dense fibrous connective tissue, and the interfascicular space was expanded by edema, reactive

fibroblasts, and infiltrated by low numbers of macrophages. These changes appear to reflect chronic collagen degeneration, and suspected replacement of collagen fibers with another unidentified proteinaceous substance. Further investigation into histologic and ultrastructural changes in the SDFT of horses with chronic tendon injuries is warranted and would provide insight into mechanisms of tendon repair.

Tuesday, November 2, 2021

8:20 a.m. – 8:30 a.m. CDT

### **SUSPECT CANINE DYSAUTONOMIA IN AN ENGLISH SPRINGER SPANIEL DOG FROM ALABAMA**

Alana Kramer<sup>1</sup>, Rachel Neto<sup>1</sup>, Jennifer Malmberg<sup>2</sup>

<sup>1</sup>Auburn University, Auburn, AL, USA, <sup>2</sup>University of Wyoming, Laramie, WY, USA

An eight-year-old, male neutered, English Springer Spaniel dog presented to Auburn University Emergency hospital for a distended abdomen and difficulty breathing with a five-day history of gastrointestinal upset including vomiting, nausea, mucopurulent nasal discharge, mydriasis, and diarrhea that resolved within 24 hours. Prior to this, the dog was healthy with no history of gastrointestinal problems. While hospitalized, the dog developed static bloating that required decompression every few hours. Radiographs and abdominal ultrasound ruled out volvulus and indicated gastric and duodenal ileus. Over two days, the dog had progressively worsening neurological signs including obtundation, absent menace response, absent PLRs, and mydriasis until he was semi-comatose and died naturally overnight. Necropsy was unremarkable and, histologically, the celiac-mesenteric ganglia and myenteric plexi in the stomach, small intestine, and large intestine had neuronal degeneration, necrosis, and loss with axonal spheroids. The ventral brain stem had rare multifocal central chromatolysis of cranial nerve nuclei and the heart had mild incidental interstitial myocarditis. Even though this patient had some atypical features (middle age, mostly indoors), histopathology coupled with the clinical picture was highly suspicious of canine dysautonomia. This is a progressive degenerative polyneuropathy of the autonomic nervous system most often reported in Europe and the Midwest United States with no known etiology.

Tuesday, November 2, 2021

8:30 a.m. – 8:40 a.m. CDT

### **GERMAN SHORTHAIRED POINTER DOGS WITH EXFOLIATIVE CUTANEOUS LUPUS ERYTHEMATOSUS DEVELOP IMMUNE-COMPLEX MEMBRANOUS GLOMERULONEPHROPATHY**

Hayley Amerman<sup>1</sup>, Rachel Cianciolo<sup>2</sup>, Margret Casal<sup>1</sup>, Elizabeth Mauldin<sup>1</sup>

<sup>1</sup>University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA, USA, <sup>2</sup>The Ohio State University College of Veterinary Medicine, Columbus, OH, USA

**Background:** German shorthaired pointer dogs (GSHP) with exfoliative cutaneous lupus erythematosus (ECLE), a rare recessive condition due a *UNC93B1* gene mutation, develop kidney disease that resembles lupus nephritis in humans. **Objective:** To characterize the kidney disease by light microscopy and electron microscopy in a population of GSHP dogs with ECLE. **Methods:** Medical records were reviewed and microscopic features (H&E, PAS, trichrome) of kidneys from 7 GSHP dogs with ECLE

were evaluated. Transmission electron microscopy of kidney from 1 dog was performed. **Results:** 5/7 dogs had proteinuria quantitated by either urine dipstick or urine protein to creatinine ratio (UPC); 2 dogs were not proteinuric. 2/7 dogs were hypoalbuminemic and none were azotemic. Histologic findings included early (2 dogs) to late (5 dogs) membranous glomerulonephropathy characterized mild to severe glomerular capillary loop thickening and tubular proteinosis. In all 7 cases, trichrome staining revealed small red granular immune deposits on the subepithelial surface of the glomerular basement membrane. Electron microscopy demonstrated subepithelial electron-dense immune deposits encircled by remodeled glomerular basement membrane. These findings are diagnostic of immune complex membranous glomerulonephropathy (MGN). **Conclusion:** This cohort of GSHP dogs with ECLE developed immune-complex MGN which is likely a manifestation of systemic lupus erythematosus. GSHP dogs with ECLE should undergo clinical evaluation of renal function for early identification and treatment.

Tuesday, November 2, 2021

8:40 a.m. – 8:50 a.m. CDT

### **A CASE OF SENNA PLANT (CASSIA OCCIDENTALIS, CASSIA OBTUSIFOLIA) POISONING IN A 4-MONTH-OLD HEIFER**

Ji-Hang Yin<sup>1</sup>, Leanne Dillard<sup>2</sup>, David Martinez Rodriguez<sup>3</sup>, Manuel Chamorro Ortega<sup>3</sup>, Russell Cattley<sup>1</sup>

<sup>1</sup>Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA, <sup>2</sup>Department of Animal Sciences, Crop, Soil, and Environmental Sciences, Auburn University,, Auburn, AL, USA, <sup>3</sup>Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL, USA

**Introduction:** Senna plant poisoning in cattle is a rapidly progressive disease with monophasic, multifocal myocyte degeneration and necrosis. Ingestion of the beans of the senna plant (*Cassia occidentalis* or *Cassia obtusifolia*) is considered a main route for intoxication. The entire plant is toxic and seeds are considered the most toxic part. The toxic principle has remained uncertain.

**Objectives:** To describe a case of Senna plants intoxication in cattle in the United States, which has been rarely reported.

**Methods:** A 4-month-old heifer was presented for a history of sternal recumbency, absent patellar and withdrawal reflexes, as well as unwillingness to move. Two other calves in the same herd exhibited similar clinical signs, and senna plant (*C. occidentalis* and *C. obtusifolia*) were found in the pasture. The heifer was humanely euthanized. Laboratory testing, and macroscopic and microscopic examinations were performed.

**Results:** Serum chemistry levels of skeletal muscle enzyme were higher than the normal ranges. Creatine phosphokinase (CK) was 83224 U/L (normal range 40-264 U/L) and aspartate aminotransferase (AST) was 2547 U/L (normal range 69-112 U/L). At necropsy, multifocal areas of skeletal muscles were extensively pale tan and dry. Senna seeds were observed in the rumen contents. On histology, over 90% of the myofibers in the affected skeletal muscles had a severe monophasic myonecrosis.

**Conclusion:** This case demonstrated the clinical presentation and pathologic findings of severe Senna plant poisoning in a 4-month-old heifer.

Tuesday, November 2, 2021

8:50 a.m. – 9:00 a.m. CDT

**AFLATOXIN INDUCED HEPATOTOXICITY FROM COMMERCIAL DRY DOG FOOD**

David Rotstein<sup>1</sup>, Kathleen Proia<sup>2</sup>, Sarah Peloquin<sup>2</sup>, Mark Glover<sup>1</sup>, Lee Anne Palmer<sup>1</sup>, Lauren Carey<sup>1</sup>, Angelica Jones<sup>2</sup>, Amanda Willey<sup>1</sup>, Justin Henson<sup>1</sup>

<sup>1</sup>FDA Center for Veterinary Medicine, Rockville, MD, USA, <sup>2</sup>FDA Center for Veterinary Medicine Veterinary Laboratory Investigation and Response Network, Laurel, MD, USA

**Background:** Microbial, chemical, and toxic animal food hazards can result in animal illnesses and deaths. FDA's Center for Veterinary Medicine (FDA CVM) conducts surveillance and investigation when animal food is potentially contaminated. Investigations involve surveillance, scientific and legal support, and coordination by the Office of Surveillance and Compliance (OSC), animal diagnostic testing by FDA CVM Veterinary Laboratory Investigation and Response Network (Vet-LIRN), and facility inspection by the FDA Office of Regulatory Affairs (ORA). We report on an investigation of two reports of animal illnesses and deaths from contaminated pet food.

**Objective:** To provide investigational findings in dogs exposed to aflatoxin through consumption of a commercial dry dog food.

**Methods:** Vet-LIRN laboratories performed necropsies and food analysis. OSC coordinated recall and case investigations. ORA conducted inspection at the commercial dry dog food facilities.

**Results:** Report 1 involved 6 dogs with elevated liver enzymes; 3 died or were euthanized. Report 2 involved multiple canine illnesses and death. Dogs exhibited jaundice and hemorrhage. Dogs were exposed to the same dog food brand. Histopathologic findings from 2 dogs included hepatocellular microlipidosis, necrosis, and regeneration as well as biliary hyperplasia. Pet food had elevated levels of aflatoxin B1 and B2. Product traceback identified a single facility was the contamination source. A large recall ensued and at inspection, the firm was cited for inadequate quality control protocols.

**Conclusions:** In this incident, the collaboration between diagnostic labs and FDA CVM Vet-LIRN led to removal of contaminated animal food highlighting the importance of a cooperative approach.

Tuesday, November 2, 2021

9:00 a.m. – 9:10 a.m. CDT

**SIMPLIFIED CARDIOPATHOLOGY PROTOCOL FOR EVALUATION OF THE CANINE HEART**

Kathleen Kelly

Penn State University, University Park, PA, USA



Canine sudden death, which is non-violent, non-traumatic, often instantaneous, and unexpected death, is infrequent but challenging to evaluate postmortem as it is associated with a variety of causes. In addition to the unexpected death of a beloved companion, sudden death is devastating for owners, veterinarians, and pathologists as there are often limitations in definitively determining a cause of death. Sudden death without an apparent postmortem correlate are “autopsy negative” and may be related to arrhythmogenic disorders. Existing protocols are comprehensive but require a relatively advanced level of familiarity with cardiac anatomy as well as numerous slides onerous in the diagnostic setting. A reproducible protocol for the systematic evaluation of the canine heart was designed for the diagnostic laboratory setting minimizing histology slide number. The protocol is a hybrid of myocardial bread-loaf and route of blood flow evaluation which leaves intact landmarks for evaluation of the conduction system. Following gross evaluation, sections of longitudinal sinoatrial node, longitudinal atrioventricular node, right ventricular free wall, left ventricular wall including papillary muscles, mid-chamber interventricular septum, and aorta were collected. This systemic cardiac evaluation revealed incidental (mild aortic coarctation, mild myocardial fibrosis, aortic osseous mineralization) and findings of undetermined significance (adipositas cordis) in a small group of dogs with a history of sudden death. This protocol can be applied the investigation of sudden/anesthetic death and characterization of suspected or confirmed cardiac arrhythmia, potentially in concert with advanced molecular testing to improve understanding of canine cardiac health.

Tuesday, November 2, 2021

9:10 a.m. – 9:20 a.m. CDT

### **VETERINARY FORENSIC POSTMORTEM STANDARDS**

Beverly McEwen<sup>1</sup>, Adam Stern<sup>2</sup>, Sean McDonough<sup>3</sup>, Tabitha Viner<sup>4</sup>, Rebecca Kagan<sup>4</sup>, Jason Brooks<sup>5</sup>, Ali Brower<sup>6</sup>

<sup>1</sup>College of Medicine, University of Florida, Gainesville, FL, USA, <sup>2</sup>College of Veterinary Medicine, University of Florida, Gainesville, FL, USA, <sup>3</sup>College of Veterinary Medicine, Cornell University, Ithaca, NY, USA, <sup>4</sup>US Fish and Wildlife Service, National Forensics Laboratory, Ashland, OR, USA, <sup>5</sup>The Pennsylvania State University, University Park, PA, USA, <sup>6</sup>College of Veterinary Medicine, Midwestern University, Glendale, AZ, USA

**Background:** Veterinary forensic postmortems are performed by veterinary pathologists and veterinary clinicians. At the 2019 International Veterinary Forensic Sciences Association (IVFSA) conference and business meeting, a motion was passed to establish standards for veterinary forensic postmortems. An ad hoc committee of 7 board-certified veterinary pathologists was struck to develop these minimum standards.

**Objective:** Veterinary forensic postmortem standards were developed to provide a process and framework to veterinarians for the postmortem examination of animal remains, and to provide a reference for legal or law enforcement professionals.

**Methods:** A survey series of itemized activities and processes of the veterinary forensic postmortem based on published human forensic autopsy standards and the veterinary forensic necropsy was completed by the pathologists. Teleconference discussions followed, and items that were agreed upon by consensus were included in the proposed

standards.

**Results:** The veterinary forensic postmortem standards document provides minimum standards for veterinarians who perform forensic postmortem examinations. Included standards cover preliminary procedures, evidence documentation, external and internal postmortem examination and documentation, lesion and injury descriptions, ancillary tests, and the postmortem examination report.

**Conclusions:** Opinions and interpretations of a forensic case made by a veterinarian must be formulated after consideration of all available information; this document lists all the information the veterinarian should consider. Most veterinarians and veterinary pathologists will exceed these minimum performance standards and are encouraged to do so.

Tuesday, November 2, 2021

9:20 a.m. – 9:30 a.m. CDT

**PATTERNS OF INJURY IN DOGS CAUSED BY VEHICULAR AND NON-ACCIDENTAL BLUNT FORCE TRAUMA**

Teresa Southard, Ayla Musciano, Sean McDonough  
Cornell University, Ithaca, NY, USA

Two of the most common causes of blunt force trauma in dogs are motor vehicle impact and non-accidental injury inflicted by humans (kicking, stomping, hitting with some sort of implement). Injuries sustained from vehicular and non-accidental trauma can be similar and include body wall contusions, fractured bones (ribs, skull, long bones, vertebrae), and visceral damage (pulmonary contusions, liver fractures). When no history is available or when the historian is unreliable, veterinary clinicians and pathologists may have to distinguish between accidental and vehicular trauma based on the type, location, and severity of the injuries. The purpose of this study was to determine whether there are differences in the patterns of injury caused by vehicular trauma and non-accidental injury using data from historical cases and from a cadaver study. In the cadaver study, six Pit Bull type dogs euthanized for other reasons were subjected to head (blow from a baseball bat) and thoracic (kick, stomp, blow from a baseball bat) trauma, and the resulting injuries were documented. Each mechanism of trauma was repeated using a force plate to determine the amount of force generated. The results suggest that paravertebral fractures are more common in non-accidental trauma while long bone fractures and mid-body rib fractures are more common in vehicular trauma. This study also provides a rough estimate of the amount of force required to cause rib and skull fractures in large dogs.

Tuesday, November 2, 2021

9:30 a.m. – 9:40 a.m. CDT

**FOREIGN BODY, GASTRIC CHORISTOMA, AND ATYPICAL CYSTIC JEJUNAL MASS IN A DOG**

Rachel Neto, Kaleigh Bush, Jessica Ball, Brad Matz  
College of Veterinary Medicine, Auburn University, Auburn, AL, USA

Choristomas are defined as well-differentiated mature tissue situated in an abnormal location in the body. Assorted heterotopic tissues have been sporadically reported in domestic animals and humans, including gastric choristomas, which are rare entities occurring at any level of the alimentary tract. An 11-year-old, castrated male, Shih Tzu dog was referred to the Teaching Hospital's Oncology Service for evaluation of a suspected mid-abdominal mass with history of frequent vomiting and weight loss. A cavitated mural jejunal mass was found on ultrasound. The mass was excised and submitted for histopathology. A multilocular, firm, 5 x 4.5 x 4 cm mass focally expanded the jejunal wall, compressing the lumen. A foreign body (artificial nail) was embedded within a partially disrupted wall, at the level of the mass. Histologically, a mural chronic jejunitis with granulation tissue was at the penetrating site, accompanied by multifocal tubular glands lined by chief, foveolar, and parietal cells within the mucosa, recapitulating gastric fundic tissue. The mass consisted of communicating ectatic cysts lined by cytokeratin-positive cuboidal, attenuated, or foveolar-like cells with variable dysplasia adjacent to patchy mural inflammation. No genuine malignant transformation was appreciated, and a compound diagnosis of penetrating injury secondary to foreign material with heterotopic gastric fundic mucosa and aberrantly lined cystic jejunal mass was concluded. Gastric choristomas are typically solitary findings, but progression to adenocarcinoma has been documented in the dog. This patient had an uneventful recovery and no pertinent clinical signs have been documented throughout the four months after surgery.

Tuesday, November 2, 2021

9:40 a.m. – 9:50 a.m. CDT

### **PROGNOSTIC CLINICAL AND HISTOPATHOLOGICAL FEATURES OF CANINE CUTANEOUS T-CELL LYMPHOMA**

Martina Dettwiler<sup>1</sup>, Elizabeth Mauldin<sup>2</sup>, Sara Jastrebski<sup>2</sup>, Deborah Gillette<sup>2</sup>, Darko Stefanovski<sup>3</sup>, Amy Durham<sup>2</sup>

<sup>1</sup>Institute of Animal Pathology, Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland, <sup>2</sup>Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA, <sup>3</sup>Department of Clinical Studies, New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA, USA

**Background/Objective.** Canine cutaneous epitheliotropic T-cell lymphoma (CETCL) is a polymorphic neoplasm arising in aged dogs that typically carries a poor prognosis. This study evaluates parameters associated with response and survival in a large group of dogs.

**Methods.** Clinical data and tissues were collected from 176 cases from the University of Pennsylvania and University of Bern (2012-2018). Histopathological evaluation used whole-slide digitized HE slides and QuPath software.

**Results.** 173 dogs were euthanized/died due to CETCL-related diseases. Cases included 107 females and 69 males, and mean age of 10.4 years. Of 48 breeds, Gordon Setters had the highest prevalence proportion (Prevalence:5.7%, 95%CI:1-15.7%). Clinical signs include erythema (n=131), crusting (n=108) and scaling (n=102). Affected

sites were haired skin (n=159), lip (n=74), nose (n=49) and paw pads (n=48). Median survival time (MST) was 95 days (1–850). Dogs had a 4.26-fold and 2.87-fold longer MST when treated with chemotherapy and prednisone, respectively, compared to dogs receiving supportive care.

In univariate analysis of parameters, haired skin involvement (HR:1.9, P=0.02), erosions/ulcerations (HR:1.7, P=0.001), crusting (HR:1.4, P=0.03), and nodules (HR:1.4, P=0.05) were significantly associated with shorter survival. Pharmacological treatment (HR:0.47, P≤0.001) and post-therapeutic partial (HR:0.39, P≤0.001) or complete (HR:0.47, P≤0.001) remission were associated with longer survival. Panniculus infiltration (HR:1.7-2.9 degree-dependent, P<0.03), coarse chromatin (HR:1.6, P=0.03), mitotic count ≥ 6.7/HPF (HR:2.9, P=0.000), cell diameter ≥ 10.0 mm (HR:2.0, P=0.005), and nuclear diameter ≥ 8.3 mm (HR: 3.7, P=0.002) were significantly associated with poorer outcomes.

Conclusions. Clinical and histomorphological parameters are associated with outcomes in dogs with CETCL.

Tuesday, November 2, 2021

9:50 a.m. – 10:00 a.m. CDT

**IMMUNOHISTOCHEMICAL EXPRESSION OF CD30 AND MULTIPLE MYELOMA ONCOGENE/INTERFERON REGULATORY FACTOR 4 (MUM1/IRF-4) IN CANINE CUTANEOUS NON-EPITHELIOTROPIC NULL CELL LYMPHOMA AND A SUBSET OF CANINE NON-EPITHELIOTROPIC CUTANEOUS B AND T CELL LYMPHOMA**

Nicholas Vetter, Jeanine Peters-Kennedy

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

Canine non-epitheliotropic cutaneous lymphoma (C-NECL) can be B-cell, T-cell, inflamed T-cell, or null cell. Null cell lymphoma (NCL) can be difficult to diagnose with routine B and T cell markers. NCL or anaplastic large cell lymphomas (ALCL) in people are characterized by large pleomorphic cells that express CD30. Although MUM1/IRF-4 is routinely used in the diagnosis of plasma cell tumors, it can be expressed in other round cell tumors such as histiocytomas and lymphoma. We hypothesized that both CD30 and MUM1/IRF-4 would be expressed in a subset of C-NECL and that CD30 expression would be beneficial in confirming a diagnosis of canine cutaneous NCL when B and T cell markers were negative. Using immunohistochemistry, we applied CD30 and MUM1/IRF-4 along with a routine panel of leukocyte markers (CD3, CD20, Pax5, IBA1) to 55 C-NECL cases. Thirty-four of 55 were non-inflamed, non-null NECL with 28 T-cell and 6 B-cell. Overall, 23/55 (42%) cases were CD30+, 28/55 (51%) were MUM1/IRF-4+ and 20/55 (36%) were CD30/MUM1/IRF-4+. Twelve of 55 (22%) were NCL, 10/12 (83%) were CD30+/ MUM1/IRF-4+, 11/12 (92%) were CD30+, and 1/12 was MUM1/IRF-4+ (CD30 was not performed). Two of 9 canine inflamed T-cell NECL cases were CD30+/MUM1/IRF-4+. These results support the hypothesis that CD30 is commonly expressed in C-NECL, specifically NCL. Interestingly, most cases of NCL were also MUM1/IRF-4+ as were a large subset of both T and B cell NECL. Ultimately, these results support the notion that multiple immunohistochemical markers are necessary to confirm an accurate diagnosis of C-NECL.

Tuesday, November 2, 2021  
10:00 a.m. – 10:10 a.m. CDT

### **AN INTRANEURAL PERINEURIOMA IN A DOG**

Ji-Hang Yin<sup>1</sup>, Brittani Sexton<sup>2</sup>, Maninder Sandey<sup>1</sup>

<sup>1</sup>Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA, <sup>2</sup>Department of Clinical Sciences, Neurology and Neurosurgery service, College of Veterinary Medicine, Auburn, AL, USA

Perineurioma is an extremely rare intraneural tumor in human and veterinary medicine. Histologically, neoplastic cells characteristically form multiple small “onion bulbs”, consisting of concentric layers of perineural cells ensheathing a central axon. Prognosis in animals remained uncertain with scarce case reports.

A 3-year-old, male castrated Beagle dog presented to Auburn University Veterinary Hospital with a 2-week history of a continued and progressive worsening of mid-lumbar pain and decreased postural reactions as well as reflexes of the pelvic limbs. Magnetic resonance imaging showed a left intradural-extramedullary mass spanning from the 5<sup>th</sup> to 7<sup>th</sup> lumbar vertebrae and severely compressing the spinal cord. Given that a cure would unlikely, the owner elected euthanasia due to quality of life concerns. At necropsy, the left spinal root containing the lumbar spinal nerves of 4<sup>th</sup> to 6<sup>th</sup> was enlarged and contained a well-demarcated, nonencapsulated, firm, tan, 2-cm x 0.5-cm x 0.5-cm, mass. Histopathology revealed a nonencapsulated, infiltrative, densely cellular neoplasm expanding and effacing the left nerve roots. The neoplasm was composed of fusiform cells arranged in a concentric lamellations ensheathing a central axon and formed pseudo-onion bulbs. Positive immunolabeling for S-100 antigen was only observed in the central core of nerve fibers. Taken together, the anatomic location, characteristic histopathological features, including pseudo-onion bulbs pattern as well as the negative immunolabeling for S-100 staining of the cell processes, a diagnosis of canine intraneural perineurioma was made.

Tuesday, November 2, 2021  
10:10 a.m. – 10:20 a.m. CDT

### **SALIVARY GLAND CARCINOMA IN A BUCKING BULL**

Kayla Alexander, Timothy Morgan, Brittany Baughman

Mississippi State University College of Veterinary Medicine, MS State, MS, USA

A 6-year-old bucking bull was presented to Mississippi State University-CVM for recurrence of an abscess at the angle of the right mandible following treatment one month prior. Due to the aggressive nature of the bull, exploration under general anesthesia was elected. The bull went into cardiopulmonary arrest at induction and was submitted for necropsy. Gross findings included a 15x15 cm encapsulated mass with a caseonecrotic center. Adjacent musculature was dry and friable with dark purple discoloration that extended from the thoracic inlet to the base of the right horn. The right paracondylar process was lytic. The tracheobronchial lymph nodes were markedly enlarged. Approximately 75-80% of the pulmonary parenchyma was consolidated and mottled dark red to purple with multifocal to coalescing, pinpoint to 3 cm, firm to caseous, white-yellow

nodules. Histologically, pulmonary and cervical masses were arranged into lobules, nests, and islands embedded within expansive fibrous stroma. Neoplastic cells had abundant eosinophilic finely granular cytoplasm and large round nuclei with coarsely stippled to vesiculated chromatin and variable nucleoli. Anisocytosis and anisokaryosis were marked. Mitotic figures averaged 16 per 2.37 mm<sup>2</sup>. Comedonecrosis and tumor embolization were frequently appreciated. Neoplastic cells demonstrated strong perimembranous immunolabeling with cytokeratin, and lobular stromal cells demonstrated perimembranous immunolabeling with smooth muscle actin ( $\alpha$ -SMA). Based on histopathologic findings, salivary carcinoma was diagnosed. Salivary neoplasms are rare in all veterinary species; however, tumors are more common in aged animals and typically develop in the parotid and mandibular glands. Histology and immunohistochemistry are key in diagnosing this disease.

Tuesday, November 2, 2021

10:20 a.m. – 10:30 a.m. CDT

### **AMELOBLASTIC FIBRO-ODONTOMA IN A BULL**

Kaylin McNulty, Brittany Baughman

Mississippi State University, Starkville, MS, USA

#### Case Report:

An approximately 2-year-old Wagyu bull is presented to Mississippi State University for a 3-week history of swelling of the right mandible. Radiographs show a severe monostotic aggressive bony lesion. The bull was non-responsive to treatment, and euthanasia was elected.

The right mandible from premolar 1 to molar 3 is expanded by a hard mass measuring 20x15x10cm. The overlying gingiva contains a large, ulcerated area with impacted feed material. The teeth are displaced. On cut surface, the lesion contains numerous cavitated spaces, occasional gelatinous areas, and multifocal hard, white irregular foci.

Microscopically, the multi-lobulated, expansile mass effaces mandibular bone and is composed of neoplastic odontogenic epithelial cells within a dense ectomesenchymal background. Neoplastic cells have scant eosinophilic cytoplasm and a small round to ovoid, usually basally located, nucleus (resembling odontoblasts). In the center of select lobules, neoplastic cells have fine stellate cytoplasm (resembling stellate reticulum). Occasionally, the mass contains scattered irregular foci of hypereosinophilic material (dentin) with usually adjacent dark purple material (enamel) and scattered hemorrhage/hemosiderophages. The overlying gingival epithelium is ulcerated to hyperplastic.

#### Discussion:

This right mandibular mass represents an ameloblastic fibro-odontoma. These dental tumors contain neoplastic odontogenic epithelium, induced ectomesenchyme, and form

dentin and enamel. Ameloblastic fibro-odontomas have been described in the dog, horse, and ox. Although they are rare in all domestic species, they are the most common odontogenic tumor in cattle. This neoplasm is not reported to metastasize but is slowly and progressively locally aggressive if not surgically removed.

Tuesday, November 2, 2021

10:30 a.m. – 10:40 a.m. CDT

### **BOVINE PAPULAR STOMATITIS IN AN ANGUS COW WITH METASTATIC JEJUNAL ADENOCARCINOMA**

Silvia Carnaccini<sup>1</sup>, Marcia Ilha<sup>1</sup>, Yung-Yi Mosley<sup>1</sup>, Hemant Naikare<sup>1</sup>, Ross Weaver<sup>2</sup>, Janemarie Hennebelle<sup>2</sup>

<sup>1</sup>University of Georgia, Tifton, GA, USA, <sup>2</sup>Georgia Department of Agriculture, Atlanta, GA, USA

Bovine papular stomatitis is a disease caused by bovine papular stomatitis virus, family Poxviridae, genus Parapoxvirus, which often causes oral lesions indistinguishable from other transboundary/foreign animal diseases. Intestinal adenocarcinomas are rare in bovine and often associated with bracken fern, heavy use of certain fertilizers, or papillomavirus. This is a case of metastatic intestinal adenocarcinoma in an adult Angus bovine with concurrent infection by bovine papular stomatitis virus (BPSV).

**Objective:** Identify the etiology of the oropharyngeal ulcers and metastatic disease.

**Methods:** PCR and sequencing were conducted on sections of oropharyngeal mucosa and tongue. Histopathology was performed on multiple organs. Field investigation was conducted to determine presence of predisposing causes and identify other affected animals. **Results:** Sequences shared 100% identity to bovine papular stomatitis virus (Genbank ID: GQ902053.1). Oral lesions were also weakly positive for Infectious bovine rhinotracheitis virus by PCR. Bovine viral diarrhea virus (BVDV), foot-and-mouth disease (FMD) virus, vesicular stomatitis virus (VSV) Indiana-1 (IND1), and VSV New Jersey (NJ) were not detected. The jejunum was effaced by an invasive proliferation of atypical epithelial cells arranged in poorly formed acini (adenocarcinoma) surrounded by a marked desmoplastic response. Visceral nodules consisted of neoplastic epithelial cell proliferations similar to the ones described for the jejunum (metastatic disease).

**Conclusion:** The study confirmed infection by bovine papular stomatitis virus and allowed excluding other transboundary/foreign animal diseases. The diagnosis of metastatic jejunal adenocarcinoma was confirmed histologically. Field investigation could not identify the presence of other affected animal within the herd nor the presence of carcinogens.

Tuesday, November 2, 2021

10:40 a.m. – 10:50 a.m. CDT

### **NOVEL PAPILOMAVIRUS ASSOCIATED WITH CUTANEOUS AND MUCOSAL PAPILOMAS IN A POLAR BEAR (URSUS MARITIMUS)**

Anna-Maria Travis<sup>1</sup>, Jennifer Luff<sup>2</sup>, Mandy Womble<sup>2</sup>, Riley Wilson<sup>3</sup>, Elise LaDouceur<sup>1</sup>

<sup>1</sup>Joint Pathology Center, Silver Spring, MD, USA, <sup>2</sup>NC State College of Veterinary Medicine, Raleigh, NC, USA, <sup>3</sup>The Pet Shop, Anchorage, AK, USA

**Background:** Papillomaviruses represent a diverse group of double stranded, DNA viruses that are associated with papillomas in many carnivore species.

**Objective:** Describe the pathologic and molecular findings of papillomas in a polar bear.

**Methods:** A cutaneous biopsy was obtained from an adult, captive polar bear with dozens of hairless, pedunculated to sessile, 0.2- to 3-cm-diameter papillomas on the haired skin (muzzle and lips) and mucosa (lips). Samples were placed in 10% neutral buffered formalin, processed routinely, sectioned at 5  $\mu$ m, and stained with hematoxylin and eosin. Genomic DNA was extracted from two, 25  $\mu$ m scrolls cut from the paraffin block. PCR was performed using three different degenerate primer pairs that amplify the L1 gene from a variety of different papillomavirus types.

**Results:** Histologically, the papillomas consisted of exophytic papillary projections of proliferative epidermis overlying thin fibrovascular stalks. The epidermis was hyperplastic with prominent spinous and granular layers with variable cytopathic effects and abundant hyperkeratosis. Two of the primer sets yielded amplicons, which were purified and sequenced. A 446-bp fragment was identified and compared to the nucleotide collection at the National Center for Biotechnology Information using BLAST. The sequence was most similar to other papillomaviruses, but it shared less than 70% nucleotide identity to any known papillomavirus sequence, which is consistent with a putatively novel polar bear papillomavirus.

**Conclusions:** There is one previously reported polar bear papillomavirus, which was associated with a glossal papilloma and is not genetically closely related to the virus described here.

Tuesday, November 2, 2021  
10:50 a.m. – 11:00 a.m. CDT

### **DIFFUSE LARGE B CELL LYMPHOMA AND A NOVEL GAMMAHERPESVIRUS IN NORTHERN ELEPHANT SEALS**

Margaret Martinez<sup>1</sup>, Emily Trumbull<sup>1,2</sup>, Emily Whitmer<sup>1</sup>, Michelle Rivard<sup>1</sup>, Nicole Stacy<sup>3</sup>, Cara Field<sup>1</sup>, Linda Archer<sup>3</sup>, James Wellehan Jr<sup>3</sup>, Salvatore Frasca Jr<sup>4</sup>, Padraig Duignan<sup>1</sup>  
<sup>1</sup>The Marine Mammal Center, Sausalito, CA, USA, <sup>2</sup>SeaWorld, San Antonio, TX, USA, <sup>3</sup>Department of Comparative, Diagnostic, and Population Medicine, University of Florida, Gainesville, FL, USA, <sup>4</sup>Connecticut Veterinary Medical Diagnostic Laboratory, Department of Pathobiology and Veterinary Science, University of Connecticut, Storrs, CT, USA



Two male northern elephant seal (NES) pups were admitted to The Marine Mammal Center and treated for malnutrition. Complete blood count showed rapid progression of marked leukocytosis characterized by a predominance of large monomorphic mononuclear cells suggestive of lymphoid origin with flower-shaped nuclei. Both seals were euthanized due to suspected neoplasia. At necropsy most lymph nodes were markedly enlarged, some with distinct white nodules, and the intestinal mucosae were thickened. On histopathology, the lymph nodes were effaced by sheets of neoplastic round cells. Individual neoplastic round cells had little cytoplasm and a single nucleus 2x the diameter of an erythrocyte. Similar neoplastic cells expanded the intestinal mucosa and bone marrow of both pups and the thymus, spleen, skin and urocytic mucosa of one pup. Immunohistochemistry revealed most of the pleomorphic round cells had CD79a, CD20 and PAX5 labeling. PCR, sequencing, and phylogenetic analysis of partial DNA polymerase gene identified a novel gammaherpesvirus from affected tissues. Based on the canine World Health Organization (WHO) classification system, this neoplasm was diagnosed as a diffuse large B cell lymphoma-immunoblastic subtype. This is the first description of B cell lymphoma with leukemic manifestation and detection of a novel gammaherpesvirus in free-living NESs. Further research regarding the prevalence of this new gammaherpesvirus and its association with pathology in NESs are necessary as this may represent a newly identified or emerging disease.

Tuesday, November 2, 2021  
11:00 a.m. – 11:10 a.m. CDT

### **MYCOTIC PNEUMONIA AND ENCEPHALITIS IN AN ADULT ALPACA WITH THIRD COMPARTMENT ULCERATION AND CHEILITIS**

Marvin Firth, Laura Setyo, Nicola Parry  
University of Surrey, Guildford, United Kingdom

A 22-month-old male alpaca was presented for post-mortem examination to the Veterinary Pathology Centre, University of Surrey (Guildford, UK), after reported rapid weight loss and weakness over a two-week period. Macroscopic post-mortem assessment revealed a focally extensive, full-thickness ulceration of the third compartment; approximately 20% of the pulmonary parenchyma had multifocal to coalescing petechiae and haemorrhage; and within the oral cavity, the gingiva, and soft and hard palates had multifocal variably sized full thickness ulcers. Subsequent histological investigation revealed a severe ulcerative and necrotising gastritis, a severe multifocal to coalescing necrotising embolic encephalitis, pneumonia and cheilitis. All lesions contained intralesional fungal hyphae that were up to 7  $\mu$ m wide and variably septate, with parallel walls and dichotomous, acute angle branching. Fungal culture of frozen tissue identified *Aspergillus* spp. Polymerase chain reaction for bovine viral diarrhoea virus (BVDv) was negative. The primary causes of depression and rapid weight loss in this animal were considered to be a combination of respiratory compromise due to the severe pneumonia and endotoxic shock associated with the ulcerative gastritis of the third gastric compartment. To the authors' knowledge, this is the first report of ulcerative gastritis of the third gastric compartment with associated embolic pneumonia and encephalitis in an alpaca. Although the exact origin of the

infection is unknown in this case, possible portals of entry could include either the oral cavity or an area of gastric ulceration.

Tuesday, November 2, 2021

11:10 a.m. – 11:20 a.m. CDT

### **ADENOVIRUS INFECTION IN RED-TAILED HAWKS (*BUTEO JAMAICENSIS*) AND A BROAD-WINGED HAWK (*BUTEO PLATYPTERUS*)**

Emma Torii<sup>1</sup>, Arno Wuenschmann<sup>1</sup>, Anibal Armien<sup>2</sup>, Sunil Mor<sup>1</sup>, Rahul Kumar<sup>1</sup>, Emma Chalupsky<sup>3</sup>, Michelle Willette<sup>3</sup>

<sup>1</sup>University of Minnesota, Veterinary Diagnostic Laboratory, St. Paul, MN, USA,

<sup>2</sup>University of California Davis, California Animal Health and Safety Laboratory

(CAHFS), Davis, CA, USA, <sup>3</sup>University of Minnesota, The Raptor Center, St. Paul, MN, USA

**Background:** Adenovirus in raptors are best described in falcons, with only a few cases reported in owls and a single case in a hawk. In raptors, adenoviral infection is most commonly characterized by necrotizing hepatitis and splenitis.

**Methods:** Five red-tailed hawks (*Buteo jamaicensis*) and a broad-winged hawk (*Buteo platypterus*) had an adenoviral infection based on history, histopathology, negative electron microscopy, and polymerase chain reaction (PCR). Additionally, sequencing of the hexon genes were performed in all but one case.

**Results:** All six birds had acute onset illness resulting in death. Microscopically, all birds had solitary, pale eosinophilic to amphophilic, intranuclear inclusion bodies within hematopoietic cells (bone marrow) and macrophages (spleen), and 5/6 birds had similar inclusions within hepatocytes and Kupffer cells. All but one bird had severe bone marrow necrosis. There was moderate splenic necrosis in 4/6, and mild to marked hepatic necrosis in 4/6 birds. Adenoviral particles were detected in bone marrow, liver, and/or spleen by electron microscopy in all birds. All were PCR positive for adenovirus in bone marrow, liver, spleen, and/or intestinal contents. Based on sequencing, three cases were clustered within the *Siadenovirus* genus and two cases were clustered within the *Aviadenovirus* genus.

**Conclusions:** This case series expands on the limited knowledge of adenovirus infections in hawks. The presence of splenic and hepatic necrosis and particularly the hitherto previously unreported bone marrow necrosis suggests that the infection is clinically relevant and potentially fatal in hawks.

Tuesday, November 2, 2021

11:20 a.m. – 11:30 a.m. CDT

### **AN OUTBREAK OF YERSINIA PSEUDOTUBERCULOSIS IN AFRICAN LIONS (*PANTHERA LEO*) WITH ABERRANT BACTERIAL MORPHOLOGY**

Mandy Womble, Megan Cabot, Tara Harrison, Tatiane Terumi Negrão Watanabe  
North Carolina State University College of Veterinary Medicine, Raleigh, NC, USA

Two African lions (*Panthera leo*) died secondary to infection with *Yersinia pseudotuberculosis* at a zoological park in central North Carolina following a 3-5 day duration of clinical signs including respiratory distress, lethargy, ataxia, and hyporexia. The lions were submitted for postmortem examination and had similar gross and

histologic findings. Macroscopically, throughout the hepatic and splenic parenchyma, there were multifocal to coalescing, semi-firm, pale tan nodules. The lungs were non-collapsed with multifocal petechial hemorrhagic foci. Microscopic examination revealed multifocal to coalescing necrotic foci with associated fibrinosuppurative cellular infiltrate in the liver, spleen, lungs, kidneys, and mesenteric lymph nodes with abundant intralosomal gram-negative bacteria. Aerobic bacterial culture of the liver, spleen, and lung of the male lion and the liver of the female lion revealed 4+ growth of *Y. pseudotuberculosis*. The observed bacterial morphology on histologic examination varied between the two lions ranging from large aggregates of coccobacilli to large rod-shaped and filamentous bacteria. Similar aberrant forms of *Y. pseudotuberculosis* have been previously described in squirrel monkeys following antibiotic administration. The source of infection with *Y. pseudotuberculosis* was not identified in this outbreak but transmission through contaminated water, soil, raw meat, or predation of wild bird or rodent reservoirs is possible. Mortality associated with *Y. pseudotuberculosis* has previously been described in an African lion cub; however, to our knowledge, this is the first report of *Y. pseudotuberculosis* infection in adult African lions with aberrant bacterial morphology.

Tuesday, November 2, 2021  
11:30 a.m. – 11:40 a.m. CDT

### **RENAL COCCIDIOSIS CAUSED BY NEPHROISOSPORA EPTESICI IN A CAPTIVE BIG BROWN BAT (EPTESICUS FUSCUS)**

Nathan Crilly, Sarah Poynton, Kathleen Gabrielson  
Johns Hopkins School of Medicine, Baltimore, MD, USA

**Background:** An adult male big brown bat presented for necropsy after being found dead two months after being captured in Maryland for use in a research colony.

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

**Methods:** Gross and histological examination was performed. H&E, Periodic acid-Schiff, and Fielgen stains were used to characterize renal parasites.

**Results:** On gross examination, there were four cystic structures in the cortex of the right kidney, containing pale tan, turbid fluid. On histological section, these cortical cysts were identified as cystic renal tubules, lined by hypertrophic and hyperplastic renal tubular epithelium containing multiple life stages of coccidian parasites. The cyst lumina contained numerous oocysts. These renal parasites were putatively identified as *Nephroisospora eptesici*.

**Conclusions:** To our knowledge, this is the first description of renal coccidiosis in a big brown bat in Maryland. Renal coccidiosis is caused by the coccidian parasite *N. eptesici*, which was first described in wild bats from Minnesota in 2010. *N. eptesici* is most closely related to *Besnoitia* spp., and has a single-host life cycle, with the complete life cycle occurring in the kidney. Renal coccidiosis is thought to be an incidental postmortem finding, which is not associated with clinically significant renal disease, although it may interfere with research.

Tuesday, November 2, 2021  
11:40 a.m. – 11:50 a.m. CDT

## **GROSS AND HISTOLOGIC LESIONS ASSOCIATED WITH NATURAL RABIES VIRUS INFECTION IN FREE-RANGING WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) IN THE EASTERN UNITED STATES (2005-2021)**

Alisia Weyna<sup>1</sup>, Mark Ruder<sup>1</sup>, Martha Frances Dalton<sup>2</sup>, Charlie Bahnson<sup>3</sup>, M. Kevin Keel<sup>4</sup>, Heather Fenton<sup>5</sup>, Jennifer Ballard<sup>6</sup>, Nicole Nemeth<sup>1</sup>

<sup>1</sup>Southeastern Cooperative Wildlife Disease Study, University of Georgia, Athens, GA, USA, <sup>2</sup>Mississippi Veterinary Research and Diagnostic Laboratory, Pearl, MS, USA,

<sup>3</sup>North Dakota Game and Fish Department, Bismarck, ND, USA, <sup>4</sup>University of California-Davis, Davis, CA, USA, <sup>5</sup>Ross University, Basseterre, Saint Kitts and Nevis,

<sup>6</sup>Arkansas Game and Fish Commission, Little Rock, AR, USA

**Background:** White-tailed deer (*Odocoileus virginianus*) are a widespread game species across North America and often share habitat with humans and domestic animals. Neurological deer are of high visibility and interest due to concerns regarding the spread of chronic wasting disease.

**Objective:** We describe demographic and diagnostic data from free-ranging white-tailed deer with rabies from across the southeastern U.S.

**Methods:** We reviewed diagnostic reports of white-tailed deer cases at the Southeastern Cooperative Wildlife Disease Study for those diagnosed with rabies from 2000-2021.

**Results:** Nine cases of rabies were diagnosed in white-tailed deer from 2005-2021. Rabies was confirmed via immunohistochemistry and/or fluorescent antibody testing. Seven (78%) of these deer were female; two (22%) were male; all were adults and came from five eastern states. Three (33%) deer were found dead and six (67%) were euthanized for abnormal behavior. Gross examination of the head of six deer revealed combinations of severe, skin changes in 6/6 deer including: forehead/periorbital alopecia, cutaneous erythema, abrasions and ulcers, and subcutaneous edema (especially periorcular). Histology was performed for 8/9 cases, all of which had intraneuronal, eosinophilic, intracytoplasmic inclusion bodies (consistent with Negri bodies) in the cerebrum and/or cerebellum. Most (6/8; 75%) had minimal to moderate, perivascular, lymphocytic or lymphoplasmacytic encephalitis. In 4/4 tested cases, the eastern raccoon rabies virus variant was identified.

**Conclusions:** Rabies should be considered a differential diagnosis for neurological disease in white-tailed deer, especially if skin lesions suggestive of head rubbing/pressing are evident. Histological lesions in the brain may be extremely subtle.

## Experimental Disease Focused Scientific Session

Tuesday, November 2, 2021 | 1:30 p.m. – 1:45 p.m. CDT

Tuesday, November 2, 2021

1:30 p.m. – 1:45 p.m. CDT

### **PULMONARY IONOCYTES ARE SPATIALLY LOCALIZED TO CARTILAGINOUS AIRWAYS IN LUNGS USING BARTTIN IMMUNOHISTOCHEMISTRY**

David Meyerholz, Lei Lei, Ian Thornell, Guillermo Romano Ibarra, David Stoltz, Paul McCray Jr.

University of Iowa, Iowa City, IA, USA

Ionocytes were recently identified as a novel, rare cell type in the lung through single cell RNA sequencing analysis of human airway epithelium. Pulmonary ionocytes are a discrete airway lineage that arises from basal cells and express the transcription factor Foxi1. Ionocytes are speculated to regulate airway surface liquid volume and composition due to the selective enrichment and co-expression of ion channel transcripts such as CFTR and CIC-K/barttin. To better understand the potential physiologic role(s) of ionocytes, we evaluated their spatial localization in the airway tree. We studied ionocyte localization and distribution in paraffin-embedded lung tissues using immunohistochemistry for the marker barttin. In humans and pigs, barttin immunostaining was detected in the trachea and bronchi, but absent in bronchioles and alveoli. Cellular immunostaining was scattered and seen primarily in the surface epithelium of cartilaginous airways with uncommon extension into submucosal gland ducts. Serous and mucous cells in acini and tubules of the submucosal glands were devoid of immunostaining. At the cellular level, barttin immunostaining was enriched on the basolateral membrane with cytoplasmic staining also observed. Our study suggests that ionocytes are preferentially localized to cartilaginous airways. This limited distribution in the airway tree parallels the localization pattern seen for submucosal glands of the lung, perhaps highlighting the regional importance of liquid regulation for homeostasis of mucociliary clearance and other host defense mechanisms. All experiments on human and animal tissues were institutionally approved.

Tuesday, November 2, 2021

1:45 p.m. – 2:00 p.m. CDT

### **ALPHA-HEMOLYSIN-MEDIATED VASCULAR INJURY AND CUTANEOUS HYPOXIA CONTRIBUTE TO THE DEVELOPMENT OF STAPHYLOCOCCUS AUREUS-INDUCED DERMONECROSIS**

Ching Yang<sup>1,2</sup>, Frank Robledo-Avila<sup>1</sup>, Santiago Partida-Sanchez<sup>1,3</sup>, Christopher Montgomery<sup>1,3</sup>

<sup>1</sup>Center for Microbial Pathogenesis, Abigail Wexner Research Institute at Nationwide Children's Hospital, Columbus, OH, USA, <sup>2</sup>Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA,

<sup>3</sup>Department of Pediatrics, College of Medicine, The Ohio State University, Columbus, OH, USA

**Background:** *Staphylococcus aureus* is a Gram-positive bacterium and the most common cause of skin and soft tissue infection in humans. The pathognomonic lesion of

cutaneous necrosis (dermonecrosis) in mouse models is thought to be the consequence of staphylococcal  $\alpha$ -hemolysin (Hla)-mediated cytotoxicity to the keratinocytes. However, Hla is also toxic toward endothelial cells, and the sequence of toxin-mediated cellular events during infection is not fully understood. We hypothesized that Hla compromises cutaneous vasculature and causes segmental ischemia leading to the development of cutaneous necrosis. **Objective:** To determine the mechanism by which dermonecrosis develops during *S. aureus* skin infection. **Methods:** BALB/c mice received control serum, Hla-neutralizing antiserum, or an inhibitor of Hla receptor (ADAM10 inhibitor) followed by subcutaneous infection by *S. aureus*. Expression of cleaved caspase-3, vascular endothelial (VE)-cadherin, epithelial (E)-cadherin, and Hypoxyprobe-1 in the cutaneous lesions using immunohistochemistry and immunofluorescence were evaluated at 3-, 6-, and 24-hours post-infection (hpi). **Results:** Hla induced endothelial apoptosis at 6 hpi followed by apoptosis in keratinocytes at 24 hpi. This was supported by the loss of VE-cadherin expression preceding the loss of E-cadherin expression. Hla induced hypoxia in the epidermis at 24 hpi after the observation of vascular lesions. Treatment with Hla-neutralizing antibody or ADAM10 inhibitor attenuated early cleavage of VE-cadherin, cutaneous hypoxia, and dermonecrosis. **Conclusions:** Our results demonstrated that Hla-mediated early vascular endothelial apoptosis with the loss of VE-cadherin contributed to cutaneous hypoxia and subsequent dermonecrosis. These findings suggest that bacterial exotoxin-mediated vascular injury with cutaneous ischemia underlies the pathogenesis of *S. aureus*-induced dermonecrosis.

Tuesday, November 2, 2021

2:00 p.m. – 2:15 p.m. CDT

### **TARGETING THE STEROL REGULATORY ELEMENT-BINDING PROTEIN PATHWAY IN PANCREATIC DUCTAL ADENOCARCINOMA**

Stephanie Myers, Meredith McGuire, Wei Shao, Chune Liu, Theodore Ewachiw, Zeshaan Rasheed, William Matsui, Toni Sepalla, Richard Burkhart, Peter Espenshade  
Johns Hopkins University, School of Medicine, Baltimore, MD, USA

**Background:** Pancreatic ductal adenocarcinoma (PDAC) is a very aggressive tumor with limited diagnostic and therapeutic options. Due to its proliferative nature and desmoplastic stroma, tumor cells are challenged with meeting a high demand for lipids in a hypoxic, lipid-poor environment. Cancer cells respond to this demand through sterol regulatory element-binding proteins (SREBPs), which are master transcriptional regulators of lipid homeostasis that require SREBP cleavage activating protein (SCAP) during signaling.

**Methods:** Using four patient-derived PDAC cell lines, *SCAP* was knocked out. All cell lines were utilized in functional growth assays in lipid-variable conditions, subcutaneous xenograft, and orthotopic xenograft experiments. A well-established PDAC mouse model, *LSL-Kras*<sup>G12D/+</sup>; *LSL-Trp53*<sup>R172H/+</sup>; *Pdx-1 Cre* (KPC), was utilized, and KPC mice lacking *Scap* in one or both alleles were generated. In all four cell lines, the following

FDA-approved drugs were applied individually and in combination: Dipyridamole, Fluvastatin, and Simvastatin.

**Results:** In lipid-poor conditions, *SCAP* knockout cells showed significantly reduced growth. In tumor xenograft models, *SCAP* knockout cells exhibited reduced tumor growth and tumor volume. KPC mice with a heterozygous loss of *Scap* exhibited a significantly increased median survival time. In combination, Dipyridamole with either statin demonstrate synergy in lipid-poor conditions.

**Conclusions:** Loss of *SCAP* in PDAC tumor cells alters the growth capability both *in vitro* and *in vivo*. Heterozygous loss of *Scap* in the KPC mouse model significantly increased survival. Finally, Dipyridamole works in synergy with statins to alter growth of tumor cells. These findings suggest that targeting the SREBP pathway has significant therapeutic potential in pancreatic cancer.

Tuesday, November 2, 2021

3:30 p.m. – 3:45 p.m. CDT

### **DEVELOPING A DEEP LEARNING CONVOLUTIONAL NEURAL NETWORK METHOD TO DETECT SMALL INTESTINE PATHOLOGY IN RAT ILEUM**

Lauren Prince<sup>1</sup>, Jogile Kuklyte<sup>2</sup>, Daniel Sammon<sup>2</sup>, Christiane Löhr<sup>3</sup>, Daniel Rudmann<sup>1</sup>

<sup>1</sup>Charles River Laboratories Inc., Ashland, OH, USA, <sup>2</sup>Deciphex, Dublin, Ireland,

<sup>3</sup>Oregon State University, Corvallis, OR, USA

**Background:** The rat is an important animal model in assessing intestinal injury after administration of drugs suspected to have radiomimetic effects. Histologic scoring methods have been used historically in the evaluation of intestine in both standard short- and long-term toxicology studies. These scoring methods are qualitative, subjective, and prone to inter- and intra-study and observer variability.

**Objective:** Develop a deep learning method using a convolutional neural network (CNN) to facilitate small intestinal lesion detection and scoring for pathologists

**Methods:** A CNN was trained to identify normal structures and four classes of lesions in rat ileum: Cell degeneration/necrosis in the mucosa or Peyer's patches, decreased cellularity in Peyer's patches, regeneration/mitoses in epithelium of the mucosa, and villous atrophy. Ileum samples from rats were sectioned, stained with hematoxylin and eosin, digitally scanned at 40x, and uploaded into a cloud server. Training annotations were completed based on a strict ground truth established by two trained scientists.

**Results:** Model performance was first assessed by comparing model masks with the original training annotations (verification step) using both visual confirmation and confusion matrix calculations. When the CNN performance reached a F value of at least 0.7, additional qualification was done using new samples from the original study (testing set) and other samples from studies not used for training (generalization). In both cases,

the performance of the algorithm was compared to annotations made by pathologists not involved in model development.

**Conclusions:** Qualification data suggest that the CNN model will be an effective decision support tool for pathologists.

Tuesday, November 2, 2021

3:45 p.m. – 4:00 p.m. CDT

### **MORBIDITY AND MORTALITY MANAGEMENT OF A NEW ZEALAND WHITE RABBIT MODEL OF STEROID-INDUCED OSTEONECROSIS OF THE FEMORAL HEAD**

Kerriann Casey, Felicity Gore, José Vilches-Moure, Masahiro Maruyama, Stuart Goodman, Yunzhi Yang, Samuel Baker  
Stanford University School of Medicine, Stanford, CA, USA

Steroid-induced osteonecrosis of the femoral head (SONFH) is a condition documented in humans and animals exposed to chronic steroid administration. The rabbit has become a preferred animal model for investigating the pathogenesis and treatment of SONFH due to its shared femoral vascular anatomy with human patients, relative size of the femoral head, and general fecundity. However, morbidities and mortalities are frequently encountered during the steroid induction period (i.e. prior to surgical manipulation) and are poorly reported and described within the literature. Herein, we report the clinical, gross, and histopathologic findings of New Zealand White (NZW) rabbits undergoing the steroid induction phase of the SONFH model. Severe weight loss (>30%), lipemia, hypercholesterolemia, hyperglycemia, and elevations in ALT and AST were consistent findings across rabbits and did not differentiate asymptomatic rabbits from those that became clinically symptomatic or experienced mortality. Euthanized and spontaneously deceased rabbits exhibited hepatomegaly, hepatic lipidosis/glycogenosis, and hepatocellular necrosis in addition to a lipid-rich and proteinaceous thoracic effusion. A subset of rabbits developed opportunistic *Bordetella bronchiseptica* and *Escherichia coli* pulmonary infections and/or small intestinal *Lawsonia intracellularis* infections superimposed on hepatic and thoracic disease. Together, these findings allowed for establishment of a clinical decision-making flowchart resulting in reduced morbidities and mortalities in a subsequent cohort of SONFH rabbits. Recognition of these model-associated morbidities is critical for providing optimal clinical care during the steroid induction phase of SONFH.

Tuesday, November 2, 2021

4:00 p.m. – 4:15 p.m. CDT

### **FETOSCOPIC IN UTERO TRACHEAL OCCLUSION AS A METHOD TO REDUCE FETAL LUNG HYPOPLASIA IN A SHEEP MODEL OF CONGENITAL DIAPHRAGMATIC HERNIA**

Renata Mammone<sup>1</sup>, Dae Young Kim<sup>1</sup>, Pamela Adkins<sup>1</sup>, John Dodam<sup>1</sup>, Emanuel Vlastos<sup>2</sup>, John Middleton<sup>1</sup>

<sup>1</sup>University of Missouri Veterinary Medical Diagnostic Laboratory, Columbia, MO, USA,

<sup>2</sup>University of Missouri, Kansas City, MO, USA



**Background:** Congenital diaphragmatic hernia (DH) is a potentially fatal malformation that occurs in 1/2500 human births. Pulmonary hypoplasia decreases survival rate due to compression of thoracic contents by abdominal viscera. Previous studies have shown that induction of congenital high airway obstruction syndrome (CHAOS) by fetoscopic insertion of a tracheal occlusion (TO) device *in utero* reduces fetal lung hypoplasia. Current methods use balloons or foam plugs with complications including pressure necrosis of the trachea, decreased type II pneumocytes, and post-natal complications due to difficulty removing the device.

**Objective:** Use a fetal lamb DH model to evaluate TO with an FDA-approved endovascular device.

**Methods:** Forty-three pregnant ewes with 45 fetuses were enrolled. Hysterotomy was performed in 25 ewes at ~75 d gestation to create a DH in the fetus. At ~110-120 d gestation, 26 fetuses had a TO device fetoscopically placed and 19 served as non-occluded controls. The device was successfully removed by fetoscopy from 8/23 fetuses at ~130-140 d gestation. Around lambing, lambs were euthanized for necropsy. In total, 37 lambs (n=7, no DH and no device placed; n=8, no DH, device placed but not removed; n=4, no DH, device placed, device removed; n=7 DH, no device placed; n=7, DH, device placed, device not removed; n=4, DH, device placed, device removed) completed the study. Tracheal necrosis, ulceration, and inflammation and lung inflammation were measured.

**Results:** Preliminary results showed no detectable differences between groups. All animals with a TO device developed CHAOS.

**Conclusion:** The endovascular device can be used for tracheal occlusion.

Tuesday, November 2, 2021

4:15 p.m. – 4:30 p.m. CDT

**INFLUENZA NEURAMINIDASE VIRUS-LIKE PARTICLE VACCINE REDUCES DISEASE, VIRUS REPLICATION AND LUNG PATHOLOGY IN SWINE**

Vasilis Pliastas<sup>1</sup>, Zach Menne<sup>2</sup>, Virginia Aida<sup>1</sup>, Ji-Hang Yin<sup>1</sup>, Maria Naskou<sup>1</sup>, Peter Neasham<sup>1</sup>, James North<sup>1</sup>, Dylan Wilson<sup>1</sup>, Sheniqua Glover<sup>1</sup>, Katharine Horzmann<sup>1</sup>, Ioanna Skountzou<sup>2</sup>, Constantinos Kyriakis<sup>1,3</sup>

<sup>1</sup>Auburn University, Auburn, AL, USA, <sup>2</sup>Emory University, Atlanta, GA, USA, <sup>3</sup>University of Georgia, Athens, GA, USA

**Background:** Influenza A viruses (IAV) are among the most significant viral pathogens of humans and animals. Seasonal influenza epidemics and the sporadic emergence of novel zoonotic IAV necessitate the development of a broadly protective universal influenza vaccine. Neuraminidase (NA), an IAV antigenic protein often underutilized in commercial influenza vaccines, has the potential to elicit broad heterologous immune responses.

**Objectives:** The goal of this study was to assess the protective efficacy of a recently developed N2 VLP vaccine platform in the swine model as a candidate universal influenza vaccine.

**Methods:** A total of 18 influenza-seronegative piglets were used in the study. They were divided into 3 groups and were prime-boost vaccinated with a 3-weeks interval with the N2 VLP vaccine, containing the NA protein from the A/Perth/16/2009 (H3N2) strain, a commercial swine IAV vaccine, or adjuvant only, respectively. Pigs were intranasally challenged four weeks post-boost with A/sw/NC/KH1552516/2016, an H3N2 swine IAV field isolate. Amino acid homology between the vaccine and challenge NA was 90.9%. Vaccine-induced protection was evaluated based on five parameters, (i) cellular immune responses, (ii) cytokine profile at euthanasia (day 5), (iii) virus titers in tissue homogenate samples, (iv) Bronchoalveolar lavage fluid (BALF) cytology, and (v) respiratory tract histopathology.

**Results:** Although neither vaccine induced sterilizing protection, the NA VLP construct significantly reduced pulmonary virus titers, BALF neutrophilic infiltration, and pulmonary histopathology compared to unvaccinated controls.

**Conclusions:** This study demonstrates that the NA VLP platform performed comparatively to the commercial vaccine and induced robust protection against heterologous challenge.

Tuesday, November 2, 2021

4:30 p.m. – 4:45 p.m. CDT

### **A SEMI-QUANTITATIVE APPROACH TO THE ANALYSIS OF SARS-COV-2 INDUCED PNEUMONIA IN THE RHESUS MACAQUE.**

Rachel Reader, Katherine Olstad, Koen Van Rompay, Amir Ardeshir  
UC Davis, Davis, CA, USA

The rhesus macaque was characterized early in the pandemic as a model of mild to moderate pneumonia induced by SARS-CoV-2 infection. Since then, they have been used as an important model for vaccine and therapeutic studies. However, there have been challenges establishing a standard approach to sample collection and scoring that both accurately characterize the lesions and predict a treatment effect. The virus is often introduced intranasally and intra-tracheally, producing multifocal to locally extensive randomly scattered lesions without clear demarcation. In addition, the larger size of macaque lungs led to challenges when trying to assess the extent and severity of disease in relation to intervention outcomes. Thus, examination of 1 or 2 sections could not be considered representative.

A semi-quantitative scoring system was developed to evaluate the lesions throughout the lungs of SARS-CoV-2 infected macaques. Initially a wide range of parameters were considered prior to selecting interstitial disease as the most relevant lesions to predict a treatment effect during acute infection. A systematic approach to lung sampling was also developed to determine the extent of the total lung affected.

The scoring approach was then used to demonstrate differences in the extent and severity of interstitial pneumonia between different treatment interventions. This analysis is most relevant to studies investigating the efficacy of a post-infection therapeutic intervention on reducing histological lung lesions, and is less applicable for prophylactic studies. If the infection is prevented, differences between groups are more dramatic (ie. lesions vs no lesions).

Tuesday, November 2, 2021

4:45 p.m. – 5:00 p.m. CDT

### **CLINICAL AND PATHOLOGIC FEATURES OF A FELINE SARS-COV-2 INFECTION MODEL ARE ANALOGOUS TO ACUTE COVID-19 IN HUMANS**

Jennifer Rudd<sup>1</sup>, Miruthula Tamil Selvan<sup>1</sup>, Shannon Cowan<sup>1</sup>, Eva Kao<sup>1</sup>, Cecily Midkiff<sup>2</sup>, Jerry Ritchey<sup>1</sup>, Craig Miller<sup>1</sup>

<sup>1</sup>Department of Veterinary Pathobiology, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, USA, <sup>2</sup>Division of Comparative Pathology, National Primate Research Center, Tulane University, Covington, LA, USA

**Background.** The emergence and ensuing dominance of COVID-19 on the world stage has emphasized the urgent need for efficient animal models to develop novel therapeutics and assess immune responses to SARS-CoV-2 infection. This study validates a feline model for SARS-CoV-2 infection that results in clinical disease and histopathologic lesions consistent with severe COVID-19 in humans.

**Methods.** Twelve age- and sex-matched domestic cats were intratracheally inoculated with  $1.26 \times 10^6$  TCID<sub>50</sub> SARS-CoV-2, isolate USA-WA1/2020. Animals were evaluated twice daily for changes in weight, temperature, activity, behavior, respiratory effort, ocular/nasal discharge, and coughing/wheezing. At 4- and 8-days post-inoculation, SARS-CoV-2 infected cats (n=6 per timepoint) were humanely euthanized and necropsied to collect tissue samples for histologic examination, immunohistochemistry (IHC), and molecular analyses.

**Results.** Intratracheal inoculation of SARS-CoV-2 caused infected cats to develop clinical disease consistent with that observed in the early exudative phase of COVID-19. A clinical scoring system for feline respiratory disease was developed, documenting significant lethargy, fever, dyspnea, and dry cough in infected cats. Histopathologic pulmonary lesions such as diffuse alveolar damage, hyaline membrane formation, fibrin deposition, and proteinaceous exudates were observed during SARS-CoV-2 infection, replicating lesions identified in hospitalized COVID-19 patients with ARDS. Viral loads and ACE2 expression were quantified in nasal turbinates, trachea, lung, and other organs, and there was a significant correlation between the degree of clinical disease and pulmonary lesions in infected cats.

Conclusion. Natural ACE2 expression, paired with clinical and pathologic correlates between this feline model and human COVID-19, encourage use of this model for future translational studies.

## **Industrial and Toxicologic Pathology Focused Scientific Session**

Tuesday, November 2, 2021 | 2:30 p.m. – 2:40 p.m. CDT

Tuesday, November 2, 2021

2:30 p.m. – 2:40 p.m. CDT

### **ASSESSMENT OF GENOTOXIC DNA DAMAGE IN DOGS FROM A HIGH POLLUTED AREA IN ITALY**

Davide De Biase, Valeria Baldassarre, Giuseppe Piegari, Ilaria d'Aquino, Francesco Prisco, Serenella Papparella, Orlando Paciello

Department of Veterinary Medicine and animal production, University of Naples "Federico II", Naples, Italy

In the last decades, many concerns have raised about the adverse effects of environmental contaminants on human population. Dogs represent a warning sentinel for human health because they share the human environment and respond to many toxic insults in ways analogous to humans. The aim of this work was to standardize and validate methods to monitor environmental damage through the evaluation of biological markers such as genotoxic DNA damage by Comet assay and Micronuclei test and the immunocytochemical expression of iNOS as a marker for oxidative stress. We conducted a cross-sectional study employing exposed dogs living in a shelter in a high polluted area in Campania region (Italy). The study was conducted with the approval of the Ethics Committee of the University of Naples (PG/2021/0030881). The study population included 15 clinically healthy dogs, aged 4 to 10 years old, randomly sampled, with a minimum two-year presence in the shelter. The control group consisted of 5 healthy dogs living in a less polluted nearby area. Blood samples were collected for Comet assay and immunocytochemistry for iNOS evaluation. Epithelial buccal cells were collected for micronuclei count. The results were compared with environmental pollution data. The expression of iNOS and genotoxic DNA damage were significantly higher in dogs from polluted area compared to controls. Our data show that dogs living in highly polluted areas have a higher risk to develop genotoxic DNA damage. Moreover, we suggest that iNOS expression, comet assay and micronuclei test may be reliable tool to assess environmental-related pathology.

Tuesday, November 2, 2021

2:40 p.m. – 2:50 p.m. CDT

### **DEVELOPMENT OF A NOVEL DECISION SUPPORT TOOL FOR DETECTION OF CARDIAC ABNORMALITIES IN THE RAT USING PATHOLYTIX AI**

T. William O'Neill<sup>1</sup>, Jogile Kuklyte<sup>2</sup>, Daniel Sammon<sup>2</sup>, Christiane Lühr<sup>3</sup>, Daniel Rudmann<sup>1</sup>

<sup>1</sup>Charles River Laboratories, Ashland, OH, USA, <sup>2</sup>Deciphex, Dublin, Ireland, <sup>3</sup>Oregon State University, Corvallis, OR, USA

**Introduction:** The fast workflow of toxicologic pathology can be more efficiently managed with decision support tools that highlight possible lesions to the reviewing pathologist.

**Objective:** To develop a decision support tool for detection of cardiac pathology in the rat.

**Methods:** We annotated a variety of lesions in digital, whole slide images (WSI) of H&E stained sections captured at 40x equivalent magnification of rat hearts with varying pathology. Patholytix AI, a deep learning, artificial intelligence platform utilizing convoluted neural networks, was deployed to automatically generate digital masks in WSI that highlight normal cardiac anatomy and possible areas of inflammation, fibrosis, valvular stromal proliferation, and cardiomyocyte necrosis/degeneration. The ground truth was established based on INHAND guidance and consultation with experts in the field. The model was assessed by examining confusion matrices, precision, sensitivity, and F1-scores with iterative training to improve scores and visual performance.

**Results:** The model is robust enough and accurately identified the majority of targeted lesions to provide a useful decision support tool. All normal and lesion classes perform with F1-scores above 0.70; cardiomyocyte degeneration/necrosis had the lowest F1-scores based on confusion with inflammatory infiltrate. However, despite confusion between lesions, the areas are identified as abnormal by the model, supporting the goal of decision support for the pathologist.

**Conclusions:** This tool will help toxicologic pathologists' efficiency in reviewing digital slides. Continuing work will focus on adding more lesion classes, ongoing improvement to the above metrics, and adaptation of the model to recognize cardiac lesions in other species.

Tuesday, November 2, 2021

2:50 p.m. – 3:00 p.m. CDT

## **DEVELOPMENT OF A NOVEL AI-BASED ALGORITHM FOR LESION DETECTION IN THE LUNG OF RATS**

Esther Crouch<sup>1</sup>, Jogile Kuklyte<sup>2</sup>, Daniel Sammon<sup>2</sup>, Daniel Rudmann<sup>1</sup>

<sup>1</sup>Charles River Laboratories, Wilmington, MA, USA, <sup>2</sup>Deciphex, Dublin, Ireland

**Background:** The evaluation of lung tissue is an important component of xenobiotic safety assessment. Toxicologic pathologists must identify pulmonary pathology related to xenobiotic administration, as well as background changes. Histologic assessment of these is time-intensive and subject to interobserver variability, therefore, strategies to improve reproducibility and efficiency are valuable.

**Objective:** The development of a deep learning artificial intelligence algorithm that will detect alveolar macrophage aggregates, and alveolar and perivascular infiltrates, with high sensitivity and specificity, providing decision support for the pathologist and increasing diagnostic quality and efficiency.

**Methods:** Whole slide images containing lung from control and rats administered test item were scanned at 40x magnification on a whole slide scanner and uploaded to a Patholytix Study Browser platform. Lung annotations were performed at 20X magnification for four normal and two abnormal classes (aggregates, alveolar macrophages, and infiltrate, mixed cell, perivascular/alveolar). A convolutional neural network (CNN) classifier was developed that produced a pixel segmentation mask that highlighted regions of interest. The classifier was qualified at the pixel level using confusion matrices and F1 scores derived from 15% of annotated data that was reserved for blinded validation.

**Results:** The CNN model identified normal pulmonary structures with 95% to 99% accuracy. The model accurately identified both abnormal tissue classes with 99% accuracy. All class F1 scores exceeded the minimal acceptable performance level of 0.70.

**Conclusion:** A novel CNN detects pulmonary lesions in rats.

Impact Statement: This work highlights the potential for a CNN to provide toxicologic pathologists decision support during microscopic pulmonary evaluation.

Tuesday, November 2, 2021

3:10 p.m. – 3:20 p.m. CDT

**DEVELOPMENT OF A DEEP LEARNING MODEL FOR THE QUANTIFICATION OF LEUCINE-RICH REPEAT KINASE 2 INHIBITOR-INDUCED PNEUMOCYTE FINDINGS IN A RAT MODEL**

Christiane Löhr<sup>1</sup>, Esther Crouch<sup>2</sup>, James Baily<sup>3</sup>

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**Background:** Inhibitors of leucine-rich repeat kinase 2 (LRRK2) activity hold promise as treatments for patients with Parkinson's disease. Vacuolation of pneumocytes has been described in animal models after oral administration of small molecule LRRK2 inhibitors. Pneumocyte vacuolation may be reversible but is currently interpreted as an adverse drug effect, and the manual quantification of these cells is both painstaking and subject to inter-observer variability. Therefore, strategies to reliably and reproducibly identify and quantify affected pneumocytes are important in the safety assessment of LRRK2 inhibitors.

**Objective:** Development of an image analysis-based, automated method for the quantification of vacuolated pneumocytes in whole slide images

**Methods:** A convolutional neural network (CNN)-based deep learning model was trained, by two investigators, on a commercial artificial intelligence-based image analysis platform (Aiforia®). Validation annotations, visual quality control of image analysis markups and model validity examination assessment were performed by two separate validators. The multi-CNN model was trained for semantic segmentation of alveolar tissue (lumen and septa) as the parent layer. A child layer, designed as object count, had vacuolated pneumocyte as the single feature. The model was developed in an iterative process of fine-tuning the ground truth and manual annotations, adding training annotations, and increasing CNN training iterations.

**Results:** As expected, the overall error rate of the model, compared to user-provided annotations, was low for alveolar tissue (area) and higher for vacuolated pneumocytes counts.

**Conclusions:** The automation of vacuolated pneumocyte quantification with the model will improve reproducibility and reduce time required for analysis.

Tuesday, November 2, 2021

3:20 p.m. – 3:30 p.m. CDT

### **DO FUNGICIDES INCREASE THE INCIDENCE OF EUROPEAN FOULBROOD DISEASE IN HONEY BEES DURING BLUEBERRY POLLINATION?**

Jenna Thebeau<sup>1</sup>, Dana Liebe<sup>1</sup>, Sarah Wood<sup>1</sup>, Allyssa Cloet<sup>1</sup>, Igor Moshynskyy<sup>1</sup>, Larhonda Sobchishin<sup>1</sup>, Ivanna Kozii<sup>1</sup>, Colby Klein<sup>1</sup>, Igor Medici de Mattos<sup>1</sup>, Michael Zabrodski<sup>1</sup>, Melanie Roulin<sup>1</sup>, Fatima Masood<sup>1</sup>, Brandele Brown<sup>1</sup>, Mateo Castano Ospina<sup>1</sup>, Lara Reitsma<sup>1</sup>, Jessica Debruyne<sup>1</sup>, Mohsen Sharafi<sup>1</sup>, Meghan Milbrath<sup>2</sup>, Geoff Wilson<sup>3</sup>, Marta Guarna<sup>4</sup>, Patricia Wolf Veiga<sup>5</sup>, Eric Gerbrandt<sup>6</sup>, Antonio Ruzzini<sup>1</sup>, Elemir Simko<sup>1</sup>

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The United States and Canada are the world's largest blueberry producers, with the majority of their blueberry crop dependent on honey bee (*Apis mellifera*) pollination. Recently, beekeepers have reported an increased incidence of European foulbrood (EFB) disease in their colonies during blueberry pollination.

EFB is a bacterial disease of honey bee larvae caused by *Melissococcus plutonius* which results in heightened larval mortality when colonies experience environmental or nutritional stress. One such stress is pesticide exposure during blueberry pollination which may predispose these colonies to EFB disease. The effects of exposure to formulated blueberry fungicides on the susceptibility of honey bee larvae to EFB is currently unknown.

To investigate the possible correlation between fungicide use and EFB, we employed an *in vitro* model of larvae infected with *M. plutonius* and tested the effects of chronic exposure to field-relevant concentrations of two blueberry fungicides, Captan and Kenja, on larval survival.

We found that exposure to Captan, Kenja or a combination of these two products did not result in a significant decrease in larval survival relative to infected controls. Instead, chronic exposure to Captan or Kenja during development significantly increased larval survival from EFB by 33% ( $P < 0.01$ ).

These *in vitro* results suggest that chronic exposure of honey bee colonies to Captan or Kenja during blueberry pollination should not predispose these colonies to EFB, although colony-level studies are imperative. Further *in vitro* experiments are underway to test the effect of additional fungicides and their combinations on the susceptibility of honey bee larvae to EFB.

Tuesday, November 2, 2021

3:40 p.m. – 3:50 p.m. CDT

### **THIAMETHOXAM TOXICITY IS DEPENDENT ON THE AGE AND CASTE OF HONEY BEES (*APIS MELLIFERA*)**

Ivanna Kozii, Sarah Barnsley, Marina Bezerra, Sarah Wood, Colby Klein, Igor de Mattos, Michael Zabrodski, Jenna Thebeau, Roney Silva, Claudia Fabela, Ihor Dvylyuk, Maud Ferrari, Elemir Simko

University of Saskatchewan, Saskatoon, SK, Canada

The honey bees are essential pollinators and their health is of global concern. Neonicotinoid insecticides have been implicated in the decreasing honey bee health worldwide. Regulatory toxicity assays focus predominantly on the worker honey bee (sterile females) toxicity, thus potentially overlooking the toxicities present in the queens and drones (reproductively active females and males respectively).

The objective of this study was to compare thiamethoxam (THI) toxicity, a commonly used neonicotinoid, between honey bee castes at different ages (larvae, newly emerged, young adult, and mature adult).

First, a single colony was manipulated to produce synchronized larvae and adult honey bees. The larvae were subjected to individual dietary exposure while adult bees of three age categories were subjected to individual contact exposure to control or incremental doses of THI.

Larval toxicity tests revealed honey bee queens to be most sensitive to THI exposure evidenced by over 60% decrease in emergence rate of queens compared to drones and workers post THI exposure. Conversely, adult queen survival was consistently higher or comparable to that of worker bees; queen survival was highest in newly emerged individuals. The drones were most sensitive to THI contact exposure.

Accordingly, the results of our study highlight that THI toxicity in honey bees is highly caste and age specific. Considering the wide contamination of the hive products with



agrochemicals we suggest that the age and caste specific toxicity assays should be employed in future toxicological screening tests to detect most vulnerable caste and age of the bees.

Tuesday, November 2, 2021

3:50 p.m. – 4:00 p.m. CDT

### **ARE DETOXIFICATION ENZYMES INDUCED SIMILARLY ACROSS HONEY BEE CASTES IN RESPONSE TO NEONICOTINOID EXPOSURE?**

Ivanna Kozii, Sarah Wood, Colby Klein, Michael Zabrodski, Jenna Thebeau, Melanie Roulin, Dana Liebe, Igor de Mattos, Mohsen Sharafi, Larhonda Sobchishin, Igor Moshynskyy, Maud Ferrari, Elemir Simko  
University of Saskatchewan, Saskatoon, SK, Canada

Neonicotinoid insecticides are implicated in decreasing honey bee health. Toxicity assays in honey bees focus on the worker caste, possibly overlooking toxicity to the queens and drones. Our previous study demonstrated that neonicotinoid toxicity is highly caste-specific. The objective of this study was to determine if there is a correlation between detoxification enzyme activity and the caste-specific susceptibility of honey bees to a commonly used neonicotinoid, thiamethoxam (THI), in response to larval, adult or combined larval and adult exposure.

We tested the induction of enzyme activity in bees in response to larval and/or adult THI exposure. Age-matched honey bee larvae received either water (control) or 25ng THI through larval food contamination. At emergence, adult bees in these groups were further subjected to contact exposure of incremental doses of THI. Activity of esterase, glutathione S-transferase (GST), and acetylcholine esterase (AChEst) were determined at emergence and 48 hours post contact exposure.

We found that enzyme activity is highly caste-specific; however, THI exposure during larval and/or adult stages did not have a significant effect on enzymes activity.

Enzyme activity was highest in worker bees, followed by drones, and then queens.

Enzyme activity of esterase, GST and AChEst does not correlate with the differential caste survival in response to THI exposure observed in our previous studies. However, our findings highlight that enzyme activity is highly variable between castes, which may affect their susceptibility to insecticide toxicity and should be considered in future toxicity studies.

Tuesday, November 2, 2021

4:00 p.m. – 4:10 p.m. CDT

### **MECHANISMS OF THE COXIELLA BURNETII WHOLE CELL VACCINE REACTOGENIC RESPONSE**

Alycia Fratzke<sup>1,2</sup>, Erin Van Schaik<sup>2</sup>, James Samuel<sup>1,2</sup>

<sup>1</sup>Texas A&M University, College Station, TX, USA, <sup>2</sup>Texas A&M Health Science Center, Bryan, TX, USA

*Coxiella burnetii* is the causative agent of Q fever. The only approved vaccine for humans, Q-VAX, a formalin-inactivated, whole cell vaccine (WCV), is not licensed in the United States due to the high rate of local and systemic reactions in previously sensitized individuals. A greater understanding of the immunological mechanisms responsible for these reactions is needed to produce safe and effective vaccines against *C. burnetii*. Our prior work showed that vaccine site reactions caused by *C. burnetii* WCV contain an influx of IFN $\gamma$ + and IL17a+ CD4 T cells. To further elucidate the immunopathogenesis of these reactions, we began by investigating the roles of CD4 T cells in WCV reactogenicity. Antibody-mediated depletion of CD4 T cells, but not CD8 T cells, in sensitized mice abrogated Cb reactogenic lesions and adoptive transfer of CD4 T cells from sensitized mice to naïve mice partially reproduced reactive lesions upon elicitation with WCV. We then went on to evaluate the roles of IFN $\gamma$  and IL17a in vaccine site reactions using antibody-mediated depletion of these cytokines during elicitation of hypersensitivity responses. Our results show that local *C. burnetii* WCV reactogenicity is a Th1/Th17-mediated hypersensitivity reaction. Understanding the immunopathogenesis of vaccine reactogenicity provides information essential for the development safe and effective novel vaccines.

Tuesday, November 2, 2021

4:10 p.m. – 4:20 p.m. CDT

### **CHARACTERIZATION OF RESISTANCE TO PRMT5 INHIBITOR THERAPY IN MANTLE CELL LYMPHOMA**

Mackenzie Long<sup>1,2</sup>, Shirsha Koirala<sup>2</sup>, Shelby Sloan<sup>2</sup>, Fiona Brown<sup>2</sup>, Kara Corps<sup>1</sup>, JoBeth Helmig-Mason<sup>2</sup>, Stacey Beck<sup>2</sup>, Ji-Hyun Chung<sup>2</sup>, Peggy Scherle<sup>3</sup>, Kris Vaddi<sup>3</sup>, Bradley Blaser<sup>2</sup>, Lapo Alinari<sup>2</sup>, Robert Baiocchi<sup>2</sup>

<sup>1</sup>The Ohio State University Department Of Veterinary Biosciences, Columbus, OH, USA, <sup>2</sup>The Ohio State University Wexner Medical Center, Columbus, OH, USA,

<sup>3</sup>Prelude Therapeutics, Wilmington, DE, USA

**Background:** Mantle cell lymphoma (MCL) is an incurable B-cell Non-Hodgkin's lymphoma. MCL patients who relapse on targeted therapies have a particularly poor prognosis. Protein arginine methyltransferase 5 (PRMT5) is an enzyme that drives symmetric dimethylation of histone arginine residues, is overexpressed in MCL and drives growth and survival. PRMT5 has emerged as an attractive therapeutic target in MCL. In collaboration with Prelude Therapeutics, we developed a small molecule PRMT5 inhibitor (PRT-382) that exhibits significant anti-MCL activity in cell lines (low nM range) and *in vivo* preclinical MCL murine models (10 mg/kg). Despite the anti-tumor activity of PRMT5 inhibition, we have observed some animals develop drug resistance leading to rapid progression of MCL. Multiple MCL cell lines show primary resistance to PRMT5 inhibition based on high IC50s of PRT-382. Prolonged culture of PRT-382 sensitive MCL lines with drug escalation produced acquired drug resistance that persists after prolonged culture (30d) in the absence of drug. **Objective:** Based on previously reported literature and our preliminary findings, this project initially evaluated the role of compensatory methylation by other PRMTs in the setting of PRMT5 inhibitor resistant MCL. **Methods/Results:** The efficacy of a type I PRMT inhibitor on PRMT5 inhibitor resistant and sensitive MCL cell lines was determined. **Conclusions:** No significant differential sensitivity to the type I PRMT inhibitor was observed between

PRMT5 inhibitor resistant and sensitive MCL cell lines. However, next generation sequencing technologies (scRNA-seq, RNA-seq, WES) has highlighted potential new targets for overcoming PRMT5 inhibitor resistance including PI3K signaling and MYC.

Tuesday, November 2, 2021

4:20 p.m. – 4:30 p.m. CDT

### **CANCER TREATMENT INDUCED COGNITIVE IMPAIRMENT AND NEUROINFLAMMATION IN MICE**

Kimberly Demos-Davies, Allison Rogich, Jessica Lawrence, Davis Seelig  
University of Minnesota, Saint Paul, MN, USA

**Background:** Cancer survivors are increasingly identified with a syndrome of neurocognitive dysfunction termed cancer-related cognitive impairment (CRCI). Side effects of cancer therapy (chemotherapy and radiation therapy) have been implicated in causing CRCI. CRCI has been best documented in breast cancer survivors. The exact mechanism for the distant brain injury following cancer treatment is unknown.

**Objective:** To investigate the underlying mechanisms by which doxorubicin, an anthracycline that is standard of care in breast carcinoma treatment, and/or extracranial radiation trigger clinical and neuroinflammatory effects of CRCI.

**Methods:** Eight to ten week old SKH1 mice were administered a battery of behavior tests before treatment and 7 days post-treatment. Mice were treated with a single dose of radiation to their right hindlimb, an intraperitoneal injection of doxorubicin or both. After euthanasia, plasma, spleen and brains were collected for cytokine and immune cell analysis by flow cytometry and immunohistochemistry for GFAP and Iba1.

**Results/Conclusion:** Mice treated with doxorubicin, radiation or both showed equivalent hippocampal dependent memory deficits compared to control mice in the novel location recognition behavior test. Neuroinflammation was evident in the brains in all three mice treatment groups compared to control mice. Mice treated with doxorubicin had a significant increase in IL-6 plasma levels and changes in splenic lymphocyte percentages compared to controls. In conclusion, both standard of care therapies used in breast cancer patients cause neuroinflammation and cognitive changes in mice. This is the first study to report that mice treated with extracranial radiation, chemotherapy or both have comparable cognitive impairment.

Tuesday, November 2, 2021

4:30 p.m. – 4:40 p.m. CDT

### **MODELING HUMAN PROSTATE CANCER METASTASIS IN MICE VIA RESECTION OF SUBCUTANEOUS ALLOGRAFTS**

Lauren Peiffer, Angelo De Marzo, Karen Sfanos, Janielle Maynard  
Johns Hopkins University School of Medicine, Baltimore, MD, USA

**Background:** More than 80% of patients with advanced prostate cancer develop bone metastases, however, bone metastases rarely occur spontaneously in murine prostate cancer models. Most prostate cancer metastatic models involve intracardiac or

intraosseous implantation of cancer cells, which bypass early stages of tumor invasion and metastasis.

**Objective:** Perform a pilot study to determine whether resection of subcutaneous allografts in immunocompetent mice results in spontaneous metastasis.

**Methods:** Three-month-old intact male FVB/NCrl mice (n=9) were inoculated subcutaneously with MyC-CaP cells. Tumors were surgically resected once they reached 1.5cm in any direction. After recovery, mice were monitored three times weekly for tumor recurrence. Animals were euthanized or died, and a full set of tissues were collected and fixed in 10% neutral buffered formalin for histopathologic examination, immunohistochemistry, and RNA in-situ hybridization.

**Results:** Tumors took an average of 44 days (range 23-61) to reach 1.5cm in any direction. All tumors were resectable. One mouse was euthanized due to multifocal dermatitis of unknown cause. The eight remaining mice were euthanized or died an average of 113 days (range 72-156) after tumor inoculation and 70 days (range 30-121) after tumor resection. Six mice developed tumor recurrence at the site of resection by 121 days post-resection and one mouse developed bone metastases positive for androgen receptor and human c-Myc. One mouse developed suspected metastases to the abdominal cavity (confirmatory stains pending).

**Conclusions:** Resection of subcutaneous allografts in mice extends time of tumor growth and results in tumor recurrence with metastases to bone and potentially the abdominal cavity.

## Natural Disease Focused Scientific Session II

Tuesday, November 2, 2021 | 1:30 p.m. – 1:45 p.m. CDT

Tuesday, November 2, 2021

1:30 p.m. – 1:45 p.m. CDT

### **PULMONARY LESIONS ASSOCIATED WITH A NOVEL EMMONSIELLOPSIS SPECIES INFECTION IN WOMBATS.**

Marina Gimeno<sup>1</sup>, Alexandra Berry<sup>1</sup>, Karrie Rose<sup>2</sup>, David Phalen<sup>1</sup>

<sup>1</sup>Sydney School of Veterinary Science, The University of Sydney, Camden, Australia,

<sup>2</sup>Australian Registry of Wildlife Health, Taronga Conservation Society Australia, Sydney, Australia

A dimorphic fungus has been reported in the lungs of all three species of wombats. The fungus was previously described as *Emmonsia parva*. However, a recent phylogenetic

study of fungi in the lungs of the northern hairy-nosed wombat (*Lasiorhinus krefftii*) found the organism to be a novel species of the geophilic genus *Emmonsiiellopsis*. Infections are usually accompanied by an interstitial pneumonia, but a cause-and-effect relationship between fungal infection and pneumonia has not been proven. In this study, we showed, using PCR and amplicon sequencing, that the *Emmonsiiellopsis* species identified in the northern hairy-nosed wombat was subsequently identified in the lungs of the southern hairy-nosed (*Lasiorhinus latifrons*) and bare-nosed wombat (*Vombatus ursinus*). Lung sections from 28 bare-nosed wombats and six southern hairy-nosed wombats were examined. Pulmonary lesions included variable degrees of interstitial pneumonia associated with increased numbers of interalveolar macrophages, giant cells and hyperplasia of the bronchus-associated lymphoid tissue (BALT). Fungal spores were frequently intrahistiocytic and found in both healthy and diseased lung. However, a significant association between the presence of fungal spores and interstitial pneumonia was found (P-value: 0.0016) and hyperplasia of the BALT was particularly prominent when there were large numbers of spores. This study presents the largest case series of *Emmonsiiellopsis* sp. infections in wombats to-date and provides evidence of its association with interstitial pneumonia in wombats. Furthermore, the study demonstrates that this fungus can, in some instances, cause lesions that would likely significantly impact the health and fitness of the infected animal.

Tuesday, November 2, 2021

1:45 p.m. – 2:00 p.m. CDT

### **CAPRINE GRANULOMATOUS MURAL FOLLICULITIS IS INFREQUENTLY ASSOCIATED WITH MALIGNANT CATARRHAL FEVER SUBGROUP MACAVIRUSES**

Thomas Westermann, Elena Demeter, Jeanine Peters-Kennedy  
Cornell University College of Veterinary Medicine, Ithaca, NY, USA

**Background:** Granulomatous mural folliculitis (GMF) is an uncommon reaction pattern, with occasional reported associations with malignant catarrhal fever (MCF) subgroup macaviruses in non-adapted ruminant hosts, including ovine herpesvirus-2 (OHV-2) in 2 goats and 1 bighorn sheep (*Ovis canadensis*), and caprine herpesvirus-2 (CpHV-2) in 2 sika deer (*Cervus nippon*).

**Objective:** Characterize caprine GMF and associated cutaneous lesions using histochemistry and immunohistochemistry and assess for associated herpesviruses using PCR and *in situ* hybridization (ISH).

**Methods:** Formal fixed paraffin-embedded (FFPE) tissues from 15 goats with and 9 without GMF underwent DNA extraction, OHV-2 qPCR, and pan-herpesvirus nested conventional PCR with sequencing. Sections from all goats with GMF were analyzed by routine histochemistry (H&E and GMS) and colorimetric ISH using RNAscope® targeting OHV-2. Immunohistochemistry (CD3, CD20, IBA1, and CKAE1/3) was performed on a subset of 7 cases.

**Results:** All animals with GMF presented with crusting dermatitis. Four (26.7%) of 15 goats with GMF were PCR positive for MCF-subgroup macaviruses, including MCF virus of white-tailed deer (MCFV-WTD) in 1 goat, and OHV-2 in 3 goats, 2 having positive OHV-2 ISH immunolabelling. Herpesvirus was not detected in any of the 9 goats without GMF. Lesions in all 15 GMF cases were comprised of macrophages, fewer T lymphocytes, and variably included eosinophils, multinucleated giant cells, and/or neutrophils.

**Conclusions:** This report describes the first association between MCFV-WTD and GMF in a goat and also demonstrates infrequent associations between MCF-subgroup macaviruses and GMF in goats, suggesting the involvement of other unknown causes.

Tuesday, November 2, 2021

2:00 p.m. – 2:15 p.m. CDT

### **PREVALENCE AND MICROSCOPIC FINDINGS OF PARELAPHOSTRONGYLUS TENUIS IN FREE-RANGING CERVIDS IN NORTH DAKOTA AND TENNESSEE**

Michelle Liu, Richard Gerhold, Nickolus Stahlman, Denae LoBato

University of Tennessee, Knoxville, TN, USA

*Parelaphostrongylus tenuis* (*P. tenuis*) rarely causes clinical disease within its natural host, the white-tailed deer (WTD). However, *P. tenuis* has been implicated as a contributing factor to neurological disease and population decline of other wild cervids throughout the eastern U.S. Here, we sought to determine the microscopic lesions and prevalence of *P. tenuis* in various free-ranging neurologic cervids. Whole brains from 20 free-ranging cervids (6 mule deer, 4 moose, and 1 WTD from North Dakota; 9 elk from Tennessee) with neurological signs were submitted from 2004-2020 to the University of Tennessee and subjected to microscopic exam and molecular testing for *P. tenuis*. Selected microscopic findings were graded for severity from 1-3. Immunohistochemistry for West Nile virus (WNV), Eastern equine encephalitis virus (EEE), and Epizootic hemorrhagic disease virus (EHD) was performed on select elk tissues. Of the 20 cervid brains examined, 12 (60%) had suspected *P. tenuis* infection, with all 6 mule deer affected. Microscopic findings supportive of *P. tenuis* infection were consistent with previous studies involving wild cervids. PCR for *P. tenuis* in suspected cases is pending. Five cervids (25%) had an undetermined cause of neurological disease, represented only by mononuclear meningitis or meningoencephalitis. Three cervids (15%), including the WTD, had no significant findings. Among the elk, immunoreactive animals included two for WNV, five for EEE, and none for EHD. *P. tenuis* should be considered a significant cause of morbidity and mortality in cervids in central and southeastern U.S. EEE and WNV are potential causes of neurological disease in elk.

Tuesday, November 2, 2021

2:15 p.m. – 2:30 p.m. CDT

### **MICROSCOPIC EVALUATION OF NECROSIS IN CANINE SOFT TISSUE TUMORS: ASSESSMENT OF INTER-PATHOLOGIST VARIATION USING WSI ANNOTATION**

Taryn Donovan<sup>1</sup>, Alexander Bartel<sup>2</sup>, Frauke Wilm<sup>3</sup>, Jean Rogers<sup>4</sup>, Frances Moore<sup>5</sup>, Donald Meuten<sup>6</sup>, Bruce Williams<sup>7</sup>, Rachel St.Vincent<sup>4</sup>, Robert Klopfleisch<sup>8</sup>, Christof Bertram<sup>9</sup>

<sup>1</sup>Department of Anatomic Pathology, Animal Medical Center, New York, NY, USA, <sup>2</sup>Institute for Veterinary Epidemiology and Biostatistics, Freie Universität Berlin, Berlin, Germany, <sup>3</sup>Pattern Recognition Lab, Computer Sciences, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany, <sup>4</sup>Department of Radiation Oncology, Animal Medical Center, New York, NY, USA, <sup>5</sup>Heska Corporation, Loveland, CO, USA, <sup>6</sup>College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA, <sup>7</sup>Joint Pathology Center, Silver Spring, MD, USA, <sup>8</sup>Freie Universität Berlin, Berlin, Germany, <sup>9</sup>Institute of Pathology, University of Veterinary Medicine, Vienna, Austria

**Background:** Soft tissue sarcomas/soft tissue tumors (STS/STT) are common canine tumors. The current grading scheme includes histologic differentiation, mitotic count and necrosis to predict tumor behavior and patient outcome. Percent tumor necrosis is included in this scheme, however, the means to evaluate and determine the percent necrosis has not been defined.

**Objective:** To quantify inter-pathologist agreement of necrosis quantification in canine STS/STT.

**Methods:** Our retrospective dataset consisted of 100 canine STS/STT (total of 178 whole slide images, WSI). In this study, 2 pathologists first estimated (increments of 10%) then measured areas of necrosis and the tumor area in WSI. Measurements were obtained with annotation software (SlideRunner).

**Results:** Cohen's Kappa scores for inter-rater agreement on presence or absence of necrosis (perfect agreement is 1.0 while no agreement is 0) was  $\kappa = 0.66$  for estimated and  $\kappa = 0.73$  for measured values. For 75 slides in which at least one pathologist identified necrosis, the agreement was  $\kappa = 0.49$  (estimated) and  $\kappa = 0.45$  (measured). Measurement of the tumor area had a much higher inter-observer agreement ( $\kappa = 0.93$ ) than measurement of the necrosis.

**Conclusions:** Pathologist identification of necrosis requires additional morphologic characterization and standardization in order to improve inter-pathologist variation, which might affect grading of STT/STS. Using annotation software to measure necrosis did not improve the ability to quantify percentages, likely due to inconsistency in classifying necrosis vs look-alikes (e.g. fibrosis, edema etc). Future studies should aim to better characterize descriptive features of necrosis and correlate with outcome data.

Tuesday, November 2, 2021

2:30 p.m. – 2:45 p.m. CDT

## **PATHOLOGY OF EPTESIPOX VIRUS IN NATURALLY INFECTED BIG BROWN BATS**

Ursula Perdrizet<sup>1,2</sup>, Janet Hill<sup>1</sup>, Champika Fernando<sup>1</sup>, Trent Bollinger<sup>1,2</sup>, Vikram Misra<sup>1</sup>

<sup>1</sup>Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada, <sup>2</sup>Canadian Wildlife Health Cooperative Western/Northern Region, Saskatoon, SK, Canada

**Background:** *Eptesipox virus* was first isolated from big brown bats associated with severe fibrinosuppurative and necrotizing tenosynovitis and osteoarthritis in Washington state in 2011.

**Objective:** Determine prevalence and pathology associated with *Eptesipox virus* infection in big brown bats submitted to the Canadian Wildlife Health Cooperative Western/Northern region.

**Methods:** Necropsy submissions of big brown bats from 2017-2021 were screened for *Eptesipox virus* using PCR targeting the DNA polymerase gene, histology was reviewed, and in-situ hybridization targeting the p39 putative membrane-associated core protein and p4b precursor performed on tissues with lesions. Virus was isolated and characterized using cell culture, electron microscopy, and next generation sequencing.

**Results:** 28/61 submissions were positive for viral DNA. The positive cases had the following distribution of lesions: 5/28 oral ulceration, 4/28 joint inflammation or proliferation, 7/28 hepatic necrosis, 7/28 necrosis of the gastric mucosa, 3/28 necrosis of the dermis and or hypodermis, 1/28 infarct of the skeletal muscle, 1/28 vasculitis, and 6/28 with 2 or more lesions. No unusual lesions in 14/28. Virus was isolated from tissue homogenate and electron microscopy of negatively stained virus and infected cells was consistent with a poxvirus. The contiguous sequences generated from de novo assembly shared 99.7% identity with 85.7% coverage of the previous isolate, *Eptesipox virus* Washington strain accession number NC\_035460. In-situ hybridization demonstrated viral RNA positive staining in 1/3 joints, 0/3 livers, 3/4 oral ulcers, and 1/1 skeletal muscle.

**Conclusion:** Natural *Eptesipox virus* is associated with a wide range of lesions with the role of viral replication remaining unclear.

Tuesday, November 2, 2021

2:45 p.m. – 3:00 p.m. CDT

**IMMUNOHISTOCHEMICAL CHARACTERIZATION OF THE IMMUNE RESPONSE IN CANINE CHRONIC HEPATITIS: IS THERE A CANINE IMMUNE-MEDIATED HEPATITIS?**

Brittany Rasche, John Cullen

North Carolina State University, Raleigh, NC, USA



**Background:** Most canine chronic hepatitis is classified as idiopathic. An immune-mediated pathogenesis has been proposed but is not well characterized.

**Objective:** This study aims to characterize the lymphocytic infiltrate and aberrant hepatocellular MHC class II expression in putative immune-mediated hepatitis compared to copper-associated hepatitis and control dogs.

**Methods:** This retrospective study includes liver biopsies from 46 dogs, including controls (15 dogs without inflammation), putative immune-mediated hepatitis (16 dogs with lymphocytic hepatitis with parenchymal activity, individual cell death, and copper grade of < 3/5), and copper-associated hepatitis (15 dogs with copper grade  $\geq$  3/5). Immunohistochemistry for CD3, CD20, and MHC class II was applied, and the number of CD3+ and CD20+ lymphocytes was counted in 10 consecutive 40x fields.

**Results:** In all cases (including controls), there was a predominance of CD3+ over CD20+ lymphocytes. Using one-way ANOVA, there was a significant difference ( $p < 0.05$ ) between the three groups in the ratio of CD3+ to CD20+ lymphocytes. In 10/16 of the putative immune-mediated hepatitis cases and in 2/15 of the copper-associated hepatitis cases, this ratio was greater than that of the control population ( $> 6:1$ ). Some aberrant MHC class II expression in hepatocytes was present in a proportion of cases from both hepatitis groups but not the control group.

**Conclusion:** An overwhelming predominance of CD3+ lymphocytes in some cases of canine chronic hepatitis may support an immune-mediated pathogenesis. Aberrant MHC class II expression in hepatocytes can be seen with both copper-associated and putative immune-mediated chronic hepatitis in dogs.

Tuesday, November 2, 2021

3:00 p.m. – 3:15 p.m. CDT

### **HISTOPATHOLOGIC AND MOLECULAR CHARACTERIZATION OF ERETHIZON DORSATUM PAPILLOMAVIRUS 1 AND ERETHIZON DORSATUM PAPILLOMAVIRUS 2 IN NORTH AMERICAN PORCUPINES**

Zoe Mack, Leonardo Caserta, Randall Renshaw, Rhea Gerdes, Diego Diel, Sara Childs-Sanford, Jeanine Peters-Kennedy

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

**Background:** *Erethizon dorsatum* papillomavirus 1 (EdPV1) and *Erethizon dorsatum* papillomavirus 2 (EdPV2) have been associated with cutaneous papillomas in North American porcupines (*Erethizon dorsatum*). **Objective:** This study aimed to define morphologic and molecular characteristics of viral papillomas in North American porcupines submitted to the New York State Animal Health Diagnostic Center.

**Methods:** Histopathology was assessed for 6 papillomas submitted from wild and captive North American porcupines in New York. Five of 6 cases were investigated for the presence of EdPV1 and EdPV2 DNA via polymerase chain reaction (PCR) and all cases ( $n=6$ ) were investigated for the detection and localization of EdPV1 and EdPV2 E6 and E7 nucleic acid via chromogenic in situ hybridization (ISH). Next generation sequencing (NGS) was pursued in 2/6 cases. **Results:** Papillomas were diagnosed on the muzzle ( $n=2$ ), caudal dorsum ( $n=1$ ), chin ( $n=1$ ), gingiva ( $n=1$ ), and nasal planum ( $n=1$ ). Histologically, the lesions consisted of hyperplastic epidermis or epithelium with orthokeratotic keratin, prominent keratohyalin granules and amphophilic intranuclear inclusion bodies. PCR identified EdPV1 in 3/5 samples and EdPV2 in 2/5 samples. NGS

identified EdPV1 in 1/2 samples and EdPV2 in 1/2 samples, with 92.1% sequence homology to the EdPV2 L1 gene, indicating a novel subtype. Hybridization patterns in 5/6 cases were characterized by strong nuclear signals in the superficial epidermis, with strong nuclear and punctate cytoplasmic signals in the stratum spinosum and basale.

**Conclusions:** This study supports the presence of EdPV1 and EdPV2 within North American porcupines in New York, and ISH can be utilized to detect infection.

Tuesday, November 2, 2021

3:15 p.m. – 3:30 p.m. CDT

## **A RETROSPECTIVE STUDY ON SURVIVAL OF DOGS DIAGNOSED WITH SPLENIC FIBROHISTIOCYTIC NODULES**

Cleide Sprohnle-Barrera, Chiara Palmieri, Rachel Allavena, Jayne McGhie, Tamsin Barnes

**Background:** Canine splenic fibrohistiocytic nodules are commonly diagnosed. A recent re-classification has considered a continuum from benign lymphoid hyperplasia to malignancy, whilst re-classifying malignant fibrous histiocytomas into histiocytic sarcoma and stromal sarcoma. However, reliable indicators of survival after splenectomy are still unknown.

**Objectives:** To estimate the frequency of lymphoid hyperplasia, complex hyperplasia, histiocytic sarcoma, and stromal sarcoma among splenectomised dogs and associated median survival times. To investigate possible associations between sex, age, and breed with diagnosis.

**Methods:** Medical records from splenectomised dogs with a histopathological diagnosis that had presented to primary care practices from 1989-2018 were sourced from the VetCompass Australia database. Possible associations between sex, age, and breed and the four diagnoses were investigated and median survival time for each diagnosis estimated.

**Results:** Overall, 693 splenectomised dogs had a histopathological diagnosis. Of these, 315 cases were diagnosed as fibrohistiocytic nodules: lymphoid hyperplasia (24.4%), complex hyperplasia (7.9%), histiocytic sarcoma (4.6%) and stromal sarcoma (8.5%). Dogs aged 8- 10 years were more likely to be diagnosed with histiocytic sarcoma, or stromal sarcoma compared to lymphoid hyperplasia. Dogs diagnosed with lymphoid hyperplasia typically lived longer than those with other diagnoses (median >2 years). Dogs diagnosed with splenic histiocytic sarcoma typically lived longer than previously reported (median 349 days) compared to those diagnosed with stromal sarcoma (median 166 days).

**Conclusions:** Findings suggest that splenectomised dogs diagnosed with histiocytic sarcoma live longer than previously reported. However, further studies are warranted to better characterise the effects of adjuvant chemotherapy on survival time.

Tuesday, November 2, 2021

3:30 p.m. – 3:45 p.m. CDT

## **IMMUNE RESPONSE TO CHLAMYDIA SPP. INFECTION IN THE MALE AND FEMALE REPRODUCTIVE SYSTEM IN KOALAS (PHASCOLARCTOS CINEREUS)**

Sara Pagliarani<sup>1,2</sup>, Stephen Johnston<sup>2</sup>, Kenneth Beagley<sup>3</sup>, Chiara Palmieri<sup>1</sup>

<sup>1</sup>School of Veterinary Science, The University of Queensland, Gatton, Australia, <sup>2</sup>School of Agriculture and Food Science, The University of Queensland, Gatton, Australia,

<sup>3</sup>School of Biomedical Sciences, Faculty of Health, Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia

### **Introduction:**

Chlamydiosis is one of the main causes of the progressive decline of koala populations in Australia. While histologic, immunologic and molecular studies have provided insight into the basic function of the koala immune system, the *in situ* immune cell signatures during chlamydial infection of the reproductive tract in koalas have not been investigated so far.

### **Materials and Methods:**

Fifty-three female koalas and sixty-two males presented to wildlife hospitals with clinical signs suggestive of *Chlamydia* infection were euthanized with the entire reproductive tract collected for histology, immunohistochemistry (IHC) for T- (CD3 $\epsilon$ , CD4 and CD8 $\alpha$ ), B- (CD79b) and HLA-DR-DP-DQ-positive cells and quantitative real time-PCR (rtPCR) for *Chlamydia pecorum*.

### **Results:**

This study demonstrated the recruitment of specific T-, B- and HLA-DR-positive cells to the lower and upper reproductive compartments in male and female koalas and the association between different CD- and HLA-DR-positive cells and PCR load. The CD4-positive cells number was positively correlated with the gross pathology score for the female koalas.

### **Conclusions:**

The recruitment of specific T-, B- and HLA-DR-positive cells to both the lower and upper reproductive tract is a clear step forward in understanding the mechanisms behind koala chlamydial infection immunopathogenesis. This work is the first comprehensive study combining the evaluation of the immune cells with a throughout investigation of the pathological lesions in the reproductive tract to get meaningful information on the ability of the immune system to resolve the infection or the inability of the animals to remove the organisms and develop instead a non-resolving chronic infection.

Tuesday, November 2, 2021

3:45 p.m. – 4:00 p.m. CDT

## **ANALYSIS OF SINGLE NUCLEOTIDE POLYMORPHISMS AND COPY NUMBER VARIATIONS IN METASTATIC FELINE MAMMARY TUMORS**

Alessandro Sammarco<sup>1,2</sup>, Serena Ferraresso<sup>1</sup>, Alessandro Calore<sup>1</sup>, Selina Iussich<sup>3</sup>, Giuseppe Sarli<sup>4</sup>, Valentina Zappulli<sup>1</sup>

<sup>1</sup>University of Padua, Padua, Italy, <sup>2</sup>Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA, <sup>3</sup>University of Turin, Turin, Italy, <sup>4</sup>University of Bologna, Bologna, Italy

**Background:** Feline mammary tumors (FMTs) are often malignant, frequently metastasize, and represent a therapeutic challenge.

**Objective:** This study aims to investigate genetic aberrations of nonmetastatic and metastatic FMTs using next-generation sequencing.

**Methods:** Whole-exome sequencing analysis was performed on nonmetastatic FMTs, metastatic FMTs, and paired lymph node (LN) metastases, in comparison with the matched healthy tissue.

**Results:** Genetic analyses revealed a significantly higher tumor mutational burden in LN metastases when compared to nonmetastatic FMTs. We identified nonsynonymous single nucleotide polymorphisms in 3 genes of nonmetastatic FMTs (HAS3, PIK3CA, and USP21), in 6 genes of metastatic FMTs (MCF2L, TXNDC15, ATXN1, MCL1, MYORG, and REXO4), and in 3 genes of LN metastases (PEX11A, RHOBTB3, and AGO4).

Focal and large-scale chromosomal aberrations were identified in all cases, with copy number (CN) gains prevailing over CN losses. A total of 95, 125, and 47 genomic imbalances were found in at least 57% of nonmetastatic FMTs, metastatic FMTs, and LN metastases, respectively. Interestingly, CN gains of chromosome portions containing cancer-associated genes MAD1L1 and ELFN1 were frequent. Notably, in metastatic FMTs, CN losses were in chromosome portions that contain metastasis-related genes LAMA2, PTPRK, TFPI, and MTUS1. A similar evolutionary CN aberration pattern was recognized in LN metastases and in metastatic tumors. Functional annotation analysis revealed that Notch, focal adhesion, metabolic, and MAPK pathways were altered in nonmetastatic and metastatic tumors.

**Conclusions:** We discovered novel genomic aberrations in cats with mammary tumors that have strong implications for the development of metastases and possibly for new therapeutic strategies.

Tuesday, November 2, 2021

4:00 p.m. – 4:15 p.m. CDT

## **BOVINE ASTROVIRUS AND ITS POTENTIAL ROLE IN BOVINE LYMPHOCYTIC ENCEPHALITIS**

Dominique Comeau<sup>1</sup>, Maria Spinato<sup>2</sup>, Robert Foster<sup>1</sup>, Davor Ojkic<sup>2</sup>, Jan Sargeant<sup>1</sup>, Jeff Caswell<sup>1</sup>

<sup>1</sup>Ontario Veterinary College, Guelph, ON, Canada, <sup>2</sup>Animal Health Laboratory, Guelph, ON, Canada

Astroviruses are a well-known cause of gastroenteritis in humans and many domestic animal species. More recently, these emerged as a cause of encephalitis in cattle and other species. Encephalitis is an economically important disease in cattle due to death of animals and potential exclusion of carcasses from the food chain. There is a zoonotic concern as many causes of encephalitis in cattle can also cause disease in humans. It is therefore essential to determine the causes of encephalitis and their relative importance in a population.

To investigate bovine astrovirus in Ontario cattle, 35 cases of idiopathic lymphocytic encephalitis were retrieved from the Animal Health Laboratory/Ontario Veterinary College archives. As controls 32 animals with non- lymphocytic encephalitis, and 42 animals with no neurologic disease or encephalitic lesions were included in the study. All animals were screened using RT-qPCR for bovine astrovirus. No animals from either control group tested positive for bovine astrovirus. Four animals with lymphocytic encephalitis are positive for bovine astrovirus; they all had lymphocytic perivascular cuffs affecting both grey and white matter of the cerebrum. All positive cases had a history of neurologic disease, and most displayed ataxia and staggering.

Tuesday, November 2, 2021

4:15 p.m. – 4:30 p.m. CDT

## **DESCRIPTIVE COMPARISON OF INDIVIDUAL HIVE AND YARD-LEVEL SAMPLING TECHNIQUES FOR SPORES OF PAENIBACILLUS LARVAE IN SASKATCHEWAN HONEY BEE OPERATIONS WITH RECENT OUTBREAKS OF AMERICAN FOULBROOD**

Michael Zabrodski<sup>1</sup>, Jessica DeBruyne<sup>1</sup>, Geoff Wilson<sup>2</sup>, Igor Moshynskyy<sup>1</sup>, Mohsen Sharafi<sup>1</sup>, Sarah Wood<sup>1</sup>, Ivanna Kozii<sup>1</sup>, Jenna Thebeau<sup>1</sup>, Colby Klein<sup>1</sup>, Igor Medici de Mattos<sup>1</sup>, LaRhonda Sobchishin<sup>1</sup>, Tasha Epp<sup>1</sup>, Antonio Ruzzini<sup>1</sup>, Elemir Simko<sup>1</sup>

<sup>1</sup>Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada, <sup>2</sup>Ministry of Agriculture, Government of Saskatchewan, Prince Albert, SK, Canada

**Background:** Three commercial honey bee operations within Saskatchewan (Canada) with recent outbreaks of American foulbrood (AFB) and recent or ongoing metaphylactic antibiotic use were intensively sampled to detect spores of *Paenibacillus larvae* during the summer of 2019.

**Methods:** Samples of brood chamber honey, honey super honey, and brood chamber bees from individual hives within yards with and without clinical evidence of AFB, as

well as pooled, yard-level honey samples from end-of-season extraction, were collected and cultured to detect and enumerate spores.

**Results:** All operations were different from one another with regard to both overall degree of spore contamination across yards and distribution of spores between clinically affected and unaffected yards. Spore concentrations in unaffected yards were significantly different from AFB affected yards in one of three operations. Only a few hives were responsible for the majority of spore contamination in any given yard across all operations. For individual hive samples, brood chamber honey was best for discriminating clinically affected yards from those unaffected ( $p = 0.001$ ), followed by honey super honey ( $p = 0.06$ ), and bees ( $p = 0.398$ ).

**Conclusions:** Honey super honey positively correlated with both brood chamber honey ( $r_s = 0.76$ ,  $p < 0.0001$ ) and bees ( $r_s = 0.50$ ,  $p < 0.0001$ ) and may be useful as a surrogate for either. Spore concentrations in pooled, extracted honey seem to have predictive potential for overall spore contamination within each operation and may have prognostic value in assessing the risk of future AFB outbreaks at the yard (or operation) level.

Tuesday, November 2, 2021

4:30 p.m. – 4:45 p.m. CDT

### **PATHOLOGICAL FEATURES OF ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY IN FOUR UNRELATED CHIMPANZEES**

Karen Terio<sup>1</sup>, Linda Lowenstine<sup>2</sup>, Trevor Gerlach<sup>3</sup>, Rita McManamon<sup>4</sup>, Sushan Han<sup>5</sup>, Marietta Danforth<sup>6</sup>, Hayley Murphy<sup>6</sup>, Gisela Martinez-Romero<sup>1</sup>

<sup>1</sup>University of Illinois at Urbana-Champaign, Brookfield, IL, USA, <sup>2</sup>University of California, Davis, CA, USA, <sup>3</sup>Veterinary Specialty Center, Buffalo Grove, IL, USA, <sup>4</sup>University of Georgia, Athens, GA, USA, <sup>5</sup>Colorado State University, Fort Collins, CO, USA, <sup>6</sup>Zoo Atlanta, Atlanta, GA, USA

Cardiovascular disease is a major cause of death in zoo great apes; however, the underlying cause(s) remains unknown. While fibrosis is the most common cardiovascular lesion, we also identified cases consistent with arrhythmogenic right ventricular cardiomyopathy (ARVC) in four unrelated male chimpanzees aged 21-43 years. In humans and small animals, ARVC is an inherited disease that can result in ventricular arrhythmias and sudden death. While histologic changes can affect all four chambers, predominant right ventricular involvement is most common variant. In the cases described here, three of four experienced acute collapse. In all cases, the right ventricle was dilated with thinning of right ventricular free wall. Histologically, the right ventricle, including the trabecular myocardium, had multiple areas of subepicardial to transmural, interstitial, or replacement fibrosis with infiltration of adipose tissue, and degeneration of entrapped cardiomyocytes. Lesions also affected the left ventricle and interventricular septum in two of four cases. While both fatty and fibrofatty infiltration has been described in humans, the fibrofatty variant was the only pattern observed in this case series. ARVC has been previously reported in two related chimpanzees. Our findings of similar disease in four additional, unrelated chimpanzees suggests that the disease may be more prevalent than previously recognized in this species. Ongoing

Great Ape Heart Project (GAHP) studies involving ARVC screening include quantitative analysis of myocardial plakoglobin, genetic analysis for cardiac desmosomal gene mutations, and critical *ante-* and *postmortem* evaluation for potential cases in other ape species.

Tuesday, November 2, 2021

4:45 p.m. – 5:00 p.m. CDT

### **RETROSPECTIVE ANALYSIS OF MYCOBACTERIOSIS IN AQUARIUM HOUSED ELASMOBRANCHS**

Mari Inohana<sup>1</sup>, Kaoru Nagaya<sup>2</sup>, Tsunehisa Komatsu<sup>2</sup>, Sakiko Kubo<sup>2</sup>, Yoshiaki Tanaka<sup>3</sup>, Keisuke Kondo<sup>4</sup>, Takeshi Komine<sup>1</sup>, Kentarou Tomaru<sup>1</sup>, Kentarou Ono<sup>1</sup>, Shinpei Wada<sup>1</sup>  
<sup>1</sup>Nippon Veterinary and Life Science University, Tokyo, Japan, <sup>2</sup>Shinagawa Aquarium, Tokyo, Japan, <sup>3</sup>Shimane Aquarium, Shimane, Japan, <sup>4</sup>MARINE WORLD uminonakamichi, Fukuoka, Japan

**Background:** Mycobacteriosis is one of the most common chronic disease in fish. On the other hand, mycobacteriosis has rarely been diagnosed in elasmobranchs. **Objective:** The retrospective study characterized the prevalence and the histologic and molecular biological features of the mycobacteriosis of elasmobranchs by examining aquarium fishes. **Methods:** Elasmobranch submissions (60) received by our laboratory from 1999 to 2021 were searched for mycobacteriosis. Histopathologic sections of various tissues from 60 individuals (22 species) were examined. Tissues with inflammatory cell infiltration were stained with acid fast stain. DNA was extracted from frozen, ethanol fixed or formalin fixed paraffin embedded tissues, which showed acid fast bacilli in lesions, and tested for *Mycobacterium* spp. by PCR targeting the *hsp65* gene with sequencing analysis. The DNA showing high nucleotide sequence identities to *M. marinum* were tested for the insertion elements IS2404 and IS2606 by PCR. Peptide nucleic acid *in situ* hybridization were performed to identify pathogen in tissues where multiple mycobacteria were detected. **Results:** Acid fast bacilli within macrophages were histologically detected in 18 out of the 60 elasmobranchs examined. Sixteen of them, including 8 species from 5 aquaria, were diagnosed as mycobacteriosis by sequencing analysis. Microscopically, lesions were typified by lymphocytic inflammation with small number of macrophages and granulomatous inflammation both without caseous necrosis. **Conclusions:** Pathologic investigations on aquarium housed elasmobranchs in Japan have revealed high prevalence of mycobacteriosis compared to the number of mycobacterial case reports in elasmobranchs. The prevalence of mycobacteriosis in elasmobranchs could be similar to that in teleost previously reported.

### **Clinical Pathology Focused Poster Session**

Saturday, October 30, 2021 | 5:00 p.m. – 6:00 p.m. CDT

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **PRECURSOR-DIRECTED IMMUNE-MEDIATED ANEMIA IN A CAT WITH PHAGOCYTOSED NUCLEAR MATERIAL IN CIRCULATING NEUTROPHILS**

Nina Randolph, Camille McAloney, Sam Evans

The Ohio State University College of Veterinary Medicine Department of Veterinary Biosciences, Columbus, OH, USA

Precursor-directed immune-mediated anemia (PIMA) is an autoimmune disorder in which erythroid hematopoietic cells are targeted and destroyed, resulting in a non-regenerative anemia. It can pose a diagnostic challenge, as the CBC findings are quite different from the classic presentation of immune-mediated hemolytic anemia (IMHA), which targets mature red blood cells. Although it is uncommon, PIMA is most frequently seen in dogs and rarely observed in cats. To the authors' knowledge, this is the first report of PIMA in a cat with phagocytosed nuclear material identified within neutrophils in peripheral blood. A 6 year old neutered male domestic short hair cat was presented for decreased appetite and lethargy; the referring veterinarian had noted icterus and elevated liver enzymes and referred to The Ohio State University's Veterinary Medical Center. The cat's CBC showed marked non-regenerative, normocytic, normochromic, anemia, marked thrombocytopenia, and mild leukocytosis characterized by mild mature neutrophilia (rare cytoplasmic basophilia consistent with toxic change) and mild monocytosis. Blood smear evaluation showed occasional round, basophilic inclusions in the neutrophils ("ragocyte"-like morphology) that were positive for DAPI staining, consistent with nuclear material. Neutrophils that displayed erythrophagia were also observed in peripheral blood. Bone marrow aspirates revealed marked erythroid hyperplasia with left shift and evidence of phagocytosis of erythroid precursors by macrophages. Histopathology of bone marrow core biopsy was supportive of a diagnosis of PIMA, and anemia and thrombocytopenia resolved with immunosuppressive therapy. These findings are consistent with a unique presentation of PIMA in a cat.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

**ADVIA HEMATOLOGY SYSTEM-PLATELET CLUMPS FLAG DUE TO INCIDENTAL ASPIRATION OF HARDERIAN GLAND IN A FEMALE CRL:CD-1 MOUSE**

Dumisani Chuchu, Ashleigh Caffull, Matthew Edward Hartness Hartness, Claudio Petterino

Sequani Ltd., Ledbury, United Kingdom

An eight-week-old female Crl:CD-1 mouse was routinely admitted for peripheral blood sampling from the orbital sinus. An ADVIA warning flag resulted indicating slight platelet clumps. Moreover, the basophil cytogram, indicated a prominent streak to the far right, below the baso noise threshold, which was below the polymorphonuclear and mononuclear region. On the perox cytogram the noise minimally affected eosinophils. This has been reported as a lipid pattern. In contrast, upon evaluation of a Wright-Giemsa stained peripheral blood smear, platelet estimation was adequate, and platelets occurred individually and disperse throughout the smear along with erythrocytes. However, on the feathered edge of the smear there were a few very large nucleated cells found individually or in small groups. These cells had poorly defined cell borders, abundant pale grey-blue cytoplasm containing numerous lipid-like clear vacuoles. These cells had round to irregularly bi-lobate nuclei occurring singly or double and had irregularly clumped and deep stained chromatin. Nucleoli were not visible. On the



background a very low amount of dark yellow to dark brown-black granules were found along with anucleate cell remnants still associated with lipid-like vacuoles and resembling lipid micelles. The flag we report was due to the presence of a few acinar nucleated and highly vacuolated epithelial cells along with some anucleate cell remnants from the incidentally aspirated Harderian gland during blood sampling. The analyser likely misidentified the large vacuolated cells, aggregates of anucleate remnants of vacuolated cells, and possibly lipid-like micelles released from ruptured cells as platelet clumps within the platelet channel.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **MELAMINE-CYANURIC-LIKE CRYSTALLURIA IN A DOG**

David Rotstein<sup>1,2</sup>, Sarah Peloquin<sup>2</sup>, Renate Reimschuessel<sup>2</sup>, Jake Guag<sup>2</sup>, Kathleen Proia<sup>2</sup>, Betsy Yakes<sup>3</sup>

<sup>1</sup>FDA Center for Veterinary Medicine, Rockville, MD, USA, <sup>2</sup>FDA Center for Veterinary Medicine Veterinary Laboratory Investigation and Response Network, Laurel, MD, USA,

<sup>3</sup>FDA Center for Food Safety and Nutrition, College Park, MD, USA

**Background:** FDA's Center for Veterinary Medicine (CVM), Veterinary Laboratory Investigation and Response Network (Vet-LIRN) initiated an investigation reported by a Vet-LIRN member lab about suspected melamine crystalluria in an 8.5-year-old FS Pit Bull Mix. The dog had intermittent urinary incontinence. Crystals had a golden brown "wagon wheel" spherulite appearance and were observed on two subsequent urine sediments. Vet-LIRN was contacted to evaluate crystal composition for melamine-cyanuric acid.

**Objective:** To investigate the crystal composition with traditional methods and Raman microspectroscopy, perform genetic testing, and determine possible dietary relationships.

**Methods:** Vet-LIRN investigation involved medical record review, exposure interview, serum chemistry analysis including uric acid and bile acids, urine uric acid: creatinine ratio, urine sediment formalin exposure microscopy and Raman spectroscopy, and genetic testing for hyperuricosuria (SLC2A9 mutation).

**Results:** Two types of crystals were detected with Raman spectroscopy – uric acid and sulfate. Urine sediment crystals dissolved within three minutes upon formalin exposure. Creatinine, BUN, uric acid, and bile acids were within reference ranges. The urine uric acid: creatinine ratio was 0.8 (ref. <0.5). The dog had the HU/HU genotype for hyperuricosuria.

**Conclusions:** The dog had a SLC2A9 mutation leading to hyperuricosuria; the dog food was unrelated. This mutation has been reported in Dalmatians, Pitbull Terrier, and American Staffordshire. Hyperuricosuria can result in formation of uric acid crystals which appear similar to melamine-cyanuric acid crystals. While spectroscopy was

diagnostic, formalin treatment of a slide of urine sediment provides a simple and accessible method for ruling out melamine-cyanuric crystalluria.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **ANAPHYLAXIS-ASSOCIATED HEMOPERITONEUM AFTER VACCINATION IN A DOG**

Bridget Garner<sup>1</sup>, Alyssa Brooker<sup>2</sup>, Amie Koenig<sup>1</sup>

<sup>1</sup>University of Georgia, Athens, GA, USA, <sup>2</sup>IDEXX Laboratories, Maple Shade, NJ, USA

A seven-year-old female spayed Yorkshire Terrier presented to the primary veterinarian for an annual wellness exam including vaccination. Within minutes of vaccination, she began vomiting and defecating; diphenhydramine and maropitant were administered. The following day, lethargy continued and melena, orthopnea and abdominal effusion were identified. The hematocrit was 57% and platelets were  $243 \times 10^3/\mu\text{L}$ . The dog was referred.

On presentation, she was quiet and alert and had abdominal distention. Thrombocytopenia ( $87 \times 10^3/\mu\text{L}$ ) with normal PT and PTT were identified. Abdominal ultrasound showed no masses and a moderate quantity of abdominal effusion. The grossly red effusion had a hematocrit of 61% (compared to the peripheral PCV of 45%), and did not clot. It consisted almost exclusively of blood with few blood-associated leukocytes and rare platelets. Given the history and the lack of overt trauma, neoplasia or coagulopathy, the hemoperitoneum, as well as the gastrointestinal signs, were attributed to vaccine-induced anaphylaxis. The dog was hospitalized overnight with near resolution of the clinical signs the next day. Within days of discharge, the owner reported the dog seemed clinically healthy and her abdominal distention had resolved.

The mechanism of anaphylaxis-induced hemoperitoneum is not known and can occur independent of coagulation abnormalities. A possible mechanism includes heparin and tryptase released from mast cell degranulation. Hepatic venous congestion and increased vascular permeability with exudation of blood and fluid or hepatosplenic engorgement with vascular rupture could also contribute. Pathologists interpreting abdominal effusions in dogs should consider anaphylaxis as a differential for hemoperitoneum.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **A CASE REPORT OF AN EOSINOPHILIC LEUKEMIA IN A HEDGEHOG**

Hamideh Esmaeilzadeh, Priscilla Serpa, Courtney Sweeney, Margaret Miller, John Christian, Andrea Santos

Purdue University, West Lafayette, IN, USA

A one year and two-months-old male hedgehog presented to Purdue University Veterinary Teaching Hospital with a history of anorexia and constipation. Fecal flotation reported no parasites. Complete blood count showed mild neutrophilia (21,700 cells/ $\mu$ L), eosinophilia (650 cells/ $\mu$ L), basophilia (2,380 cells/ $\mu$ L) and "other cells" (1,080 cells/ $\mu$ L) that appeared immature eosinophil precursors. Erythron and thrombon were within reference intervals. After four months, he became lethargic and weak in his hindlimb, and developed hematochezia. Follow-up blood work showed regenerative anemia and increased numbers of eosinophils, basophils, and eosinophilic precursors. Due to the grave prognosis, the owner opted for humanly euthanasia. Histopathologic findings were consistent with a systemic eosinophilic neoplasm and a suspicion of eosinophilic leukemia. Bone marrow evaluation showed excessive numbers of eosinophils and eosinophilic precursors. Widespread eosinophilic infiltration was found in the lungs, liver, spleen, and intestines. Differential diagnosis include reactive eosinophilia, which occurs in response to an underlying cause, such as infectious agents and hypersensitivity or neoplastic conditions. Clonal eosinophilia can be seen with chronic eosinophilic leukemia and myelodysplastic syndrome. Idiopathic eosinophilia is a diagnosis of exclusion. In eosinophilic leukemia, left-shift is present as far as myeloblasts, with some dysplasia. Given the bone marrow evaluation (excessive eosinophilic precursors), and the presence of more immature forms in the peripheral blood and organs, the diagnosis of this case is eosinophilic leukemia with paraneoplastic basophilia. Hedgehogs with eosinophilic leukemia usually have mild to moderate anemia or low normal hematocrit. In the case herein, the regenerative anemia was likely due to blood loss.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **SEBACEOUS ADENOCARCINOMA IN AN 11 YEAR OLD COCKER SPANIEL WITH PULMONARY METASTASIS**

Karena Tang, Andrea Santos, Craig Thompson  
Purdue University, West Lafayette, IN, USA

**Background:** An 11-year-old castrated male cocker spaniel presented to his referring veterinarian for further evaluation of a rapidly growing mass 7.3 x 4.6 x 5.9 cm neck mass. A CT scan also revealed a small, irregularly marginated pulmonary nodule at the periphery of the left caudal lung lobe. Cytologic evaluation of a fine needle aspirate from the pulmonary nodule revealed a population of large, cohesive-appearing cells measuring 15-35  $\mu$ m in diameter. They had an abundant amount of pink, grainy cytoplasm that was often markedly expanded by variably-sized, round, colorless vacuoles that displaced the nucleus peripherally. Observed atypia included marked anisocytosis, moderate anisokaryosis, rare binucleation, and 1-2 large, prominent, round nucleoli. Differential diagnoses included sebaceous adenocarcinoma, liposarcoma, and pulmonary adenocarcinoma. The histopathological evaluation of the neck mass was most consistent with sebaceous adenocarcinoma due to the formation of structures reminiscent of ductules.

**Objective:** Our objective was to use special staining (immunohistochemistry, cytochemistry, and immunocytochemistry) to further differentiate the origin of the neoplasm and highlight cytologic similarities between our differential diagnoses.

**Methods:** Cytology aspirates are currently being stained with pan-CK, vimentin, CD18, and Oil red O. Histopathologic sections of the mass are currently being stained with pan-CK, vimentin, and CDK18.

**Results:** Histopathologic immunohistochemistry and special staining of the cytology aspirates are currently in process to further support sebaceous carcinoma with metastasis and determine consistency between the neck mass and the lung aspirate.

**Conclusions:** Sebaceous adenocarcinoma has cytologic similarities to other neoplasms and can be further differentiated with special staining techniques.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

## **HEPATOCTE DERIVED CFA-MICRORNAS AS SERUM BIOMARKERS OF HEPATIC FIBROSIS OR CIRRHOSIS IN DOGS**

Ahmed El-Sebaey<sup>1,2</sup>, Pavel Abramov<sup>1</sup>, Natalia Slesarenko<sup>1</sup>, Seidfatima Borunova<sup>1</sup>, Sergey Pozyabin<sup>1</sup>

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**Background:** Liver fibrosis or cirrhosis are major complications of prolonged chronic hepatitis in which most dogs remain asymptomatic until progressive fatal liver failure or hepatocellular carcinoma (HCC) is developed. **Objective:** The goal of this prospective study was to evaluate the possible use of hepatocyte-derived *Canine familiaris* (cfa)-miRNAs-122, -21, 34a, 126, and -200c as accurate serum biomarkers to diagnose the early onset of fibrotic and cirrhotic complications in dogs diagnosed with chronic liver damage. **Methods:** Based on ultrasonographic, computed tomographic and histopathological findings, the relative expression of selected cfa-miRNAs was estimated in 60 serum samples [(20 healthy controls, 20 with liver fibrosis, 20 with cirrhosis] using quantitative RT-PCR and Qiagen® PCR kit. **Results:** Cfa-miRNA-122 and -21 were upregulated in dogs with hepatic fibrosis at significance levels of  $P < 0.01$  and  $P < 0.001$ , respectively compared to cirrhosis and control groups and at an area under the curve (AUC) of 0.96 and 0.99, respectively exhibited potential roles in distinguishing dogs with fibrosis from the control. Cfa-miRNA-200c was significantly ( $P < 0.01$ ) expressed only in dogs with cirrhosis compared to the fibrosis and control groups and at AUC of 0.87 showed high diagnostic performance in differentiating dogs with cirrhosis from the healthy group. **Conclusions:** Cfa-miRNA-122 and -21 are reliable biomarkers for diagnosis of liver fibrosis while cfa-miRNA-200c is a diagnostic biomarker of cirrhosis, particularly at early stages which could help the clinician for early prediction or prevention of hepatic fibrosis leaden to end-stage cirrhosis-associated HCC in dogs.

Keywords: dogs, fibrosis, cirrhosis, RT-PCR, AUC, cfa- miRNAs.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **SYSMEX XT-2000iV AND PROCYTE DX SCATTERGRAM FINDINGS IN A DOG WITH MASTOCYTEMIA**

Jelena Palic, Annabelle Heier, Els Acke

Vet Med Labor GmbH Division of IDEXX Laboratories, Kornwestheim, Germany

**Background:** Complete blood count (CBC) with morphologic evaluation was performed in the assessment of a 10-year-old female Golden Retriever with history of completely removed low-grade complex mammary carcinoma two months prior. In-house ProCyte Dx automated counts revealed moderate non-regenerative anemia, moderate eosinophilia and mild monocytosis.

**Objective:** To describe scattergrams from ProCyte Dx and Sysmex XT-2000iV with blood smear findings in a dog with mastocytomia.

**Methods:** CBC analysis performed on ProCyte Dx and Sysmex XT-2000iV, followed by blood smear evaluation.

**Results:** ProCyte Dx WBC scattergram showed the second cloud parallel and to the right from the monocyte dot plot location. Cells in that cloud were classified as either monocytes or neutrophils with no clear separation. On Sysmex XT-2000iV DIFF scattergram, neutrophil and eosinophil dot plots were present at the respective locations, appeared separated but could not be differentiated. Lymphocyte and monocyte dot plots were abnormal. In addition to the normal lymphocyte dot plot location, the second cloud of cells classified as lymphocytes was displayed to the right of the monocyte dot plot area. Cluster was also present in basophil dot plot area. Blood smear assessment detected mastocytomia with 16% degranulated mast cells. They had round nuclei with finely stippled chromatin, moderate amount of cytoplasm with numerous small uniform vacuoles and rare small magenta granules.

**Conclusion:** This is the first description of abnormal scattergrams from ProCyte Dx and Sysmex XT-2000iV correlated with blood smear examination from a dog with mastocytomia. In cases with abnormalities detected on scattergrams, blood smear examination is essential.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **METASTATIC RHABDOMYOSARCOMA IN A GOLDEN DOODLE**

Stacey Woods<sup>1</sup>, Jim Meinkoth<sup>1</sup>, Alexa Hunter<sup>1</sup>, Timothy Snider<sup>1</sup>, Jana Gordon<sup>2</sup>

<sup>1</sup>Oklahoma State University, Stillwater, OK, USA, <sup>2</sup>Oregon State University, Corvallis, OR, USA

**Case Presentation:** A 1.5 year old dog presented for evaluation of vomiting and lethargy. Physical exam showed multiple large, firm, subcutaneous masses on the right maxilla, ventral abdomen, and right hindlimb. Thoracic radiographs revealed numerous,

variably sized pulmonary nodules. AFAST showed abdominal effusion and multiple large, targetoid hepatic masses.

**Diagnostic Findings:** Cytology of subcutaneous masses and prescapular lymph node revealed many undifferentiated, discrete cells. They were primitive in appearance, with high N:C ratios and a single round to bi-lobed nucleus, coarse chromatin, and a scant amount of basophilic cytoplasm. Few cells showed evidence of cohesion. Anisocytosis and anisokaryosis was moderate and rare multinucleated cells were identified. Differentials included a rhabdomyosarcoma or primitive neuroectodermal tumor. The patient was euthanized due to a poor prognosis. Necropsy showed many neoplastic masses diffusely affecting the lungs and omentum, with solitary masses in the heart, lymph nodes, kidney, liver, pancreas, and stomach. Histopathology revealed a highly anaplastic cell population, similar in morphology to those described on cytology. Few cells with “strap” cell morphology were seen. Neoplastic cells were strongly immunopositive for vimentin and desmin. They were negative for cytokeratin, myoglobin, SMA, Melan-A, NSE, lymphoid, and histiocytic markers. Based on the immunohistochemistry results, this is consistent with a rhabdomyosarcoma. While an uncommon tumor, rhabdomyosarcomas should be a differential for a poorly differentiated neoplasm in young dogs. Undifferentiated rhabdomyosarcomas are often misdiagnosed as lymphoma or other primitive tumors and a panel of IHCs is recommended for definitive diagnosis.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **DUODENAL PERFORATION AND HYPOGLYCEMIA AS PARANEOPLASTIC SYNDROMES ASSOCIATED WITH CHRONIC BASOPHILIC LEUKEMIA IN A DOG**

Sergio Vázquez<sup>1,2</sup>, Mónica Bragado-Cuesta<sup>2</sup>, José Espinosa<sup>1,3</sup>, Valentín Pérez<sup>1,3</sup>

<sup>1</sup>Department of Animal Health, University of León, León, Spain, <sup>2</sup>Small Animal Medicine, Veterinary Teaching Hospital-University of León, León, Spain, <sup>3</sup>Pathology, Veterinary Teaching Hospital-University of León, León, Spain

*Background:* An 8-year-old male greyhound presented with lethargy, abdominal pain and vomiting. Neutrophilia (19810 cells/ $\mu$ L) and marked basophilia (740 cells/ $\mu$ L) was observed together with a neutrophilic abdominal exudate. At exploratory laparotomy, perforation in the duodenum was found. Two weeks later, he came back with no improvement of the clinical signs. Splenomegaly and hepatomegaly, as well as moderate anemia (23.1%), significant basophilia (1160 cells/ $\mu$ L), monocytosis (56060 cells/ $\mu$ L) and thrombocytopenia (51000 cells/ $\mu$ L) were detected. Seizures appeared, consequence of severe hypoglycaemia (28 mg/dL) and euthanasia was decided.

*Objective:* This study reports and describes the main clinical and pathological findings of basophilic leukaemia in a dog.

*Methods:* Peripheral blood smears were stained with Diff-Quick. Histological examination of several organ samples, stained with H-E, toluidine blue and immunohistochemically for c-kit, was performed.

*Results:* Myeloid leukaemia was suspected due to the detection of abundant cells containing basophilic granules in blood smears. The duodenal perforated area was resected and no neoplastic cells found. At necropsy, bone marrow was red in color. Hepatomegaly and splenomegaly, with a diffuse whitish stippling, were observed. Intraabdominal and sternal lymph nodes were also enlarged. Microscopically, a diffuse infiltrate formed by round cells with moderate anisocytosis and anisokaryosis, containing intracytoplasmic granules, metachromatically stained with toluidine blue, was observed. These cells were negatively immunostained for c-kit receptor, discarding a mast cell neoplasia.

*Conclusions:* As in the present case, hypoglycemia and a duodenal ulcer have been reported in humans associated with chronic basophilic leukemia, but not in dogs, where this disease has been scarcely reported.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **NASAL COLONIZATION BY ENTAMOEBIA GINGIVALIS IN A 13-YEAR-OLD ITALIAN GREYHOUND**

Nina Kristen Randolph<sup>1</sup>, Camille McAloney<sup>1</sup>, Robert Ossiboff<sup>2</sup>, Matthew Cook<sup>1</sup>, Betsy Hernandez<sup>1</sup>, Jessica Hokamp<sup>1</sup>

<sup>1</sup>The Ohio State University, Columbus, OH, USA, <sup>2</sup>University of Florida, Gainesville, FL, USA

A 13-year-old 4.6 kg castrated male Italian Greyhound was presented for evaluation of bilateral epistaxis and mucoid nasal discharge of approximately 3 months duration. At presentation, the patient's CBC was unremarkable. His biochemical profile revealed mild azotemia as well as mild electrolyte abnormalities. Severe periodontal disease was noted on physical exam. Rhinoscopy revealed a tan plaque present in the left dorsal meatus of the nasal cavity. Vigorous flushing yielded granular white particulates suspended in the saline, which were cytologically examined. Marked neutrophilic inflammation, a diverse bacterial population, and organisms consistent with amoeba were identified. The remaining fluid was analyzed by PCR identifying *Entamoeba gingivalis*. The dog's clinical signs improved dramatically after initiating treatment with clindamycin. 6 weeks after diagnosis a dental cleaning with multiple extractions was performed. Four weeks after the dental procedure the dog was clinically normal. This report describes the clinical, hematologic, cytologic, and gross findings in a case of *Entamoeba gingivalis* infection in a dog.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **A CASE OF PRIMARY CNS B-CELL LYMPHOMA IN A DOMESTIC SHORTHAIRED CAT**

Mara Varvil, Jaya Mehra, Rothman Reyes, Margaret Miller, Stephanie Thomovsky, R Bentley, John Christian

Purdue University, West Lafayette, IN, USA

A 14-year-old spayed female domestic shorthair cat presented with a several day history of pacing and vomiting. Results of hematology, serum chemistry, thoracic radiography, and abdominal radiography were unremarkable. Neurologic evaluation revealed a left > right forebrain neurolocalization. A mass in the left forebrain, detected on magnetic resonance imaging, was surgically excised. Cytologically, impression smears had many small lymphocytes intermixed with a population of large round cells with a high nucleus to cytoplasm ratio and discrete colorless vacuoles. Histologically, the neoplastic cells resembled those in cytologic preparations, with large round to oval hyperchromatic nuclei, an irregular nuclear membrane, and 5-6 mitotic figures/0.237 mm<sup>2</sup>. These cells were concentrated in the cerebral leptomeninges but focally invaded the brain. The neoplastic cells had strong immunohistochemical expression of B-lymphocyte markers CD20 and Pax-5 and were negative for CD-3 and Iba-1, consistent with a B-cell lymphoma. No evidence of neoplasia was detected extracranially. The cat's condition improved after surgery and chemotherapy utilizing lomustine, L-asparaginase, and prednisolone. Three months post-surgery, the cat developed persistent anisocoria, but was otherwise stable. Eight months post-surgery, weight loss, decreased muscle mass, and renal azotemia were seen, with no neurologic symptoms. Though hospitalized, the cat's condition continued to decline, and humane euthanasia was elected. On autopsy, no evidence of lymphoma was identified, and hypertrophic cardiomyopathy was considered the cause for decline and ultimately euthanasia.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **CALCIUM AND PHOSPHORUS HOMEOSTASIS IN ADVANCED STAGES OF CANINE LEISHMANIASIS**

MARIO ALBERTO GONZÁLEZ, RAFAEL BARRERA, MARÍA GIL-MOLINO, BEATRIZ MACÍAS, JOSÉ IGNACIO CRISTÓBAL, PALOMA NICOLÁS-BARCELÓ, ÁNGELA DURÁN, ANA BELÉN GARCÍA, FRANCISCO JAVIER DUQUE

Departamento Medicina Animal, Universidad de Extremadura, Cáceres, Spain

**Background:** Chronic kidney disease (CKD) secondary to canine leishmaniasis (CL) is frequently associated with mineral and bone disorders (MBD). In CL, information about that is very scarce. Correct knowledge of calcium and phosphorus homeostasis is essential to improve the management of advanced CKD. Our objectives were: 1) To assess CKD-MBD in patients with advanced stages of LC, and 2) To evaluate the diagnostic utility of biomarkers analyzed.



**Methods:** Sixteen dogs classified in Leishvet (n(III)=7; n(IV)=9) were compared with control group (n(CG)=6). Hematology, biochemistry with plasmatic total calcium (PtCa) and phosphorus (PPho), and urinalysis were performed. CL was diagnosed by ELISA. Parathyroid hormone (PTH) (Human, Abcam®, UK) was determined using a sandwich ELISA kit; fibroblast growth factor-23 (FGF23) and osteocalcin (OC) were analyzed using the same method (Canine, Mybiosource®, U.S.A.). 25-hydroxyvitamin-D (VD) was measured using electrochemiluminescence immunoassay (Human, 800 Cobas® Roche Diagnostics, Switzerland). One-way ANOVA was used to compare CL groups (III and IV) with CG. A p-value of <0.05 was considered statistically significant.

**Results:** PPho (mg/dL) showed differences between CG and IV, (GC=4.2±0.7;IV=10.1±5.5); FGF23 (pg/mL) had differences between CG and IV (10478,6±9937,7 and 148,6±134,1, respectively); finally, for VD (ng/mL), III and IV presented differences compare with CG (CG=39,5±9,8;III=15,5±9,6;IV=9,6±3,4).

**Conclusions:** III and IV groups presented marked hypovitaminosis D. Marked phosphorus disorders were presented in IV group, exactly hyperphosphatemia and increased levels of FGF23. These biomarkers present a great potential for diagnosis and management of MBD in advanced stages of CL.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

## **DEEP LEARNING-BASED ALGORITHMS CAN BE USED TO PREDICT CYTOLOGIC TOTAL HEMOSIDERIN SCORES**

Christof A Bertram<sup>1,2</sup>, Christian Marzahl<sup>3,4</sup>, Alexander Bartel<sup>5</sup>, Jason Stayt<sup>6</sup>, Federico Bonsembiante<sup>7,8</sup>, Janet Beeler-Marfisi<sup>9</sup>, Ann Kristin Barton<sup>10</sup>, Ginevra Brocca<sup>7</sup>, Maria Elena Gelain<sup>7</sup>, Agnes Gläsel<sup>11</sup>, Kelly du Preez<sup>12</sup>, Kristina Weiler<sup>11</sup>, Katharina Breininger<sup>3,13</sup>, Marc Aubreville<sup>14</sup>, Andreas Maier<sup>3</sup>, Robert Klopffleisch<sup>2</sup>, Jenny Hill<sup>6</sup>

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**Background:** Exercise-Induced Pulmonary Hemorrhage (EIPH) is a common condition in horses that is associated with reduced race performance. The gold standard for diagnosis is cytologic quantification of hemosiderophages in bronchoalveolar lavage fluid using the Total Hemosiderin Score (THS). For this scoring system, 300 alveolar macrophages are graded into 5 classes based on their hemosiderin content. However, routine application of the THS is hampered by the high time investment. **Objective:** To evaluate the performance of a deep learning-based algorithm for automated THS analysis. **Methods:** Fifty-two cytopsin preparations stained with special iron stains were collected from 26 horses with clinically suspected EIPH. The THS was determined in whole-slide images by ten pathologists (scoring 300 cells) using annotation software (SlideRunner or EXACT) and a deep learning-based algorithm (scoring all cells in the whole-slide images). **Results:** Correlation of the mean pathologists' THS ( $r = 0.98$ ) or the individual pathologist's THS ( $r$  ranging between 0.95 and 0.98) with the algorithmic THS had almost perfect agreement. Accuracy for determining a value above the diagnostic THS cut-off of 75 (as compared to a ground truth dataset) was 92.3% for the algorithm and 63.4-92.3% for the individual pathologists. Whereas the algorithm analyzed entire whole-slide images in less than 2 minutes, pathologists required on average 15 minutes to score 300 hemosiderophages. **Conclusion:** As compared to the pathologists, the evaluated algorithms had very high accuracy and reproducibility. Deep learning-based algorithms are a promising tool to facilitate widespread use of the THS for routine diagnostics.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **SUMMARY OF PROCESS AND CHALLENGES TO HARMONISATION OF A NETWORK OF VETERINARY HEMATOLOGY ANALYZERS**

Susan Daly<sup>1,2</sup>, Kathleen Freeman<sup>3</sup>, Peter Graham<sup>2</sup>

<sup>1</sup>Synlab VPG Cork, Cork, Ireland, <sup>2</sup>University of Nottingham, Nottingham, United Kingdom, <sup>3</sup>Synlab VPG Exeter, Exeter, United Kingdom

**Background:** Harmonization is a necessary step to equivalent results within a network of laboratories, providing confidence that patient results are not misclassified between analytical systems. This presentation summarizes the process and challenges associated with implementation and maintenance of a harmonized network of 6 veterinary Sysmex XT 2000V hematology analyzers.

**Objectives:** To summarize harmonization of 6 hematology analyzers and methods of assessment and those aspects of harmonization found to be most difficult within our laboratory system.

**Methods:** Performance evaluation was undertaken using ASVCP total error goals, biological variation-based quality goals and Sigma metrics as the anchors of quality assessment. QC validation was determined using commercial available software. Excel spreadsheets was used to develop worksheets for ongoing assessments.

**Results:** The harmonization of the 6 analyzers was possible since desirable biologic variation goals were achieved. The use of a 1-2.5s rule for QC was validated for the commercial quality control material. A Sigma metric < 5.5 was used as an indicator for instrument service. The use of a reference analyzer within the group provided the standard for comparison, with ongoing excellent performance. Challenges included the need to ensure standardization of instrument processes across the laboratories, as well as the development of multiple spreadsheets and associated standard operating procedures and policies for ongoing use and documentation of the quality system.

**Conclusions:** Harmonization across a group of veterinary hematology analyzers was successfully achieved and maintained. Challenges associated with this were extensive, but can be overcome with perseverance and creativity.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **THE DIAZYME FREE LIGHT CHAIN ASSAY MAY NOT BE HELPFUL IN DETECTING LYMPHOID NEOPLASMS IN DOGS**

Cecelia Chapman, A Russell Moore

Colorado State University, Fort Collins, CO, USA

**Background:** Dogs and humans produce immunoglobulin kappa (Ig-k) and lambda (Ig- $\lambda$ ) light chains. These proteins are over-produced and secreted by plasma cells and B cells, resulting in low concentrations of serum free light chains (fLC). The ratio of serum  $\kappa$ -fLC to  $\lambda$ -fLC (fk:f $\lambda$  ratio) is used in human to immunoglobulin producing tumors. The serum fk:f $\lambda$  ratio has not been evaluated in dogs but may help diagnose secretory B cell neoplasms, including multiple myeloma.

**Objective:** Determine the utility of an automated human free light chain ratio assay to detect secretory lymphoid neoplasia in dogs

**Methods:** Forty dog serum samples were assigned to four groups based on clinical data: normal, infectious, multiple myeloma, and non-secretory lymphoid. Serum protein electrophoresis and immunofixation confirmed or ruled out monoclonal immunoglobulin secretion. The Diazyme fk:f $\lambda$  ratio assay was modified to increase diagnostic sensitivity and performed on all samples. Diagnostic performance statistics were calculated from

patient samples and manufacturer controls. The  $\text{fk:f}\lambda$  ratio was compared between groups using a Welch's one way ANOVA test.

**Results:** Intra-run CV of control material was 3.71%. Limit of blank for  $\lambda$ -fLC was 0.6 mg/L and for  $\kappa$ -fLC was 0.2 mg/L. Inter-run CV of patient samples for  $\lambda$ -fLC was 17.2% and for  $\kappa$ -fLC was 35.9%. Although there was a visual difference in the multiple myeloma sub-group, the ANOVA statistical test did not document a statistical difference amongst the groups ( $p=0.168$  to  $0.925$ ).

**Conclusions:** The Diazyme  $\text{fk:f}\lambda$  ratio assay may not be useful to diagnose lymphoid neoplasms in dogs

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **COMPARISON BETWEEN RBC VARIABLES IN CATS ACCORING TO FIV/FELV INFECTION STATUS**

Laura Victoria Contreras, Fernanda Andreola, Vanessa Eder, Ana Paula Borenstein, Kauana Kaefer, Taynara Fogaça, Lina Bilhalva, Bruno Almeida, Stella Valle  
Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

**Background:** Automated analyzers are widely used as the first source of information regarding CBC in clinics and hospitals. Leukocytes variables on FIV or FeLV infection have been described; however, there is less information about RBC variables.

**Objective:** To explore the difference between RBC variables of cats tested for FIV and FeLV using a rapid test (Snap FIV/FeLV combo test, IDEXX) in a veterinary teaching hospital.

**Methods:** Results of Snap tests and automated CBC (ProCyte Dx, IDEXX) from cats tested for FIV and FeLV from September of 2018 to June of 2021 were retrospectively evaluated. Data included records from 584 cats and RBC, HCT, HGB, MCV, MCH, MCHC, RDW, %RETIC, Reticulocyte count (RETIC) and reticulocyte hemoglobin (RETIC-HB) were considered. CBCs performed more than five days before or after the retroviral test were excluded. Cats were divided into four groups: Negative (G1), positive for FIV (G2), positive for FeLV (G3) and positive for both (G4). Kruskal-Wallis test was used to compare the distribution of variables between groups.

**Results:** Groups were composed as: G1: 448 cats (76.8%), G2: 30 (5.1%), G3: 94 (16.1%) and G4: 12 (2%). A difference was observed between G1-G2 and G3-G4 in RBC, PCV and HCT. MCHC was different between G1 vs G3-G4. MCV, MCH and RETIC-HB were different between G3 vs G1-G2.

**Conclusion:** This study provides information on RBC parameters on a wide population, which contribute to understand the link between retroviral infections and certain RBC alterations, and the potential use of these parameters as indicators of disease.

## **Diagnostic Pathology Focused Poster Session**

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Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **CERVICAL COMPRESSIVE MYELOPATHY SECONDARY TO GUDAIR VACCINE ASSOCIATED GRANULOMAS IN SHEEP**

Leah Manning<sup>1</sup>, Katie Eager<sup>1</sup>, Brendon O'Rourke<sup>1</sup>, Eve Hall<sup>2</sup>, Keith Walker<sup>1</sup>, Erika Bunker<sup>1</sup>, Angel Ngo<sup>1</sup>, Zoe Spiers<sup>1</sup>, Mukesh Srivastava<sup>1</sup>, Pedro Pinczowski<sup>1</sup>

<sup>1</sup>Elizabeth Macarthur Agricultural Institute, Department of Primary Industries, Menangle, NSW, Australia, <sup>2</sup>Murray Local Land Services, Holbrook, NSW, Australia

A commercial and stud sheep property in the Riverina region of New South Wales, Australia had a three-year history of gradually progressive neurological signs affecting approximately 1% of six to 12-month-old sheep. Animals had a wide-based stance, hindlimb weakness and ataxia, occasional forelimb knuckling and recumbency. Histopathology revealed Wallerian degeneration of the spinal cord of variable severity. Based on these findings, 'Cooma ataxia', a likely inherited degenerative thoracic myelopathy reported in sheep in NSW, was initially suspected. Subsequent necropsy on six affected sheep found all animals to have multifocal to coalescing, soft to firm, pale brown, variably demarcated masses within the deep cervical musculature, extending within the spinal canal causing marked compression of the cranial cervical spinal cord. Histologically these masses were granulomas with variable mineralisation and rare lipid vacuoles containing small numbers of acid-fast bacteria. There was marked Wallerian degeneration, most pronounced in the cranial cervical spinal cord. Aerobic cultures of the granulomas yielded no significant findings. A High Throughput Johnes (HTJ) PCR of granulomas from 2 animals was positive for *Mycobacterium avium* subspecies *paratuberculosis*. The HTJ PCR was also positive for cervical spinal cord from 5 of 6 affected animals, and negative for spinal cord from both vaccinated and unvaccinated control animals. It was concluded that the granulomas were due to improper vaccination technique of the Gudair vaccine, used for management of ovine Johne's disease. No clinically affected animals have been reported since changes to the vaccine procedure were implemented.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **ABERRANT (ECTOPIC) CD3 EXPRESSION IN A FELINE EXTRAMEDULLARY CUTANEOUS PLASMACYTOMA**

Michael Childress<sup>1</sup>, Caitlin Brown<sup>1</sup>, Tsang Lin<sup>1</sup>, Emily Hartman<sup>1</sup>, Peter Moore<sup>2</sup>, José Ramos-Vara<sup>1</sup>

<sup>1</sup>Purdue University, West Lafayette, IN, USA, <sup>2</sup>University of California Davis, Davis, CA, USA

Aberrant CD3 lineage expression has been reported in humans and rarely in dogs in a variety of B-cell lymphomas and plasmacytomas. We report a case of feline extramedullary plasmacytoma (FEP) with aberrant CD3 expression. A 14-year-old, neutered, Domestic Shorthair cat developed a subcutaneous mass on the abdomen. The original punch biopsy specimens had a neoplastic proliferation of discrete round cells with variable amount of eosinophilic to amphophilic cytoplasm and slightly eccentric round nucleus with nucleolus. Rare binucleated cells were present. Mitotic count was 2-14 per 400x field (0.237 mm<sup>2</sup>). The differential diagnosis included extramedullary plasmacytoma (EP) with lymphoma or histiocytic sarcoma considered less likely. Immunohistochemistry for MUM 1 was positive in the majority of neoplastic cells (nuclear), whereas Iba 1 and Pax 5 were negative. A month later, and based on cytologic features and CD3 positivity, an excisional biopsy of this mass was classified as a T-cell lymphoma. The neoplastic cells also expressed MUM 1. Molecular clonality analysis of IgH2 and IgH3 (B cell) yielded clonal rearrangements; analysis of TRG (T cell) revealed a somewhat reduced repertoire, but ultimately polyclonal rearrangements in both biopsy specimens. Based on the histopathological, immunohistochemical, and molecular findings, this tumor was diagnosed as a plasmacytoma with aberrant expression of CD3. To our knowledge, this is the first report of FEP with aberrant (ectopic) CD3 expression.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **PERFORMANCE OF A BLOOD-BASED ‘LIQUID BIOPSY’ MULTI-CANCER EARLY DETECTION (MCED) TEST IN SEVEN COMMON CANINE CANCERS**

Andi Flory, Jason Chibuk, Lauren Holtvoigt, Katherine Lytle, Dana Tsui, Ilya Chorny, Jill Rafalko, Daniel Grosu, Kristina Kruglyak  
PetDx, La Jolla, CA, USA

**Background/Objective:** Certain types of cancer are more common in dogs and account for the majority of cancer mortality in the species, notably: lymphoma, hemangiosarcoma, soft-tissue sarcoma, mast cell tumor, osteosarcoma, mammary gland carcinoma, and malignant melanoma. Recently, a blood-based multi-cancer early detection (MCED) test using next-generation sequencing was developed for use in dogs, and its performance for the detection of these seven common cancers was evaluated.

**Methods:** Blood samples from an all-comers cohort of 191 cancer-diagnosed dogs and 188 presumably cancer-free dogs were subjected to DNA extraction, proprietary library preparation, and next-generation sequencing. Sequencing data were analyzed using an internally developed bioinformatics pipeline to detect genomic alterations associated with the presence of cancer. The testing laboratory was blinded to the cancer status and type of cancer in these patients until after test results were issued.

**Results:** In 191 cancer-diagnosed subjects, the seven ‘common’ cancer types noted above accounted for 60% of cases (n=114). The test’s overall detection rate across these cancers was 63% (72 of 114). Of 188 presumably cancer-free dogs, 2 were

excluded after a cancer diagnosis and 6 were putative false positives, corresponding to a minimum specificity of 97%.

**Conclusions:** Genomic testing solutions being developed for canine cancer screening should enable detection of frequently encountered and clinically relevant malignancies. A novel blood-based MCED test has demonstrated the ability to detect some of the most common canine cancers with high sensitivity and specificity. This test has several potential applications, including use as a routine screening tool for common malignancies.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **RETROSPECTIVE ANALYSIS OF REPRODUCTIVE LESIONS IN REPTILES**

Kelsie Dougherty, Brigid Troan, Greg Lewbart

North Carolina State University College of Veterinary Medicine, Raleigh, NC, USA

Reptiles including crested geckos (*Correlophus ciliates*), leopard geckos (*Eublepharis macularius*), and several species of turtles and snakes are commonly utilized in the pet trade. Increasing demands for exotic pets encourage captive breeding, which is fairly successful in some species such as the crested gecko. Captive breeding encourages better trade practices, including improved biosecurity and reduced capture demands from the wild. Understanding the reproductive health of captive reptiles is essential for captive breeding programs and to encourage best practices in caring for them. A retrospective analysis of reproductive lesions over the past 10 years in all reptiles seen through the North Carolina State University Diagnostic Laboratory Service was conducted. 48 cases were examined (37 snakes, 1 tortoise, 1 frilled lizard, and 11 geckos) with several lesion types represented including bacterial salpingitis and oophoritis (7/48), egg yolk coelomitis (4/48), neoplasia (10/48), and oviductal torsion (1/48). Granulomatous lesions were the most uncommon lesion found (2/48). The lesions represented were not necessarily the cause of death in animals examined, but were often deemed co-morbidities. During this investigation, it was found characterizing neoplasia in reptiles, especially in reproductive tissues, can be difficult and should be further explored to better understand their behavior. In conclusion, the most common reproductive lesion found in reptiles was neoplasia (primary or metastatic), followed closely by bacterial salpingitis and oophoritis, and reproductive cysts (ovarian and testicular).

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **PAUCIBACILLARY MYCOBACTERIOSIS IN RELATED CAPTIVE RED SISKINS (CARDUELIS CUCULLATA)**

Brittany Beavis<sup>1</sup>, Andrew Cartoceti<sup>2</sup>, Steven Kubiski<sup>3</sup>, Jason Thornton<sup>1</sup>, Elise LaDouceur<sup>1</sup>

<sup>1</sup>Joint Pathology Center, Silver Spring, MD, USA, <sup>2</sup>Smithsonian National Zoo, Washington, DC, USA, <sup>3</sup>San Diego Zoo, San Diego, CA, USA

**Background:** Red siskins (*Carduelis cucullata*) are a species of finch in the Fringillidae family of Passeriformes. From 2016 to 2020, four related individuals within a captive breeding flock died or were euthanized and had histologic evidence of multi-systemic histiocytic disease.

**Methods:** Tissue samples were fixed in 10% neutral buffered formalin, processed routinely for histology, and stained with hematoxylin and eosin, Giemsa, Ziehl-Neelsen, and Fite-Faraco stains. Polymerase chain reaction (PCR) was performed on paraffin embedded tissue scrolls from all cases using degenerate *Mycobacterium* spp. primers.

**Results:** Histiocytic inflammation was present in coelomic membranes (4/4 cases), liver (4/4 cases), lungs (4/4 cases), heart (2/4 cases), dermis (2/4 cases), large vessels (1/4 cases), kidneys (1/4 cases), proventriculus (1/4 cases), skeletal muscle (1/4 cases), sinuses/nasal mucosa (1/4 cases), bone marrow (1/4 cases), adrenal gland (1/3 cases), and spleen (1/3 cases). Histiocytic inflammation was mild to moderate, except in the coelomic membranes of case Nos. 1 and 2, and in the heart and spleen of case 3, in which inflammation was severe. Acid-fast staining identified rare, intrahistiocytic acid-fast bacilli in all cases. PCR amplified mycobacterial DNA in only one case (19-0213) and had 85.4% similarity to *M. florentium*.

**Conclusion:** Paucibacillary mycobacteriosis has not been reported in passerines, which typically present with multibacillary infections. Coelomic membranes were frequently the most severely inflamed anatomic location. PCR could not definitively identify the causative mycobacterial species, likely due to low numbers of organisms present in these samples.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **ACUTE CEREBRAL TOXOPLASMOSIS IN A 2-YEAR-OLD QUARTER HORSE**

Andrew Oates, Terry Spraker

Colorado State University Veterinary Diagnostic Laboratory, Fort Collins, CO, USA

#### **Background:**

A 2-year-old quarter horse mare was submitted for necropsy to the Colorado State University Veterinary Diagnostic Laboratory with a 5-day history of progressive neurologic signs including head pressing, right-sided head tilt, circling to the right, and lack of menace response. On gross examination of the brain, both the right and left lateral ventricles were filled and expanded by well-demarcated, pale green to tan masses. These were ~3 cm in diameter and gelatinous to firm with gritty mineralized foci.

#### **Methods:**

The brain was immersion fixed in 10% neutral buffered formalin for >72 hours. Sections of the masses were trimmed and routinely processed to produce slides stained with hematoxylin and eosin (H&E), Brown-Hopps modified gram, Grocott's Methenamine Silver (GMS), acid fast, Luna, and Periodic acid Schiff (PAS). Immunohistochemistry for



*Toxoplasma gondii* and *Neospora* were performed by the Animal Health Diagnostic Center at Cornell University, College of Veterinary Medicine.

### **Results:**

Histopathology of the lesion demonstrated severe, regionally extensive, necrotizing and granulomatous encephalitis with many intralesional tachyzoites. These organisms stained negative with gram, GMS, and acid fast; and stained positive with PAS, Luna, and with immunohistochemistry for *Toxoplasma gondii* antigen.

### **Conclusion:**

This case represents a rare instance of fulminant equine cerebral toxoplasmosis with intralesional tachyzoites.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **RESPIRATORY CRYPTOCOCCOSIS IN AN UMBRELLA COCKATOO (CACATUA ALBA)**

Camille Cordero-Aponte, Denae LoBato

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

A 45-year-old female intact Umbrella Cockatoo (*Cacatua alba*) died after presenting for respiratory distress and was submitted for necropsy to The University of Tennessee College of Veterinary Medicine. Gross post-mortem findings revealed bilateral translucent mucoid material within the oral cavity, and multiple soft, pale yellow, translucent, myxomatous nodules expanding the air sacs and lungs. Microscopically, these nodules consisted of extracellular round to oval yeasts with a large clear capsule morphologically consistent with *Cryptococcus* spp., with variable numbers of mixed inflammatory cells. *Cryptococcus neoformans* was confirmed as the infective species through polymerase-chain reaction (PCR). Previous reports of cryptococcosis in psittacine in other countries have described proliferative lesions affecting the beak with disseminated infection to coelomic organs; lesions were restricted to the lungs and air sacs in this case. The source of inoculation in this cockatoo is unknown. To our knowledge, we provide the first report of *Cryptococcus neoformans* infection in a psittacine in the U.S. In wild and domestic avian species with respiratory disease, cryptococcosis should be considered, even in the absence of proliferative beak lesions. Furthermore, this report can raise awareness for prevention of outbreaks and potential zoonotic transmission.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **OSTEOFUOROSIS IN A FREE RANGING CALIFORNIA SEA LION**

Margaret Martinez<sup>1</sup>, Michelle Rivard<sup>1</sup>, Jaclyn Isbell<sup>1</sup>, Chelsea Sykes<sup>2</sup>, Robert Poppenga<sup>2</sup>, Pdraig Duignan<sup>1</sup>

<sup>1</sup>The Marine Mammal Center, Sausalito, CA, USA, <sup>2</sup>California Animal Health and Food Safety Laboratory, School of Veterinary Medicine, University of California, Davis, CA, USA

A free ranging subadult male California sea lion (CSL) was admitted to The Marine Mammal Center with a primary clinical differential of a musculoskeletal disease. Radiographs demonstrated periosteal new bone proliferation on the mandible and both humeri. Despite care, the animal continued to decline, and euthanasia was elected. Necropsy revealed segmental circumferential periosteal new bone proliferation on the diaphysis of both femurs and humeri. New bone proliferation was also on both mandibles and the cranium. Histopathology established that the periosteal woven bone was formed from endochondral and a lesser extent intramembranous ossification radiating perpendicular to the pre-existing cortical bone, entrapping atrophied muscles bundles at the periphery, and with remodeling of the inner two thirds of the new bone trabeculae. Differentials for hyperostosis included a toxicity, congenital disease, and hypertrophic osteopathy. Total fluoride levels within various bones of the case ranged from 4,000 – 9,700 ppm dw, 2-3x higher than levels found to cause hyperostosis in adult cattle. Ribs from 4 CSLs of various ages and both sexes had an average total fluoride of 1,575 ppm dw, which is twice as high as the bone fluoride levels of normal adult cattle, but 6x lower than the rib fluoride levels in the present case. Therefore, this is the first case of osteofluorosis in a free ranging marine mammal. As the growth plates and teeth were not affected, this animal was likely exposed later in life; however, the exact source is not known as male CSLs have a large range and varied diet.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **EQUINE PLACENTAL MIXED GERM CELL TUMOR WITH METASTASIS TO THE FETAL LIVER**

Abigail Finley, JungKeun Lee, Mary White, Danielle Gordon, Sarah Matyjaszek  
Midwestern University, Glendale, AZ, USA

An adult Quarter horse mare gave birth to a full-term stillborn filly. The mare is in good health. The owner submitted the placenta and fetal filly for postmortem examination. Impression smears on the placenta and fetal liver were performed for cytologic evaluation. Routine samples were submitted for histopathologic evaluation. Immunohistochemistry on the placenta and fetal liver included pancytokeratin AE1/3, vimentin, synaptophysin, S100, CD3, CD20, CD79a, and OCT3/4.

Grossly, there were numerous masses on the placenta, umbilicus, and fetal liver. Cytologically, the placental and fetal hepatic masses consisted of an atypical round cell population, few epithelial cells, rare mesenchymal cells, and necrosis. The masses in the placenta and liver are composed of primitive germ cells that differentiate to neural tissue and epithelial tissue, microscopically. The neural tissue consisted of immature to ciliated ependymal cells on an immature neuroparenchyma embedded with glial cells. The epithelial tissue consisted of keratinizing squamous epithelial cells or cords of

cuboidal to columnar epithelial cells. Pancytokeratin had specific, strong immunoreactivity for neoplastic cells with epithelial differentiation while vimentin had unexpected immunoreactivity for neoplastic cells with neural differentiation. The neoplastic cells displayed no immunoreactivity for synaptophysin, S100, CD3, CD20, and CD79a. OCT3/4 displayed non-specific, diffuse cytoplasmic staining across multiple cell types with more specific, nuclear staining of neoplastic cells.

Gross, microscopic, and immunohistochemical examination of the placental and fetal liver masses supports the diagnosis of placental mixed germ cell tumor. This is a rare case of equine placental mixed germ cell tumor with metastasis to the fetal liver.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **CAPRINE PEMPHIGUS FOLIACEUS: A CASE REPORT**

Adam Powers<sup>1</sup>, Emma Stapley<sup>1</sup>, Daniela Bedenice<sup>1</sup>, Amanda Prisk<sup>1</sup>, Elizabeth Larsen<sup>1</sup>, David Gardiner<sup>2</sup>, Ramón Almela<sup>1</sup>

<sup>1</sup>Tufts University Cummings School of Veterinary Medicine, North Grafton, MA, USA,

<sup>2</sup>Zoetis Reference Laboratories, Parsippany-Troy, NJ, USA

**Background:** Pemphigus foliaceus (PF) is an antibody-mediated autoimmune disorder affecting the epidermal keratinocyte desmosomes. In dogs, auto-antibodies attack Desmocollin 1, resulting in superficial acantholysis and sub/intracorneal pustule formation usually spanning several hair follicles. **Objective:** The goal of this case report is to add to the scant existing information in the literature on PF in goats, which is currently best described in humans and domestic canines and felines. **Method:** Four 8 mm punch biopsies of skin were formalin fixed and processed before paraffin embedding. Paraffin embedded tissue was sectioned into 5 µm slices and stained with hematoxylin, and eosin and gram stains. **Results:** A 2.5-year-old, Nigerian Dwarf wether presented with a 3—4-week history of progressive, diffuse alopecia and crusting, with pruritus, and multifocal erythematous patches across the dorsum, abdomen, head, neck, and limbs. Systemic signs include fever and multi-limb lameness without apparent effusion or swelling. No improvement was seen with doramectin, oxytetracycline, flunixin meglumine, or vitamin B therapy. Sections of haired skin were microscopically examined and contained discrete, intracorneal, superficial neutrophilic pustules spanning multiple hair follicles and containing acantholytic keratinocytes. There was also mild perivascular neutrophilic and lymphocytic inflammation with occasional eosinophils, and an absence of parasitic or infectious organisms. Clinical symptoms improved prior to initiation of immunosuppressive therapy and therefore drug-related pemphigus foliaceus must also be considered. **Conclusions:** Microscopic changes are similar in caprine species to those seen in humans and other domestic animals, however further investigation is needed to identify targeted auto-antigens in caprine species.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **HEMANGIOBLASTOMA IN THE BRAINSTEM OF A DOG**

Kirsten Landsgaard<sup>1</sup>, Samantha St. Jean<sup>1</sup>, Jonathan Levine<sup>1</sup>, Stephanie Lovell<sup>1</sup>, Brian Summers<sup>2</sup>, Raquel Rech<sup>1</sup>

<sup>1</sup>Texas A&M University, College Station, TX, USA, <sup>2</sup>Cornell University, Ithaca, NY, USA

A 3-year-old, castrated male, American Pit Bull Terrier presented to the Texas A&M Veterinary hospital for a 6-month history of mixed cerebellar and proprioceptive ataxia, circling, a head tilt, and dull mentation. Neurologic examination revealed delayed postural reactions in the right limbs, delayed oculocephalic reflex bilaterally, and positional nystagmus, all consistent with vestibular and mesencephalic dysfunction. MRI showed a mass within the brainstem. Necropsy revealed a focal, 1.1x1x0.8 cm, soft, dark red, well-circumscribed mass in the left side of the brainstem, extending from the crus cerebri of the midbrain caudally to the pons. Histologically, the neoplasm was an interstitial population of cells located between prominent capillary network. Neoplastic cells had spindle, fusiform to stellate shapes with indistinct cell borders, a moderate amount of eosinophilic cytoplasm, a single oval to elongate nucleus with finely stippled chromatin and two variably distinct nucleoli. Anisocytosis and anisokaryosis were moderate, and the mitotic count was 7 in ten 40X fields. Immunohistochemistry revealed that the interstitial cells were diffusely positive for neuron-specific enolase (NSE) and variably positive for glial fibrillary acidic protein (GFAP), while vascular endothelial cells were diffusely positive for vWF. In humans, hemangioblastomas usually develop in the cerebellum, and less frequently in the brain stem, as observed in this case. Only six cases of hemangioblastoma have been reported in dogs (most 6-9 years of age), five of which were in the spinal cord and one in the rostral cerebrum.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **LYMPHOCYTIC PLEOCYTOSIS CAUSED BY LISTERIA MONOCYTOGENES IN A HORSE**

Shannon Phelps, Deborah Chong, Sean Spagnoli, Elena Gorman  
Oregon State University, Corvallis, OR, USA

**Background:** *Listeria monocytogenes* infection is typically acinical in adult horses, and cases of meningoencephalitis are rare. Mononuclear pleocytosis is commonly found on CSF evaluation of clinically affected ruminants, and their signs typically localize to the brainstem or vestibular system. Reports of CSF findings in horses with confirmed listeriosis appear to be lacking.

Case description: A 16-year-old Quarter Horse gelding presented to the OSU large animal hospital for a severe and sudden onset of central neurologic signs, including disorientation, circling, and profuse sweating. No cranial nerve deficits were noted but the patient was severely ataxic, anxious, and hyperaesthetic. CSF was collected and the fluid was colorless. Fluid analysis revealed a pleocytosis (67 cells/uL, microprotein=40 mg/dL). Microscopic evaluation revealed a lymphocytic pleocytosis (88% small

lymphocytes, 12% large mononuclear cells). Viral testing was negative. The patient continued to deteriorate, and humane euthanasia was elected. Gross necropsy was performed and did not reveal an explanation for the clinical signs. Rabies virus FA was negative. Histologic examination of the caudal brainstem revealed nonsuppurative encephalitis with microabscessation, strongly suggestive of listeriosis. Bacterial culture of the caudal brainstem resulted in pure growth of *Listeria monocytogenes*.

Discussion: Results of bacterial culture confirm the histologic suspicion of *Listeria monocytogenes*. Clinical signs secondary to this infection are rare in adult horses, and the signs localizing primarily to the central nervous system rather than the brainstem were unique in this case. Although rare, *Listeria monocytogenes* should be considered in horses with lymphocytic pleocytosis, especially if viral testing is negative.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **HISTIOCYTIC DISORDER IN A CAPTIVE NORTH AMERICAN RIVER OTTER**

Panchan Sitthicharoenchai<sup>1</sup>, Claire Andreasen<sup>1</sup>, Andrew Gall<sup>2</sup>

<sup>1</sup>Iowa State University, Ames, IA, USA, <sup>2</sup>Blank Park Zoo, Des Moines, IA, USA

**Background:** A 2-year-old intact male captive North American river otter presented with progressive lethargy and decreased appetite. Examination revealed a marked decline of hematocrit from 52% at the previous annual examination to 21-29%. There were increased liver enzymes (e.g., ALT, ALP and AST), mild hypocalcemia, hypoalbuminemia, and hypoproteinemia. An enzyme-linked vector-borne disease immunoassay (4DxSNAP®) was negative, hemoparasites were not identified on a blood smear, and serology was negative for *Leptospira* serovars. Treatment, including a whole blood transfusion, was initiated. The otter died during the transfusion and was submitted for postmortem examination. **Methods and Results:** On gross examination, a moderate degree of splenomegaly and small tan foci on the subpleural surface of the lung were noted. Microscopically, there was evidence of erythrophagocytosis in the spleen, liver and bone marrow; submassive hepatic dissociation with centrilobular hepatic necrosis and regeneration; and myeloid hyperplasia in the bone marrow. Aggregates of mitotically active, erythrophagocytic, atypical histiocytic cells were noted in hepatic vessels, pulmonary vessels, and splenic sinusoids; the bone marrow was 70-75% effaced by atypical histiocytes. Differential diagnoses included immune-mediated hemolytic anemia (IMHA), histiocytic disorders, and heavy metal oxidative injury. A toxic element panel was unremarkable. Immunohistochemical diffuse positive staining with Iba-1 was present in aggregates of numerous atypical histiocytic cells in the liver, lung, spleen and bone marrow. **Conclusion:** Erythrophagocytic histiocytic proliferative disorder was diagnosed. This entity has been reported in canine, feline and human patients with clinical symptoms mimicking IMHA. This is the first suspected case in an otter.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **CUTANEOUS REACTIVE ANGIOENDOTHELIOMATOSIS IN A GUINEA PIG (*CAVIA PORCELLUS*)**

Gayathriy Balamayooran<sup>1</sup>, Matthew Kuhar<sup>2</sup>, Nicole McCrea<sup>3</sup>, Cinthya Cirqueira<sup>4</sup>, Josue Diaz-Delgado<sup>1</sup>

<sup>1</sup>Texas Veterinary Medical Diagnostic Laboratory, College Station, TX, USA, <sup>2</sup>New Jersey Animal Health Diagnostic Laboratory, Erwing Township, NJ, USA, <sup>3</sup>Madison Animal Hospital, Madison, NJ, USA, <sup>4</sup>Instituto Adolfo Lutz (IAL), Centro de Patologia, São Paulo, Brazil

A 1.8-year-old, intact male Guinea pig (*Cavia porcellus*) presented for recurrent hematuria and progressive erythema and swelling of the caudal abdomen and hind legs. Due to unresponsiveness to therapy and poor prognosis, euthanasia was elected, and a necropsy followed. The main gross findings were severe subcutaneous and muscular thickening of the hind legs due to edema; portal vein thrombosis; and cloudy urine with sandy sludge. Microscopically, there was multisystemic inflammation characterized by pleocellular meningitis, interstitial pneumonia, tracheitis, gastritis, adrenalitis and pancarditis with intravascular gram-negative bacteria. The portal vein had obliterative and recanalizing thrombosis. In the skin of hind legs, there was pleocellular dermatitis with vasculitis and intralesional gram-negative and gram-positive bacteria, intimately associated with multifocal to coalescing, non-neoplastic, infiltrative vascular growths that exhibited nodular and diffuse patterns. Proliferating endothelial cells immunoexpressed factor VIII. These findings indicated septicemia and concurrent cutaneous reactive angioendotheliomatosis (RAE). RAE is rare in veterinary species, including limited reports in cats and cattle infected with *Bartonella* species and a steer infected with bovine viral diarrhea virus. In humans, RAE is more prevalent in the extremities and is associated with infectious and autoimmune diseases, vasculopathies, and hemo-lymphoproliferative disorders. Angiogenic cytokines released after vascular occlusion and local hypoxia may induce endothelial cell proliferation and are thought to be key pathogenic factors in RAE. To the authors' knowledge, this is the first documentation of RAE in rodents. These findings suggest RAE should be considered in the differential diagnosis of integumentary disease in rodents.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **CONGENITAL TUBULAR DEFECTS CONSISTENT WITH FANCONI-LIKE SYNDROME IN A SPHINX CAT WITH ACIDURIA AND PARADOXICAL GLUCOSURIA**

Michaela Botts<sup>1</sup>, Petra Cerna<sup>1</sup>, Tawfik Aboellail<sup>1</sup>, Sarah Shropshire<sup>1</sup>, Rachel Cianciolo<sup>2</sup>, Jessica Hokamp<sup>2</sup>, Petra Werner<sup>3</sup>, Margret Casal<sup>3</sup>

<sup>1</sup>Colorado State University Veterinary Diagnostic Laboratory, Fort Collins, CO, USA, <sup>2</sup>The Ohio State University, Columbus, OH, USA, <sup>3</sup>University of Pennsylvania, Philadelphia, PA, USA

**Background:** Spontaneous renal tubular defects are sporadically reported in certain breeds of dogs but have never been reported in felids. A 3.5-year-old castrated male

Sphinx cat presented with persistently elevated liver enzymes and chronic kidney disease. A urinalysis performed at the University of Pennsylvania confirmed a mild amino aciduria and glucosuria consistent with Fanconi-like syndrome. Two months following an initial biopsy, the patient presented to Colorado State University Veterinary Diagnostic Laboratory for necropsy.

**Methods:** Sections were stained with hematoxylin and eosin (H&E), Congo Red, Rhodanine stain, Grocott's Methenamine Silver (GMS), Masson's trichrome, Periodic acid Schiff (PAS), and Phosphotungstic Acid Hematoxylin (PTAH). Semithin sections were prepared for transmission EM.

**Results:** A biopsy read at the Ohio State University (OSU) 2 months prior to euthanasia showed acute tubular injury, atrophy and multifocal karyomegaly of lining tubular epithelial cells with variable interstitial replacement fibrosis. Electron microscopy at the OSU did not identify significant glomerular lesions. On necropsy, both kidneys were markedly pale, puckered and smaller than average. Histologically, a moderate-to-severe chronic interstitial fibrosis with tubular degeneration and atrophy, mild glomerulosclerosis, multifocal interstitial lymphoplasmacytic nephritis, large vessel amyloidosis (multifocal and mild) and tubular epithelial cell karyomegaly were evident. The liver had moderate diffuse Zone 1 hepatocellular atrophy, periportal fibrosis, biliary hyperplasia, mild amyloidosis and karyomegaly.

**Conclusion:** All causes of acquired Fanconi-like syndrome were ruled-out. This is presented as the first reported case of congenital Fanconi-like syndrome in a cat. Genetic analysis to determine whether the syndrome has a familial basis is underway.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **ITRACONAZOLE-ASSOCIATED VASCULITIS IN A DOG WITH CUTANEOUS PARALAGENIDIOSIS**

Emily Brinker, Karly Hicks, Amelia White, Robert Kennis, Rachel Neto

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

**Background:** A 4-year-old, intact male, Labrador Retriever presented for well-demarcated, crusted to ulcerated, mostly symmetrical lesions on the face, axillae, and distal limbs. The dog was previously diagnosed with focal *Paralagenidium sp.* papular dermatitis with histology, immunohistochemistry, culture, and polymerase chain reaction (PCR). The *Paralagenidium sp.* lesion was non-progressive and maintained with oral itraconazole (10.1 mg/kg/day). The new crusted lesions regressed with decreased itraconazole dosage (6.8 mg/kg/day).

**Objective:** Our objective was to histologically examine and describe the crusted lesions to support the clinical suspicion of vasculitis.

**Methods:** Skin biopsies were performed and routinely processed and stained with hematoxylin and eosin. Infectious agents were screened for using routine Gram, Periodic acid-Schiff (PAS), and Grocott's methenamine silver stains. Fungal tissue culture of the dermal lesion was performed. PCR and serology were performed to rule out tick-borne disease.

**Results:** Within all layers of the minimally edematous and hemorrhagic dermis, the increased numbers of small-caliber blood vessels are lined by, often disrupted, hypertrophic endothelial cells. The walls of these blood vessels are thickened with and surrounded by a small amount of smudgy to fibrillar homogeneous eosinophilic material, neutrophils, and karyorrhectic cellular debris (leukocytoclastic vasculitis). Fibrin thrombi occlude few blood vessels. The superficial collagen bundles are variably smudged. Tick titers were not indicative of disease and no oomycetes were cultured.

**Conclusions:** Higher doses of itraconazole can potentially trigger vasculitis, with resolution of lesions with decreased dosage. Vasculitis should be investigated in cases of dogs with new dermal lesions being treated for cutaneous oomycete infections.

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## **CHONDRO-OSSEOUS RESPIRATORY EPITHELIAL ADENOMATOID HAMARTOMA IN AN EIGHT-YEAR-OLD MIXED-BREED DOG**

Cynthia de Vries<sup>1</sup>, Jerzy Gawor<sup>2</sup>

<sup>1</sup>LABOKLIN GmbH & Co.KG, Bad Kissingen, Germany, <sup>2</sup>Klinika Weterynaryjna Arka, Kraków, Poland

**Background:** Chondro-osseous respiratory epithelial adenomatoid hamartomas (COREAH) are extremely rare tumors of the nasal cavity, nasopharynx and paranasal sinuses, with only three canine cases reported so far. An eight-year-old, male castrated, mixed-breed dog presented with a four-month history of sneezing and unilateral decreased airflow. Previous treatment with prednisone (0.5 mg/kg) once daily for fourteen days and amoxicillin with clavulanic acid (12.5 mg/kg) twice daily for ten days was ineffective.

**Objective:** To report the fourth case of COREAH in a dog and describe the clinical, macroscopical and histological lesions.



**Methods:** 3-D cone beam computer tomography (CBCT) was performed. The mass was surgically excised and tissue samples were fixed in 10% buffered formalin and routinely processed for histology.

**Results:** A large, unilateral, intranasal mass caused increased density and obliteration of the majority of the left nasal cavity on 3-D CBCT. Nasal turbinates were osteolytic. Grossly, the polypoid smooth mass had a brown color on cut surface and firm consistency. Histologically, the mass was composed of large folds and polypoid to adenomatoid proliferations covered by a single layer of well-differentiated, pseudostratified, columnar ciliated epithelium. The epithelium covered central cores of variably edematous fibrovascular stroma, with diffuse mild infiltration of lymphocytes, plasma cells and neutrophils and multifocal hemorrhages. Multifocal areas of trabecular bone, with chondroid parts, lined by osteoblasts and osteoclasts involve about 40% of the mass. There was no evidence of vascular invasion or metastasis.

**Conclusion:** Although rare, COREAH should be considered as a possible differential in canine nasal masses.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **PRIMARY INTESTINAL LYMPHOMA IN RABBITS**

Daniel Bland<sup>1</sup>, Michael Garner<sup>2</sup>, Jessica Magnotti<sup>3</sup>, Alana Frum<sup>3</sup>, Jennifer Phinney<sup>4</sup>, Brandon Plattner<sup>4</sup>, Elise LaDouceur<sup>1,2</sup>

<sup>1</sup>Joint Pathology Center, Silver Spring, MD, USA, <sup>2</sup>Northwest ZooPath, Monroe, WA, USA, <sup>3</sup>Stahl Exotic Animal Veterinary Services, Fairfax, VA, USA, <sup>4</sup>Kansas State Veterinary Diagnostic Laboratory, Manhattan, KS, USA

**Background:** Alimentary tract involvement of disseminated lymphoma is occasionally encountered in rabbits, but primary intestinal lymphoma is rare, with only one previously reported case.

**Objective:** Describe pathologic findings of primary intestinal lymphoma in rabbits.

**Methods:** Northwest ZooPath archives were searched for biopsy and necropsy samples of neoplasia in rabbits. Cases were reviewed of primary intestinal lymphoma (defined as lymphoma that was confined to the intestines +/- regional lymph nodes), and immunohistochemistry (CD79a and CD3) was performed.

**Results:** Neoplasia was diagnosed in 737 rabbit specimens, 26 (3.5%) of which had lymphoma. Lymphoma was disseminated/visceral in 12 cases (12/26; 46.2%), primary cutaneous in 10 cases (10/26; 38.5%), and primary intestinal in four cases (case Nos. 1-4; 4/26; 15.4%). Case Nos. 1 and 2 were composed of large lymphocytes with oval to reniform nuclei; transmural effacement of the small intestine and invasion of the mesentery were evident, and mitotic counts were 80 and 98 per 2.37 mm<sup>2</sup>, respectively. Case Nos. 3 and 4 were composed of variably sized lymphocytes (case No. 3 large cell and case No. 4 small cell) with round nuclei; infiltration was limited to the mucosa and submucosa of the cecum, and mitotic counts were 35 and 45 per 2.37 mm<sup>2</sup>, respectively. Neoplastic lymphocytes in all cases had strong, multifocal, membranous immunoreactivity to CD79a and were negative for CD3.

**Conclusions:** All primary intestinal lymphomas were B cell origin, likely arising from GALT. Cecal lymphomas did not invade beyond the submucosa. Small intestinal lymphomas, however, had transmural effacement.

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## **DETECTING FUSION GENE TRANSCRIPTS IN CANINE PERIPHERAL T CELL LYMPHOMA**

Eileen Larsen, Lauren Harris, Robert Burnett, Anne Avery  
Colorado State University, Fort Collins, CO, USA

**Background:** Peripheral T-cell lymphoma (PTCL) is a poorly understood heterogeneous group of T-cell neoplasms associated with short survival times in humans and dogs. The discovery of several recurrent gene fusions has illuminated the molecular pathways involved in human PTCL lymphomagenesis and survival and subsequently impacted its diagnosis, prognosis, and treatment. Information on the occurrence of such chromosomal rearrangements in canine PTCL is lacking.

**Objective:** Develop a multiplexed assay to screen canine PTCL patients for fusion gene transcripts predicted from RNA-seq derived transcriptome data.

**Methods:** PCR primers targeting mRNA sequences for the B2M-GNAS, JPT1-GATD3A, MROH-GATD3A, TPD5-GATD3A, MCM5-PTMA, NCL-PTMA, RGS10-PTMA, NFkB2-TRIM8, SRSF5-LMO4, and TOX2-LMO4 fusions predicted from canine PTCL RNAseq data were designed using the Primer3 tool and validated with IDT gBlocks™ containing the predicted fusion sequences. PCR for these fusions was performed on 10 dogs with a diagnosis of PTCL confirmed by flow cytometry. gBlocks™ on a background of pooled cDNA from normal dogs were used as positive controls for the fusion primers.

**Results:** At least one of the predicted fusions was present in 7/10 dogs with PTCL, with 4/10 dogs having multiple of the predicted fusions. The SRSF5-LMO4 and NCL-PTMA fusions occurred most frequently, with each detected in 5/10 dogs.

**Conclusion:** Our findings support the recurrence of particular gene fusions in canine PTCL similar to what has been described in human PTCL, and that a multiplexed PCR assay utilizing gBlocks™ as positive controls is a useful tool for the validation and rapid detection of recurrent fusions.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

**MYCOBACTERIAL ENCEPHALITIS, PNEUMONIA AND SPLENITIS WITH SPLENIC INTRAHISTIOCYTIC CRYSTAL DEPOSITION IN A DOMESTIC RABBIT (ORYCTOLAGUS CUNICULUS)**

Nicolas Decelles, Maegan Hester, Lara Cusack, Shannon Martinson  
Atlantic Veterinary College, Charlottetown, PE, Canada

A 6-year-old, female intact domestic rabbit had a recurring clinical history of left-sided head tilt, inability to use its hind legs, depression and weight loss. The rabbit eventually succumbed to illness and was submitted for postmortem examination. Gross findings included: decreased muscle mass and fat stores; mottled tan to pale pink, rubbery lungs with numerous, randomly distributed, 1 mm, well-demarcated, tan nodules; numerous 1-2 mm in diameter, depressions on the cortical surface of both kidneys; and a segmentally markedly enlarged left oviduct. The brain and spinal cord, which appeared unremarkable grossly, had frequent granulomas distributed in the white and gray matter. Granulomas were also detected in the lung and spleen. A Ziehl-Neelsen acid-fast stain revealed large numbers of acid-fast bacilli in the cytoplasm of epithelioid macrophages and multinucleated giant cells. Birefringent crystals were detected within epithelioid macrophages in the splenic granulomas. Real-time polymerase chain reaction was performed on brain tissue and was positive for *Mycobacteria* but negative for *Mycobacterium tuberculosis* complex and *Mycobacterium avium*. In addition, 16s ribosomal RNA (rRNA) sequencing failed to identify the mycobacterial species. Mycobacterial culture is pending and is negative after 4 weeks of incubation.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

**METASTATIC TESTICULAR TERATOMA IN A FLORIDA MANATEE (TRICHECHUS MANATUS LATIROSTRIS)**

David Rotstein<sup>1</sup>, Martine deWit<sup>2</sup>, Nicole Stacy<sup>3</sup>

<sup>1</sup>Marine Mammal Pathology Services, Olney, MD, USA, <sup>2</sup>Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute, St. Petersburg, FL, USA, <sup>3</sup>University of Florida College of Veterinary Medicine, Gainesville, FL, USA

**Background:** The Florida manatee (*Trichechus manatus latirostris*) is a threatened population that is closely monitored. Threats to the population include anthropogenic and environmental causes; individual animals have succumbed to infectious disease and rarely neoplasia. Reproductive neoplasia has been reported in females, but to date, not in males.

**Objective:** Describe gross and histopathologic findings of a testicular teratoma in an approximately 4-year old male manatee.

**Methods:** Tissue samples from a necropsied free-ranging adult male manatee were processed and stained with hematoxylin and eosin.

**Results:** The 239 cm and 243 kg male was found dead. The manatee was known and had Passive Integrated Transponders (PIT tags). At necropsy, an irregular, 34.5 cm X 29.0 cm X 18.0 cm mass was associated with the left testis. The mass had calcified foci, resolving clots, cystic spaces, and firm yellow and white layers. Similar pulmonary nodules were present. Other findings included, serous atrophy of fat, intestinal displacement, and a flipper abscess. Histopathologic findings from the primary mass and lung metastases included presence of neoplastic mesenchymal and epithelial cells forming cartilage, bone, dermoid cysts, adipose, and myeloid cells.

**Conclusions:** Teratomas are an uncommon tumor type with metastases rarely reported. While the testis and ovary are the typical site, extragonadal sites include the thyroid gland, and in a dolphin, adrenal gland. Teratomas are parthenogenic tumors arising from a germ cell undergoing the first, but not second meiotic division. This case provides additional information on neoplasia in manatees.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **EVALUATION OF GENOMIC HETEROGENEITY IN CANINE CANCER BY NGS-BASED ANALYSIS OF TISSUE AND BLOOD SAMPLES**

Dana Tsui, Andi Flory, Daniel Grosu, Jason Chibuk, Lauren Holtvoigt, Katherine Lytle, Jill Rafalko, Kristina Kruglyak, Ilya Chorny  
PetDx, La Jolla, CA, USA

**Background/Objective:** Surgical biopsy in canine patients is associated with variable levels of risk dependent upon the site of the mass and characteristics of the procedure. Therefore, evaluation of suspected malignancy is often restricted to a single biopsy from one tumor site, limiting the assessment of intra-patient and intra-tumor genomic heterogeneity. Liquid biopsy offers the ability to capture the diversity of genomic alterations in a given cancer patient and may serve as a complement to traditional tissue biopsy.

**Methods:** A blood sample from an 11-year-old female spayed Border Collie with anal sac adenocarcinoma and lymph node metastases was subjected to cell-free DNA (cfDNA) extraction, proprietary library preparation, and next-generation sequencing (NGS). Sequencing data were analyzed using an internally developed bioinformatics

pipeline to detect cancer-associated genomic alterations. Results were compared with multiple spatially-separated tissue samples (two within the primary anal sac tumor, two within an affected lymph node).

**Results:** Four copy number variants (CNVs) were shared across all four tissue samples (on chromosomes 9, 13, 18, 22), while other CNVs were specific to either the lymph node sites, or one of the two anal sac tumor samples. Both shared and site-specific CNVs were detected in cfDNA from the pre-operative blood sample. No small genomic alterations were detected.

**Conclusions:** Blood-based liquid biopsy captures the diversity of genomic alterations derived from spatially-separated sites within a primary tumor or across metastatic locations. This information may be helpful for the selection of targeted therapies, assessment of minimal residual disease following surgical resection, or treatment response monitoring.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **CASE REPORT: PRIMARY NEUROLYMPHOMATOSIS IN A DOG**

Elizabeth Green, Renata Mammone, Elizabeth Curtis, Fred Williams III, Gayle Johnson  
University of Missouri Veterinary Medical Diagnostic Laboratory, Columbia, MO, USA

**Background:** Neurolymphomatosis is a rare condition characterized by invasion of malignant lymphocytes into peripheral nerves. It is suspected to be a manifestation of lymphosarcoma and can involve either B or T lymphocytes.

**Objective:** To describe the findings of an adult mix-breed canine diagnosed post-mortem with a neurotropic lymphosarcoma.

**Methods:** An 11-year-old, male castrated, mixed breed dog was presented for difficult and painful walking. A full neurologic evaluation was performed. Magnetic resonance imaging (MRI) of the thoracolumbar spine was performed. Cerebrospinal fluid was submitted for cytology. Subsequently, a gross necropsy examination was performed, and tissues were prepared for microscopic evaluation. Immunohistochemical stains CD3 and CD79 were used to further characterize the lesion.

**Results:** A painful and progressive peripheral polyneuropathy was identified on physical examination. Cytology of the cerebrospinal fluid revealed a lymphocytic pleocytosis. MRI showed thickening and hyperintensity of the lumbar nerve roots and sciatic nerves. Relevant gross findings include accumulation of opaque white fluid in the meninges and

a soft tan splenic mass. Microscopic findings revealed small lymphocyte infiltrates into spinal and sciatic nerves, along the lumbar dura, and in the meninges. Neoplastic small lymphocytes were confined to neural tissues. Degenerative changes in the sciatic and lumbar spinal nerves were severe. Lymphocytes were diffusely positive for CD3 and negative CD79a. Additionally, a splenic histiocytic sarcoma was identified.

**Conclusion:** This case report explores a unique manifestation of lymphosarcoma with likely primary peripheral nerve involvement. Neurolymphomatosis is rarely described in veterinary species; thus, it is helpful to further characterize the condition.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **DISORDERS OF SEXUAL DEVELOPMENT IN FREE RANGING CALIFORNIA SEA LIONS**

Margaret Martinez<sup>1</sup>, Michelle Rivard<sup>1</sup>, Emily Trumbull<sup>1,2</sup>, Barbie Halaska<sup>1</sup>, Robert Foster<sup>3</sup>, Padraig Duignan<sup>1</sup>

<sup>1</sup>The Marine Mammal Center, Sausalito, CA, USA, <sup>2</sup>SeaWorld, San Antonio, TX, USA,

<sup>3</sup>Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

Disorders of sexual development (DSD) are common in domestic animals but have not been documented in free ranging California sea lions (CSLs). Since 2016, 4 cases of disorders of sexual development were recorded at The Marine Mammal Center out of the 1,349 postmortems (prevalence of 0.3%). The cases included two phenotypically juvenile males and two adult females. The males had normal prepubertal testes and internal and external genitalia though with persistent penile frenulum and one a persistent paramesonephric duct (rudimentary uterus). The females had normal ovaries and external genitalia but internally one had a persistent uterine horn frenulum (nonunion) and the other segmental aplasia of the paramesonephric duct with no cervix, nor a uterine body and unilateral horn ostia. Three of the cases were incidental findings, while the male with a persistent paramesonephric duct (uterus) had posthitis, endometritis, cystitis, and pyelonephritis as the cause of death. DSDs occur due to sex chromosome abnormalities, hormonal or receptor disruptions, or exogenous substances such as endocrine disrupting chemicals in the environment. The cause of DSDs in these sea lions is not known; further investigation is ongoing to characterize the sex chromosomes and presence of SRY. It is also likely that pollutants within the food chain could be responsible for the lesions, as endocrine disrupting chemicals within the environment are shown to induce DSDs in fish and other wildlife; and several chemicals that cause significant disease, such as PCBs and DDTs, have already been detected in CSLs.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **POLYORCHIDISM IN A CAT WITH FOUR INTRAABDOMINAL TESTICLES**

Bryan Lohr<sup>1</sup>, Danielle Lieske<sup>2</sup>, Nicola Parry<sup>3</sup>

<sup>1</sup>Middle Road Animal Clinic, LLC, Glenshaw, PA, USA, <sup>2</sup>IDEXX Laboratories, Westbrook, ME, USA, <sup>3</sup>University of Surrey, Guildford, United Kingdom

A 4.14 kg, 9-month-old, intact male domestic shorthair cat was presented for castration surgery. Two weeks prior, the cat had begun to exhibit mounting behavior with the owner's intact female cats. On physical exam, the cat was bilaterally cryptorchid, with no palpable testicles in the scrotum or inguinal subcutaneous tissue, but exhibited penile spines. The cat was anesthetized, prepared for exploratory abdominal surgery to confirm the cryptorchidism, and a midline laparotomy was performed. Upon exploration, four nodules of tissue were located and excised from the abdominal cavity. Two nodules were located near the kidney on the left side, and the other two were close to the inguinal ring on the right side. Grossly, all nodules had the macroscopic features of testicles, and ranged from 7 x 5 x 5 mm to 12 x 6 x 7 mm. Each nodule appeared to have associated epididymal tissue. The nodules on the left side were associated with vascular structures resembling vasa deferentia. Histopathologic examination confirmed the presence of seminiferous and epididymal tubules in all four nodules consistent with testicular tissue and supporting a diagnosis of polyorchidism. There was no evidence of neoplastic, infectious, or inflammatory disease. Mounting behavior had ceased four weeks after laparotomy. Polyorchidism is a rare finding in cats, dogs, and horses, with few prior accounts reported in veterinary literature. Although rare, this case highlights the need to consider the possibility of polyorchidism in cryptorchid animals.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **AMELOBLASTIC FIBRO-ODONTOMA IN AN OX**

Sofia Rosales-Martinez<sup>1</sup>, Jesus Zavaleta-Hernandez<sup>2</sup>, Lucia Garcia-Camacho<sup>1</sup>, Ignacio Rangel-Rodriguez<sup>1</sup>

<sup>1</sup>Facultad de Estudios Superiores Cuautitlan. Universidad Nacional Autonoma de Mexico, Cuautitlan, Mexico, <sup>2</sup>Laboratorios Tornel, Aguascalientes, Mexico

An eighteen-month-old steer was presented with history of difficulties to eat during the last month due to a progressively growing mandibular mass that displaced the first and second incisors. The mass was 9 cm in length, ulcerated, and was sent for histopathologic evaluation after a marginal excision. Grossly, it was multinodular, white-gray, hyaline, and firm with tiny cystic spaces and several tan, irregular hard areas that protruded through the gum, requiring decalcification. Microscopically, there was a partially circumscribed tumor composed of sheets, trabeculae and buds of odontogenic epithelium embedded in abundant loose amphophilic ectomesenchyme. Both components displayed mild anisocytosis and anisokariosis. Some epithelial buds comprise enamel organ, and were sometimes associated with layers of homogeneous basophilic to eosinophilic acellular matrix, interpreted as enamel and/or dentin. Aggregates of basophilic matrix with lacunae and reverse lines, compatible with cementum/osteodentin, were also present. Some of these arrangements seemed to form primitive or disorganized dental structures. Mitotic figures were rare. The main differential diagnosis for this mixed histological pattern are ameloblastic fibro-odontoma and a developing complex odontoma, whose differentiation is not always possible with histopathology alone. The former diagnosis was made according to the age of

presentation (after completion of odontogenesis), anatomic location and frequency of ameloblastic fibromas/fibro-odontomas in this species. Clinically, the distinction of both neoplasms is relevant as it is assumed that the potential of growth of the ameloblastic fibro-odontoma is higher than the complex odontoma. The mass had incomplete margins and it relapsed a month after excision.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **LEPTOMENINGEAL GLIOMATOSIS IN DOGS AND CATS**

Vicente Avila Reyes<sup>1</sup>, Chloe Goodwin<sup>1</sup>, Sarah Schneider<sup>1</sup>, Elizabeth Howerth<sup>1</sup>, Kaori Sakamoto<sup>1</sup>, Taryn Donovan<sup>2</sup>, Andrew Miller<sup>3</sup>, Daniel Rissi<sup>1</sup>

<sup>1</sup>University of Georgia, Athens, GA, USA, <sup>2</sup>Animal Medical Center, New York, NY, USA,

<sup>3</sup>Cornell University, Ithaca, NY, USA

**Background:** Leptomeningeal gliomatosis (LG) is a rare form of glioma characterized by widespread dissemination of neoplastic glial cells throughout the leptomeninges either without an intra-axial glioma (primary LG) or with an intraparenchymal glioma (secondary LG).

**Objectives:** To describe the clinical and pathologic features of canine and feline LG.

**Methods:** A retrospective database search for cases of canine and feline LG was performed. Retrieved cases were reviewed and classified as primary or secondary LG.

**Results:** Seven cases were retrieved (5 dogs and 2 cats). The mean age of affected dogs was 7.4 years, and 4 dogs were brachycephalic; the mean age of affected cats was 11 years. The main clinical signs included pelvic limb weakness (2 dogs and 2 cats) and acute onset seizures (1 dog). The most common magnetic resonance imaging findings were leptomeningeal enhancement of affected areas. Grossly, 3 dogs had a telencephalic glioma and one dog had areas of intradural thickening in the spinal cord. Histologically, widespread leptomeningeal neoplastic glial cell infiltration was present in the spinal cord (5 dogs and 2 cats) and/or telencephalon (5 dogs). Six cases (5 dogs and 1 cat) had a concomitant intra-axial glioma in the prosencephalon (3 dogs), spinal cord (one dog and one cat), and midbrain (one dog), consistent with secondary LG.

**Conclusions:** Most cases of LG in this study were characterized as secondary LG based on the concurrence with an intra-axial glioma.



Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **PARACONIOTHYRIUM CYCLOTHYRIOIDES DERMATITIS IN A DOG AND A CAT**

Mayane Faccin, Courtney Meason-Smith, Aline Rodrigues-Hoffmann

Texas A&M University, College Station, TX, USA

**Clinical History:** An 11-year-old, castrated male, Boxer dog and a 13-year-old, castrated male, Domestic Shorthair cat presented with masses on their feet. The dog had a mass on the left hind foot of unknown duration. The cat presented with ulcerated, non-healing, swollen areas in all four feet that started about three years ago within the right front foot. **Histologic Findings:** Histologically, the skin in both cases presented with pyogranulomatous inflammation containing numerous, 5-10 µm in diameter, basophilic hyphae with acute-angle non-dichotomous branching, parallel walls, and frequent septation. In one of the cases, the hyphae presented bulbous swelling forming chains, similar to pseudohyphae. **Fungal identification:** DNA from formalin-fixed paraffin-embedded samples was extracted and used for panfungal PCR targeting the large ribosomal unit (LSU) region. Upon analysis with the NCBI BLAST database, the sequences matched *Paraconiothyrium cyclothyrioides* with 100% identity. The dog sample was also submitted for next generation sequencing (NGS) and *Paraconiothyrium cyclothyrioides/thysanolaenae* was the main fungus identified, further supporting the fungal identification in this case. **Discussion:** *Paraconiothyrium cyclothyrioides* is a coelomycetous fungal species that is ubiquitous in the environment. It has been rarely identified as a cause of infection in immunocompromised humans, mostly due to traumatic inoculation of contaminated plant material or soil. The disease tends to involve primarily the skin, but there are reports of systemic disease involving the lungs, eye, and brain. To the authors' knowledge, this is the first report of this agent in the veterinary literature.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **MUTATIONS IN EXONS 8 AND 11 OF C-KIT IN CANINE SUBCUTANEOUS MAST CELL TUMORS AND THEIR ASSOCIATION WITH OTHER PROGNOSTIC MARKERS**

Yue Xiang (Polly) Chen<sup>1</sup>, Laura Marconato<sup>2</sup>, Silvia Sabattini<sup>2</sup>, Matti Kiupel<sup>1</sup>

<sup>1</sup>Michigan State University Veterinary Diagnostic Laboratory and Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Lansing, MI, USA, <sup>2</sup>Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy

**Background:** The prognostic significance of internal tandem duplication (ITD) mutations in exons 8 and 11 of *c-kit* has been well established for canine cutaneous mast cell tumors (MCTs) but similar mutations have rarely been reported in subcutaneous MCTs. **Objective:** Identify the prevalence of ITD mutations in exons 8 and 11 of *c-kit* in canine subcutaneous MCT and investigate its association with

histological grade, KIT pattern and proliferation markers. **Methods:** Using archival data, ITD mutations in exons 8 and 11 of *c-kit*, mitotic count, Ki67 index, AgNOR number, Ki67xAgNOR score, KIT pattern, and histologic grade (2-tier system) were recorded for 216 dogs with subcutaneous MCTs. **Results:** ITD mutations in exon 8 and 11 of *c-kit* were detected in 23 (10.6%) and 12 (5.56%) subcutaneous MCTs, respectively. Exon 11 mutations were significantly associated with high grade ( $P < 0.001$ ) and increased mitotic count ( $P < 0.001$ ) compared to subcutaneous MCTs with no mutations in exons 8 or 11 or to subcutaneous MCTs with a mutation in exon 8 ( $P = 0.002$ ,  $P = 0.001$ ). There was no significant association of either ITD mutation with KIT patterns or proliferation activity. **Conclusions:** This study identified a higher prevalence of both exon 8 and exon 11 ITD mutations of *c-kit* in subcutaneous MCTs than previously reported. Like their cutaneous counterpart, subcutaneous MCTs with ITD mutations in exon 11 had a higher likelihood of being high grade and having a higher mitotic count, while such associations were not observed in subcutaneous MCTs with exon 8 mutations.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **ORAL HISTIOCYTIC SARCOMA IN A CAT WITH MANDIBULAR INVASION AND REGIONAL LYMPH NODE METASTASIS**

Stephani Ruppert, Sylvia Ferguson, Jason Struthers, Teela Jones  
Midwestern University, Glendale, AZ, USA

An 11-year-old female spayed domestic medium hair feline presented for a dental prophylaxis, at which time no oral mass was appreciated. Fifteen days post the dental cleaning, a mass expanding the oral mucosa overlying the rostral mandible was identified. An incisional biopsy revealed that the oral mucosa was infiltrated by neoplastic round to spindloid mesenchymal cells arranged in streams and small, dense aggregates consistent with an undifferentiated sarcoma. The patient was managed medically for approximately 6 months following the diagnosis and due to declining health, euthanasia with postmortem examination was elected. On postmortem examination, the previously described neoplastic cells were infiltrating the rostral mandible and had metastasized to the right submandibular lymph node. Immunohistochemistry performed during the post-mortem examination found that neoplastic cells were positive for Iba-1, CD18, CD204, and negative for MUM-1, S100, Melan-A, E-cadherin, favoring a diagnosis of oral histiocytic sarcoma. Although recently recognized in cats, feline oral histiocytic sarcoma is rare, the tumor's immunohistochemical profile is unstandardized, the tumor's behavior and prognosis is unclear, and the diagnosis is challenging if small incisional biopsies are submitted and the neoplasm is poorly differentiated. This case report discusses the clinical, macroscopic, microscopic, and immunohistochemical features of oral histiocytic sarcoma in a cat with mandibular invasion and submandibular lymph node metastasis.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **ALOBAR HOLOPROSENCEPHALY IN AN ABORTED AMERICAN QUARTER HORSE FETUS**

Luan Henker<sup>1</sup>, Marina Lorenzetti<sup>1</sup>, Manoela Marchezan Piva<sup>1</sup>, Júlia Wronski<sup>1</sup>, Danilo Giorgi Abranches de Andrade<sup>2</sup>, Alexandre Secorun Borges<sup>2</sup>, David Driemeier<sup>1</sup>, José Paes de Oliveira-Filho<sup>2</sup>, Saulo Petinatti Pavarini<sup>1</sup>

<sup>1</sup>Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil,

<sup>2</sup>Universidade Estadual Paulista "Júlio de Mesquita Filho" (Unesp), Botucatu, Brazil

Holoprosencephaly is a central nervous system malformation, characterized by incomplete (semilobar, lobar and inter-hemispheric fusion variants) or total lack of division of prosencephalon hemispheres (alobar variant), which is usually accompanied by facial malformations. Holoprosencephaly is considered the most common malformation of the forebrain in humans, and the condition has been occasionally reported in several animal species. The etiology has been associated with teratogens and numerous genetic anomalies; however, the cause remains undetermined in many cases. An aborted, American Quarter Horse female fetus, with 9 months of gestation, was submitted for postmortem examination. The fetus lacked haircoat, presented partially rotated fore and hind limbs, and had severe facial malformations, including marked shortening or absence of the incisive, nasal and maxillary bones, anophthalmia, and cheiloschisis. The prosencephalon was small and nearly spherical, represented by a single lobe, with no visible separation between cerebral hemispheres (absence of inter-hemispheric fissure). The olfactory bulbs, piriform lobes, and the optic chiasm were absent. At cross-sectioning of the prosencephalon, the inner structures of the brain were completely absent, and replaced by a single contiguous cavity lined by the remaining compressed cortex. As mutations in the *sonic hedgehog (SHH)* gene are associated with certain cases of human holoprosencephaly, the three *SHH* coding exons were sequenced using liver DNA of the aborted foal. The obtained *SHH* sequence was similar to the *Equus caballus SHH* mRNA sequence deposited in Genbank™ (XM\_023640069.1); therefore, no polymorphism justifying the phenotype was observed. This is the first report of alobar holoprosencephaly in horses.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **CLINICAL AND PATHOLOGIC CHARACTERIZATION OF FELINE OCULAR POST-TRAUMATIC LYMPHOMA**

Alexandra Harvey<sup>1</sup>, Vanessa Holly<sup>1</sup>, Leandro Teixeira<sup>2</sup>, Richard Dubielzig<sup>2</sup>, Amy Durham<sup>1</sup>

<sup>1</sup>University of Pennsylvania, Philadelphia, PA, USA, <sup>2</sup>University of Wisconsin – Madison, Madison, WI, USA

Background. Three morphologic variants of feline ocular post-traumatic sarcoma are spindle cell, osteo-/chondrosarcoma, and round cell. The round cell variant is recognized as lymphoma, but further characterization is needed.

**Objective.** To characterize the clinical, histological, immunohistochemical features, and outcomes of feline ocular post-traumatic lymphoma (FOPT-LSA).

**Methods.** Tissues were collected from 96 cases with FOPT-LSA from Comparative Ocular Pathology Laboratory of Wisconsin (2003-2017). Histopathologic and immunohistochemical (CD3, PAX5, CD79b) evaluation included lymphoma distribution and classification, and concurrent ocular disease. Clinical data were collected from submission forms (n=96) and follow-up surveys (n=42).

**Results.** The mean age was 11.8 years (4-19). In 81 cases with clinical data, 74 (91%) reported a history of chronic ocular disease and/or trauma. Frequent concurrent ocular diseases included uveitis (n=95;99%), keratitis (n=83;86%), and cataract (n=76;79%). Intraocular distribution included panuveal blanketing/infiltration (n=87;91%), with retinal involvement in 95 cases (99%). Lymphomas were categorized based on cell size [large cell n=66 (68%), intermediate n=26 (28%), small n=4 (4%)], mitotic count [high-grade >10 n=53 (55%), mid-grade 6-10 n=31 (32%), low-grade 2-5 n=12 (13%)], and immunophenotype (B-cell n=59 (62%), T-cell n=30 (31%), double-negative n=7 (7%)). Systemic involvement was evident at diagnosis in 5 cases according to submission forms. In the 42 cases with follow-up, 13 cats had systemic disease at last appointment, suggesting 8 cats developed progressive disease post-enucleation. 19 cats died within 6 months post-diagnosis, and 28 cats died within 1 year.

**Conclusions.** FOPT-LSA has unique clinical and histologic presentations characterized by a panuveal/retinal distribution and a predominance of large B-cell lymphoma.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **DIFFUSE COLONIC DILATATION IN A CYNOMOLGUS MACAQUE (MACACA FASCICULARIS)**

Michael McKinney, Katherine Shuster, Katherine Gibson-Corley, Christopher Pinelli  
Vanderbilt University Medical Center, Nashville, TN, USA

A 15-year-old, female cynomolgus macaque presented with a 1-week history of progressive hyporexia and constipation following recovery from a routine semi-annual exam. The animal had no relevant experimental history. Abdominal radiographs revealed marked colonic dilatation secondary to intraluminal gas. Given the anesthetic risks, rapid progression of clinical signs, and poor prognosis, humane euthanasia was elected. Grossly, there was severe, diffuse colonic dilatation filling most of the abdominal cavity. The dilatation was present in all segments of the colon and secondary to a mix of luminal gas and feces. The colorectal junction appeared narrow with no mucosal or serosal lesions present. Additionally, there was torsion and ischemic necrosis of the left lateral liver lobe. Samples of liver and colon were harvested and fixed in 10% NBF, routinely processed, embedded, and stained with HE. Histologically, there was regionally extensive serosal edema and hemorrhage of the colon as well as a mild to moderate typhlocolitis. This inflammatory infiltrate was made up primarily of lymphocytes and plasma cells within the lamina propria, a common finding in aged laboratory macaques. Due to the absence of a specific etiology, idiopathic megacolon

was suspected. Idiopathic megacolon, or acute colonic pseudo-obstruction, is defined as pathologic dilatation of the colon without any underlying mechanical cause. Many risk factors have been identified but few publications exist in cynomolgus macaques. Although the sample size is small, females appear to be overrepresented. This case illustrates the potential severity of idiopathic megacolon and raises questions regarding its unusual pathogenesis.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

**IMMUNOHISTOCHEMICAL CHARACTERIZATION OF DUODENAL ADENOCARCINOMA WITH PULMONARY, HEPATIC, AND PARAPATELLAR METASTASES IN A COMMON MARMOSET (*CALLITHRIX JACCHUS*)**

Cornelia Peterson, Jessica Plunkard, Andrew Johanson, Jessica Izzi, Joseph Mankowski, Kathy Gabrielson

Johns Hopkins University, School of Medicine, Baltimore, MD, USA

**Background.** An eleven-year-old male common marmoset (*Callithrix jacchus*) presented with chronic, progressive weight loss and diarrhea. Response to treatment with nutritional supplementation, antibiotics, and immunosuppressants was modest and transient, and the animal was humanely euthanized. **Methods.** A diagnostic necropsy was performed. Tissues were formalin-fixed and routinely processed. All tissues were evaluated with routine H&E sections, and affected tissues were further evaluated by PAS and Warthin Starry staining and immunolabeling for MUC2, pancytokeratin, IBA1, CD3, and CD20. Immunohistochemistry was also performed on histologically-normal marmoset duodenum. **Results.** At necropsy, the proximal 8 cm of duodenum was diffusely pale, and transmural thickening was observed on cross section. The lungs contained coalescing pale, firm nodules, measuring up to 4 mm in diameter. Histologic examination revealed infiltrative mucinous adenocarcinoma of the duodenum with extensive metastases to the lungs, liver, and left parapatellar adipose. The mucinous matrix secreted by the primary and metastatic lesions was strongly PAS-positive. Pancytokeratin expression was lost in the primary tumor as well as the metastases, correlating to a poorly-differentiated phenotype. **Conclusions.** Previous case reports have documented small intestinal adenocarcinomas in the marmoset; however, the proximal duodenal localization is atypical, and extensive metastatic disease arising from these tumors has not been reported.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

**LIPID KERATOPATHY IN A BOSTON TERRIER**

Sarai Milliron, Erin Scott, Lucien Vallone, Laura Bryan

College of Veterinary Medicine & Biomedical Science, Texas A&M University, College Station, TX, USA

Lipid keratopathy is an acquired disease in humans and dogs characterized by corneal lipid deposits and keratocyte degeneration. Lipid keratopathy is associated with corneal vascularization and can be focal if associated with localized proliferative diseases or more diffuse when associated with generalized anterior segment inflammation. Here we

present a case of lipid keratopathy in a Boston Terrier. A 15-year-old, female spayed, Boston Terrier was presented to the Texas A&M University Veterinary Teaching Hospital for evaluation of mature cataracts OU. Phacoemulsification with intraocular lens placement OU was performed. Approximately one month post-op the patient developed progressive lipid corneal degeneration OU followed by progressive pigmentary keratitis two years post-op. In vivo confocal microscopy revealed hyper-reflective spicules within the anterior stroma, consistent with lipid, and scattered leukocytes within the superficial epithelium. Disease progressed in the right eye along with uncontrolled secondary glaucoma leading to enucleation. Histopathology showed focally extensive areas of corneal pigmentation with abundant small caliber vessels and a small number of lipid-laden macrophages infiltrating the corneal stroma. Other significant findings include fibrovascular membrane formation, anterior and posterior synechiae, chronic intraocular hemorrhage, complete retinal detachment, optic nerve atrophy, and chorioretinal vasculopathy. The histologic findings are consistent with lipid keratopathy secondary to intraocular disease.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **TYPE-1 DIABETES MELLITUS IN A YOUNG CAT**

César Arroyo-Perea, Aline Luna-Sánchez, Rubén Méndez-Medina, Karla Mollinedo-Beltrán

Facultad de Medicina Veterinaria y Zootecnia - Universidad Nacional Autónoma de México (FMVZ-UNAM), Ciudad de México, Mexico

**Background.** Type-1 diabetes mellitus (DM) is uncommon in cats.

**Objective.** Description of clinicopathological and pathological features of a case of type-1 DM in a cat.

**Methods.** An eleven-month-old, neutered male Siamese cat was presented to the FMVZ-UNAM small animal teaching hospital with a history of depression and polyuria-polydipsia, as well as vomiting and anorexia for two days. Blood samples were submitted for analysis, then necropsy was performed.

**Results.** Physical examination showed stupor, poor body condition (1/5), pale mucous membranes, weak pulse, and dehydration. CBC revealed relative erythrocytosis (0.56 L/L) and leukocytosis ( $19.9 \times 10^9/L$ ) because of neutrophilia ( $18.7 \times 10^9/L$ ) and lymphopenia ( $1.0 \times 10^9/L$ ) due to stress. Serum biochemistry showed marked hyperglycemia (27.1 mmol/L) suggestive of DM, as well as hyponatremia (125 mmol/L), increased urea (15.1 mmol/L) secondary to decreased glomerular filtration rate, hyperbilirubinemia (21.6  $\mu\text{mol/L}$ ) due to cholestasis, increased ALT (417 U/L) and AST (280 U/L) indicative of hepatocellular damage, hypochloremia (80 mmol/L) secondary to vomiting, hypokalemia (2.8 mmol/L) related to anorexia, metabolic acidosis (anion gap 28 mmol/L) due to accumulation of ketones, and metabolic alkalosis because of vomiting. Urinalysis revealed glycosuria, ketonuria and bilirubinuria. Fructosamine was also increased (512  $\mu\text{mol/L}$ ). Diabetic ketoacidosis was diagnosed. Necropsy was

performed and histopathology showed severe hepatic lipidosis, pancreatic atrophy with absence of islets, and membranoproliferative glomerulonephritis.

Conclusion. Although the most common presentation in cats is type-2 DM, the young age and the absence of islets of the patient indicated type-1 DM; glomerular lesions indicate that it was of autoimmune origin.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **BORDETELLA BRONCHISEPTICA PRESENTING AS A FIBRINOUS AND NECROTIZING PLEUROPNEUMONIA IN A RABBIT KIT**

Ashleigh Shoemaker, Jessica Herrod, Jessica Izzi, Kathleen Gabrielson  
Johns Hopkins University, Baltimore, MD, USA

**Background:** At 1-month of age, a previously healthy New Zealand White rabbit kit presented for quiet mentation and bilateral nasal discharge. On physical examination, the kit had a mild increase in respiratory effort with wheezes and crackles auscultated in the cranial lung lobes. There was mucoid discharge present in both nares, as well as extensive fecal and urine staining on the perineum. After obtaining a culture swab from the nasal cavity, the kit was anesthetized and subsequently euthanized by intracardiac euthasol injection due to a grave prognosis. No additional kits in the litter had evidence of clinical disease.

**Methods:** A diagnostic necropsy was requested to determine a cause of death.

**Results:** Gross and histopathological examinations revealed a fibrinous and necrotizing pleuropneumonia, more severe in the left lung. Antemortem culture revealed heavy growth of *Bordetella bronchiseptica*. Other histopathological findings included a necrosuppurative sinusitis, glossitis, tracheitis, and myocarditis with intralesional coccobacilli. A mild osteomyelitis of the tibial metaphysis suggested the presence of septicemia.

**Conclusions:** While a fibrinopurulent bronchopneumonia has been described in association with primary *B. bronchiseptica* infection in young rabbits, fibrinous pleuritis and pericarditis are frequently identified only in kits the presence of *Pasteurella multocida*. Therefore, we present an unusual and severe case of *Bordetella* infection in a rabbit kit with fibrinous and necrotizing pleuropneumonia.

### **Education Focused Poster Session**

Saturday, October 30, 2021 | 5:00 p.m. – 6:00 p.m. CDT

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **PILOTING A CLINICAL ENTRUSTMENT SCALE AND FEEDBACK FORM IN AN ACADEMIC MEDICAL CENTER**

Erin Burton, Laura Molgaard, Aaron Rendahl  
University of Minnesota, St Paul, MN, USA

**Introduction:** Entrustable Professional Activities (EPAs) have recently garnered interest in veterinary education. EPAs describe the routine clinical activities of a health professional and provide a context for observation and assessment of the learner's abilities in the veterinary curriculum.

**Objectives:** Evaluate the acceptability and perceived utility of a brief entrustment form to provide frequent, coaching feedback in the clinical year using a feedback form.

**Methods:** Using the CBVE EPAs a feedback form was created which included demographic information, a 5-point entrustment scale, and two prompts for narrative feedback. Clinical services were recruited to pilot the learner feedback form in their service during the 2018-2019 academic year. Raters and learners were provided training prior to using form. Bi-weekly surveys were administered for each group on efficiency and perception of the quality of feedback given and received. At the conclusion of the study learners and raters were invited to participate in focus group sessions.

**Results:** Most raters could complete the rating form in less than 2-5 minutes, felt that it improved the overall quality of feedback they provided in comparison to before. Learners reported that the amount of feedback received was more frequent than on services not using the form and that this feedback allowed reflection on personal performance.

**Conclusion:** A short EPA-based rating form can provide a platform for immediate feedback in a way that is both feasible and valuable in a busy academic veterinary medical center.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **A SEMI-COLLABORATIVE APPROACH TO MIDTERM AND FINAL EXAMINATIONS IN A CLINICAL PATHOLOGY CORE CURRICULUM COURSE**

Maxey Wellman, Jessica Hokamp, Samantha Evans, Mary Jo Burkhard, Jennifer Gonya, Cheryl Ray

College of Veterinary Medicine The Ohio State University, Columbus, OH, USA

The clinical pathology course at The Ohio State University is taught autumn semester year. In an effort to add opportunities for peer teaching, reinforcement of key concepts, and group case discussion to improve student learning, we introduced a new semi-collaborative approach to the 1<sup>st</sup> midterm examination, based on discussions with our Office of Teaching and Learning. Students were given 2 attempts to take the examination. The 1<sup>st</sup> attempt was in-class, closed resource, and the 2<sup>nd</sup> attempt was in-class, open resource, open discussion, for 75% and 25% of the grade, respectively.\* Students could choose their own groups for the 2<sup>nd</sup> attempt, could work with classmates, and did not have access to their 1<sup>st</sup> attempt scores prior to the 2<sup>nd</sup> attempt. All student scores either stayed the same (14%) or improved (86%) from the 1<sup>st</sup> to the 2<sup>nd</sup> attempt. Mean scores were 91% and 100% after the 1<sup>st</sup> and 2<sup>nd</sup> attempts, respectively, showing that when given the opportunity to discuss questions with



classmates and use resources, students were able to determine the correct responses on their own. The feedback on this hybrid examination format was so overwhelmingly positive that the same format was requested for the remaining examinations in this course and for other core curriculum courses. Students indicated that the new format helped them understand material that they had found difficult, solidify key concepts, and made learning fun. Faculty and staff thought it was rewarding to see the students' energy working together in this learning format.

\*2019, prior to the pandemic.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

## **WOMEN REPRESENTATION AND GENDER EQUALITY IN DIFFERENT ACADEMIC LEVELS IN VETERINARY SCIENCE**

Xinyue Liu, Rebecca Dunlop, Rachel Allavena, Chiara Palmieri

School of Veterinary Science, The University of Queensland, Gatton, Australia

### Background

Women's participation and completion at veterinary schools has increased globally for the past few decades. However, increased female graduates have not translated into similar patterns of academic staffing.

### Objectives

This study aimed to evaluate, and compare, the proportion of female academic staff in veterinary science faculties in Australia and New Zealand, Europe, and North America. The study assessed workplace gender distribution within each academic level and compared distributions between academic level in different veterinary science faculties and countries.

### Methods

The gender distribution within each academic level at eight accredited veterinary faculties in Australia and New Zealand, 38 accredited faculties in USA and Canada, and 98 accredited faculties in Europe were analyzed. For all analyses, general linear models were used, assuming a binomial distribution and with gender as the binomial response variable.

### Results

Women occupied 47.9%, 45.5% and 47.5% of the academic positions in Australia/New Zealand, USA/Canada and Europe, respectively. Compared to their male counterparts, female academics were more likely to hold the lower ranked positions. The gender distribution skewed toward men in the senior positions at or above Associate Professor level in all analyzed regions.

### Conclusions

The findings of this study confirm gender inequality in academic progression meaning there is a continued need to develop strategies to eliminate inequity in veterinary science faculties worldwide.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

**ONLINE MENDELIAN INHERITANCE IN ANIMALS (OMIA): A REVIEW OF SUSPECTED OR KNOWN INHERITED NEUROLOGIC CONDITIONS IN SHEEP**

Leah Manning<sup>1,2</sup>, Brendon O'Rourke<sup>1</sup>, Frank Nicholas<sup>3</sup>, Mark Krockenberger<sup>3</sup>, Imke Tammen<sup>2</sup>

<sup>1</sup>Elizabeth Macarthur Agricultural Institute, Department of Primary Industries, Menangle, NSW, Australia, <sup>2</sup>Sydney School of Veterinary Science, The University of Sydney, Camden, NSW, Australia, <sup>3</sup>Sydney School of Veterinary Science, The University of Sydney, Camperdown, NSW, Australia

**Background:** Online Mendelian Inheritance in Animals (OMIA, <https://omia.org/home/>) is a free online resource made available by The University of Sydney, documenting phenes (disease as well as non-disease traits that are suspected or known to be inherited) in more than 250 vertebrate species. Each phene-species entry includes references and where relevant, information on mode of inheritance, causative variant(s) and summaries of the clinical, pathologic and/or molecular features. There are cross links within OMIA to similar phenes in other species, as well as hyperlinks to possibly relevant human trait(s) and/or gene(s) in Online Mendelian Inheritance in Man (OMIM, <https://www.omim.org/>). New references are regularly added to the database as they become available via PubMed.

**Methods:** We conducted a structured literature review of neurologic phenotypes in sheep using PubMed and Web of Science searches with the aim to improve OMIA curation for these traits.

**Results:** A review of OMIA revealed that 58 ovine neurological phenes are listed in OMIA. Each phene was assessed for new references. Ongoing curation focuses on adding summaries for clinical signs and relevant gross or histopathology findings that assist in characterising the particular disorder, as well as any recent molecular advances. The literature was also reviewed to identify any additional suspected or known inherited neurologic phenes in sheep, allowing addition of at least four new phenes.

**Conclusion:** OMIA is an invaluable online resource that has far reaching applications including teaching, diagnostic investigations and genetic counselling in relation to suspected and confirmed inherited conditions and animal models of human disease.

## Natural Disease Focused Poster Session

Sunday, October 31, 2021 | 12:00 p.m. – 1:00 p.m. CDT

Sunday, October 31, 2021

12:00 p.m. – 1:00 p.m. CDT

### **EX VIVO IDENTIFICATION OF NATURALLY OCCURRING PRECLINICAL LESIONS OF OSTEOCHONDRITIS DISSECANANS BY MORPHOLOGICAL AND QUANTITATIVE 10.5T MRI IN PIGS**

Alexandra Armstrong<sup>1</sup>, Stefan Zbyn<sup>2</sup>, Gregory Metzger<sup>3</sup>, Jutta Ellermann<sup>2,3</sup>, Cathy Carlson<sup>1</sup>, Ferenc Toth<sup>1</sup>

<sup>1</sup>Department of Veterinary Clinical Sciences, University of Minnesota, St. Paul, MN, USA, <sup>2</sup>Center for Magnetic Resonance Research, University of Minnesota, St. Paul, MN, USA, <sup>3</sup>Department of Radiology, University of Minnesota, St. Paul, MN, USA

**Background:** Juvenile osteochondritis dissecans (OCD) is a developmental orthopedic disorder most commonly affecting the knee and elbow joints of children and young animals. The existence of preclinical OCD lesions (osteochondrosis latens [OCL] and osteochondrosis manifesta [OCM]) is well recognized in animals. Recent identification of similar lesions in human pediatric cadaveric specimens suggests a shared pathogenesis between animals and humans. MRI has not identified naturally occurring OCL lesions. **Objective:** We hypothesized that 10.5T MRI can identify naturally occurring OCL and OCM lesions in elbow and knee joints of juvenile pigs. **Methods:** Unilateral elbows (n=3) and knees (stifles) (n=3) were harvested from 3 pigs aged 4, 8 and 12 weeks, and scanned in a whole-body 10.5T MRI. Morphological images were acquired with a 3D DESS and T2-weighted images with a 2D multiecho spin echo sequence. **Results:** Areas with increased signal intensities (3D DESS) and T2 values (T2 maps) were considered suspicious of preclinical OCD and were identified by MRI in one distal femur and all three humeri. Histological sections confirmed the presence of preclinical OCD lesions at these sites. Histological findings included necrotic vascular profiles associated with areas of chondronecrosis either confined to the epiphyseal cartilage (OCL, 4- and 8-week-old specimens) or resulting in a delay in endochondral ossification (OCM, 12-week-old specimen). **Conclusions:** Morphological and quantitative 10.5T MRI identified all preclinical OCD lesions. Translating these imaging methods to clinically relevant field strength and to human cadaveric specimens will pave the way towards clinical application in early diagnosis of juvenile OCD in humans.

Sunday, October 31, 2021

12:00 p.m. – 1:00 p.m. CDT

### **SEVERE PNEUMONIA AND MYOCARDITIS CAUSED BY SARS-COV-2 INFECTION IN A CAT WITH HYPERTROPHIC CARDIOMYOPATHY**

Francisco Carvallo<sup>1</sup>, Mathias Martins<sup>2</sup>, Lok Joshi<sup>2</sup>, Leonardo Caserta<sup>2</sup>, Patrick Mitchell<sup>2</sup>, Thomas Cecere<sup>1</sup>, Sandy Hancock<sup>1</sup>, Erin Goodrich<sup>2</sup>, Julia Murphy<sup>3</sup>, Diego Diel<sup>2</sup>

<sup>1</sup>Virginia Tech, Blacksburg, VA, USA, <sup>2</sup>Cornell University, Ithaca, NY, USA, <sup>3</sup>Virginia Department of Health, Richmond, VA, USA

Coronavirus disease 19 (COVID-19), has claimed millions of human lives worldwide since the emergence of the zoonotic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in China in December 2019. Most severe and fatal SARS-CoV-2 infections in humans have been associated with underlying clinical conditions, including diabetes, hypertension and heart diseases. An autopsy was performed on a 4.5-year-old, male castrated domestic medium hair cat died after a five-day long history of severe progressive respiratory distress that was not responsive to therapy. The cat came from a household in which two people tested positive for SARS-CoV-2. Approximately 15 mL of red-tinged fluid were collected from the thorax. The lungs were diffusely mottled red and slightly firm. The left ventricular wall was moderately thickened, with a narrow ventricular cavity and the heart weighing 22 g. Histologically, broncho-interstitial pneumonia and multifocal capillaritis was noted, with segmental proliferation of type-II pneumocytes and occasional hyaline membranes. Acute myocardial degeneration and necrosis was also identified. SARS-CoV-2 was detected in pharyngeal, nasal, tracheal and rectal swabs, and the virus was detected and isolated from the lung and heart. This is a case of severe SARS-CoV-2 infection in a domestic cat that presented with hypertrophic cardiomyopathy, a chronic heart condition that has been described as a comorbidity of COVID-19 in humans and that is prevalent in domestic cats. This case provides important information that may contribute to the development of a feline model with the potential to recapitulate the clinical outcomes of severe COVID-19 in humans.

Sunday, October 31, 2021

12:00 p.m. – 1:00 p.m. CDT

### **REVIEW OF NEOPLASIA IN THE ALIMENTARY TRACT OF CAPTIVE BEARDED DRAGONS (POGONA SPP.)**

Elise LaDouceur<sup>1,2</sup>, Alexandria Argue<sup>1</sup>, Michael Garner<sup>2</sup>

<sup>1</sup>Joint Pathology Center, Silver Spring, MD, USA, <sup>2</sup>Northwest ZooPath, Monroe, WA, USA

**Background:** There are multiple reports of gastric neuroendocrine carcinoma in bearded dragons. Other types of alimentary neoplasia are rarely reported in this species. **Objective:** Describe the anatomic distribution and types of alimentary neoplasia in bearded dragons. **Methods:** Biopsy and autopsy specimens of alimentary neoplasia in captive bearded dragons were collated from the Northwest ZooPath and Joint Pathology Center archives. **Results:** Fifty one cases were identified in the stomach (n=26), oral cavity (n=18), intestines (n=13), esophagus (n=3), and cloaca (n=2). Round cell neoplasia was diagnosed in the alimentary tract in 14 cases, all of which had extra-alimentary involvement, including from most to least commonly in the liver, lung, spleen, kidney, heart, pancreas, gonad, skeletal muscle, trachea, adipose tissue, brain, eye, blood vessels, bone marrow, adrenal gland, thyroid gland, bone, nasal cavity, and mesentery. Oral neoplasms included sarcoma, adenomatous polyp, round cell neoplasia, fibromatous epulis of periodontal ligament origin, and myxoma. Esophageal neoplasms included round cell neoplasia. Gastric neoplasms included neuroendocrine carcinoma, round cell neoplasia, adenocarcinoma, and sarcoma. Enteric neoplasms included round cell neoplasia, sarcoma, adenocarcinoma, and metastatic sarcoma. Cloacal neoplasms included round cell neoplasia and squamous cell carcinoma. **Conclusions:** Round cell neoplasia and gastric neuroendocrine

carcinoma were the most common diagnoses. Round cell neoplasia was consistently present in extra-alimentary sites; all other neoplasms were primary alimentary neoplasms except for one metastatic sarcoma. Sarcomas and polyps were common in the oral cavity and rare to absent elsewhere. Adenocarcinoma was rare and only identified in the stomach and intestines.

Sunday, October 31, 2021

12:00 p.m. – 1:00 p.m. CDT

## **HISTOPATHOLOGICAL SUBTYPING, MOLECULAR AND VIRAL CHARACTERIZATION OF EQUINE GENITAL AND PERIOULAR SQUAMOUS CELL CARCINOMAS**

Kevin O'Brien<sup>1</sup>, Alejandro Suárez-Bonnet<sup>1</sup>, Tim Mair<sup>2</sup>, Hardeep Mudhar<sup>3</sup>, Patricia Pesavento<sup>4</sup>, Simon Priestnall<sup>1</sup>

<sup>1</sup>Department of Pathobiology and Population Sciences, The Royal Veterinary College, Hatfield, United Kingdom, <sup>2</sup>Bell Equine Veterinary Clinic, Maidstone, United Kingdom, <sup>3</sup>National Specialist Ophthalmic Pathology Service (NSOPS), Royal Hallamshire Hospital, Sheffield, United Kingdom, <sup>4</sup>School of Veterinary Medicine, UC Davis, Davis, CA, USA

**Background:** Equine genital and periocular squamous cell carcinomas (eSCCs) have a guarded to poor prognosis, and a significant proportion are likely induced by equine papillomavirus. Accurate prediction of clinical outcomes is challenging with no recognized prognostic criteria or consistent histopathological classification scheme for eSCC. **Objective:** To histopathologically subtype eSCC and correlate with p16, HER-2 expression, and equine papillomavirus infection. **Methods:** One hundred and nine cases (59 genital and 50 periocular) eSCC were examined and subtyped histologically. HER-2 and p16 immunohistochemistry and *in-situ* hybridization for the E6/E7 oncogenes of *Equus caballus* papillomavirus 2 (EcPV2) were performed on 77 and 30 cases respectively. Results were semi-quantitatively assessed. **Results:** Invasive was the most common histopathological subtype, 74 of 109 (67.9%), with 19 (17.4%) verrucous, 3 (2.8%) papillary, 3 (2.8%) warty, 2 (1.8%) basaloid, 2 (1.8%) pseudoglandular, and 6 (5.5%) mixed. HER-2 was widely expressed (70/77) with 49/70 demonstrating weak to moderate and 21/70 strong membranous labelling. p16 was less widely expressed (26/77) with 23/26 demonstrating weak to moderate and 3/26 strong cytoplasmic labelling. Robust E6/E7 probe hybridization was detected in 16/30 cases. **Conclusions:** Seven WHO histological subtypes of SCC were identified, with the usual/invasive type being most frequent. This is the first evidence of p16 expression in eSCC. HER-2 over-expression occurs in SCC and precursor lesions, identifying this receptor as a potential therapeutic target. This study contributes to evidence supporting a causal role of EcPV2 in a subset of genital and periocular SCCs in horses.

Sunday, October 31, 2021  
12:00 p.m. – 1:00 p.m. CDT

### **INVOLVEMENT OF FELIS CATUS PAPILLOMAVIRUS TYPE 2 IN THE TUMORIGENESIS OF FELINE MERKEL CELL CARCINOMA**

Soma Ito<sup>1</sup>, James Chambers<sup>1</sup>, Ayumi Sumi<sup>1</sup>, Nanako Yamashita-Kawanishi<sup>1</sup>, Tetsuo Omachi<sup>2</sup>, Takeshi Haga<sup>1</sup>, Hiroyuki Nakayama<sup>1</sup>, Kazuyuki Uchida<sup>1</sup>

<sup>1</sup>University of Tokyo, Tokyo, Japan, <sup>2</sup>Diagnostic Laboratory Patho Labo, Shizuoka, Japan

**Background:** Merkel cell carcinoma (MCC) is a cutaneous neuroendocrine tumor and caused by polyomavirus in human. We recently demonstrated that cats with MCC often have other proliferative cutaneous lesions, such as Bowenoid *in situ* carcinoma (BISC), squamous cell carcinoma (SCC) and basal cell carcinoma (BCC). **Objective:** Based on this finding, we hypothesized that *Felis catus* papillomavirus (FcaPV) is involved in the pathogenesis of MCC, similar to SCC and BCC. In this study, we aimed to elucidate the relationship between feline MCC and FcaPV. **Methods:** Twenty-one feline MCC cases were examined. FcaPV2-specific PCR was carried out using DNA extracted from formalin-fixed paraffin-embedded tissues. To localize FcaPV2 oncogenes, *in situ* hybridization (ISH) was performed using a DNA probe for *E7*. Also, *E6* and *E7* mRNA expression was assessed by RNAscope ISH. Furthermore, immunoreactivity for tumor suppressor proteins was evaluated by immunohistochemistry. **Results:** PCR detected FcaPV2 DNA in 20/21 MCC tissues. ISH for *E7* revealed punctate nuclear signals within tumor cells in 19/21 MCC, and RNAscope revealed multiple dot-like nuclear and cytoplasmic signals in 15/21 MCC. Increased immunoreactivity for p16<sup>CDKN2A</sup> and decreased immunoreactivity for retinoblastoma protein (pRb) and p53 were immunohistochemically confirmed in 20/21 MCC. These findings were also observed in BISC, SCC, and BCC of MCC-affected cats. **Conclusions:** The present study suggests that FcaPV2 infection may be a major etiological factor of MCC in cats. Similar to other FcaPV-induced tumors, subsequent inhibition of pRb and p53 induced by integration and expression of the viral oncogenes may be associated with feline MCC tumorigenesis.

Sunday, October 31, 2021  
12:00 p.m. – 1:00 p.m. CDT

### **STEPHANOFILARIAL DERMATITIS IN CATTLE FROM TEXAS**

Clinson Lui<sup>1</sup>, Matthew Kulpa<sup>1</sup>, Guilherme Verocai<sup>1</sup>, Erin Edwards<sup>2</sup>, Dominique Wiener<sup>1</sup>, Raquel Rech<sup>1</sup>

<sup>1</sup>Texas A&M University, College Station, TX, USA, <sup>2</sup>Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, TX, USA

**Background:** *Stephanofilaria stilesi*, a vector-borne filarioid nematode, is a common parasite of cattle in the southern United States. Transmission to cattle occurs via the hematophagous horn fly (*Haemotobia irritans*), an intermediate host. **Objective:** To characterize the cutaneous lesions caused by *S. stilesi*. **Methods:** Skin of the ventral abdominal midline from 22 cattle (*Bos taurus taurus* and *Bos taurus indicus*) with lesions suggestive of stephanofilariasis, between the ages of 8 months and 12 years old, were obtained through the autopsy service at Texas A&M University and Texas

A&M Veterinary Medical Diagnostic Laboratory. Skin samples were processed for DNA extraction, and subsequent PCR and sequence targeting multiple molecular markers.

**Results:** Grossly, lesions ranged from 5x4 cm to 36x10 cm, and presented as one large single lesion, or three to four ovoid areas at the ventral abdominal midline, surrounding the umbilicus. Each lesion presented as an ulcerative dermatitis with serocellular crusts, or alopecic and lichenified areas. Histologically, an eosinophilic and neutrophilic ulcerative dermatitis with furunculosis and folliculitis with epidermal hyperplasia was observed. Adult nematodes were identified (13/22 cases) within intact follicles, sebaceous ducts, crusts, and areas of furunculosis. Microfilariae were observed (5/13) within vitelline membranes in the superficial dermis and crusts. Sequences were successfully characterized from the cytochrome c oxidase subunit 1 (cox1) of the mitochondrial DNA and the 28S rDNA of the nuclear DNA. **Conclusions:** Adult nematodes were observed in 59% of the cases. Phylogenetic analyses were performed to understand the relationship of *S. stilesi* and other filarioid nematodes.

Sunday, October 31, 2021

12:00 p.m. – 1:00 p.m. CDT

### **SYSTEMIC AVIAN POXVIRUS INFECTIONS ASSOCIATED WITH THE B1 SUBCLADE OF CANARYPOX VIRUS**

Devinn Sinnott<sup>1,2</sup>, Jennifer Burchell<sup>2</sup>, Carmel Witte<sup>2</sup>, Rachel Burns<sup>2</sup>, Steven Kubiski<sup>2</sup>

<sup>1</sup>University of California, Davis, Davis, CA, USA, <sup>2</sup>San Diego Zoo Wildlife Alliance, San Diego, CA, USA

**Background:** Avian poxviruses classically manifest as two forms: cutaneous (“dry”) pox affecting the skin, and diphtheritic (“wet”) pox affecting the oropharyngeal and upper respiratory and gastrointestinal mucosa. Systemic viral spread beyond the skin and mucous membranes is rarely reported.

**Objective:** We evaluated the histopathologic lesions of systemic avian poxvirus over a 20-year period at a zoological institution and assessed strain and host factor variations between systemic and dry/wet poxvirus infections.

**Methods:** Hematoxylin and eosin-stained slides were reviewed for all suspected systemic avian poxvirus cases identified in a medical record search. In-situ hybridization was used to assess the intralesional presence of poxvirus DNA. Polymerase chain reaction targeting two loci (REV LTR flanking region and core P4b protein gene) was performed using frozen tissues to identify viral strains.

**Results:** Twenty-two cases of systemic avian poxvirus were identified. Two histopathologic patterns emerged: 1) histiocytic inflammation in multiple organs with intrahistiocytic viral inclusions, and 2) severe, localized dry or wet pox lesions with viral inclusions within dermal and subepithelial macrophages. In-situ hybridization confirmed the presence of poxvirus DNA within macrophages in both patterns. Sequences of the REV LTR flanking region from all systemic avian poxvirus cases were identical to a previously described condorpox virus. Sequences of the core P4b protein gene from all systemic avian poxvirus cases grouped into cluster 2 of the B1 subclade of canarypox viruses.

**Conclusion:** The definitive factors leading to systemic avian poxvirus infections are uncertain but may involve particular strain variations in combination with various possible host factors.

Sunday, October 31, 2021

12:00 p.m. – 1:00 p.m. CDT

## **HISTOLOGIC FINDINGS IN EQUINE TEETH SUBMITTED FROM AN EQUINE REFERRAL HOSPITAL**

Sarah Cudd<sup>1</sup>, Brian Murphy<sup>2</sup>, James Brown<sup>3</sup>, Charles Schwarten<sup>1</sup>, Elise LaDouceur<sup>1</sup>  
<sup>1</sup>Joint Pathology Center, Silver Spring, MD, USA, <sup>2</sup>UC Davis School of Veterinary Medicine, Davis, CA, USA, <sup>3</sup>Virginia Tech, Marion DuPont Scott Equine Medical Center, Leesburg, VA, USA

**Background:** The histologic analysis of equine teeth by pathologists is increasingly common due to the rapidly growing field of equine dentistry. However, there are few references to aid in evaluating these teeth.

**Objective:** Review histologic findings in equine tooth biopsies to provide reference material for pathologists.

**Methods:** For control specimens, incisors and cheek teeth were removed from horses that were euthanized with no history of dental disease. Control and biopsy equine teeth were fixed whole in 10% neutral buffered formalin and decalcified in a rapid acid decalcification solution (RDO). Incisors, canine, and wolf teeth (vestigial MaxPM1) were decalcified 7-10 days. Cheek teeth were decalcified up to 21 days. Teeth were sectioned in coronal sections at occlusal, mid, and apical levels, processed routinely, sectioned at 5µm thickness, and stained with hematoxylin and eosin.

**Results:** One hundred and forty teeth, including 83 incisors, 6 canines, 1 wolf (PM1), 17 premolars, and 33 molars, were examined from 78 horses ranging from 3-31 years old (mean 14 years old). The most common diagnoses per horse were periodontitis (38.5% [30/78]), equine odontoclastic tooth resorption and hypercementosis (EOTRH; 37.2% [29/78]), pulpitis/pulp necrosis (29.5% [23/78]), reactive hypercementosis (19.2% [15/78]), fracture (17.9% [14/78]), caries (16.7% [13/78]), pulp stones (6.4% [5/78]), bacterial infection (5.1% [4/78]), within normal limits (5.1% [4/78]), ameloblastoma (1.3% [1/78]), squamous cell carcinoma (1.3% [1/78]), and odontodysplasia (1.3% [1/78]).

**Conclusions:** Inflammation and EOTRH were common diagnoses, while neoplasia and odontodysplasia were rare. Reactive hypercementosis was considered secondary to inflammation and/or fracture. Pulp stones were presumed incidental.



Sunday, October 31, 2021  
12:00 p.m. – 1:00 p.m. CDT

## **LACK OF THE REPORTED GENE MUTATIONS OF CONGENITAL IMMUNODEFICIENCY IN TWO JUVENILE DOGS WITH FATAL BACTERIAL INFECTIONS AND SYSTEMIC LYMPHOID DEPLETION**

Ikki Mitsui<sup>1</sup>, Chika Inoue<sup>2</sup>, Akiko Uemura<sup>3</sup>, Md Islam<sup>4</sup>, Osamu Yamato<sup>4</sup>

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### Background

Severe combined immunodeficiency (SCID) is a genetic defect in cell-mediated and humoral immune responses. SCID patients have hypoplastic primary and secondary lymphoid organs and tend to succumb to various infectious diseases.

### Objective

Our objective was to examine whether a congenital immunodeficiency such as SCID was involved in the death of two dogs with immunodeficiency phenotypes.

### Methods

Full autopsy, histopathology, and bacterial culture were performed for 2 unrelated dogs. DNA from FFPE liver was sequenced and analyzed for the presence of known immunodeficiency-related mutations listed in Online Mendelian Inheritance in Animals (OMIA).

### Results

An intact female Shiba Inu (dog 1) with a 1-month history of diarrhea and an intact female Cavalier King Charles Spaniel (dog 2) with a 9-month history of persistent coughing were unresponsive to empirical treatment including antibiotics before death at ages 7-month and 1-year respectively. Both dogs were current on combined vaccines. Both dogs had severe lymphoid depletion in the thymus, spleen, and lymph nodes. Dog 1 had diffuse alveolar damage and suppurative hemorrhagic cerebral/cerebellar meningitis. Dog 1's bacterial isolates were *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (lung) and *Escherichia coli* and *P. aeruginosa* (meninges). Dog 2 had severe suppurative bronchopneumonia and tracheitis with pulmonary isolation of *Micrococcus* sp. Lesions suggestive of viral or fungal infection were absent in both dogs. No reported mutation was detected in *RAG1* (c.2893G>T), *PRKDC* (c.10879G>T), or *IL2RG* (4-bp deletion and 1-bp insertion) genes.

## Conclusions

The results necessitate continued accumulation of phenotypic and genetic information for detection of animal cases of congenital immunodeficiency.

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### **HISTOLOGIC LESIONS OF CESTODIASIS IN OCTOPUSES**

Daniel Finnegan<sup>1</sup>, Michael Murray<sup>2</sup>, Salvatore Frasca, Jr.<sup>3</sup>, Michael Garner<sup>4</sup>, Elise LaDouceur<sup>1,4</sup>

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**Background:** Previous reports of cestodiasis in cephalopods describe the anatomic location of infection and geographic location of the infected specimen. Although cestodiasis is well documented in cephalopods, particularly European species, there are no reports of detailed histologic findings of this infection.

Hypothesis: Cestodiasis causes histologic lesions in octopuses.

**Methods:** Northwest ZooPath archives were searched for cases of octopuses with cestode infections; HE slides were reviewed.

**Results:** Eight octopuses with cestodiasis were identified, including three common octopuses (*Octopus vulgaris*), two Caribbean reef octopuses (*Octopus briareus*), two two-spot octopuses (*Octopus bimaculoides*), and one giant Pacific octopus (*Enteroctopus dofleini*). Cestodes were present in the cecum (4/4 cases; 100%), intestines (4/6 cases; 67%), digestive gland (3/7 cases; 43%), renal appendage (1/5 cases; 20%), and chitinous alimentary tract (1/6 cases; 17%). In four cases (4/8; 50%), cestodes were invading tissue and associated with hemocytic inflammation and necrotic tracts in the digestive gland (3/3 cases), renal appendage (1/1 case), and/or intestines (2/4 cases). Inflammation was commonly mild. Cestodes were confined to the lumen without tissue invasion when present in the cecum and chitinous alimentary tract. The most common concurrent diseases included enteric *Aggregata* sp. infection, branchial rickettsial-like infection, enteric larval nematode infection, and digestive gland atrophy.

**Conclusions:** Cestodes can be pathogens in octopuses, particularly in the digestive gland, intestines, and renal appendage, where infection is often associated with mild inflammation and necrosis. Cestode infection in other portions of the alimentary tract was not associated with inflammation or tissue invasion.

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## **A NOVEL IDIOPATHIC HEPATITIS SYNDROME IN TEN HORSES FROM INDIANA**

Patrick Huang<sup>1,2</sup>, Margaret Miller<sup>1</sup>, Janice Kritchevsky<sup>1</sup>, Carla Olave<sup>1</sup>, Sandra Taylor<sup>1</sup>  
<sup>1</sup>Purdue University, West Lafayette, IN, USA, <sup>2</sup>NIH Comparative Biomedical Scientist Training Program, Bethesda, MD, USA

**Background:** Hepatitis of unknown cause was diagnosed in 10 horses between January and March of 2021, increased from 1-5 cases/year in the preceding 5 years.

**Objective:** Correlation of histologic with clinical features in horses with idiopathic hepatitis.

**Methods:** Inclusion criteria were fever and increased serum gamma-glutamyl transferase (GGT) activity. Liver biopsies from 8/10 horses were histologically reviewed and graded for hepatocellular death, biliary epithelial injury, inflammation, and fibrosis.

**Results:** Five mares and 5 geldings (median age 14 years) representing 6 breeds presented to the Purdue University from 10 Indiana premises. Fever was at least biphasic in 9/10 horses. Besides increased GGT activity, common biochemical abnormalities included hyperbilirubinemia (8/10), hyperfibrinogenemia (7/9), increased sorbitol dehydrogenase activity (3/4), and increased serum amyloid A concentration (3/3). Histologic impression of the liver biopsies was chronic hepatitis. Lesions included piecemeal (7/8) and/or random hepatic necrosis (5/8) with mainly portal lymphohistiocytic inflammation and fibrosis (8/8), increased biliary profiles (6/8), and ductular reaction (1/8). Laboratory tests were negative for known causes of equine hepatitis. Hospitalized horses (6/10) received nonsteroidal anti-inflammatory drugs, acetaminophen, antibiotics, and/or omeprazole, with clinical resolution (10/10) over 4-12 weeks. However, histologic lesions persisted in 3 horses evaluated on follow-up examination up to 3 months after clinical resolution.

**Conclusions:** The diagnosis of chronic hepatitis in febrile horses suggests viral or other infection, but a cause was not identified despite culture and molecular tests for microbial pathogens. Further investigation is required to determine the pathogenesis and prognosis of this idiopathic equine hepatitis syndrome.

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## **GROSS AND MICROSCOPIC PATHOLOGY OF WHITE SPOTTED LIVER IN WILD EUROPEAN RABBITS IN THE UNITED KINGDOM**

Diana Bochyńska, Sheelagh Lloyd, Olivier Restif, Katherine Hughes  
University of Cambridge, Cambridge, United Kingdom

In rabbits a grossly white spotted liver can be indicative of one of several disease processes, frequently caused by parasites. Several of the causative infectious agents may be transmitted to domestic rabbits via contaminated vegetation. The prevalence of white spotted liver in wild rabbits, *Oryctolagus cuniculus*, in the United Kingdom (UK) is currently undetermined. The aim of this study was to evaluate the prevalence and main parasitic etiologies of white spotted liver in a population of wild rabbits in the UK. Eighty seven wild rabbits were shot in Cambridgeshire for population control and cadavers were donated for research. Post mortem examination was undertaken including gross and histological hepatic examination. A striking 53% (46/87) of rabbits examined exhibited gross lesions consistent with white spotted liver. The majority of these lesions were considered to be mild based on the gross appearance. For 59% (27/46) of the rabbits exhibiting gross hepatic lesions, an etiological agent was apparent histologically. Overall, *Eimeria stiedae* was detected in 24% (21/87) of rabbits and *Calodium hepaticum* (synonym *Capillaria hepatica*) was detected in 8% (7/87). The occurrence of white spotted liver was marginally lower in adults than in juveniles (47% vs 76%,  $p=0.053$ ,  $N=59$ ) in the subset of rabbits caught in the summer. No gross hepatic lesions were observed in 47% (41/87) of the rabbits. For adult rabbits with no gross hepatic lesions, the median and mean liver weight as a percentage of overall body weight was 2.97% in both cases.

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## **HEMATOPOIETIC ROUND CELL NEOPLASIA IN AXOLOTLS (AMBYSTOMA MEXICANUM): A MAJOR CAUSE OF MORTALITY IN A CAPTIVE POPULATION**

Ming Lo<sup>1</sup>, Peter DiGeronimo<sup>2</sup>, Megan Caudill<sup>1</sup>, Marley Iredale<sup>1</sup>, Bryce Miller<sup>1</sup>, Nicolette Aquilino<sup>2</sup>, Robert Ossiboff<sup>1</sup>

<sup>1</sup>University of Florida, Gainesville, FL, USA, <sup>2</sup>Adventure Aquarium, Camden, NJ, USA

**Background:** The axolotl (*Ambystoma mexicanum*) is a salamander with remarkable regenerative capacity that has served as an animal model for genetics, developmental biology, and regenerative medicine. Hematopoietic neoplasia is common in most animal species, but is infrequently reported in amphibians.

**Objective:** This report documents a series of naturally occurring hematopoietic neoplasms accounted for 60% of the mortalities in a population of 20 captive axolotls in a two-year period.

**Results and Conclusions:** Between July 2019 and June 2021, a total of ten co-housed, genetically-related, adult, black axolotls from an aquarium were serially submitted to the Aquatic, Amphibian and Reptile Pathology diagnostic service at the University of

Florida. Among these, six cases were diagnosed with multicentric and circulating round cell neoplasia of hematopoietic cell origin. Five of the six cases died spontaneously. Postmortem findings included splenomegaly, hepatomegaly, hepatic or cardiac masses, hydrocoelom and edema. Immunohistochemistry (IHC) for cell surface markers CD3 (T lymphocytes) and IBA-1 (histiocytes) was attempted on four of the six cases. There was negative CD3 immunoreactivity in all four cases and negative immunoreactivity to IBA-1 in all but one case. Given this, a non-T cell, non-histiocytic origin was indicated for the majority of the cases. Based on the morphology, the neoplastic cells were suspected to be of an NK-cell or B cell origin. Although less likely, a poorly differentiated myeloid origin or erythroid origin cannot be ruled out. Definitive differentiation of the lineage of the neoplastic cells and exploration of a possible genetic or viral cause requires further investigation.

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### **AN OUTBREAK OF CHLAMYDIOSIS IN CRITICALLY ENDANGERED CAPTIVE BREEDING PUERTO RICAN PARROTS (*AMAZONA VITTATA*)**

HL Shivaprasad<sup>1</sup>, Karine Laroucau<sup>2</sup>, Rachid Aaziz<sup>2</sup>, Fabien Vorimore<sup>2</sup>, Chiara Palmieri<sup>3</sup>

<sup>1</sup>California Animal Health and Food Safety Laboratory System - Tulare branch, University of California, Davis, Tulare, CA, USA, <sup>2</sup>Anses, Animal Health Laboratory, Bacterial Zoonoses Unit, Maisons-Alfort, France, <sup>3</sup>School of Veterinary Science, The University of Queensland, Gatton, Australia

#### Background

Puerto Rican parrots (*Amazona vittata*) is the only remaining native parrot in Puerto Rico, listed as critically endangered species since 1994. Conservation efforts are thus critical to save this bird from extinction, including prompt identification and treatment of infectious threats.

#### Objective

This study summarizes the main pathologic findings, diagnostic plan and treatment of chlamydiosis in Puerto Rican Parrots.

#### Methods

An outbreak of chlamydiosis was diagnosed by gross examination, histopathology, IHC, and combined-PCR/high-resolution melting assay in an aviary containing 250 adults and 100 juvenile critically endangered Puerto Rican Parrots housed in captive breeding program in the territory of Puerto Rico.

#### Results

The disease was characterized by depression, ruffled feathers, dehydration and death of 20 parrots over a period of two weeks. Postmortem examination revealed fibrinous

thickening of the air sacs, hepatomegaly, splenomegaly and occasionally thickening of the pericardial sac and renomegaly. Histopathology revealed fibrinous airsacculitis, pericarditis, conjunctivitis, hepatitis, splenitis, nephritis, synovitis, osteomyelitis, sinusitis, associated with elementary bodies of *Chlamydia* sp. in the cytoplasm of macrophages and epithelial cells, further confirmed by IHC. *Chlamydia psittaci* genotype A was isolated in cell culture and identified as group III pigeon by PCR/high resolution melting assay. Treatment of birds with Doxycycline in the drinking water for 45 days eradicated the disease.

## Conclusions

Chlamydiosis should be considered as one of the differential diagnosis in Puerto Rican parrots showing non-specific clinical signs and unexplained mortality. Pigeons in the vicinity that had access to the aviary were probably the most likely source of chlamydia to the parrots.

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## **PATHOLOGICAL FINDINGS ASSOCIATED TO MICROFILARIAE IN HOWLER MONKEYS (*ALOUATTA* SP.)**

Juliana Guerra<sup>1</sup>, Ticiania Ervedosa<sup>1</sup>, Eduardo Ferreira-Machado<sup>1,2</sup>, Pedro Enrique Suarez<sup>1,2</sup>, Alessandra dos Santos<sup>1,2</sup>, Isis de Jesus<sup>1</sup>, Ana Carolina de Carvalho<sup>1</sup>, Ketlyn Figueiredo<sup>1</sup>, Josué Díaz-Delgado<sup>3</sup>, Natália Fernandes<sup>1,2</sup>

<sup>1</sup>Instituto Adolfo Lutz, São Paulo, Brazil, <sup>2</sup>Universidade de São Paulo, São Paulo, Brazil,

<sup>3</sup>Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, TX, USA

**Background:** Several species of filarial nematodes, especially those from genus *Dipetalonema*, *Brugia* and *Mansonella* (subgenus *Tetrapetalonema*), have been described in deep connective or subcutaneous tissues, body cavities, and cardiovascular or lymphatic systems of Neotropical non-human primates.

**Objective:** To characterize the histopathological findings associated with filarial nematodes in free-ranging howler monkeys from São Paulo State, Brazil.

**Methods:** Histopathological reports for *Alouatta* sp. assessed between 2016 and 2020 (n=1,138) were retrospectively searched for adult filariids or microfilariae.

**Results:** Seventy-eight (6.9%, n=78/1138) howler monkeys presented microfilariae, most commonly in spleen (60.3%), followed by lungs (15.4%), liver (15.4%), kidneys (7.7%), heart (6.4%), brain (1.9%) and lymph node (1.2%). The most common histopathological findings (by decreasing order of occurrence) were: mild to moderate nodular eosinophilic and histiocytic splenitis with microfilariae (58.9%); mild to moderate proliferative pleuritis, pericarditis and/or peritonitis 53.8% and scattered eosinophilic and histiocytic infiltrates in liver (11.5%), kidneys (10.3%) and lymph node (1.3%) associated with microfilariae. Thrombosis with intravascular microfilariae was observed in one

case. Concurrent filariasis and yellow fever or toxoplasmosis were diagnosed in 18 and one case, respectively.

**Conclusions:** Our findings highlight that mild to moderate inflammatory lesions in serosal surfaces, as well as splenic and hepatic parenchyma are frequently associated with filariasis in howler monkeys in São Paulo. In few cases, filariasis could be clinically significant, particularly those with extensive serosal inflammation. Further studies aimed at identification of these filariids combining molecular and morphological features are warranted to clarify species' diversity with emphasis on zoonotic potential.

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### **PREVALENCE AND DISEASE ASSOCIATION OF AMDOPARVOVIRUS INFECTION IN RED PANDAS (*AILURUS FULGENS*)**

Charles Alex<sup>1</sup>, Steven Kubiski<sup>2</sup>, Kenneth Jackson<sup>1</sup>, Raymund Wack<sup>1</sup>, Michael Garner<sup>3</sup>, Patricia Pesavento<sup>1</sup>

<sup>1</sup>School of Veterinary Medicine, University of California - Davis, Davis, CA, USA, <sup>2</sup>San Diego Zoo Wildlife Alliance, San Diego, CA, USA, <sup>3</sup>Northwest ZooPath, Monroe, WA, USA

**Background:** Amdoparvovirus infections are recognized in many carnivore species, with an enigmatic spectrum of outcomes ranging from asymptomatic persistence to fatal disease. In 2018, we reported a novel Amdoparvovirus infecting a zoo cohort of endangered red pandas (*Ailurus fulgens*).

**Objective:** We aimed to establish the population-level impact of Red Panda Amdoparvovirus (RPAV) by (1) evaluating prevalence and persistence of infection in US zoo-housed red pandas and (2) analyzing the presence, tissue distribution, and disease association of RPAV in archived necropsy cases.

**Methods:** Prevalence and persistence of infection were evaluated by PCR testing of fecal DNA. To evaluate disease association, we identified necropsy cases representing the spectrum of recognized Amdoparvovirus-associated diseases and major causes of mortality in red pandas. We detected infections by PCR of FFPE spleen tissue and examined tissue distribution using *in situ* hybridization.

**Results:** Infections were detected in 52/104 red pandas tested (50%). Virus was persistently shed by a cohort of asymptomatic animals over a four year period. Infection status was significantly associated with red panda subspecies ( $p=0.0005$ ), and there was a trend toward increased infections among older animals ( $p=0.13$ ). In necropsied animals, RPAV was found in putative sites of asymptomatic persistence (spleen,

gastrointestinal tract), and in association with significant lesions including myocarditis, tubulointerstitial nephritis, pharyngitis, and interstitial pneumonia.

**Conclusions:** RPAV infection is widespread in US zoo-housed red pandas. Infection can be persistent and clinically silent, but can also contribute to significant disease in this endangered species.

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### **MANNHEIMIA HAEMOLYTICA RESPIRATORY DISEASE IN MATURE DAIRY COWS**

DeLenn Burrows<sup>1</sup>, Cynthia Miltenburg<sup>2</sup>, Andrew Brooks<sup>3</sup>, Jeff Caswell<sup>1</sup>

<sup>1</sup>Department of Pathobiology, Guelph, ON, Canada, <sup>2</sup>Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, ON, Canada, <sup>3</sup>Animal Health Lab, Guelph, ON, Canada

**Background:** *M. haemolytica* pneumonia outbreaks in mature dairy cows are anecdotally increasing in frequency. Despite welfare and economic impacts, the literature on pneumonia in mature dairy cows is sparse.

**Objective:** Describe risk factors, herd characteristics, seasonality, duration, and main pathogens involved in pneumonia in mature dairy cows, and to identify changes over time in laboratory accessions.

**Methods:** 725 cases from 2007-2020 were retrieved from the laboratory database. Search criteria included bovine, lung, suppurative or fibrinous inflammation, *Mannheimia*, *Histophilus*, or *Pasteurella*. Inclusion criteria were dairy cows  $\geq 2$  years of age with pneumonia as the proximate cause of death. Aspiration and embolic pneumonia cases were excluded.

**Results:** 115 cases were included in the study. Of 100 cases with bacterial culture data, *M. haemolytica* was isolated from 61 cases, *H. somni* from 4, *P. multocida* from 29, *T. pyogenes* from 30, and other bacteria from 14. Of 55 cases tested for BRSV, 6 were positive. None of the 14 cases tested for BVDV were positive. Of 57 cases tested for other viruses, 2 were positive for BHV-1. Common risk factors included mixing of animals, weather changes, and peripartum period. Year of submission was not associated with number of cases.

**Conclusions:** An increase in laboratory diagnoses of *M. haemolytica* pneumonia was not observed. *Mannheimia haemolytica* was the most commonly isolated bacteria. This



study identifies the major pathogens and associated risk factors for pneumonia in mature dairy cows, as a basis for understanding why outbreaks occur and for implementing appropriate therapy.

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### **SPONTANEOUS DISEASES IN CAPTIVE CAPYBARAS (HYDROCHOERUS HYDROCHAERIS)**

Dallas Clontz, Alexander Aceino, Sharman Hoppes, Laura Bryan, Brian Porter, Raquel Rech

Texas A&M University, College Station, TX, USA

A retrospective study was performed to investigate the spontaneously occurring diseases in captive capybaras (*Hydrochoerus hydrochaeris*) submitted for necropsy to Texas A&M University from 2011 to 2021. Twenty-four capybaras were submitted including 9 neonates to adolescents (< 6 months), 5 juveniles to young adults (6 months to <2 years), 6 adults (2 years to <5 years), and 4 seniors (5 years to 11 years). In many animals, multiple disease processes contributed to clinical decline. Causes for mortality included chronic hepatopathy (33.3%), myocardial degeneration/ necrosis (25%), dental disease (20.8%), dystocia/fetal distress (16.7%), sepsis (16.7%), pneumonia (12.5%), enteritis (4.2%), hypovitaminosis C (4.2%), intestinal leiomyosarcoma (4.2%), ankylosing spondylosis (4.2%), and trauma/diaphragmatic hernia (4.2%). In neonates, the most common causes for mortality were dystocia/fetal distress (44.4%) and sepsis (33.3%). The most common cause for mortality in juveniles to young adults was cardiomyopathy (50%) characterized by acute myocardial degeneration and necrosis. Encephalomyocarditis virus was confirmed via real time PCR in one capybara. The most common lesion of adult and senior capybaras was a chronic hepatopathy (50% and 100% respectively) distinguished by a nodular liver with lobular collapse, bridging fibrosis, biliary hyperplasia, and hepatocellular regeneration.

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### **PYTHIOSIS: AN EMERGING DISEASE OF DOGS IN TRINIDAD AND TOBAGO**

Karelma Frontera-Acevedo<sup>1</sup>, Lana Gyan<sup>2</sup>, Stacy Rajh<sup>1</sup>, Rod Suepaul<sup>1</sup>, Devina Supersad<sup>1</sup>, Alissa Bally<sup>1</sup>, Indira Pargass<sup>1</sup>

<sup>1</sup>University of the West Indies, St. Augustine, Trinidad and Tobago, <sup>2</sup>Caribbean Veterinary Diagnostics, St. Augustine, Trinidad and Tobago

*Pythium insidiosum* is an oomycete which can be found mainly, but not exclusively, in tropical, subtropical and temperate areas and is pathogenic in mammals, most frequently horses, dogs, and humans causing the disease pythiosis. It can be found in both aquatic environments and soil. Pythiosis is severe, progressive, and frequently fatal. The objective of this study was to characterize an ongoing emerging outbreak of gastrointestinal and cutaneous disease in multiple young dogs in Trinidad and identify the organism as *P. insidiosum*. Biopsy (endoscopic and sectional) and autopsy samples were submitted for histopathologic examinations. Most of the cases had sufficient material to also perform ancillary stains such as PAS and Gomori methenamine silver

(GMS). Samples were also submitted for confirmation via PCR. From late 2019 to present, 14 cases have been identified as suspect for *P. insidiosum*, and two have been confirmed so far using PCR. Cases were evenly split between male (7) and female (7) dogs. The most common breed identified was the Husky, followed by Pitbulls and mixed breed dogs, with age ranges between 9 months to 5 years. Most cases involved the gastrointestinal tract, except one cutaneous case. No case so far has had both cutaneous and gastrointestinal manifestations. These cases highlight the presence of a previously unreported disease in Trinidad and Tobago, and thus the importance for clinicians of including pythiosis on a differential list for dogs (especially young ones) with chronic, progressive gastrointestinal clinical signs including palpable abdominal masses.

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### **RNA-SEQ REVEALS THE CELL OF ORIGIN OF T-ZONE LYMPHOMA TO BE AN ACTIVATED T-CELL.**

Evan Conaway, Kelly Hughes, Emily Garrison, Lauren Harris, Anne Avery  
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#### **Introduction:**

T-zone lymphoma (TZL) is a common T-cell neoplasm of dogs. TZL is unique in its indolent disease course and high incidence among certain breeds. A defining feature of TZL is lack of CD45 expression. TZL can express CD4, CD8, or neither antigen, and consistently expresses high levels of class II MHC and often CD25. This phenotype led us to hypothesize that the normal counterpart of TZL is an activated, mature T cell. Here we used gene set enrichment analysis (GSEA) and functional assays to determine the cell of origin of TZL.

#### **Methods:**

RNA sequencing of 6 CD8<sup>+</sup> T-zone cases from sorted lymph nodes was compared to sorted CD8<sup>+</sup> T-cells from control lymph nodes. GSEA was performed to look for gene sets indicative of the cell of origin of TZL. T-zone cells were stimulated *in vitro* with PMA and ionomycin and stained for intracellular production of TNF $\alpha$  and IFN $\gamma$ .

#### **Results:**

GSEA revealed enrichment of gene sets expressed by activated T-cells. This observation was further supported by the high level of TNF $\alpha$  and IFN $\gamma$  detected by intracellular staining of *in vitro* stimulated T zone cells. Although transcriptomics were performed on CD8 T cells, high levels of expression of the CD4 transcription factors ThPOK and GATA3 were identified, but not the CD8-specific transcription factor EOMES.

#### **Conclusion:**

We conclude that the normal counterpart of TZL is an activated T cell, supported by GSEA and cytokine production. The paradoxical expression of T helper transcription factors merits further investigation.

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## **MORTALITY EVENTS IN PASSERINES ASSOCIATED WITH SALMONELLA ENTERICA TYPE B SEROVAR TYPHIMURIUM IN THE SOUTHEASTERN UNITED STATES**

Rebecca Radisic<sup>1,2</sup>, Caitlin Burrell<sup>1</sup>, Melanie Kunkel<sup>1</sup>, Alisia Weyna<sup>1,2</sup>, Susan Sanchez<sup>2</sup>, Mark Ruder<sup>1</sup>, Sonia Hernandez<sup>1,2</sup>, Nicole Nemeth<sup>1,2</sup>

<sup>1</sup>Southeastern Cooperative Wildlife Disease Study, Athens, GA, USA, <sup>2</sup>University of Georgia, Athens, GA, USA

*Background:* Mortality due to salmonellosis affected numerous passerine species across the U.S. in 2020-21.

*Objectives:* To describe geographical distribution, species affected, lesion patterns and coinfections of passerines submitted to the Southeastern Cooperative Wildlife Disease Study.

*Methods:* Postmortem diagnostic evaluation, including gross and histopathology and select ancillary tests, was performed on 40 passerine carcasses collected from the southeastern U.S. from January 1, 2020-May 31, 2021.

*Results:* Bird carcasses of six species were collected from nine states, with highest numbers from North Carolina (11/40), extending west to Louisiana and north to West Virginia. Most deaths (78%; 31/40) were attributed to salmonellosis; isolates were confirmed as zoonotic *Salmonella enterica* group B serovar *typhimurium* in 22/31 via serology. Pine siskin (*Spinus pinus*) was the species most often diagnosed (9/22), followed by American goldfinch (7/22; *Spinus tristis*), brown headed cowbird (3/22; *Molothrus ater*), cedar waxwing (1/22; *Bombycilla cedrorum*), house finch (1/22; *Haemorrhous mexicanus*) and northern mockingbird (1/22; *Mimus polyglottos*). Salmonellosis was usually systemic (25/40), with most severe lesions in crop/esophagus (i.e., fibrinonecrotizing, heterophilic ingluvititis). Concurrent diagnoses included trichomonosis (2/22), fungal pneumonia (1/22) and trauma (1/22).

*Conclusion:* *Salmonella enterica* caused recent mortality events in passerines across the U.S. Pine siskins and American goldfinches are disproportionately affected, possibly facilitated by high density congregations (e.g., at birdfeeders, agricultural sites). Lesion patterns underscore the uniqueness (i.e., lack of intestinal disease) of passerine salmonellosis. Co-infection with other anthropogenic pathogens (e.g., trichomonosis)

can contribute to mortality. Risk to avian and human health justifies monitoring and epidemiological assessments of these outbreaks.

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### **KLEBSIELLA PNEUMONIAE-ASSOCIATED NECROSUPPURATIVE LYMPHADENITIS AND PERITONITIS IN JUVENILE RACCOONS**

Zoe Mack, Elizabeth Buckles, Elena Demeter

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**Background:** *Klebsiella pneumoniae* is a Gram-negative, facultative anaerobic, heavily encapsulated bacterium, associated with a number of disease manifestations in veterinary and human medicine, including nosocomial infections.

**Objective:** This study aimed to characterize a series of unusual cases of necrosuppurative lymphadenitis and peritonitis diagnosed in juvenile raccoons (*Procyon lotor*) under rehabilitator care, submitted to the Cornell University College of Veterinary Medicine New York State Animal Health Diagnostic Center.

**Methods:** Gross examination and histopathologic evaluation, employing hematoxylin and eosin (H&E) and Gram stains, were performed in 6 wild juvenile raccoons who died while under rehabilitator care in the states of New York and New Jersey. Aerobic culture was performed in 5 out of 6 cases to discern the etiology.

**Results:** In 5 out of 6 cases, lymphadenomegaly was noted, correlating to necrosuppurative lymphadenitis histologically. Additionally, in 5 out of 6 cases pyoabdomen was diagnosed at gross necropsy and correlated to peritonitis of variable severity. Bacteria were present in 4 out of 6 cases; Gram stain revealed large numbers of Gram-negative bacteria with morphology ranging from short rods to coccobacilli. Aerobic culture performed in 5 out of 6 cases revealed *Klebsiella pneumoniae* and *Klebsiella pneumoniae* subspecies *ozaenae*. In one case, many *Psychrobacter* sp. were additionally isolated.

**Conclusions:** This study supports *Klebsiella pneumoniae* as a differential for bacterial lymphadenitis and peritonitis in raccoons. The disease may be associated with nosocomial infection in this species given the development of lesions while under clinical care.

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### **HEPATIC HEMOSIDEROSIS IN CAPTIVE ENDANGERED PYGMY RABBITS (BRACHYLAGUS IDAHOENSIS): A PILOT STUDY AND PROPOSED HEPATIC IRON GRADING SCHEME**

Jessica Wong, Gay Lynn Clyde, Lisa Shipley, Patricia Talcott, Laura White

Washington State University, Pullman, WA, USA

**Background:** The Columbia Basin (CB) pygmy rabbit is a federally endangered distinct population segment in Washington. In 2001, a captive breeding and reintroduction

program was established with 16 founders from the last remaining CB wild population. CB pygmy rabbits were outbred with pygmy rabbits from Idaho. Some animals were housed at an intensively managed facility for foraging behavior and nutrition studies and were fed a commercial rabbit diet; others were housed in a semi-captive environment with access to their native sagebrush diet supplemented with a commercial diet ad libitum.

**Objective:** To characterize and establish a grading scheme for hepatic iron accumulation in captive and semi-captive pygmy rabbits.

**Methods:** In this retrospective case series, 26 pygmy rabbit necropsies (16 captive; 10 semi-captive) submitted to the Washington Animal Disease Diagnostic Laboratory between 2011-2016 were evaluated. Liver sections were assessed by hematoxylin and eosin and Perl's Prussian Blue stains. A modified grading scheme for parenchymal and Kupffer cells was developed to semi-quantify iron accumulation (grade 0-3). For a subset of animals, hepatic iron levels were quantified.

**Results:** Compared to semi-captive pygmy rabbits, captive animals exhibited significantly greater iron accumulation in both parenchymal ( $P= 0.004$ ) and Kupffer cells ( $P= 0.004$ ). Quantified hepatic iron levels were associated with histologic grade. Additionally, most severely affected rabbits displayed hepatocellular degeneration, bridging portal fibrosis, and nodular regeneration, suggestive of hemochromatosis.

**Conclusions:** Continued monitoring of iron levels in captive pygmy rabbits may be critical for effective management and to better support the reestablishment of this species in the wild.

Sunday, October 31, 2021  
12:00 p.m. – 1:00 p.m. CDT

## **COMPLEX FETLOCK BREAKDOWNS IN CALIFORNIA RACEHORSES**

Monika Samol<sup>1</sup>, Susan Stover<sup>2</sup>, Rick Arthur<sup>3</sup>, Francisco Uzal<sup>1</sup>

<sup>1</sup>California Animal Health and Food Safety Laboratory, University of California Davis, San Bernardino, CA, USA, <sup>2</sup>J.D. Wheat Veterinary Orthopedic Research Laboratory, Veterinary Medicine Teaching Hospital, University of California Davis, Davis, CA, USA, <sup>3</sup>Veterinary Medicine Teaching Hospital, University of California Davis, Davis, CA, USA

Fetlock breakdowns (FBs) remain the leading cause of euthanasia among California racehorses. Little is known about complex fetlock breakdowns (CFBs), characterized by simultaneous metacarpal/tarsal lateral condylar fracture, proximal sesamoid bone (PSB) fracture(s), and proximal phalangeal (P1) fracture. CAHFS (California-Animal-Health & Food-Safety-Laboratory) necropsy reports of racehorses with 1) CFB (n=25) 2) FB with PSB fracture(s) only, with pre-existing lesion (PEL) in the medial PSB (PSB, n=66) 3) musculoskeletal injury other than FB (nonFB-MSI, n=93) from August 2018 through February 2021 were reviewed and compared. Horses with CFB had biaxial (19) or lateral (6) PSB fractures, lateral condylar fracture (25), and P1 chip (12) or highly comminuted (13) fractures. Most occurred while racing (60%), on dirt (48%), turf (32%), and among 4- and ≥5 year-olds (52%, 28%), whereas other MSI (PSB, nonFB) were

least common on turf (9%, 8%). Four and  $\geq 5$ -year-olds were least frequently affected by nonFB-MSI (19%, 16%), and 3-year-olds the most in PSB group (41%). Most CFBs were unilateral (96%) with significantly higher proportion of hindlimbs (36%) affected compared to other MSI (PSB 0%, nonFB 10%). In CFB horses, evidence of PELs was observed in 2 of 13 P1 fractures, none in PSBs, and in all lateral condylar fractures associated with palmar osteochondral disease (44%) or focal bone remodeling in the parasagittal groove region (56%). Racehorses  $\geq 4$ -years of age with evidence of bone remodeling in the lateral condyle should be considered at risk for CFB. High proportion of CFBs on turf and in hindlimbs appears unique to other MSI.

Sunday, October 31, 2021

12:00 p.m. – 1:00 p.m. CDT

### **A SURVEY OF PATHOLOGY IN 26 CAPTIVE MEXICAN GRAY WOLVES (*CANIS LUPUS BAILEYI*) FROM ARIZONA, 2016-2021**

Jennifer Buczek, Jason Struthers

Animal Health Institute, Department of Pathology and Population Medicine, Midwestern University, Glendale, AZ, USA

The Mexican gray wolf (*Canis lupus baileyi*) (MGW) is an endangered species with 186 individuals in the wild as of 2020. Once extinct in the SW USA, the U.S. Fish and Wildlife service have led species recovery efforts through captive breeding and reintroduction programs, which require healthy individuals. Between 2016-2021, 26 captive MGW from two institutions were autopsied at Midwestern University's Diagnostic Pathology Center. There were 11 females and 15 males that ranged from 11 to 17 y-o (mean 14.38 y-o). 30.7% (8/26) were found dead (n=7) or died under anesthesia (n=1); 75.0% (6/8) of these deaths were attributed to gastric dilatation and volvulus (GDV) or gastric bloat. Additionally, one wolf was euthanized due to GDV. 57.7% (15/26) of wolves had neoplasms; specifically, 80% (12/15) had malignant neoplasia, which warranted euthanasia in 9 animals. Metastatic oral melanoma was the most frequent malignancy (n=3). Otherwise, there were singular cases of nasal squamous cell carcinoma, hepatocellular carcinoma, adrenocortical carcinoma, adrenal neuroendocrine tumor, thyroid carcinoma, malignant Sertoli cell tumor, intestinal lymphoma (T cell), and malignant pancreatic gastrinoma with Zollinger-Ellison syndrome. Common benign neoplasms were leiomyoma and adrenocortical adenoma. 88.5% (23/26) of wolves had degenerative changes, most frequently, chronic kidney disease, valvular endocardiosis, and vertebral and appendicular degenerative joint disease. Six (23%) wolves were euthanized because of osteoarthritis and/or intervertebral disc disease. Common significant morbidities in this aged population of MGW were malignant neoplasia, degenerative diseases, and GDV/bloat, in which the latter frequently resulted in natural mortality. Infectious disease was absent.

Sunday, October 31, 2021

12:00 p.m. – 1:00 p.m. CDT

### **A RETROSPECTIVE STUDY OF MYOCARDITIS IN 19 PUPPIES**

Chaunte Lewis, Victoria Watson

Michigan State University, East Lansing, MI, USA

**Background:** Myocarditis is a rare histologic lesion linked to numerous causes in puppies; however, the cause often remains unknown. **Objectives:** The purpose of this retrospective study is to determine the common causes of myocarditis in puppies and determine if there is a link between the cause and type of inflammation in the heart. **Methods:** Nineteen dogs under 7-months old which had previously been diagnosed with myocarditis on the necropsy floor over the last 5 years were identified. Formalin-fixed, paraffin-embedded heart tissues were trimmed, and DNA or RNA was extracted from the samples to run PCR for parvovirus, apicomplexan, West Nile virus, Bartonella and Leptospira. Tissues were also evaluated histologically for characterization of inflammatory lesions. **Results:** Four of the nineteen cases were diagnosed as Parvovirus, three were individually diagnosed as Canine herpes virus-1 (CHV-1) via PCR, two were diagnosed as apicomplexan, one was diagnosed as West Nile virus, one was diagnosed as Leptospira, one was individually diagnosed as Clostridium septicum via anaerobic culture, and seven were undiagnosed. The histologic lesions generally included lymphocytic necrotizing myocarditis, severe pyogranulomatous necrotizing myocarditis, suppurative myocarditis, and myocarditis with mineralization. **Conclusion:** Parvovirus was the most common cause of myocarditis and tended to show necrotizing and lymphocytic lesions. Cases associated with CHV-1 showed necrotizing myocarditis with histiocytic and neutrophilic inflammation. Cases associated with toxoplasmosis showed lymphohistiocytic lesions. Although there were trends in the type of inflammation linked to cause, a significant link between cause and inflammation type could not be demonstrated in these nineteen cases.

Sunday, October 31, 2021

12:00 p.m. – 1:00 p.m. CDT

## **TRAUMATIC FINDINGS IN POSTMORTEM INVESTIGATIONS OF ROADKILL WILD MAMMALS OF BRAZIL**

Pedro Navas-Suárez<sup>1</sup>, Josué Diaz-Delgado<sup>2</sup>, Débora Yogui<sup>3</sup>, Mario Alves<sup>3</sup>, Mayara Grego Caiaffa<sup>1</sup>, Julia Ferraz Cereda<sup>1</sup>, Marina Pellegrino da Silva<sup>1</sup>, Mauricio Candido da Silva<sup>4</sup>, Marta Cremer<sup>5</sup>, Patricia Medici<sup>6</sup>, Arnaud Desbiez<sup>3</sup>, José Catão-Dias<sup>1</sup>

<sup>1</sup>Laboratório de Patologia Comparada de Animais Selvagens, Departamento de Patologia, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, Brazil, <sup>2</sup>Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, TX, USA, <sup>3</sup>Instituto de Conservação de Animais Silvestres (ICAS), Campo Grande, Brazil, <sup>4</sup>Museu de Anatomia Veterinária, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, Brazil, <sup>5</sup>Laboratório de Ecologia e Conservação de Tetrápodes Marinhos e Costeiros, Universidade da Região de Joinville, São Francisco do Sul, Brazil, <sup>6</sup>Instituto de Pesquisas Ecológicas (IPE), Nazaré Paulista, Brazil

**Background:** Direct mortality from motor vehicle collisions (MVC) is one of the greatest road-related threats to wildlife. In the next 30 years, it is expected that at least 25 million kilometers of new roads will be built, and 90% of these projects are planned in developing countries of tropical regions such as Brazil. Furthermore, MVC pose a threat to human safety and represent a great economic cost to society. **Objective:** To identify, characterize and categorize traumatic injuries related to MVC in Brazilian wild mammals. **Methods:** Over a three-year period, carcasses from wild mammals were

collected on roads in five Brazilian states, representing three biomes (Brazilian savannah, Atlantic forest, Brazilian wetlands). Necropsy and sample collection for ancillary laboratory analyses was performed on all carcasses. Traumatic injuries were classified topographically. **Results:** MVC was documented in 453 individuals corresponding to 47 species and ten classes: Artiodactyla (5%, n=24), Carnivora (32%, n=146), Chiroptera (1%, n=2), Cingulata (13%, n=57), Didelphimorphia (5%, n=21), Lagomorpha (1%, n=3), Perissodactyla (8%, n=36), Pilosa (24%, n=109), Primates (4%, n=20), and Rodentia (8%, n=35). MVC-traumatic injuries occurred in thorax (82%, n=372), head/neck (80%, n=363), pelvis/abdomen (84%, n=381), appendicular (64%, n=290) and undetermined (5%, n=21). July (13%, n=57), October (11%, n=49), and December (10%, n=47) had the highest mortality. **Conclusions:** These results characterized and delineated MVC-associated traumatic injuries in a large number of wild mammals of Brazil, ratifying the severity of this conservation issue and providing foundational knowledge to improve the assessment of MVC in wild mammals.

Sunday, October 31, 2021

12:00 p.m. – 1:00 p.m. CDT

### **IMPROVING METHODS OF CONTROL OF AMERICAN FOULBROOD IN HONEY BEES IN SASKATCHEWAN**

Michael Zabrodski<sup>1</sup>, Geoff Wilson<sup>2</sup>, Igor Moshynskyy<sup>1</sup>, Mohsen Sharafi<sup>1</sup>, Lara Reitsma<sup>1</sup>, Mateo Castano Ospina<sup>1</sup>, Jessica DeBruyne<sup>1</sup>, Alexandra Wentzell<sup>1</sup>, Sarah Wood<sup>1</sup>, Ivanna Kozii<sup>1</sup>, Colby Klein<sup>1</sup>, Jenna Thebeau<sup>1</sup>, Fatima Masood<sup>1</sup>, Igor Medici de Mattos<sup>1</sup>, Allyssa Cloet<sup>1</sup>, Brandele Brown<sup>1</sup>, Melanie Roulin<sup>1</sup>, Dana Liebe<sup>1</sup>, LaRhonda Sobchishin<sup>1</sup>, Tasha Epp<sup>1</sup>, Antonio Ruzzini<sup>1</sup>, Elemir Simko<sup>1</sup>

<sup>1</sup>Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada, <sup>2</sup>Ministry of Agriculture, Government of Saskatchewan, Prince Albert, SK, Canada

**Background:** American foulbrood (AFB) is a devastating disease of honey bee larvae caused by the spore-forming bacterium, *Paenibacillus larvae*. Disease often results in colony death by either natural disease progression or destruction by the beekeeper to limit the spread of spores to other colonies. North American beekeepers rely heavily on routine antimicrobial metaphylaxis to prevent disease, but treatment fails to eliminate infectious spores. With the emergence of antibiotic-resistant strains of *P. larvae*, there is a need for alternative, evidence-based management tools to reduce reliance on antibiotics while maintaining sustainable beekeeping operations.

**Methods:** Province-wide surveillance of *P. larvae* spores in honey may be a proxy for evaluating yard-level AFB risk. Accordingly, we analyzed the spore content of pooled, extracted honey from 52 large-scale and 72 small-scale Saskatchewan (Canada) beekeepers, representing over 70,000 of the province's 110,000 honey bee colonies.

**Objective:** These results, in conjunction with data from an accompanying questionnaire, will establish prognostic reference ranges for honey to identify the immediate risk of AFB outbreaks in antibiotic-dependent management systems.

**Results:** To date, spores have been detected in 52.2% of large-scale honey samples



at low (66.5%), medium (26.8%), or high (6.7%) concentrations. For small-scale samples, 28.2% have detectable spores at low (84.1%), medium (13.6%), or high (2.3%) concentrations.

**Conclusions:** Subsequent incidence of AFB was observed in 1/1 small-scale and 3/6 large-scale beekeepers with high spore concentrations, and 3/14 large-scale beekeepers with medium spore concentrations. Consistent with our current risk criteria, AFB has not been reported in beekeepers with low spore concentrations.

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12:00 p.m. – 1:00 p.m. CDT

### **HISTIOCYTIC AND NEUTROPHILIC HEPATITIS: A NOVEL FORM OF CHRONIC CANINE HEPATITIS**

Andeliene Croce, John Cullen

North Carolina State University, Raleigh, NC, USA

**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 dead 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.

Sunday, October 31, 2021

12:00 p.m. – 1:00 p.m. CDT

### **'FELINE TRIADITIS': A RETROSPECTIVE HISTOPATHOLOGICAL ANALYSIS**

Belen Hernandez, Hailey Devries, Lauren McKeen, Shankumar Mooyottu

Iowa State University, Ames, IA, USA

'Feline triaditis' is a multi-organ disease involving inflammation of the small intestine, pancreas, and liver. The multifactorial nature of this disease makes the diagnosis and

treatment challenging. Moreover, the disease complex of 'triaditis' has yet to be validated as a distinct histopathologic entity. Therefore, the present study aims to explore the histologic characteristics of triaditis and its constituent comorbidities in relation to relevant clinicopathologic parameters. A twenty-year retrospective study was performed on cats admitted to the Lloyd Veterinary Medical Center at Iowa State University. Sixty-six cats were identified with a minimum of two out of three histologically confirmed inflammatory comorbidities of hepatitis (H), enteritis (E) or pancreatitis (P), in which all three tissues were examined. Twenty-six of the 61 cats were diagnosed with 'triaditis' (HPE). The most common clinical parameters reported in this cohort included abnormal defecation (constipation or diarrhea), hyperphosphatemia, hypocalcemia, hypokalemia, and lymphopenia. Furthermore, lymphoplasmacytic inflammation was the major lesion type in all organs. Multinomial logistic regression model suggested an increased probability of finding hepatitis and pancreatitis (HP) combination in older cats; whereas, in young cats, it was hepatitis and enteritis (HE). However, the probability of finding HPE stayed the same regardless of age. Overall, the occurrence of HPE was significantly higher compared to PE and HP (p-value= 0.003). However, no difference was noticed in the occurrence of HPE and HE within the cohort. To conclude, the results from this study did not provide sufficient evidence to distinguish 'triaditis' as a distinct disease entity in cats.

Sunday, October 31, 2021

12:00 p.m. – 1:00 p.m. CDT

### **HISTOCHEMICAL, IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL COMPARISON OF BOVINE, FELINE AND OPOSSUM GLOBULE LEUKOCYTES**

Alisha Muscatwala, Elizabeth Howerth  
University of Georgia, Athens, GA, USA

Globule leukocytes (GL) are intraepithelial cells with large eosinophilic cytoplasmic granules found in the mucosa of the gastrointestinal and respiratory tract, often in association with parasitic infection. Their origin is controversial. In sheep and mice, evidence suggests they are related to mast cells and, in mice, have been termed intraepithelial mast cells. We investigated the histochemical, immunohistochemical and ultrastructural features of GL from animals of three different orders to better understand the origin of these cells, or at least potential relatedness to mast cells. Tissues from cattle, cats and opossums containing GL were stained by a variety of histochemical stains (PTAH, Luna, PAS, giemsa, toluidine blue, alcian blue) and a variety of immunohistochemical stains for lymphoid markers (CD3, CD79, CD21) and mast cell markers (CD117, tryptase, beta-chymase). In addition, GL were evaluated by electron microscopy. In all three species, globules stained positive for Luna and PTAH, and were not metachromatic; only bovine globules stained with the PAS reaction. Cells from all species were negative for B and T cell markers; only cattle GL stained for CD117, tryptase and beta-chymase. GL had a similar ultrastructural appearance among species, characterized by electron dense and membrane bound globules. Bovine GL appear to be strongly related to mast cells, but additional immunohistochemical staining is needed to elucidate the contents of feline and opossum GL.

## Experimental Disease Focused Poster Session

Tuesday, November 2, 2021 | 12:30 p.m. – 1:30 p.m. CDT

Tuesday, November 2, 2021

12:30 p.m. – 1:30 p.m. CDT

### **A NOVEL MODEL OF ESTROGEN RECEPTOR-POSITIVE BREAST CANCER BONE METASTASIS WITH ANTIESTROGEN RESPONSIVENESS**

Kendall Langsten<sup>1</sup>, Lihong Shi<sup>1</sup>, Adam Wilson<sup>2</sup>, Brian Westwood<sup>2</sup>, Maria Xie<sup>1</sup>, Victoria Surratt<sup>1</sup>, JoLyn Turner<sup>1</sup>, Ravi Singh<sup>1,3</sup>, Katherine Cook<sup>1,2,3</sup>, Bethany Kerr<sup>1,3</sup>

<sup>1</sup>Department of Cancer Biology, Wake Forest School of Medicine, Winston-Salem, NC, USA, <sup>2</sup>Department of Surgery, Wake Forest School of Medicine, Winston-Salem, NC, USA, <sup>3</sup>Wake Forest Baptist Comprehensive Cancer Center, Winston-Salem, NC, USA

Estrogen receptor alpha positive (ER+) breast cancer (BC) is the most common subtype of BC. When it metastasizes to bone, it becomes incurable. Little advancement has occurred in the treatment of bone metastasis from ER+ BC, partly due to the lack of animal models. To establish an animal model of ER+ BC, we genetically modified two triple-negative (TN) BC cell lines to express ER $\alpha$  and injected the cell lines into murine mammary glands. Mice were treated with standard antiestrogen therapies, the selective estrogen receptor modulator (SERM) tamoxifen or the selective estrogen receptor degrader (SERD) ICI 182,780. We found that compared to mice with TN BC, mice with ER+ BC developed bone metastases and were responsive to antiestrogen therapy. This model allows for further exploration of bone metastasis mechanisms and for the development of new therapeutics, translating into improved clinical outcomes for women with bone metastasis from ER+ BC.

Tuesday, November 2, 2021

12:30 p.m. – 1:30 p.m. CDT

### **TARGETING THE STEROL REGULATORY ELEMENT-BINDING PROTEIN PATHWAY IN PANCREATIC DUCTAL ADENOCARCINOMA**

Stephanie Myers, Meredith McGuire, Wei Shao, Chune Liu, Theodore Ewachiw, Zeshaan Rasheed, William Matsui, Toni Sepalla, Richard Burkhart, Peter Espenshade  
Johns Hopkins University, School of Medicine, Baltimore, MD, USA

**Background:** Pancreatic ductal adenocarcinoma (PDAC) is a very aggressive tumor with limited diagnostic and therapeutic options. Due to its proliferative nature and desmoplastic stroma, tumor cells are challenged with meeting a high demand for lipids in a hypoxic, lipid-poor environment. Cancer cells respond to this demand through sterol regulatory element-binding proteins (SREBPs), which are master transcriptional regulators of lipid homeostasis that require SREBP cleavage activating protein (SCAP) during signaling.

**Methods:** Using four patient-derived PDAC cell lines, *SCAP* was knocked out. All cell lines were utilized in functional growth assays in lipid-variable conditions, subcutaneous xenograft, and orthotopic xenograft experiments. A well-established PDAC mouse

model, *LSL-Kras*<sup>G12D/+</sup>; *LSL-Trp53*<sup>R172H/+</sup>; *Pdx-1 Cre* (KPC), was utilized, and KPC mice lacking *Scap* in one or both alleles were generated. In all four cell lines, the following FDA-approved drugs were applied individually and in combination: Dipyridamole, Fluvastatin, and Simvastatin.

**Results:** In lipid-poor conditions, *SCAP* knockout cells showed significantly reduced growth. In tumor xenograft models, *SCAP* knockout cells exhibited reduced tumor growth and tumor volume. KPC mice with a heterozygous loss of *Scap* exhibited a significantly increased median survival time. In combination, Dipyridamole with either statin demonstrate synergy in lipid-poor conditions.

**Conclusions:** Loss of *SCAP* in PDAC tumor cells alters the growth capability both *in vitro* and *in vivo*. Heterozygous loss of *Scap* in the KPC mouse model significantly increased survival. Finally, Dipyridamole works in synergy with statins to alter growth of tumor cells. These findings suggest that targeting the SREBP pathway has significant therapeutic potential in pancreatic cancer.

Tuesday, November 2, 2021

12:30 p.m. – 1:30 p.m. CDT

### **BI-TRANSGENIC CTLA4+/- PDC1-/- MOUSE AS A MODEL FOR LONG TERM OUTCOMES IN IMMUNE CHECKPOINT THERAPY**

Rachael Wolters, Matthew Fleming, Dev Patel, Javid Moslehi, Christopher Pinelli  
Vanderbilt University Medical Center, Nashville, TN, USA

**Background:** Immune checkpoint inhibitors (ICI) (anti-CTLA-4 and anti-PD-1/PD-L1 antibodies) have transformed oncology care; however, ICI can lead to immune-related adverse events (irAE) that can affect any organ. Combination therapy (e.g., anti-CTLA-4 plus anti-PD-1 therapies) potentiates both anti-tumor efficacy as well as toxicities. Long-term sequelae of ICI are not well understood, though this is relevant given the increasing number of patients who both respond to and survive from treatment. Our lab developed a transgenic *Ctla4+/-;Pdc1-/-* mouse on a C57BL/6J background to mimic these irAE. These mice have fulminant myocarditis and early mortality, although only about 50% of *Ctla4+/- Pdc1-/-* are affected.

**Objective:** To better determine the long-term effects of anti-PD-1 and anti-CTLA4 treatment we assessed middle-aged transgenic mice that did not succumb to acute toxicities.

**Methods:** 10-14-month-old male *Ctla4+/-;PD-1-/-* mice and age/sex-matched controls were evaluated with pre-mortem cardiac functional tests, and postmortem pathological investigation.

**Results:** Electrocardiographic and echocardiographic studies revealed little to no functional cardiac differences between the two groups. However, The *Ctla4+/-;PD-1-/-*

mice have significantly lower total body weight and lean body weight. Histologically there is mild myocarditis in 50% (2/4) of the transgenic mice.

**Conclusion:** We show mild myocarditis in 50% of the transgenic mice, and reduced body weight in all transgenic mice as compared to the controls. Further characterization of the mechanism and clinical implications of these findings is necessary to fully understand long-term outcomes for patients receiving ICI therapy.

Tuesday, November 2, 2021

12:30 p.m. – 1:30 p.m. CDT

## **SYSTEMIC AND RESPIRATORY INFLAMMATORY RESPONSES FROM PREDISPOSING FACTORS OF BOVINE RESPIRATORY DISEASE IN BEEF CALVES**

Seyed Saeid Tabatabaei<sup>1</sup>, DeLenn Burrows<sup>1</sup>, Monica Baquero<sup>1</sup>, Ksenia Vulikh<sup>1</sup>, Derek Haley<sup>2</sup>, Jeff Caswell<sup>1</sup>

<sup>1</sup>Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, <sup>2</sup>Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

**Background:** The literature suggests that predisposing factors for bovine respiratory disease (BRD) such as weaning, transport, and viral infection cause immunosuppression, which predisposes to subsequent bacterial infection. However, our recent work suggests that a dysregulated pro-inflammatory response plays a role in BRD development.

**Objective:** Characterize systemic and local respiratory inflammatory responses after transport or abrupt weaning in calves, to determine if these risk factors cause a heightened response to inflammatory stimuli.

**Methods:** Three groups of calves were considered: two-stage (low-stress) weaning with nose flaps (n=5), abrupt weaning (n=6), and abrupt weaning and transportation (n=6). Blood and bronchoalveolar lavage fluid (BALF) samples were collected on arrival and 24h after an aerosolized inflammatory stimulus on day 1. Differential cell counts were performed on blood. Cell counts, cytokine concentrations (IL-1b, IL-6, IL-8), T cell subpopulations, and total MHC II expression were evaluated in BALF.

**Results:** In BALF, there were higher IL-8 concentrations, percentages of neutrophils and gamma delta T cells, and MHC II expression on leukocytes in abruptly weaned and transported calves compared to those with two-stage weaning.

**Conclusions:** Our results suggest that abrupt weaning and transportation promote an increased response to an aerosolized inflammatory stimulus. These findings suggest a novel mechanism by which well-known risk factors predispose to BRD.

Tuesday, November 2, 2021

12:30 p.m. – 1:30 p.m. CDT

## **CLINICOPATHOLOGICAL CHANGES IN A MURINE MODEL OF HEPATIC ENCEPHALOPATHY**

MILENE RACHID

DEPARTAMENTO DE PATOLOGIA GERAL, INSTITUTO DE CIÊNCIAS BIOLÓGICAS,  
UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE, Brazil

**Background:** Hepatic encephalopathy (HE) has been described as a spectrum of neuropsychiatric abnormalities characterized by apathy, mental disorder, seizures, coma, and even death in dogs and cats with liver dysfunction. Murine models have been used to study the pathophysiology of the disease and the literature about behavioral deficits associated with neuropathology and neurochemical changes from mice with HE induced by thioacetamide (TAA) are scarce. **Objective:** The aim of the present study was to investigate the clinicopathological changes and the levels of BDNF, GDNF and NGF at different brain regions of female C57BL/6 mice with HE induced by TAA. **Methods:** Acute liver failure was induced by an intraperitoneal injection of a single dose of 600mg/kg of TAA. Locomotor activity was assessed by Open Field test. After euthanasia, brains were removed for histopathological and immunohistochemical analysis. The concentrations of BDNF, GDNF and NGF were measured from frontal cortex, striatum and hippocampus by ELISA (protocol number 393/2017). **Results:** HE animals presented decreased locomotor activity one day after induction, that was reversed 6 days later. HE animals exhibited hemorrhagic foci in the cerebellum, focal areas of astrogliosis and several Iba-1 immunopositive microglia with amoeboid aspect in the hippocampus and cerebral cortex. The levels of BDNF were significantly reduced in the frontal cortex of HE mice compared with control mice. The concentrations of GDNF and NGF were similar in both groups. **Conclusion:** Our data demonstrated that hepatic encephalopathy induced by TAA promoted locomotor deficits associated with neuropathological changes and down-regulation of BDNF in mice.

Tuesday, November 2, 2021

12:30 p.m. – 1:30 p.m. CDT

## **TRANSCRIPTOME ANALYSIS OF DUCK AND CHICKEN BRAINS INFECTED WITH AQUATIC BIRD BORNAVIRUS-1 (ABBV-1) DEMONSTRATES DIFFERENTIAL TIMING AND TYPE OF IMMUNE RESPONSE BETWEEN HOSTS**

Phuc (John) Pham, Teodora Tockovska, Alexander Leacy, Melanie Iverson, Nicole Ricker, Leonardo Susta

University of Guelph, Guelph, ON, Canada

### Background

Aquatic bird bornavirus-1 (ABBV-1) is a neurotropic virus that causes persistent infection in nervous tissues. Recently, we have demonstrated that ABBV-1 can infect day-old White Leghorn chickens and Muscovy ducks leading to persistent infection in the brain.

### Objectives and Methods

Transcriptome profiles of ABBV-1 infected chicken and duck brains were examined. Total RNA from brains of seven intracranial infected and control chickens and ducks at 4 and 12 weeks post-infection (wpi) was used for paired-end sequencing using an Illumina NovaSeq S4 (28 birds/species).

## Results

There were more differentially expressed genes (DEGs) and enriched gene ontology (GO) terms in the brain of ducks at 4 compared to 12 wpi. In chickens, more DEGs and GO terms were seen at 12 compared to 4 wpi. Within the top 20 enriched GO terms, there was overrepresentation of those associated with immune function for both species, which for ducks, were mainly related to innate immunity, whereas chickens had both innate and adaptive immune terms. KEGG pathway analysis showed enrichment of many similar pathways between ducks and chickens, including but not limited to cytokine-cytokine receptor interaction, NOD-like receptor signaling, and Toll-like receptor signaling.

## Conclusions

These results show that the immune response to ABBV-1 infection is activated earlier in ducks compared to chickens, despite the virus being present in the brain of both species at 4 wpi. Earlier response in ducks is consistent with development of non-heterophilic encephalitis at 4 wpi. Despite activation of the immune response, persistent infection was not prevented in either host.

Tuesday, November 2, 2021

12:30 p.m. – 1:30 p.m. CDT

### **EXPERIMENTAL INFECTION OF AQUATIC BIRD BORNAVIRUS-1 IN CHICKENS**

Melanie Iverson<sup>1</sup>, Alexander Leacy<sup>1</sup>, Phuc Pham<sup>1</sup>, Eva Nagy<sup>1</sup>, Emily Brouwer<sup>2</sup>, Brandon Lillie<sup>1</sup>, Leonardo Susta<sup>1</sup>

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**Background:** Aquatic bird bornavirus-1 (ABBV-1), a novel orthobornavirus, was identified in 2009 in several wild waterfowl species, and has been associated with neurologic disease and non-suppurative inflammation of the nervous system. The virus has been since identified in multiple avian taxa. No experimental infection has been attempted to document the pathogenesis of ABBV-1 in commercial poultry.

**Objective:** Evaluate the ability of ABBV-1 to infect, replicate, and cause disease in experimentally-infected White Leghorn chickens (*Gallus gallus domesticus*).

**Methods:** Day-old chicks ( $n = 160$ ) were divided into 4 groups (40/group), and inoculated with ABBV-1 by one of four routes: intracranial (IC,  $6.6 \times 10^4$  FFUs [focus-

forming units]/bird), intramuscular (IM,  $1.3 \times 10^5$  FFUs/bird), oral (PO,  $1.3 \times 10^5$  FFUs/bird), and control (sham-infected). At 1, 4, 8, and 12 weeks post infection (wpi), 10 birds from each group were euthanized for tissue collection.

**Results:** No clinical signs or gross lesions were observed. By 12 wpi, all brains (8/8) and most spinal cords (6/8) from IC inoculated chickens were positive for ABBV-1 by RT-qPCR. By the same time point, 3/9 spinal cords and 2/10 brains from IM inoculated chickens tested positive. Lymphocytic perivascular inflammation was identified in the brain of all IC inoculated chickens at 8 and 12 wpi, with increased severity at 12 wpi. Immunohistochemistry confirmed the presence of the virus within tissues.

**Conclusions:** ABBV-1 delivered intracranially and intramuscularly, but not orally, can infect chickens. Chickens are susceptible to ABBV-1 infection and development of microscopic lesions, although clinical signs may require more time to appear.

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### **KIDNEY INTERCALATED CELLS ACTIVATE INNATE IMMUNE DEFENSES IN RESPONSE TO UROPATHOGENIC ESCHERICHIA COLI.**

Sarah Linn<sup>1,2</sup>, Laura Schwartz<sup>2</sup>, John David Spencer<sup>2,3</sup>

<sup>1</sup>The Ohio State University College of Veterinary Medicine, Columbus, OH, USA, <sup>2</sup>The Abigail Wexner Research Institute at Nationwide Children's Hospital, Columbus, OH, USA, <sup>3</sup>The Ohio State University College of Medicine, Columbus, OH, USA

**Background:** Urinary tract infections (UTI) are common in domestic species. Intercalated cells (IC) in the renal collecting duct prevent and combat UTI by secreting antimicrobial peptides (AMPs) into the urine. Mechanisms regulating IC AMP production during UTI are unclear. Here, we challenged ICs *in vitro* with uropathogenic *E. coli* (UPEC) or bacterial cell membrane components to define pathways that control AMP production during UTI.

**Methods:** ICs were infected with UPEC or challenged with lipopolysaccharide (LPS), muramyl dipeptide (MDP) and  $\gamma$ -D-Glu-mDAP (iE-DAP). Following stimulation, IC lysates were collected, and 87 immune genes were profiled using an antimicrobial response PCR array or targeted qRT-PCR. Immunoblotting was performed to validate pathway activation.

**Results:** In response to UPEC, ICs activate immunomodulatory pathways and AMPs. Analysis of PCR array data identified 15 upregulated genes associated with Toll-like receptor (TLR), NOD-like receptor (NLR), and NF- $\kappa$ B signaling 4 hours post infection. Immunoblotting confirmed activation of downstream targets in these pathways. qRT-PCR identified that AMPs, like *Lcn2*, are activated while *Rhase8* was suppressed. Upon stimulation with LPS and MDP, qRT-PCR showed upregulation of select



AMPs suggesting that TLR4 and NOD2 activation, respectively, may regulate their expression. AMP expression did not change with the NOD1 agonist, iE-DAP.

**Conclusions:** During UPEC infection, TLR, NLR, and NF- $\kappa$ B responses are activated in ICs and their activation may induce AMPs. Confirmation studies are needed to determine how these pathways regulate AMP expression and identify potential regulatory nodes which may serve as future targets to increase AMP production to treat UTI across species.

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### **NEONATAL RHESUS MACAQUE MODEL OF TUBERCULOSIS AND THE PRESENCE OF INDOLEAMINE 2, 3-DIOXYGENASE**

Katherine Turnbull, Eunice Vincent, Peter Didier, Robert Blair, Chad Roy, Lara Doyle-Meyers, Widade Ziani, Huanbin Xu, Ronald Veazey, Xiaolei Wang  
Tulane National Primate Research Center, Covington, LA, USA

#### Background/Objective

Tuberculosis (TB) is a disease caused by the bacteria *Mycobacterium tuberculosis* (*Mtb*) and is the cause of more deaths per year than any other infectious agent. Adult macaque models of TB have demonstrated that the suppression of indoleamine 2, 3-dioxygenase (IDO) reduced bacterial burden and pathology and increased host survival, but the role of IDO in the disease process in *Mtb* infected infants remains unknown.

#### Methods

Our initial attempt for aerosol mediated *Mtb* infection in four infant non-human primates (NHPs) observing physical and blood parameters before necropsy and pathological and histological changes after.

#### Results

We showed that TB symptoms in all 4 *Mtb*-exposed infants were more acute in onset and had concurrent pulmonary and exclusively extrapulmonary TB (EPTB) mimicking clinical findings in human infants. This is distinct from adult NHPs infected with the same dose and the same strain of *Mtb* that had asymptomatic TB infection only. We also observed that these TB infants presented with high levels of IDO expression in pulmonary granulomas co-localized within the band of epithelioid macrophages in the granuloma, suggesting inhibition of tryptophan metabolism via IDO blockade may enhance immune-mediated control of TB diseases.

#### Conclusions

Combined, we successfully established an aerosol neonatal model in NHPs that mimics clinical and bacteriological characteristics of *Mtb* infection as seen in human newborns/infants, which can be used to determine whether treatment with an IDO

inhibitor will induce better formation of lymphoid tissues and increase the killing ability of macrophages leading to improvement of TB disease in pediatric host.

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### **INFECTING AMBLYOMMA AMERICANUM TICKS WITH CYTAUXZON FELIS VIA DIRECT INJECTION**

Tzushan Yang<sup>1</sup>, Mason Reichard<sup>2</sup>, Henry Marr<sup>1</sup>, Leah Cohn<sup>3</sup>, Laura Nafe<sup>2,3</sup>, Nathan Whitehurst<sup>1</sup>, Adam Birkenheuer<sup>1</sup>

<sup>1</sup>North Carolina State University, Raleigh, NC, USA, <sup>2</sup>Oklahoma State University, Stillwater, OK, USA, <sup>3</sup>University of Missouri, Columbia, MO, USA

**Background:** *Cytauxzoon felis* is a tick-borne hemoprotozoa that causes life-threatening disease in cats in the United States. Currently, platforms for *C. felis* research are limited to natural or experimental infection of domestic cats.

**Objective:** Develop an alternative model by infecting *Amblyomma americanum* ticks with *C. felis* via direct injection.

**Methods:** *A. americanum* adults were injected with *C. felis*-infected feline erythrocytes through the anal pore or percutaneously into the hemocoel. RNAscope<sup>®</sup> *in situ* hybridization (ISH) was used to visualize the parasites within the ticks at different time points after injection. Eight weeks after injection, ticks were tested for *C. felis* RNA via RT-qPCR and fed on 3 naïve cats to assess their ability to transmit *C. felis*.

**Results:** ISH signals were observed in ticks up to 3 weeks after injection, but the number of hybridization signals notably decreased over time. Prior to the transmission challenge, 50% of the sampled ticks were positive for *C. felis* RNA. Despite successful tick attachment and feeding activity during the transmission challenge, none of the cats became infected with *C. felis*.

**Conclusion:** In this study, the injected *C. felis* remained alive in ticks but failed to progress to infective sporozoites after injection. This outcome may be associated with uncharacterized differences in *C. felis* life cycle or the lack of the feeding and molting process in our model. Nonetheless, our study demonstrated the potential of using ticks to study *C. felis*, and the utility and concept of such model should continue to be refined and explored.

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### **PATHOLOGY OF LYMPHOPROLIFERATIVE DISEASE VIRUS INFECTION IN NATURALLY AND EXPERIMENTALLY INFECTED TURKEYS**

Chloe Goodwin<sup>1,2</sup>, Kayla Adcock<sup>2</sup>, Mark Ruder<sup>2</sup>, Rebecca Poulson<sup>2</sup>, Kevin Niedringhaus<sup>3</sup>, Daniel Mead<sup>2</sup>, Heather Fenton<sup>4</sup>, Kevin Keel<sup>3</sup>, Nicole Nemeth<sup>1,2</sup>

<sup>1</sup>Department of Pathology, University of Georgia, Athens, GA, USA, <sup>2</sup>Southeastern Cooperative Wildlife Disease Study, Athens, GA, USA, <sup>3</sup>Veterinary Medical Teaching

Hospital, School of Veterinary Medicine, University of California-Davis, Davis, CA, USA,  
4School of Veterinary Medicine, Ross University, Basseterre, Saint Kitts and Nevis

**Background:** Lymphoproliferative disease virus (LPDV) is an oncogenic retrovirus associated with lymphoid proliferation and round cell neoplasia in wild and domestic turkeys. Since its initial detection in wild turkeys in 2009, high LPDV prevalence among apparently healthy wild turkeys across eastern North America has raised questions about transmission, pathogenesis, and risk to population health.

**Objective:** To experimentally reproduce LPDV infection and disease in domestic turkeys and compare lesions with naturally infected, wild turkeys.

**Methods:** Twenty-five, 4-week-old, domestic turkeys were LPDV-inoculated via various routes: oral, nasal, crop, or subcutaneous or coelomic injection. Blood was collected weekly until euthanasia and necropsy at 12 weeks post-inoculation. Pathology from diagnostic cases of 195 wild turkeys that were LPDV PCR-positive from 2009 to 2021 were reviewed.

**Results:** 11/25 (48%) inoculated birds had DNAemia at varying times from 1-12 weeks post-infection, representing all inoculation routes. All birds (sham and inoculated) displayed lymphoid proliferation including perivascular lymphocytic cuffs to discrete lymphoid follicles, most frequently liver, adrenal gland, and pancreas. Three inoculated birds had round cell neoplasia in spleen, intestine, adrenal gland, and/or bone marrow (27%). Sixty-three (32%) wild turkeys exhibited similar lesions but with skin most commonly affected (34/63; 54%). Round cell neoplasia was in 30/195 (15%) naturally infected wild turkeys.

**Conclusions:** Experimental LPDV infection was achieved via multiple routes in domestic turkeys and caused similar pathology to naturally infected wild turkeys. As with spontaneous LPDV infection in wild turkeys, neoplastic lesions were uncommon but raise questions about LPDV pathogenesis, diagnostic strategies, and epidemiology in wild turkeys.

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### **CLINICAL AND MORPHOLOGIC CHARACTERIZATION OF THE CENTRAL NERVOUS SYSTEM OF MICE INFECTED WITH VARIOUS STRAINS OF CHIKUNGUNYA VIRUS**

Jonathan Nagel<sup>1,2</sup>, Audrey Knight<sup>3</sup>, Elizabeth Anderson<sup>1</sup>, Hannah Atkins<sup>1,3</sup>, Stephanie Montgomery<sup>1,3</sup>, Victoria Baxter<sup>1,3</sup>

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<sup>2</sup>Department of Population Health and Pathobiology, North Carolina State University, Raleigh, NC, USA, <sup>3</sup>Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC, USA

**Background:** Chikungunya virus (CHIKV) infection in people often manifests as rash and arthritis and occasionally encephalomyelitis. CHIKV neurovirulence is poorly understood and lacks a well-established preclinical model.

**Objective:** We aim to establish a model of CHIKV neurovirulence by examining the clinical and histomorphologic manifestations of intracranial inoculation with different CHIKV strains in mice.

**Methods:** 4-6-week-old C57BL/6J mice were intracranially inoculated with PBS or one of three clinical CHIKV strains: SL15649, AF15561, SM2013, or neuroadapted SL15649 (MANV). Mice were monitored daily for clinical disease, and brains and spinal cords were harvested 3-29 days post-infection. Tissues were assessed for viral titers and histomorphology, including H&E and immunohistochemistry [cleaved caspase 3 (cC3), IBA1, GFAP, B220, CD4/CD8].

**Results:** Neurological signs (hunched posture, abnormal gait, abnormal tail posture) and weight loss were more prominent in mice infected with SL15649 than AF15561 and SM2013. Microscopic examination revealed brain inflammation was primarily lymphohistiocytic with variable amounts of CD4 and CD8 positive cells. Inflammation was focused in the corpus callosum and more severe in mice infected with SL15649 and AF15561. Other brain morphologic findings included cavitation, astrocyte and microglia proliferation, apoptosis (cC3 positive), and neuronal necrosis (cC3 negative). Spinal cord inflammation was rare, mild, and predominantly lymphohistiocytic. Mice infected with MANV developed more severe weight loss, neurological signs, and encephalitis with higher peak titers than clinical CHIKV strains.

**Conclusions:** Clinical and morphologic findings in CHIKV-infected mice vary by CHIKV strain and are more severe following neuroadaptation, aiding in the development of a pre-clinical CHIKV model.

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### **DEVELOPMENT OF OCULAR PATHOLOGY IN A CANINE MODEL OF MUCOPOLYSACCHARIDOSIS IIIB**

Ariel Nenninger<sup>1</sup>, Tyler Harm<sup>1</sup>, Bethann Valentine<sup>1</sup>, N. Ellinwood<sup>2</sup>, Jodi Smith<sup>1</sup>

<sup>1</sup>Iowa State University, Ames, IA, USA, <sup>2</sup>National MPS Society, Durham, NC, USA

**Background:** Mucopolysaccharidosis (MPS) type IIIB is a rare lysosomal storage disease caused by a deficiency in the enzyme  $\alpha$ -N-acetylglucosaminidase. This enzyme deficiency results in accumulation of the glycosaminoglycan (GAG) heparan sulfate in numerous tissues and organs, including the retina and optic nerve. Ocular

manifestations of MPS IIIB include retinopathy, pigmentary retinal degeneration, optic atrophy, and optic disc swelling. Retinopathy is the most common finding in MPS IIIB patients. As modern therapies, such as enzyme replacement therapy, have the potential to improve and extend the lives of MPS IIIB patients, addressing ocular disease has taken on greater significance.

**Objective:** The objective of the current study is to characterize ocular pathology in a canine model of MPS IIIB. This data will improve our understanding of the pathogenesis of this disease and identify outcome measures for future therapeutic studies.

**Methods:** Entire globes from 2 to 26-month-old MPS IIIB affected dogs and age-matched controls were collected and evaluated microscopically for morphologic changes and accumulation of metabolic storage material.

**Results:** PAS-positive and Luxol fast blue-positive cytoplasmic inclusions were identified in vacuolated cells within various layers of the retina suggesting primary storage of GAG and secondary accumulation of gangliosides in MPS IIIB affected dogs. Lysosomal integral membrane protein 2 (LIMP-2) immunohistochemistry was performed to assess lysosomal volume within the retinal cells of MPS IIIB affected animals.

**Conclusions:** Results of this study characterize ocular pathology in the canine model of MPS IIIB and provide foundational data for future therapeutic efficacy studies.

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## **DOES BLUEBERRY POLLEN PREDISPOSE HONEY BEES TO EUROPEAN FOULBROOD DISEASE?**

Ivanna Kozii<sup>1</sup>, Igor de Mattos<sup>1</sup>, Melanie Roulin<sup>1</sup>, Sarah Wood<sup>1</sup>, Colby Klein<sup>1</sup>, Michael Zabrodski<sup>1</sup>, Mohsen Sharafi<sup>1</sup>, Jenna Thebeau<sup>1</sup>, Dana Liebe<sup>1</sup>, Jessica Debruyne<sup>1</sup>, Brandele Brown<sup>1</sup>, Fatima Masood<sup>1</sup>, Larhonda Sobchishin<sup>1</sup>, Igor Moshynskyy<sup>1</sup>, Meghan Millbrath<sup>2</sup>, Robyn McCallum<sup>3</sup>, Marta Guarna<sup>4</sup>, Patricia Wolf Veiga<sup>5</sup>, Eric Gerbrandt<sup>6</sup>, Elemir Simko<sup>1</sup>

<sup>1</sup>University of Saskatchewan, Saskatoon, SK, Canada, <sup>2</sup>Michigan State University, East Lansing, MI, USA, <sup>3</sup>Atlantic Tech Transfer Team for Apiculture, Perennia, NS, Canada, <sup>4</sup>Agriculture and Agri-Food Canada, Beaverlodge, AB, Canada, <sup>5</sup>National Bee Diagnostic Centre, Beaverlodge, AB, Canada, <sup>6</sup>British Columbia Blueberry Council, Abbotsford, BC, Canada

European foulbrood (EFB) is an important disease of honey bees caused by *Melissococcus plutonius*, a bacterium that infects larval midgut and outcompetes the

larva for nutrients. The recent increased incidence of EFB in honey bee colonies involved in blueberry pollination has been associated with lower acidity and protein contents of blueberry pollen compared to polyfloral pollen. However, this association is putative and has not been tested.

Our objective was to *i)* establish an in-vivo model of EFB infection and *ii)* compare the effects of blueberry and polyfloral pollen on survival and weight of bees inoculated with incremental doses of *M. plutonius*.

We inoculated 5-day-old honey bee larvae with 0, 5000 and 45,000 colony forming units (CFU) of *M. plutonius*. These larvae were raised within restricted mesh-lined compartments inside a hive containing either blueberry or polyfloral pollen. Capping rate (survival) and weight at emergence were recorded.

We have successfully established an *in-vivo* model for EFB research. We found that larval capping rate inversely correlated with *M. plutonius* infection doses. Additionally, bees inoculated with the highest dose of *M. plutonius* and raised on blueberry pollen had a significant 6.6% decreased survival rate compared to those raised on polyfloral pollen.

Our *in vivo* model of EFB is well suited to study *M. plutonius* infection in honey bees. The results of this study suggest that blueberry pollen diet may be associated with increased mortality of bee larvae exposed to high infectious dose of *M. plutonius*.

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**DISCOVERY PROTEOMICS FOR THE DETECTION OF MARKERS FOR ERADICATION OF INFECTION IN AN EXPERIMENTAL MODEL OF EQUINE SEPTIC ARTHRITIS USING LC-MS/MS**

Roman Koziy, Joe Bracamonte, Seiji Yoshimura, Paulos Chumala, George Katselis, Elemir Simko

University of Saskatchewan, Saskatoon, SK, Canada

Bacterial joint infection in horses is a debilitating condition, which may lead to long-lasting adverse effects. Despite its high clinical significance, identification of eradication of infection in equine septic arthritis (SA) remains challenging. The goal of this study was the discovery of putative protein biomarkers for the eradication of joint infection in horses. We performed global proteomics analysis of plasma and synovial fluid collected from horses with experimental SA, non-septic lipopolysaccharide-induced arthritis, as well as control horses. The point of eradication of infection in horses with SA was previously determined using standard diagnostic techniques. We compared the protein spectral intensities between groups, as well as before and after the eradication of infection. Twenty-six differentially expressed proteins were identified which were upregulated in the synovial fluid of horses with SA compared to controls and non-septic arthritis, as well as compared to the same horses post eradication of infection. In plasma differentially expressed proteins were not identified. The differentially expressed proteins (n=26) in the synovial fluid were predominantly of cellular origin and had various biological functions. The difference in their relative abundance between

experimental groups was at least 10-fold or higher, which is more than the abundance that would be expected based on the difference in cell count alone (2-fold). Since the majority of cells in joints with bacterial infection are neutrophils, we suggest that the variable expression of neutrophil- and cell-associated proteins may represent potential biomarkers of eradication of infection in equine SA.

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### **CONTINUOUS ANTIBIOTIC ADMINISTRATION AMELIORATES COLITIS IN AN IL10 KNOCKOUT IBD MOUSE MODEL**

Viju Vijayan Pillai, Shiyu Li, Lavanya Reddivari, Abigail Cox  
Purdue University, West Lafayette, IN, USA

**Background:** Inflammatory bowel disease (IBD) has been linked to antibiotic administration. Most of the observations on antibiotic administration and development of IBD have been incidental with limited studies on temporal pharmacodynamics of antibiotic administration and development of IBD.

**Objective:** To elucidate the association between antibiotic use and IBD development.

**Methods:** An IL-10 knockout mouse model (IL-10<sup>-/-</sup>) that develops spontaneous colitis was used to examine the effects of continuous or intermittent antibiotic treatment on colitis development. Antibiotics (1.0 mg/mL ampicillin and 0.5 mg/mL neomycin) were administered orally in drinking water, either continuously or intermittently (alternate weeks) for 20-30 weeks. Proximal and distal colons were histologically assessed for severity of colitis by evaluating a set of six histomorphologic criteria and assigning scores from 0-3 based on progressing severity.

**Results:** Intermittent antibiotic treatment resulted in similar colonic histopathologic changes compared to control group without antibiotic treatment, whereas continuous antibiotic treatment showed no obvious colonic alterations. Histologic score and severity of colitis were higher in the intermittent antibiotic treatment group compared to continuous antibiotic treatment and did not differ from the control group. The correlation of histologic grading and colitis severity was further verified by measuring intestinal permeability post administration of FITC-dextran. FITC-dextran levels in the serum of IL-10<sup>-/-</sup> mice with intermittent antibiotic treatment were markedly higher than in mice with continuous antibiotic treatment but were similar to the control.

**Conclusions:** Continuous antibiotic administration resulted in lower histologic scores and therefore ameliorates colitis in an IL-10<sup>-/-</sup> knockout IBD mouse model.

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### **ALTERATION OF COLONIC MUCIN GLYCOSYLATION AND CYTOKINE EXPRESSION IN SWINE DYSENTERY**

Susanne Je-Han Lin, Emme Helm, Nicholas Gabler, Eric Burrough  
Iowa State University, Ames, IA, USA

## **Background:**

Infection with the strongly beta-hemolytic *Brachyspira hyodysenteriae* (Bhyo) causes swine dysentery (SD) in grower-finisher pigs associated with mucohemorrhagic diarrhea and typhlocolitis. Previous studies showed upregulation of interleukin-17A (IL-17A) may induce mucin production in infected pigs, and Bhyo binding ability was associated with higher abundance of N-glycolylneuraminic acid (NeuGc)-based structures on mucins instead of N-acetyl neuraminic acid (NeuAc).

## **Objectives:**

To evaluate the histochemical and immunohistochemical characteristics of colonic mucin and investigate cytokine expression in colon specimens of pigs with and without SD by RNA in situ hybridization (RNA ISH).

## **Materials and Methods:**

Formalin fixed spiral colon samples were obtained from a total of 36 gilts (12 controls and 24 inoculated with Bhyo). Histochemical staining of *Limax flavus* lectin stain targeting NeuAc, immunohistochemical staining of NeuGc, mucin 2, mucin 5 AC, and RNA ISH for IL-17A was performed. Standardized images were captured, and quantification of staining specific for the above targets was analyzed using a commercial software program.

**Results:** Pigs infected with SD showed significantly lower expression of NeuAc, and a numerical trend of decreased expression of NeuGc. RNA ISH revealed significantly higher expression of IL-17A in pigs infected with SD, but without correlation with any mucin expression.

**Conclusions:** Pigs that developed SD showed upregulation of IL-17A in the colonic tissues. The reduction in NeuAc concurrent with decreased expression of NeuGc in pigs infected with SD suggests that mucin glycosylation changes during SD development.

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## **EQUINE ALVEOLAR MACROPHAGES AND BLOOD MONOCYTE-DERIVED MACROPHAGES RESPOND DIFFERENTLY TO BARN DUST-ANALOGUE CHALLENGE**

Heng Kang, Gary Lee, Dorothee Bienzle, Janet Beeler-Marfisi



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

## Background

Severe equine asthma (SEA) is a non-infectious inflammatory disease of the lower respiratory tract caused by exposure to respirable barn dusts. Inhalation of a mixture of *Aspergillus fumigatus* spores, lipopolysaccharide, and silica microspheres (FLS) as barn dust analogue causes SEA exacerbation in susceptible horses. Alveolar macrophages (AMs) are lung-resident macrophages and the predominant immune cell in the healthy equine lower respiratory tract. Additionally, blood monocytes are recruited to inflamed tissues, including the lung, where they differentiate into monocyte-derived macrophages (MDMs). However, responses of AMs and MDMs to barn dust mimics are unclear.

## Objectives

To assess how AMs and MDMs react to FLS exposure.

## Methods

AMs and MDMs from six healthy horses were incubated with FLS in serum-free cell culture medium for six hours, using medium without FLS as control. Cell surface markers and cytokine production after FLS exposure were analyzed by flow cytometry and direct cytokine measurement, respectively.

## Results

Flow cytometry results showed that MDMs but not AMs had reduced surface expression of CD206 after FLS exposure. Cell culture supernatant from non-FLS exposed AMs, but not MDMs contained IL-8, indicating constitutive production by AMs. FLS-exposure induced production of TNF- $\alpha$ , IL-8, IL-1 $\beta$  and INF- $\gamma$  in AMs, and production of TNF- $\alpha$ , IL-8 and IL-10 in MDMs.

## Conclusions

These results indicate unique immunophenotypes and cytokine profiles in two different types of equine macrophages. The results emphasize that AMs and MDMs are not interchangeable for *in vitro* investigations of the pathogenesis of SEA, and provide a rationale for further functional characterization.

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## **UPREGULATED INFLAMMATORY PATHWAYS IN THE SPINAL CORD OF SIV-INFECTED MACAQUES**

Kathleen Mulka<sup>1</sup>, Suzanne Queen<sup>1</sup>, Audrey Knight<sup>1,2</sup>, Lisa Mangus<sup>1</sup>, Sarah Beck<sup>1</sup>, Megan McCarron<sup>1</sup>, Clarisse Solis<sup>1</sup>, Carlo Colantuoni<sup>1</sup>, Joseph Mankowski<sup>1</sup>

<sup>1</sup>Johns Hopkins University, Baltimore, MD, USA, <sup>2</sup>University of North Carolina, Chapel Hill, NC, USA

**Background:** HIV-associated peripheral neuropathy is one of the most prevalent clinical manifestations of neurological disease associated with HIV infection even with anti-retroviral treatment. The spinal cord is a key relay point for sensory pathways, and studies have shown increased glial activation and pro-inflammatory signaling in HIV-infected patients although evaluation of this tissue in humans has been limited. In contrast, the SIV/macaca model allows for evaluation of the CNS at strategic time points throughout infection. Our previous work has shown myelitis and increased CD68 expression by microglia in the spinal cord of infected animals.

**Objective:** Our objective was to compare gene expression patterns in the spinal cord of SIV-infected and uninfected macaques by global RNA sequencing to characterize immunoregulatory pathways.

**Methods:** We isolated RNA from the spinal cord of SIV-infected and uninfected animals, prepared cDNA libraries followed by sequencing, and analyzed the relative mRNA expression in these groups.

**Results:** Differential expression analysis using DESeq2 revealed 355 genes upregulated in SIV infection and 219 downregulated genes. Pathway analysis using Enrichr indicated that upregulated genes in SIV-infected spinal cord were aligned with interferon and viral response pathways. Additionally, this upregulated gene set significantly overlapped with those expressed in myeloid cells and microglia. Downregulated genes were involved in cholesterol and collagen biosynthesis, and TGF-beta regulation of extracellular matrix.

**Conclusions:** These findings identify activated immune pathways in the spinal cord immunologic compartment that may contribute to neuropathy, and implicate microglia and myeloid cells as key players involved in the spinal cord response to SIV.

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## **THE ROLE OF THE CLOSTRIDIUM PERFRINGENS AGR-LIKE QUORUM SENSING (QS) SYSTEM IN A MOUSE MODEL OF GAS GANGRENE**

Mauricio Navarro<sup>1</sup>, Jihong Li<sup>2</sup>, Juliann Beingesser<sup>1</sup>, Bruce McClane<sup>2</sup>, Francisco Uzal<sup>1</sup>

<sup>1</sup>University of California, Davis, San Bernardino, CA, USA, <sup>2</sup>University of Pittsburgh, Pittsburgh, PA, USA

**Background:** *Clostridium perfringens* type A causes gas gangrene in humans and animals. Alpha toxin (CPA) and perfringolysin (PFO) are the main virulence factors involved. Recent *in vitro* studies identified an Agr-like quorum-sensing (QS) system in *C. perfringens* that regulates the production of both toxins. A key component of this system is a membrane transporter named AgrB. In addition, a synthetic peptide named 6-R has been shown to interfere with this system *in vitro*, reducing toxin production.

**Objective:** To explore the role of this *C. perfringens* Agr-like QS system in a mouse model of gas gangrene.

**Methods:** An *agrB*-null mutant of *C. perfringens* type A was constructed by an insertional mutagenesis method. A complementing strain was then prepared. BALB/c mice were directly inoculated in the left hind leg with similar numbers of the wild-type, the *agrB*-null mutant, and the complementing strains. In addition, mice received the wild-type strain with or without the 6-R synthetic peptide. At 4h, animals were euthanized, and samples of skeletal muscle collected.

**Results:** The wild-type and complementing strains induced severe microscopic lesions in the inoculated skeletal muscle. Lesions in mice inoculated with the *agrB* mutant were significantly less severe. A similar significant difference was also observed in mice receiving the wild-type strain in combination with the 6-R peptide compared with the wild-type strain alone.

**Conclusions:** The Agr-like QS system is important in the pathogenesis of *C. perfringens* type A-mediated gas gangrene *in vivo*, and disruption of this system may represent a novel therapeutic target for this disease.

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12:30 p.m. – 1:30 p.m. CDT

### **PATHOGENESIS OF AQUATIC BIRD BORNAVIRUS-1 (ABBV-1) IN PEKIN DUCKS**

Fernanda Ampuero<sup>1</sup>, Alexander Leacy<sup>1</sup>, Phuc Pham<sup>1</sup>, Eva Nagy<sup>1</sup>, Brandon Lillie<sup>1</sup>,  
Pauline Delnatte<sup>2</sup>, Leonardo Susta<sup>1</sup>

<sup>1</sup>University of Guelph, Guelph, ON, Canada, <sup>2</sup>Toronto Zoo, Toronto, ON, Canada

**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 (Muscovy ducks and chickens).

**Objective:** Investigate the pathogenesis of ABBV-1 in Pekin ducks, selected as the most common waterfowl species raised worldwide.

**Methods:** Day-old ducklings (n=121) were divided into four groups and inoculated with ABBV-1 through one of three routes: intracranial (IC, 1.45x10<sup>4</sup> FFUs [focus-forming units]/bird), intramuscular (IM, 2.9x10<sup>4</sup> FFUs/bird), and intranasal (IN, 2.9x10<sup>4</sup> FFUs/bird). Controls received carrier only. At 1, 12 and 20 weeks post-infection (wpi), 7-14 birds were scheduled to be euthanized from each group.

**Results:** Neurological signs were present in 2/32, 8/30, and 1/30 ducks from the IC, IM, and IN groups, respectively. Infection of the brain was detected (RT-qPCR) in IC birds at 1 (6/7), 12 (7/7), and 20 wpi (14/14); 8/10 and 13/13 of IM birds were positive by 12 and 20 wpi, respectively. Peripheral tissues (proventriculus, kidneys, and gonads) were positive in 70-100% of IC birds at 12 and 20 wpi, and 40-70% of IM birds at 20 wpi. Only

1 IN bird became infected. Encephalitis/myelitis were identified in >75% of the IC and IM birds at 12 wpi, and less frequently at 20 wpi (< 30%).

**Conclusions:** Intracranial and intramuscular are efficient routes of ABBV-1 infection in Pekin ducks, leading to centripetal and centrifugal spread with systemic virus distribution. Inflammation of the nervous system was transient and did not clear persistent infection.

Tuesday, November 2, 2021

12:30 p.m. – 1:30 p.m. CDT

### **A DEEP LEARNING MODEL FOR QUANTIFICATION OF RETINAL ATROPHY IN A RAT MODEL OF BLUE LIGHT-INDUCED RETINAL DAMAGE**

Christiane Löhr<sup>1</sup>, Typhaine Lejeune<sup>2</sup>, Virginie Piccicuto<sup>2</sup>, Lindsey Smith<sup>3</sup>, Aleksandra Zuraw<sup>4</sup>

<sup>1</sup>Oregon State University, Corvallis, OR, USA, <sup>2</sup>Charles River Montreal ULC, Montreal, QC, Canada, <sup>3</sup>Aiforia Technologies Oy, Helsinki, Finland, <sup>4</sup>Charles River Laboratories Inc., Frederick, MD, USA

**Background:** Rodent models of retinal damage are used to evaluate the efficacy of candidate drugs in preventing retinal atrophy. Outer retina atrophy (ORA) is quantified following initial injury and administration of a buffer or test item(s). Currently, ORA severity is determined by manual or semi-automated measurements of outer nuclear layer (ONL) thickness or manual counting of ONL nuclei. This analysis is time consuming and manual counts prone to error.

**Objective:** Develop an image analysis-based, automated method of ORA assessment in whole slide images

**Methods:** A convolutional neural network (CNN)-based deep learning model was trained using a commercial artificial intelligence-based image analysis platform (Aiforia®). The model was trained by one investigator; validation performed by three independent validators. The multi-CNN model was trained for semantic segmentation of retina as parent layer. A child layer was designed as object count, with ONL nuclear count as the single feature. Model development was an iterative process.

**Results:** F1-scores of inter-observer concordance of manual annotations exceeded 97.7% for retina area and 92% for ONL count. Performance of the algorithm in comparison to manual annotations of individual validators yielded F1-scores between 95.1% and 95.6% for retina area and between 89% and 92.9% for ONL counts.

**Conclusions:** Performance of the ORA quantification deep learning model is non-inferior to that of current manual and semi-automated ORA scoring approaches. Our model provides total counts of ONL nuclei in addition to retina area and improves reproducibility and reduce time of the evaluation.

## Diagnostic Pathology Focused Poster Session

Saturday, October 30, 2021 | 5:00 p.m. – 6:00 p.m. CDT

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **A DEEP LEARNING APPROACH TO TUMOR VS. NORMAL DETECTION IN DIGITIZED HISTOPATHOLOGY SLIDES OF HUMAN SOFT TISSUE LEIOMYOSARCOMA**

James Cronin, Asmaa Aljuhani, Monika Karera, Xiaoyan Cui, Raghu Machiraju, David Liebner

The Ohio State University College of Medicine, Columbus, OH, USA

**Background:** Deep learning methods for digital histopathology analysis can achieve accurate and reproducible diagnoses, particularly in the domain of oncology.

**Objective:** We sought to implement a deep convolutional neural network on digitized histopathology images to discriminate tissue regions of soft tissue leiomyosarcoma from peri-tumoral non-neoplastic tissue.

**Methods:** 120 H&E stained whole slide images from 51 human soft tissue (non-uterine) leiomyosarcoma patients were collected from The Cancer Genome Atlas and Clinical Proteomic Tumor Analysis Consortium. A board-certified pathologist exhaustively annotated the slides for regions of leiomyosarcoma and peri-tumoral non-neoplastic tissue. The annotated images were then processed into non-overlapping patches, which were assigned labels based on the pixel level annotation masks. Oversampling was implemented to overcome class imbalance in our training dataset (192,549 leiomyosarcoma patches vs. 32,385 non-neoplastic patches). We manipulated the following experimental conditions to maximize performance: patch size and magnification, normalization technique, Otsu's binarization threshold, oversampling strategy, network architecture, training epochs, and learning rate.

**Results:** Our best performing algorithm (a pre-trained ResNet18) achieved 94.5% accuracy, 96.5% F1-score, 94.9% precision, and 98.1% recall in the validation set for the classification of leiomyosarcoma vs. non-neoplastic tissue at the patch level.

**Conclusions:** Our network achieved good performance in classifying leiomyosarcoma vs. peri-tumoral non-neoplastic tissue. The most common source of error was in distinguishing well-differentiated leiomyosarcoma from surrounding normal smooth muscle, which can be a challenging distinction for pathologists. This work serves as an initial step in a computational pathology analysis pipeline for risk stratification in human soft tissue sarcoma.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **QUANTIFICATION OF MUCOSAL LYMPHOCYTES IN FELINE SMALL INTESTINAL BIOPSIES USING ARTIFICIAL INTELLIGENCE**

Judit Wulcan<sup>1</sup>, Jonatan Wulcan<sup>2</sup>, Peter Moore<sup>1</sup>, Kevin Keel<sup>1</sup>, Stefan Keller<sup>1</sup>

<sup>1</sup>University of California Davis, Davis, CA, USA, <sup>2</sup>Technogarden, Malmö, Sweden

**Background:** Semi-quantitative grading of mucosal inflammation in veterinary diagnostic pathology is time-consuming and poorly reproducible, which compromises confidence in the method. The use of artificial intelligence (AI) for whole slide analysis allows the identification and quantification of tissues and cells and has the potential to improve the speed and reproducibility of lymphocyte quantification in small intestinal biopsies. **Objectives:** To assess the performance of AI-based quantification of lymphocytes in small intestinal biopsies from cats. **Methods:** Whole slide images of randomly selected biopsy specimens from the small intestine of cats were used to train, validate and test a convolutional neural network. Separate models for the recognition of regions (epithelium and lamina propria) and objects (lymphocytes) were developed and results were merged post-analysis. **Results:** In comparing computer-generated area and object recognition, to manual test set annotations, best performance metrics were obtained for epithelium (88% recall, 86 % precision and 86% F1-score) followed by lamina propria (85% recall, 75% precision and 78% F1-score) and lymphocytes (77% recall, 84% precision and 77% F1-score). In addition, a reference dataset for features such as tissue area, lymphocyte density and distance was created based on the analysis of 348 slides. **Conclusion:** The generated algorithm performed well in object and region recognition and might provide a more reproducible alternative to manual quantification of mucosal inflammation. The generated dataset can be used as an objective reference for assessing the degree of abnormality for individual biopsies.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **COMPARISON OF ANTI-MULLERIAN HORMONE AND INHIBIN IMMUNOLABELING IN CANINE AND EQUINE GRANULOSA CELL TUMORS**

Sophie Nelissen, Andrew Miller

Department of Biomedical Sciences, Section of Anatomic Pathology, Cornell University College of Veterinary Medicine, Ithaca, NY, USA

**Background:** Granulosa cell tumors (GCTs) are common ovarian neoplasms in the mare and bitch. While in mares, the classic honeycomb pattern imparted by the cystic histomorphology of those tumors usually allows for a straightforward diagnosis, canine GCTs often present in various histomorphologic patterns and can represent a diagnostic challenge. Inhibin has long been the standard immunohistochemical marker for granulosa cell tumors; however, anti-Mullerian hormone (AMH) has not been evaluated as a diagnostic IHC in the dog and horse.

**Objective:** This study aims to compare the efficacy of AMH and inhibin as immunohistochemical markers in canine and equine GCTs.

**Methods:** We performed a retrospective search of equine and canine GCTs in the histologic database of the New York State Animal Health Diagnostic Center. A total of 18 equine and 15 canine cases were selected based on histomorphological criteria assessed on hematoxylin and eosin.

**Results:** Virtually all equine tumors were dominated by a cystic pattern, while canine tumors had a more solid, follicular pattern. Both inhibin and AMH had a cytoplasmic, granular pattern of immunolabeling. Labeling for AMH occurred in 12/15 canine cases (varying from 1-50% of cells) and 18/18 equine cases ( $\geq 75\%$  of cells). Labeling for inhibin occurred in 15/15 canine cases (from 50 - 75% of cells) and 18/18 equine cases (25 - 75% of cells). Distribution and intensity of the labeling were unrelated to histomorphologic pattern.

**Conclusions:** While inhibin and AMH performed comparably in dogs, AMH had more diffuse immunolabeling than inhibin in mares.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

**A TALE OF TWO BEARS: HISTOLOGIC AND IMMUNOHISTOCHEMICAL FEATURES OF TUMORS OF THE PERIPHERAL NERVOUS SYSTEM IN TWO FREE-RANGING AMERICAN BLACK BEARS (*URSUS AMERICANUS*)**

Deborah Chong<sup>1</sup>, Robert Bildfell<sup>1</sup>, Kurt Licence<sup>2</sup>, Julia Burco<sup>2</sup>, Colin Gillin<sup>2</sup>, Peregrine Wolff<sup>3</sup>, Christiane Löhr<sup>1</sup>

<sup>1</sup>Oregon State University, Corvallis, OR, USA, <sup>2</sup>Oregon Department of Fish and Wildlife, Corvallis, OR, USA, <sup>3</sup>Wildlife Disease Association, Moorpark, CA, USA

**Background:** Reports of tumors of the nervous system in ursids are rare. Here we present two cases of peripheral nervous system tumors in two free-ranging American black bears (*Ursus americanus*). Case 1: Adult male with a subcutaneous, right cheek mass. Histologic and immunohistochemical findings: An infiltrative mass composed of mildly anisokaryotic, fusiform to spindle cells, arranged in haphazard streams and cords to an Antoni type B pattern. No mitoses were observed. Extensive areas of necrosis were present. Neoplastic cells were variably immunopositive for Sox-10, S100 and vimentin, and immunonegative for laminin, neurofilament, neuron specific enolase and GFAP. Diagnosis: Malignant nerve sheath tumor. Case 2: Adult female with a mass associated with the left trigeminal ganglion. Histologic and immunohistochemical findings: A well-encapsulated mass composed of a few variably-differentiated ganglion cells (<5% of the neoplastic cells) and packets of mildly anisokaryotic, polygonal cells with wispy amphophilic cytoplasm and a round nucleus with finely stippled chromatin and prominent nucleolus. They are supported by a fine fibrovascular and pale basophilic stroma. Mitotic count was 1.5 per 400x field (2.37mm<sup>2</sup>). Extensive areas of necrosis were present. Neoplastic cells were variably immunopositive for Sox-10, S100, vimentin, neuron specific enolase and synaptophysin, and immunonegative for laminin, neurofilament, and GFAP.

Diagnosis: Neuroblastoma, poorly differentiated subtype

Normal tissues from adult American black bears used as tissue controls for immunohistochemistry performed as expected.

**Conclusion:** These findings help document the range of ursid neoplastic diseases and the utility of specific immunohistochemical markers in American black bears.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **A PRESUMPTIVE CASE OF MUCOLIPIDOSIS IN A PIT BULL TERRIER**

Rebecca Bacon<sup>1</sup>, Brian Porter<sup>1</sup>, Margret Casal<sup>2</sup>, Petra Werner<sup>2</sup>, Carmen Lau<sup>1</sup>

<sup>1</sup>Texas A&M University College of Veterinary Medicine, College Station, TX, USA,

<sup>2</sup>University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA, USA

A 7-year-old, male castrated Pit Bull Terrier presented for respiratory distress with a history of difficulty breathing for 3 months, a one-year history of urinary incontinence, and a several year history of seizure-like activity following exercise. Following radiographs and bloodwork, the dog was diagnosed with megaesophagus and chronic aspiration pneumonia. Despite aggressive antimicrobial and oxygen therapy, the dog continued to decompensate and was humanely euthanized. Necropsy examination revealed a severe megaesophagus, aspiration pneumonia, and a markedly enlarged vagus nerve. Histopathology demonstrated variably sized accumulations of eosinophilic intracytoplasmic material within neurons of the brainstem, spinal cord, esophageal ganglia, and vagus nerve, as well as within hepatocytes and histiocytes throughout the liver, intestine, and lymph nodes. These accumulations stained strongly with Luxol fast blue, lightly with Periodic-acid Schiff, and did not stain with Alcian blue pH 2.5. On transmission electron microscopy, the accumulations were confirmed as intracytoplasmic, membrane-bound aggregates of storage material which were arranged in concentric lamellations or parallel arrays. Enzyme analysis showed greatly elevated levels of many enzymes typically associated with mucopolysaccharidoses, gangliosidoses, and mannosidoses, suggesting a form of mucolipidosis. Mucolipidoses have been reported only in cats, mice, and humans. While most forms of mucolipidosis in animals are severe, with affected animals dying at a young age, some forms in humans are less severe and patients may live well into adulthood. Genetic analysis is pending. This is the first report of a presumptive mucolipidosis in a dog.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **SARS-COV-2 INFECTION IN TWO FARMED MINKS (NEOVISON VISON)**

Ryan Yanez, Dodd Sledge, Matti Kiupel

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

A facility with 17,000 minks (Neovison vison) had 12 mink die on 9/27/2020 and 7 die on 9/28/2020. Coughing, anorexia and bloody nasal discharge were reported prior to death. The facility owner reported that these clinical signs were present for several weeks in the facility. Two male minks were submitted for necropsy to Michigan State University Veterinary Diagnostic Laboratory. In general, the gross findings were unremarkable. Histologically, the nasal epithelium was eroded to ulcerated and had a lymphoplasmacytic to suppurative infiltrate. The turbinates were coated by mucus, hemorrhage and numerous degenerate neutrophils and foamy macrophages. In the lungs, large to small sized veins and arteries had prominent lymphoplasmacytic cuffing with vasculitis and fibrinoid necrosis. Extensive loss of terminal bronchiolar epithelium was a prominent feature. The alveolar interstitium had a lymphoplasmacytic to



suppurative inflammation and fibrin thrombi within alveolar capillaries. The formation of hyaline membranes was not observed. PCR for SARS-CoV-2 was performed on nasal turbinate and lung samples from both minks and was found to be positive and confirmatory testing performed by the National Veterinary Services Laboratory (NVSL) in Ames, Iowa was also positive. Immunohistochemistry for the SARS coronavirus antigen was performed. Strong cytoplasmic labeling for the SARS coronavirus antigen was observed within mononuclear inflammatory cells, the nasal epithelium, tracheal epithelium and bronchial and terminal bronchiolar epithelium. Rarely, there was cytoplasmic labeling of endothelial cells in the subepithelial stroma of the turbinates. This report documents unique histologic lesions and positive endothelial immunohistochemical labeling in farmed minks diagnosed with SARS-Cov-2.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **ANGIOSTRONGYLUS CANTONENSIS IN A RED-RUFFED LEMUR**

Jessica Rizer<sup>1</sup>, Ryan Yanez<sup>1</sup>, Shannon Gonsoulin<sup>2</sup>, Matti Kiupel<sup>1</sup>

<sup>1</sup>Michigan State University Veterinary Diagnostic Laboratory and Department of Pathobiology and Diagnostic Investigation, East Lansing, MI, USA, <sup>2</sup>All Creatures Veterinary Hospital, New Iberia, LA, USA

*Angiostrongylus cantonensis* is a metastrongyloid nematode that has recently become endemic in the Southeastern United States. Rats are the definitive and gastropod species are the intermediate host. *A. cantonensis* is a common cause of eosinophilic meningitis in humans in Southeast Asia and the Pacific Islands and also affects nonhuman primates. A 9-year-old male Red-ruffed lemur (*Varecia rubra*), from Louisiana, was euthanized for worsening hind limb paresis. Sections of the brain and spinal cord were collected at necropsy for microscopic examination. Microscopically, within the subarachnoid space and neuropil of the cerebellum and brainstem, there were numerous transverse and longitudinal sections of adult nematodes. These nematodes were 50-70µm in diameter and had a 3-4µm thick smooth eosinophilic cuticle. The nematodes had a coelomyarian musculature, a pseudocoelom, a reproductive tract, and an intestine lined by uni-nucleated cells and intraluminal eosinophilic to brown flocculent material. The surrounding neuropil and affected cerebellar folia were effaced by hemorrhage and small numbers of eosinophils, neutrophils, macrophages, and glial cells. A single adult nematode was observed within the subdural space of the thoracic spinal cord. Another nematode had regionally effaced the dorsal horn in a section of the lumbar spinal cord causing regional axonal degeneration. The leptomeninges of the cerebellum and spinal cord were expanded by numerous eosinophils and fewer neutrophils and macrophages. The morphologic features of the nematodes were consistent with *Angiostrongylus spec.* Considering the affected species, the associated pathology, and the geographic origin of the lemur, these nematodes were most consistent with *A. cantonensis*.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **TRYPANOSOMA CRUZI INFECTION IN A JUVENILE ASIAN-SMALL-CLAWED OTTER (AONYX CINEREUS) AND A JUVENILE TWO-TOED SLOTH (CHOLOEPUS SPP.)**

Samantha Hughes, Brittany Baughman

Mississippi State University College of Veterinary Medicine, Mississippi State, MS, USA

In April 2021, a 9-week-old Asian Small-Clawed Otter with limited clinical history was submitted to the Mississippi State University College of Veterinary Medicine for necropsy. No gross lesions were appreciated on gross examination. Histopathology revealed a multiorgan protozoal infection, and frozen tissue was PCR-positive for *Trypanosoma cruzi*. After the diagnosis, the owner mentioned that a 4-month-old Two-Toed Sloth died in December 2020 and was submitted to the Mississippi State Diagnostic Laboratory where it was diagnosed with a protozoal myocarditis. Formalin fixed paraffin embedded tissue scrolls from the sloth's heart revealed a PCR-positive result for *Trypanosoma cruzi*. Additional history indicated that the otter and sloth were housed in the same enclosure at an exotic animal park in southern Mississippi, but were purchased from facilities in different states months apart. This additional information was interesting as it suggests these animals may have become infected on site in Mississippi. *Trypanosoma cruzi* is a protozoal parasite that is transmitted by blood-sucking triatomine bugs, also known as "kissing bugs." These insect vectors are known to inhabit Mississippi; however, natural *Trypanosoma cruzi* infections have not been documented in humans or domestic species. These cases are notable because they are the first documented cases of *Trypanosoma cruzi* infections in exotic animal species within the state of Mississippi.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **GERMAN SHORTHAIRED POINTER DOGS WITH EXFOLIATIVE CUTANEOUS LUPUS ERYTHEMATOSUS DEVELOP IMMUNE-COMPLEX MEMBRANOUS GLOMERULONEPHROPATHY**

Hayley Amerman<sup>1</sup>, Rachel Cianciolo<sup>2</sup>, Margret Casal<sup>1</sup>, Elizabeth Mauldin<sup>1</sup>

<sup>1</sup>University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA, USA, <sup>2</sup>The Ohio State University College of Veterinary Medicine, Columbus, OH, USA

**Background:** German shorthaired pointer dogs (GSHP) with exfoliative cutaneous lupus erythematosus (ECLE), a rare recessive condition due a *UNC93B1* gene mutation, develop kidney disease that resembles lupus nephritis in humans. **Objective:** To characterize the kidney disease by light microscopy and electron microscopy in a population of GSHP dogs with ECLE. **Methods:** Medical records were reviewed and microscopic features (H&E, PAS, trichrome) of kidneys from 7 GSHP dogs with ECLE were evaluated. Transmission electron microscopy of kidney from 1 dog was performed. **Results:** 5/7 dogs had proteinuria quantitated by either urine dipstick or urine protein to creatinine ratio (UPC); 2 dogs were not proteinuric. 2/7 dogs were hypoalbuminemic and none were azotemic. Histologic findings included early (2 dogs) to late (5 dogs) membranous glomerulonephropathy characterized mild to severe

glomerular capillary loop thickening and tubular proteinosis. In all 7 cases, trichrome staining revealed small red granular immune deposits on the subepithelial surface of the glomerular basement membrane. Electron microscopy demonstrated subepithelial electron-dense immune deposits encircled by remodeled glomerular basement membrane. These findings are diagnostic of immune complex membranous glomerulonephropathy (MGN). **Conclusion:** This cohort of GSHP dogs with ECLE developed immune-complex MGN which is likely a manifestation of systemic lupus erythematosus. GSHP dogs with ECLE should undergo clinical evaluation of renal function for early identification and treatment.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **A CASE OF SENNA PLANT (CASSIA OCCIDENTALIS, CASSIA OBTUSIFOLIA) POISONING IN A 4-MONTH-OLD HEIFER**

Ji-Hang Yin<sup>1</sup>, Leanne Dillard<sup>2</sup>, David Martinez Rodriguez<sup>3</sup>, Manuel Chamorro Ortega<sup>3</sup>, Russell Cattley<sup>1</sup>

<sup>1</sup>Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA, <sup>2</sup>Department of Animal Sciences, Crop, Soil, and Environmental Sciences, Auburn University,, Auburn, AL, USA, <sup>3</sup>Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL, USA

**Introduction:** Senna plant poisoning in cattle is a rapidly progressive disease with monophasic, multifocal myocyte degeneration and necrosis. Ingestion of the beans of the senna plant (*Cassia occidentalis* or *Cassia obtusifolia*) is considered a main route for intoxication. The entire plant is toxic and seeds are considered the most toxic part. The toxic principle has remained uncertain.

**Objectives:** To describe a case of Senna plants intoxication in cattle in the United States, which has been rarely reported.

**Methods:** A 4-month-old heifer was presented for a history of sternal recumbency, absent patellar and withdrawal reflexes, as well as unwillingness to move. Two other calves in the same herd exhibited similar clinical signs, and senna plant (*C. occidentalis* and *C. obtusifolia*) were found in the pasture. The heifer was humanely euthanized. Laboratory testing, and macroscopic and microscopic examinations were performed.

**Results:** Serum chemistry levels of skeletal muscle enzyme were higher than the normal ranges. Creatine phosphokinase (CK) was 83224 U/L (normal range 40-264 U/L) and aspartate aminotransferase (AST) was 2547 U/L (normal range 69-112 U/L). At necropsy, multifocal areas of skeletal muscles were extensively pale tan and dry. Senna seeds were observed in the rumen contents. On histology, over 90% of the myofibers in the affected skeletal muscles had a severe monophasic myonecrosis.

**Conclusion:** This case demonstrated the clinical presentation and pathologic findings of severe Senna plant poisoning in a 4-month-old heifer.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **AN INTRANEURAL PERINEURIOMA IN A DOG**

Ji-Hang Yin<sup>1</sup>, Brittani Sexton<sup>2</sup>, Maninder Sandey<sup>1</sup>

<sup>1</sup>Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA, <sup>2</sup>Department of Clinical Sciences, Neurology and Neurosurgery service, College of Veterinary Medicine, Auburn, AL, USA

Perineurioma is an extremely rare intraneural tumor in human and veterinary medicine. Histologically, neoplastic cells characteristically form multiple small “onion bulbs”, consisting of concentric layers of perineural cells ensheathing a central axon. Prognosis in animals remained uncertain with scarce case reports.

A 3-year-old, male castrated Beagle dog presented to Auburn University Veterinary Hospital with a 2-week history of a continued and progressive worsening of mid-lumbar pain and decreased postural reactions as well as reflexes of the pelvic limbs. Magnetic resonance imaging showed a left intradural-extramedullary mass spanning from the 5<sup>th</sup> to 7<sup>th</sup> lumbar vertebrae and severely compressing the spinal cord. Given that a cure would unlikely, the owner elected euthanasia due to quality of life concerns. At necropsy, the left spinal root containing the lumbar spinal nerves of 4<sup>th</sup> to 6<sup>th</sup> was enlarged and contained a well-demarcated, nonencapsulated, firm, tan, 2-cm x 0.5-cm x 0.5-cm, mass. Histopathology revealed a nonencapsulated, infiltrative, densely cellular neoplasm expanding and effacing the left nerve roots. The neoplasm was composed of fusiform cells arranged in a concentric lamellations ensheathing a central axon and formed pseudo-onion bulbs. Positive immunolabeling for S-100 antigen was only observed in the central core of nerve fibers. Taken together, the anatomic location, characteristic histopathological features, including pseudo-onion bulbs pattern as well as the negative immunolabeling for S-100 staining of the cell processes, a diagnosis of canine intraneural perineurioma was made.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **SALIVARY GLAND CARCINOMA IN A BUCKING BULL**

Kayla Alexander, Timothy Morgan, Brittany Baughman

Mississippi State University College of Veterinary Medicine, MS State, MS, USA

A 6-year-old bucking bull was presented to Mississippi State University-CVM for recurrence of an abscess at the angle of the right mandible following treatment one month prior. Due to the aggressive nature of the bull, exploration under general anesthesia was elected. The bull went into cardiopulmonary arrest at induction and was submitted for necropsy. Gross findings included a 15x15 cm encapsulated mass with a caseonecrotic center. Adjacent musculature was dry and friable with dark purple discoloration that extended from the thoracic inlet to the base of the right horn. The right paracondylar process was lytic. The tracheobronchial lymph nodes were markedly enlarged. Approximately 75-80% of the pulmonary parenchyma was consolidated and mottled dark red to purple with multifocal to coalescing, pinpoint to 3 cm, firm to caseous, white-yellow

nodules. Histologically, pulmonary and cervical masses were arranged into lobules, nests, and islands embedded within expansive fibrous stroma. Neoplastic cells had abundant eosinophilic finely granular cytoplasm and large round nuclei with coarsely stippled to vesiculated chromatin and variable nucleoli. Anisocytosis and anisokaryosis were marked. Mitotic figures averaged 16 per 2.37 mm<sup>2</sup>. Comedonecrosis and tumor embolization were frequently appreciated. Neoplastic cells demonstrated strong perimembranous immunolabeling with cytokeratin, and lobular stromal cells demonstrated perimembranous immunolabeling with smooth muscle actin ( $\alpha$ -SMA). Based on histopathologic findings, salivary carcinoma was diagnosed. Salivary neoplasms are rare in all veterinary species; however, tumors are more common in aged animals and typically develop in the parotid and mandibular glands. Histology and immunohistochemistry are key in diagnosing this disease.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **AMELOBLASTIC FIBRO-ODONTOMA IN A BULL**

Kaylin McNulty, Brittany Baughman

Mississippi State University, Starkville, MS, USA

#### Case Report:

An approximately 2-year-old Wagyu bull is presented to Mississippi State University for a 3-week history of swelling of the right mandible. Radiographs show a severe monostotic aggressive bony lesion. The bull was non-responsive to treatment, and euthanasia was elected.

The right mandible from premolar 1 to molar 3 is expanded by a hard mass measuring 20x15x10cm. The overlying gingiva contains a large, ulcerated area with impacted feed material. The teeth are displaced. On cut surface, the lesion contains numerous cavitated spaces, occasional gelatinous areas, and multifocal hard, white irregular foci.

Microscopically, the multi-lobulated, expansile mass effaces mandibular bone and is composed of neoplastic odontogenic epithelial cells within a dense ectomesenchymal background. Neoplastic cells have scant eosinophilic cytoplasm and a small round to ovoid, usually basally located, nucleus (resembling odontoblasts). In the center of select lobules, neoplastic cells have fine stellate cytoplasm (resembling stellate reticulum). Occasionally, the mass contains scattered irregular foci of hypereosinophilic material (dentin) with usually adjacent dark purple material (enamel) and scattered hemorrhage/hemosiderophages. The overlying gingival epithelium is ulcerated to hyperplastic.

#### Discussion:

This right mandibular mass represents an ameloblastic fibro-odontoma. These dental tumors contain neoplastic odontogenic epithelium, induced ectomesenchyme, and form

dentin and enamel. Ameloblastic fibro-odontomas have been described in the dog, horse, and ox. Although they are rare in all domestic species, they are the most common odontogenic tumor in cattle. This neoplasm is not reported to metastasize but is slowly and progressively locally aggressive if not surgically removed.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **MYCOTIC PNEUMONIA AND ENCEPHALITIS IN AN ADULT ALPACA WITH THIRD COMPARTMENT ULCERATION AND CHEILITIS**

Marvin Firth, Laura Setyo, Nicola Parry

University of Surrey, Guildford, United Kingdom

A 22-month-old male alpaca was presented for post-mortem examination to the Veterinary Pathology Centre, University of Surrey (Guildford, UK), after reported rapid weight loss and weakness over a two-week period. Macroscopic post-mortem assessment revealed a focally extensive, full-thickness ulceration of the third compartment; approximately 20% of the pulmonary parenchyma had multifocal to coalescing petechiae and haemorrhage; and within the oral cavity, the gingiva, and soft and hard palates had multifocal variably sized full thickness ulcers. Subsequent histological investigation revealed a severe ulcerative and necrotising gastritis, a severe multifocal to coalescing necrotising embolic encephalitis, pneumonia and cheilitis. All lesions contained intralesional fungal hyphae that were up to 7  $\mu$ m wide and variably septate, with parallel walls and dichotomous, acute angle branching. Fungal culture of frozen tissue identified *Aspergillus* spp. Polymerase chain reaction for bovine viral diarrhoea virus (BVDv) was negative. The primary causes of depression and rapid weight loss in this animal were considered to be a combination of respiratory compromise due to the severe pneumonia and endotoxic shock associated with the ulcerative gastritis of the third gastric compartment. To the authors' knowledge, this is the first report of ulcerative gastritis of the third gastric compartment with associated embolic pneumonia and encephalitis in an alpaca. Although the exact origin of the infection is unknown in this case, possible portals of entry could include either the oral cavity or an area of gastric ulceration.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **ADENOVIRUS INFECTION IN RED-TAILED HAWKS (*BUTEO JAMAICENSIS*) AND A BROAD-WINGED HAWK (*BUTEO PLATYPTERUS*)**

Emma Torii<sup>1</sup>, Arno Wuenschmann<sup>1</sup>, Anibal Armien<sup>2</sup>, Sunil Mor<sup>1</sup>, Rahul Kumar<sup>1</sup>, Emma Chalupsky<sup>3</sup>, Michelle Willette<sup>3</sup>

<sup>1</sup>University of Minnesota, Veterinary Diagnostic Laboratory, St. Paul, MN, USA,

<sup>2</sup>University of California Davis, California Animal Health and Safety Laboratory

(CAHFS), Davis, CA, USA, <sup>3</sup>University of Minnesota, The Raptor Center, St. Paul, MN, USA

**Background:** Adenovirus in raptors are best described in falcons, with only a few cases reported in owls and a single case in a hawk. In raptors, adenoviral infection is most commonly characterized by necrotizing hepatitis and splenitis.

**Methods:** Five red-tailed hawks (*Buteo jamaicensis*) and a broad-winged hawk (*Buteo platypterus*) had an adenoviral infection based on history, histopathology, negative electron microscopy, and polymerase chain reaction (PCR). Additionally, sequencing of the hexon genes were performed in all but one case.

**Results:** All six birds had acute onset illness resulting in death. Microscopically, all birds had solitary, pale eosinophilic to amphophilic, intranuclear inclusion bodies within hematopoietic cells (bone marrow) and macrophages (spleen), and 5/6 birds had similar inclusions within hepatocytes and Kupffer cells. All but one bird had severe bone marrow necrosis. There was moderate splenic necrosis in 4/6, and mild to marked hepatic necrosis in 4/6 birds. Adenoviral particles were detected in bone marrow, liver, and/or spleen by electron microscopy in all birds. All were PCR positive for adenovirus in bone marrow, liver, spleen, and/or intestinal contents. Based on sequencing, three cases were clustered within the *Siadenovirus* genus and two cases were clustered within the *Aviadenovirus* genus.

**Conclusions:** This case series expands on the limited knowledge of adenovirus infections in hawks. The presence of splenic and hepatic necrosis and particularly the hitherto previously unreported bone marrow necrosis suggests that the infection is clinically relevant and potentially fatal in hawks.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **AN OUTBREAK OF YERSINIA PSEUDOTUBERCULOSIS IN AFRICAN LIONS (PANTHERA LEO) WITH ABERRANT BACTERIAL MORPHOLOGY**

Mandy Womble, Megan Cabot, Tara Harrison, Tatiane Terumi Negrão Watanabe  
North Carolina State University College of Veterinary Medicine, Raleigh, NC, USA

Two African lions (*Panthera leo*) died secondary to infection with *Yersinia pseudotuberculosis* at a zoological park in central North Carolina following a 3-5 day duration of clinical signs including respiratory distress, lethargy, ataxia, and hyporexia. The lions were submitted for postmortem examination and had similar gross and histologic findings. Macroscopically, throughout the hepatic and splenic parenchyma, there were multifocal to coalescing, semi-firm, pale tan nodules. The lungs were non-collapsed with multifocal petechial hemorrhagic foci. Microscopic examination revealed multifocal to coalescing necrotic foci with associated fibrinosuppurative cellular infiltrate in the liver, spleen, lungs, kidneys, and mesenteric lymph nodes with abundant intralesional gram-negative bacteria. Aerobic bacterial culture of the liver, spleen, and lung of the male lion and the liver of the female lion revealed 4+ growth of *Y. pseudotuberculosis*. The observed bacterial morphology on histologic examination varied between the two lions ranging from large aggregates of coccobacilli to large rod-shaped and filamentous bacteria. Similar aberrant forms of *Y. pseudotuberculosis* have been previously described in squirrel monkeys following antibiotic administration. The source of infection with *Y. pseudotuberculosis* was not identified in this outbreak but transmission through contaminated water, soil, raw meat, or predation of wild bird or rodent reservoirs is possible. Mortality associated with *Y. pseudotuberculosis* has previously been described in an African lion cub; however, to our knowledge, this is the first report of *Y. pseudotuberculosis* infection in adult African lions with aberrant bacterial morphology.

Saturday, October 30, 2021

5:00 p.m. – 6:00 p.m. CDT

### **RENAL COCCIDIOSIS CAUSED BY NEPHROISOSPORA EPTESICI IN A CAPTIVE BIG BROWN BAT (EPTESICUS FUSCUS)**

Nathan Crilly, Sarah Poynton, Kathleen Gabrielson  
Johns Hopkins School of Medicine, Baltimore, MD, USA

**Background:** An adult male big brown bat presented for necropsy after being found dead two months after being captured in Maryland for use in a research colony.

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

**Methods:** Gross and histological examination was performed. H&E, Periodic acid-Schiff, and Fielgen stains were used to characterize renal parasites.

**Results:** On gross examination, there were four cystic structures in the cortex of the right kidney, containing pale tan, turbid fluid. On histological section, these cortical cysts were identified as cystic renal tubules, lined by hypertrophic and hyperplastic renal tubular epithelium containing multiple life stages of coccidian parasites. The cyst lumina contained numerous oocysts. These renal parasites were putatively identified as *Nephroisospora eptesici*.

**Conclusions:** To our knowledge, this is the first description of renal coccidiosis in a big brown bat in Maryland. Renal coccidiosis is caused by the coccidian parasite *N. eptesici*, which was first described in wild bats from Minnesota in 2010. *N. eptesici* is most closely related to *Besnoitia* spp., and has a single-host life cycle, with the complete life cycle occurring in the kidney. Renal coccidiosis is thought to be an incidental postmortem finding, which is not associated with clinically significant renal disease, although it may interfere with research.

### **Natural Disease Focused Poster Session**

Sunday, October 31, 2021 | 12:00 p.m. – 1:00 p.m. CDT

Sunday, October 31, 2021

12:00 p.m. – 1:00 p.m. CDT

### **DIAGNOSTIC IMMUNOHISTOCHEMISTRY OF CANINE GLIOMA**

Gregory Krane<sup>1,2,3</sup>, Carly O'Dea<sup>4</sup>, David Malarkey<sup>1,2</sup>, Andrew Miller<sup>5</sup>, C. Miller<sup>6</sup>, Debra Tokarz<sup>1,7</sup>, Heather Jensen<sup>2</sup>, Kyathanahalli Janardhan<sup>8,9</sup>, Keith Shockley<sup>2</sup>, Christopher Mariani<sup>1</sup>

<sup>1</sup>North Carolina State University - College of Veterinary Medicine, Raleigh, NC, USA,

<sup>2</sup>National Institute of Environmental Health Sciences - National Toxicology Program, Research Triangle Park, NC, USA, <sup>3</sup>Charles River Laboratories, Shrewsbury, MA, USA,

<sup>4</sup>Charles River Laboratories - PAI, Durham, NC, USA, <sup>5</sup>Cornell University - College of Veterinary Medicine, Ithaca, NY, USA, <sup>6</sup>University of Alabama at Birmingham - School

of Medicine, Birmingham, AL, USA, <sup>7</sup>Experimental Pathology Laboratories, Research Triangle Park, NC, USA, <sup>8</sup>Integrated Laboratory Systems, Research Triangle Park, NC, USA, <sup>9</sup>Abbvie, North Chicago, IL, USA



Glioma is a devastating cancer with diverse histologic features that can create diagnostic difficulty and generate inter-pathologist diagnostic disagreement. Immunohistochemistry, which has been shown to increase inter-pathologist diagnostic agreement of canine brain tumors, can help diagnose canine gliomas. Though the literature describes qualitative canine glioma immunolabeling, quantitative assessment has not been reported. We report immunolabeling characteristics for 73 canine gliomas diagnosed with consensus by a five-pathologist panel utilizing NCI diagnostic recommendations with access to H&E, Olig2, GFAP, and CNPase slides. Cases were reported as positive or negative for immunolabeling based on manual examination by a single evaluator, and area fractions were measured by digital analysis. Astrocytoma had greater frequency of tumors positive for GFAP immunolabeling than oligodendroglioma (83 vs 17%;  $p < 0.01$ , Chi-squared test), and oligodendroglioma had greater frequency of tumors positive for CNPase immunolabeling than astrocytoma (55 vs 17%;  $p = 0.01$ , Chi-squared test). Olig2 median area fraction was higher in oligodendroglioma than in astrocytoma (16 vs 1.5%;  $p < 0.01$ , Mann-Whitney test). GFAP median area fraction was higher in pooled low-grade than in pooled high-grade tumors (45 vs 21%;  $p < 0.01$ , Mann-Whitney test), and GFAP median area fraction was higher in astrocytoma than in oligodendroglioma (52 vs 21%;  $p < 0.01$ , Mann-Whitney test). These data reinforce the utility of these markers in helping to differentiate oligodendroglioma from astrocytoma. Future studies are indicated to determine to what degree these immunohistochemical profiles predict prognosis and response to therapy for canine patients with glioma.

Sunday, October 31, 2021

12:00 p.m. – 1:00 p.m. CDT

## RETROSPECTIVE ANALYSIS OF MYCOBACTERIOSIS IN AQUARIUM HOUSED ELASMOBRANCHS

Mari Inohana<sup>1</sup>, Kaoru Nagaya<sup>2</sup>, Tsunehisa Komatsu<sup>2</sup>, Sakiko Kubo<sup>2</sup>, Yoshiaki Tanaka<sup>3</sup>, Keisuke Kondo<sup>4</sup>, Takeshi Komine<sup>1</sup>, Kentarou Tomaru<sup>1</sup>, Kentarou Ono<sup>1</sup>, Shinpei Wada<sup>1</sup>  
<sup>1</sup>Nippon Veterinary and Life Science University, Tokyo, Japan, <sup>2</sup>Shinagawa Aquarium, Tokyo, Japan, <sup>3</sup>Shimane Aquarium, Shimane, Japan, <sup>4</sup>MARINE WORLD uminonakamichi, Fukuoka, Japan

**Background:** Mycobacteriosis is one of the most common chronic disease in fish. On the other hand, mycobacteriosis has rarely been diagnosed in elasmobranchs. **Objective:** The retrospective study characterized the prevalence and the histologic and molecular biological features of the mycobacteriosis of elasmobranchs by examining aquarium fishes. **Methods:** Elasmobranch submissions (60) received by our laboratory from 1999 to 2021 were searched for mycobacteriosis. Histopathologic sections of various tissues from 60 individuals (22 species) were examined. Tissues with inflammatory cell infiltration were stained with acid fast stain. DNA was extracted from frozen, ethanol fixed or formalin fixed paraffin embedded tissues, which showed acid fast bacilli in lesions, and tested for *Mycobacterium* spp. by PCR targeting the *hsp65* gene with sequencing analysis. The DNA showing high nucleotide sequence identities to *M. marinum* were tested for the insertion elements IS2404 and IS2606 by PCR. Peptide nucleic acid *in situ* hybridization were performed to identify pathogen in tissues where multiple mycobacteria were detected. **Results:** Acid fast bacilli within macrophages were histologically detected in 18 out of the 60

elasmobranchs examined. Sixteen of them, including 8 species from 5 aquaria, were diagnosed as mycobacteriosis by sequencing analysis. Microscopically, lesions were typified by lymphocytic inflammation with small number of macrophages and granulomatous inflammation both without caseous necrosis. **Conclusions:** Pathologic investigations on aquarium housed elasmobranchs in Japan have revealed high prevalence of mycobacteriosis compared to the number of mycobacterial case reports in elasmobranchs. The prevalence of mycobacteriosis in elasmobranchs could be similar to that in teleost previously reported.

Sunday, October 31, 2021

12:00 p.m. – 1:00 p.m. CDT

### **PREVALENCE AND MICROSCOPIC FINDINGS OF PARELAPHOSTRONGYLUS TENUIS IN FREE-RANGING CERVIDS IN NORTH DAKOTA AND TENNESSEE**

Michelle Liu, Richard Gerhold, Nickolus Stahlman, Denae LoBato  
University of Tennessee, Knoxville, TN, USA

*Parelaphostrongylus tenuis* (*P. tenuis*) rarely causes clinical disease within its natural host, the white-tailed deer (WTD). However, *P. tenuis* has been implicated as a contributing factor to neurological disease and population decline of other wild cervids throughout the eastern U.S. Here, we sought to determine the microscopic lesions and prevalence of *P. tenuis* in various free-ranging neurologic cervids. Whole brains from 20 free-ranging cervids (6 mule deer, 4 moose, and 1 WTD from North Dakota; 9 elk from Tennessee) with neurological signs were submitted from 2004-2020 to the University of Tennessee and subjected to microscopic exam and molecular testing for *P. tenuis*. Selected microscopic findings were graded for severity from 1-3. Immunohistochemistry for West Nile virus (WNV), Eastern equine encephalitis virus (EEE), and Epizootic hemorrhagic disease virus (EHD) was performed on select elk tissues. Of the 20 cervid brains examined, 12 (60%) had suspected *P. tenuis* infection, with all 6 mule deer affected. Microscopic findings supportive of *P. tenuis* infection were consistent with previous studies involving wild cervids. PCR for *P. tenuis* in suspected cases is pending. Five cervids (25%) had an undetermined cause of neurological disease, represented only by mononuclear meningitis or meningoencephalitis. Three cervids (15%), including the WTD, had no significant findings. Among the elk, immunoreactive animals included two for WNV, five for EEE, and none for EHD. *P. tenuis* should be considered a significant cause of morbidity and mortality in cervids in central and southeastern U.S. EEE and WNV are potential causes of neurological disease in elk.

Sunday, October 31, 2021

12:00 p.m. – 1:00 p.m. CDT

### **IMMUNE RESPONSE TO CHLAMYDIA SPP. INFECTION IN THE MALE AND FEMALE REPRODUCTIVE SYSTEM IN KOALAS (PHASCOLARCTOS CINEREUS)**

Sara Pagliarani<sup>1,2</sup>, Stephen Johnston<sup>2</sup>, Kenneth Beagley<sup>3</sup>, Chiara Palmieri<sup>1</sup>

<sup>1</sup>School of Veterinary Science, The University of Queensland, Gatton, Australia, <sup>2</sup>School of Agriculture and Food Science, The University of Queensland, Gatton, Australia,

<sup>3</sup>School of Biomedical Sciences, Faculty of Health, Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia

**Introduction:**

Chlamydiosis is one of the main causes of the progressive decline of koala populations in Australia. While histologic, immunologic and molecular studies have provided insight into the basic function of the koala immune system, the *in situ* immune cell signatures during chlamydial infection of the reproductive tract in koalas have not been investigated so far.

**Materials and Methods:**

Fifty-three female koalas and sixty-two males presented to wildlife hospitals with clinical signs suggestive of *Chlamydia* infection were euthanized with the entire reproductive tract collected for histology, immunohistochemistry (IHC) for T- (CD3 $\epsilon$ , CD4 and CD8 $\alpha$ ), B- (CD79b) and HLA-DR-DP-DQ-positive cells and quantitative real time-PCR (rtPCR) for *Chlamydia pecorum*.

**Results:**

This study demonstrated the recruitment of specific T-, B- and HLA-DR-positive cells to the lower and upper reproductive compartments in male and female koalas and the association between different CD- and HLA-DR-positive cells and PCR load. The CD4-positive cells number was positively correlated with the gross pathology score for the female koalas.

**Conclusions:**

The recruitment of specific T-, B- and HLA-DR-positive cells to both the lower and upper reproductive tract is a clear step forward in understanding the mechanisms behind koala chlamydial infection immunopathogenesis. This work is the first comprehensive study combining the evaluation of the immune cells with a throughout investigation of the pathological lesions in the reproductive tract to get meaningful information on the ability of the immune system to resolve the infection or the inability of the animals to remove the organisms and develop instead a non-resolving chronic infection.

Sunday, October 31, 2021

12:00 p.m. – 1:00 p.m. CDT

**PATHOLOGY OF EPTESIPOX VIRUS IN NATURALLY INFECTED BIG BROWN BATS**

Ursula Perdrizet<sup>1,2</sup>, Janet Hill<sup>1</sup>, Champika Fernando<sup>1</sup>, Trent Bollinger<sup>1,2</sup>, Vikram Misra<sup>1</sup>

<sup>1</sup>Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada, <sup>2</sup>Canadian Wildlife Health Cooperative Western/Northern Region, Saskatoon, SK, Canada

**Background:** *Eptesipox virus* was first isolated from big brown bats associated with severe fibrinosuppurative and necrotizing tenosynovitis and osteoarthritis in Washington state in 2011.

**Objective:** Determine prevalence and pathology associated with *Eptesipox virus* infection in big brown bats submitted to the Canadian Wildlife Health Cooperative Western/Northern region.

**Methods:** Necropsy submissions of big brown bats from 2017-2021 were screened for *Eptesipox virus* using PCR targeting the DNA polymerase gene, histology was reviewed, and in-situ hybridization targeting the p39 putative membrane-associated core protein and p4b precursor performed on tissues with lesions. Virus was isolated and characterized using cell culture, electron microscopy, and next generation sequencing.

**Results:** 28/61 submissions were positive for viral DNA. The positive cases had the following distribution of lesions: 5/28 oral ulceration, 4/28 joint inflammation or proliferation, 7/28 hepatic necrosis, 7/28 necrosis of the gastric mucosa, 3/28 necrosis of the dermis and or hypodermis, 1/28 infarct of the skeletal muscle, 1/28 vasculitis, and 6/28 with 2 or more lesions. No unusual lesions in 14/28. Virus was isolated from tissue homogenate and electron microscopy of negatively stained virus and infected cells was consistent with a poxvirus. The contiguous sequences generated from de novo assembly shared 99.7% identity with 85.7% coverage of the previous isolate, *Eptesipox virus* Washington strain accession number NC\_035460. In-situ hybridization demonstrated viral RNA positive staining in 1/3 joints, 0/3 livers, 3/4 oral ulcers, and 1/1 skeletal muscle.

**Conclusion:** Natural *Eptesipox virus* is associated with a wide range of lesions with the role of viral replication remaining unclear.

Sunday, October 31, 2021  
12:00 p.m. – 1:00 p.m. CDT

### **MOLECULAR AND MORPHOLOGIC CHARACTERIZATION OF FUNGAL ENCEPHALITIS IN DOGS SUBMITTED TO NEW YORK STATE DIAGNOSTIC LABORATORY FROM 1996-2020**

Cristy Rocío González Barrientos<sup>1</sup>, Andrew David Miller<sup>1</sup>, Courtney Meason-Smith<sup>2</sup>, Aline Rodrigues Hoffmann<sup>2</sup>, Eric Glass<sup>3</sup>, James Hammond<sup>4</sup>, Elena Alina Demeter<sup>1</sup>  
<sup>1</sup>Cornell University College of Veterinary Medicine, Ithaca, NY, USA, <sup>2</sup>College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA, <sup>3</sup>Red Bank Veterinary Hospital, Tinton Falls, NJ, USA, <sup>4</sup>Pieper Memorial Veterinary Center, Middletown, CT, USA

**Background:** Fungal infections of the CNS are under-characterized causes of neurologic disease in dogs. **Objective:** Characterize cases of fungal encephalitis in

dogs (1996-2020) by histopathology and molecular identification. **Methods:** Formalin-fixed paraffin-embedded (FFPE) tissue from 19 dogs was evaluated applying H&E, GMS, Fontana-Masson stains and immunohistochemistry (IHC) for canine distemper virus (CDV). DNA was extracted from FFPE blocks. Panfungal PCR targeting the internal transcribed spacer (ITS) and large (28S) subunit (LSU) regions was followed by sequencing and comparison to NCBI database. **Results:** The age range of affected dogs was 1-10 years, with most cases being 2-4 years. German Shepherd (7/19, 36.8%) and mixed breed dogs (5/19, 26.3%) were primarily affected. Histopathologic changes included necrosis (18/19, 94.7%), encephalitis (17/19, 89.5%), meningitis (16/19, 84.2%), perivascular cuffing (14/19, 73.7%), and vasculitis (12/19, 63.2%). Nine (47.4%) of 19 cases had yeast morphologically compatible with *Cryptococcus neoformans* (6/19, 31.6%) and *Blastomyces dermatitidis* (3/19, 15.8%). Ten of 19 cases had pigmented (4/19, 21.1 %) and hyaline hyphae (6/19, 31.6%). Fungal identification was achieved in 9 cases: *Cladophialophora bantiana* (3/19, 15.8%), *Coniochaeta* sp. (2/19, 10.5%), *Cryptococcus neoformans* (2/19, 10.5%), *Blastomyces dermatitidis* (1/19, 5.3%) and *Phialemonium obovatum* (1/19, 5.3%). CDV IHC was positive in one case co-infected with *Cladophialophora bantiana*. **Conclusions:** Fungal agents are important causes of encephalitis in dogs younger than 4 years and German shepherds are overrepresented. Morphologically, *Cryptococcus neoformans* and hyaline septate fungi were most common, while *Cladophialophora bantiana*, a dematiaceous fungus, was the most commonly confirmed by molecular techniques. Fungal coinfection with CDV was infrequent.

Sunday, October 31, 2021

12:00 p.m. – 1:00 p.m. CDT

## **BOVINE ASTROVIRUS AND ITS POTENTIAL ROLE IN BOVINE LYMPHOCYTIC ENCEPHALITIS**

Dominique Comeau<sup>1</sup>, Maria Spinato<sup>2</sup>, Robert Foster<sup>1</sup>, Davor Ojkic<sup>2</sup>, Jan Sargeant<sup>1</sup>, Jeff Caswell<sup>1</sup>

<sup>1</sup>Ontario Veterinary College, Guelph, ON, Canada, <sup>2</sup>Animal Health Laboratory, Guelph, ON, Canada

Astroviruses are a well-known cause of gastroenteritis in humans and many domestic animal species. More recently, these emerged as a cause of encephalitis in cattle and other species. Encephalitis is an economically important disease in cattle due to death of animals and potential exclusion of carcasses from the food chain. There is a zoonotic concern as many causes of encephalitis in cattle can also cause disease in humans. It is therefore essential to determine the causes of encephalitis and their relative importance in a population.

To investigate bovine astrovirus in Ontario cattle, 35 cases of idiopathic lymphocytic encephalitis were retrieved from the Animal Health Laboratory/Ontario Veterinary College archives. As controls 32 animals with non- lymphocytic encephalitis, and 42 animals with no neurologic disease or encephalitic lesions were included in the study. All animals were screened using RT-qPCR for bovine astrovirus. No animals from either control group tested positive for bovine astrovirus. Four animals with lymphocytic encephalitis are positive for bovine astrovirus; they all had lymphocytic perivascular

cuffs affecting both grey and white matter of the cerebrum. All positive cases had a history of neurologic disease, and most displayed ataxia and staggering.

Sunday, October 31, 2021

12:00 p.m. – 1:00 p.m. CDT

## **DESCRIPTIVE COMPARISON OF INDIVIDUAL HIVE AND YARD-LEVEL SAMPLING TECHNIQUES FOR SPORES OF PAENIBACILLUS LARVAE IN SASKATCHEWAN HONEY BEE OPERATIONS WITH RECENT OUTBREAKS OF AMERICAN FOULBROOD**

Michael Zabrodski<sup>1</sup>, Jessica DeBruyne<sup>1</sup>, Geoff Wilson<sup>2</sup>, Igor Moshynskyy<sup>1</sup>, Mohsen Sharafi<sup>1</sup>, Sarah Wood<sup>1</sup>, Ivanna Kozii<sup>1</sup>, Jenna Thebeau<sup>1</sup>, Colby Klein<sup>1</sup>, Igor Medici de Mattos<sup>1</sup>, LaRhonda Sobchishin<sup>1</sup>, Tasha Epp<sup>1</sup>, Antonio Ruzzini<sup>1</sup>, Elemir Simko<sup>1</sup>

<sup>1</sup>Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada, <sup>2</sup>Ministry of Agriculture, Government of Saskatchewan, Prince Albert, SK, Canada

**Background:** Three commercial honey bee operations within Saskatchewan (Canada) with recent outbreaks of American foulbrood (AFB) and recent or ongoing metaphylactic antibiotic use were intensively sampled to detect spores of *Paenibacillus larvae* during the summer of 2019.

**Methods:** Samples of brood chamber honey, honey super honey, and brood chamber bees from individual hives within yards with and without clinical evidence of AFB, as well as pooled, yard-level honey samples from end-of-season extraction, were collected and cultured to detect and enumerate spores.

**Results:** All operations were different from one another with regard to both overall degree of spore contamination across yards and distribution of spores between clinically affected and unaffected yards. Spore concentrations in unaffected yards were significantly different from AFB affected yards in one of three operations. Only a few hives were responsible for the majority of spore contamination in any given yard across all operations. For individual hive samples, brood chamber honey was best for discriminating clinically affected yards from those unaffected ( $p = 0.001$ ), followed by honey super honey ( $p = 0.06$ ), and bees ( $p = 0.398$ ).

**Conclusions:** Honey super honey positively correlated with both brood chamber honey ( $r_s = 0.76$ ,  $p < 0.0001$ ) and bees ( $r_s = 0.50$ ,  $p < 0.0001$ ) and may be useful as a surrogate for either. Spore concentrations in pooled, extracted honey seem to have predictive potential for overall spore contamination within each operation and may have prognostic value in assessing the risk of future AFB outbreaks at the yard (or operation) level.

Sunday, October 31, 2021  
12:00 p.m. – 1:00 p.m. CDT

### **IMMUNOHISTOCHEMICAL CHARACTERIZATION OF THE IMMUNE RESPONSE IN CANINE CHRONIC HEPATITIS: IS THERE A CANINE IMMUNE-MEDIATED HEPATITIS?**

Brittany Rasche, John Cullen  
North Carolina State University, Raleigh, NC, USA

**Background:** Most canine chronic hepatitis is classified as idiopathic. An immune-mediated pathogenesis has been proposed but is not well characterized.

**Objective:** This study aims to characterize the lymphocytic infiltrate and aberrant hepatocellular MHC class II expression in putative immune-mediated hepatitis compared to copper-associated hepatitis and control dogs.

**Methods:** This retrospective study includes liver biopsies from 46 dogs, including controls (15 dogs without inflammation), putative immune-mediated hepatitis (16 dogs with lymphocytic hepatitis with parenchymal activity, individual cell death, and copper grade of < 3/5), and copper-associated hepatitis (15 dogs with copper grade  $\geq$  3/5). Immunohistochemistry for CD3, CD20, and MHC class II was applied, and the number of CD3+ and CD20+ lymphocytes was counted in 10 consecutive 40x fields.

**Results:** In all cases (including controls), there was a predominance of CD3+ over CD20+ lymphocytes. Using one-way ANOVA, there was a significant difference ( $p < 0.05$ ) between the three groups in the ratio of CD3+ to CD20+ lymphocytes. In 10/16 of the putative immune-mediated hepatitis cases and in 2/15 of the copper-associated hepatitis cases, this ratio was greater than that of the control population ( $> 6:1$ ). Some aberrant MHC class II expression in hepatocytes was present in a proportion of cases from both hepatitis groups but not the control group.

**Conclusion:** An overwhelming predominance of CD3+ lymphocytes in some cases of canine chronic hepatitis may support an immune-mediated pathogenesis. Aberrant MHC class II expression in hepatocytes can be seen with both copper-associated and putative immune-mediated chronic hepatitis in dogs.

### **Industrial and Toxicologic Pathology Focused Poster Session**

Tuesday, November 2, 2021 | 12:30 p.m. – 1:30 p.m. CDT

Tuesday, November 2, 2021  
12:30 p.m. – 1:30 p.m. CDT

### **CHARACTERIZATION OF RESISTANCE TO PRMT5 INHIBITOR THERAPY IN MANTLE CELL LYMPHOMA**

Mackenzie Long<sup>1,2</sup>, Shirsha Koirala<sup>2</sup>, Shelby Sloan<sup>2</sup>, Fiona Brown<sup>2</sup>, Kara Corps<sup>1</sup>, JoBeth Helmig-Mason<sup>2</sup>, Stacey Beck<sup>2</sup>, Ji-Hyun Chung<sup>2</sup>, Peggy Scherle<sup>3</sup>, Kris Vaddi<sup>3</sup>, Bradley Blaser<sup>2</sup>, Lapo Alinari<sup>2</sup>, Robert Baiocchi<sup>2</sup>

<sup>1</sup>The Ohio State University Department Of Veterinary Biosciences, Columbus, OH, USA, <sup>2</sup>The Ohio State University Wexner Medical Center, Columbus, OH, USA,

<sup>3</sup>Prelude Therapeutics, Wilmington, DE, USA

**Background:** Mantle cell lymphoma (MCL) is an incurable B-cell Non-Hodgkin's lymphoma. MCL patients who relapse on targeted therapies have a particularly poor

prognosis. Protein arginine methyltransferase 5 (PRMT5) is an enzyme that drives symmetric dimethylation of histone arginine residues, is overexpressed in MCL and drives growth and survival. PRMT5 has emerged as an attractive therapeutic target in MCL. In collaboration with Prelude Therapeutics, we developed a small molecule PRMT5 inhibitor (PRT-382) that exhibits significant anti-MCL activity in cell lines (low nM range) and *in vivo* preclinical MCL murine models (10 mg/kg). Despite the anti-tumor activity of PRMT5 inhibition, we have observed some animals develop drug resistance leading to rapid progression of MCL. Multiple MCL cell lines show primary resistance to PRMT5 inhibition based on high IC50s of PRT-382. Prolonged culture of PRT-382 sensitive MCL lines with drug escalation produced acquired drug resistance that persists after prolonged culture (30d) in the absence of drug. **Objective:** Based on previously reported literature and our preliminary findings, this project initially evaluated the role of compensatory methylation by other PRMTs in the setting of PRMT5 inhibitor resistant MCL. **Methods/Results:** The efficacy of a type I PRMT inhibitor on PRMT5 inhibitor resistant and sensitive MCL cell lines was determined. **Conclusions:** No significant differential sensitivity to the type I PRMT inhibitor was observed between PRMT5 inhibitor resistant and sensitive MCL cell lines. However, next generation sequencing technologies (scRNA-seq, RNA-seq, WES) has highlighted potential new targets for overcoming PRMT5 inhibitor resistance including PI3K signaling and MYC.

Tuesday, November 2, 2021

12:30 p.m. – 1:30 p.m. CDT

## **MECHANISMS OF THE COXIELLA BURNETII WHOLE CELL VACCINE REACTOGENIC RESPONSE**

Alycia Fratzke<sup>1,2</sup>, Erin Van Schaik<sup>2</sup>, James Samuel<sup>1,2</sup>

<sup>1</sup>Texas A&M University, College Station, TX, USA, <sup>2</sup>Texas A&M Health Science Center, Bryan, TX, USA

*Coxiella burnetii* is the causative agent of Q fever. The only approved vaccine for humans, Q-VAX, a formalin-inactivated, whole cell vaccine (WCV), is not licensed in the United States due to the high rate of local and systemic reactions in previously sensitized individuals. A greater understanding of the immunological mechanisms responsible for these reactions is needed to produce safe and effective vaccines against *C. burnetii*. Our prior work showed that vaccine site reactions caused by *C. burnetii* WCV contain an influx of IFN $\gamma$ + and IL17a+ CD4 T cells. To further elucidate the immunopathogenesis of these reactions, we began by investigating the roles of CD4 T cells in WCV reactogenicity. Antibody-mediated depletion of CD4 T cells, but not CD8 T cells, in sensitized mice abrogated Cb reactogenic lesions and adoptive transfer of CD4 T cells from sensitized mice to naïve mice partially reproduced reactive lesions upon elicitation with WCV. We then went on to evaluate the roles of IFN $\gamma$  and IL17a in vaccine site reactions using antibody-mediated depletion of these cytokines during elicitation of hypersensitivity responses. Our results show that local *C. burnetii* WCV reactogenicity is a Th1/Th17-mediated hypersensitivity reaction. Understanding the immunopathogenesis of vaccine reactogenicity provides information essential for the development safe and effective novel vaccines.



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## **DO FUNGICIDES INCREASE THE INCIDENCE OF EUROPEAN FOULBROOD DISEASE IN HONEY BEES DURING BLUEBERRY POLLINATION?**

Jenna Thebeau<sup>1</sup>, Dana Liebe<sup>1</sup>, Sarah Wood<sup>1</sup>, Allyssa Cloet<sup>1</sup>, Igor Moshynskyy<sup>1</sup>, Larhonda Sobchishin<sup>1</sup>, Ivanna Kozii<sup>1</sup>, Colby Klein<sup>1</sup>, Igor Medici de Mattos<sup>1</sup>, Michael Zabrodski<sup>1</sup>, Melanie Roulin<sup>1</sup>, Fatima Masood<sup>1</sup>, Brandele Brown<sup>1</sup>, Mateo Castano Ospina<sup>1</sup>, Lara Reitsma<sup>1</sup>, Jessica Debruyne<sup>1</sup>, Mohsen Sharafi<sup>1</sup>, Meghan Milbrath<sup>2</sup>, Geoff Wilson<sup>3</sup>, Marta Guarna<sup>4</sup>, Patricia Wolf Veiga<sup>5</sup>, Eric Gerbrandt<sup>6</sup>, Antonio Ruzzini<sup>1</sup>, Elemir Simko<sup>1</sup>

<sup>1</sup>Western College of Veterinary Medicine, Saskatoon, SK, Canada, <sup>2</sup>Michigan State University, East Lansing, MI, USA, <sup>3</sup>Government of Saskatchewan, Prince Albert, SK, Canada, <sup>4</sup>Agriculture and Agri-Food Canada, Beaverlodge, AB, Canada, <sup>5</sup>Grand Prairie Regional College, Beaverlodge, AB, Canada, <sup>6</sup>British Columbia Blueberry Council, Delta, BC, Canada

The United States and Canada are the world's largest blueberry producers, with the majority of their blueberry crop dependent on honey bee (*Apis mellifera*) pollination. Recently, beekeepers have reported an increased incidence of European foulbrood (EFB) disease in their colonies during blueberry pollination.

EFB is a bacterial disease of honey bee larvae caused by *Melissococcus plutonius* which results in heightened larval mortality when colonies experience environmental or nutritional stress. One such stress is pesticide exposure during blueberry pollination which may predispose these colonies to EFB disease. The effects of exposure to formulated blueberry fungicides on the susceptibility of honey bee larvae to EFB is currently unknown.

To investigate the possible correlation between fungicide use and EFB, we employed an *in vitro* model of larvae infected with *M. plutonius* and tested the effects of chronic exposure to field-relevant concentrations of two blueberry fungicides, Captan and Kenja, on larval survival.

We found that exposure to Captan, Kenja or a combination of these two products did not result in a significant decrease in larval survival relative to infected controls. Instead, chronic exposure to Captan or Kenja during development significantly increased larval survival from EFB by 33% ( $P < 0.01$ ).

These *in vitro* results suggest that chronic exposure of honey bee colonies to Captan or Kenja during blueberry pollination should not predispose these colonies to EFB, although colony-level studies are imperative. Further *in vitro* experiments are underway to test the effect of additional fungicides and their combinations on the susceptibility of honey bee larvae to EFB.

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### **ARE DETOXIFICATION ENZYMES INDUCED SIMILARLY ACROSS HONEY BEE CASTES IN RESPONSE TO NEONICOTINOID EXPOSURE?**

Ivanna Kozii, Sarah Wood, Colby Klein, Michael Zabrodski, Jenna Thebeau, Melanie Roulin, Dana Liebe, Igor de Mattos, Mohsen Sharafi, Larhonda Sobchishin, Igor Moshynskyy, Maud Ferrari, Elemir Simko  
University of Saskatchewan, Saskatoon, SK, Canada

Neonicotinoid insecticides are implicated in decreasing honey bee health. Toxicity assays in honey bees focus on the worker caste, possibly overlooking toxicity to the queens and drones. Our previous study demonstrated that neonicotinoid toxicity is highly caste-specific. The objective of this study was to determine if there is a correlation between detoxification enzyme activity and the caste-specific susceptibility of honey bees to a commonly used neonicotinoid, thiamethoxam (THI), in response to larval, adult or combined larval and adult exposure.

We tested the induction of enzyme activity in bees in response to larval and/or adult THI exposure. Age-matched honey bee larvae received either water (control) or 25ng THI through larval food contamination. At emergence, adult bees in these groups were further subjected to contact exposure of incremental doses of THI. Activity of esterase, glutathione S-transferase (GST), and acetylcholine esterase (AChEst) were determined at emergence and 48 hours post contact exposure.

We found that enzyme activity is highly caste-specific; however, THI exposure during larval and/or adult stages did not have a significant effect on enzymes activity.

Enzyme activity was highest in worker bees, followed by drones, and then queens.

Enzyme activity of esterase, GST and AChEst does not correlate with the differential caste survival in response to THI exposure observed in our previous studies. However, our findings highlight that enzyme activity is highly variable between castes, which may affect their susceptibility to insecticide toxicity and should be considered in future toxicity studies.

Tuesday, November 2, 2021  
12:30 p.m. – 1:30 p.m. CDT

### **THIAMETHOXAM TOXICITY IS DEPENDENT ON THE AGE AND CASTE OF HONEY BEES (APIS MELLIFERA)**

Ivanna Kozii, Sarah Barnsley, Marina Bezerra, Sarah Wood, Colby Klein, Igor de Mattos, Michael Zabrodski, Jenna Thebeau, Roney Silva, Claudia Fabela, Ihor Dvylyuk, Maud Ferrari, Elemir Simko  
University of Saskatchewan, Saskatoon, SK, Canada

The honey bees are essential pollinators and their health is of global concern. Neonicotinoid insecticides have been implicated in the decreasing honey bee health worldwide. Regulatory toxicity assays focus predominantly on the worker honey bee

(sterile females) toxicity, thus potentially overlooking the toxicities present in the queens and drones (reproductively active females and males respectively).

The objective of this study was to compare thiamethoxam (THI) toxicity, a commonly used neonicotinoid, between honey bee castes at different ages (larvae, newly emerged, young adult, and mature adult).

First, a single colony was manipulated to produce synchronized larvae and adult honey bees. The larvae were subjected to individual dietary exposure while adult bees of three age categories were subjected to individual contact exposure to control or incremental doses of THI.

Larval toxicity tests revealed honey bee queens to be most sensitive to THI exposure evidenced by over 60% decrease in emergence rate of queens compared to drones and workers post THI exposure. Conversely, adult queen survival was consistently higher or comparable to that of worker bees; queen survival was highest in newly emerged individuals. The drones were most sensitive to THI contact exposure.

Accordingly, the results of our study highlight that THI toxicity in honey bees is highly caste and age specific. Considering the wide contamination of the hive products with agrochemicals we suggest that the age and caste specific toxicity assays should be employed in future toxicological screening tests to detect most vulnerable caste and age of the bees.

Tuesday, November 2, 2021

12:30 p.m. – 1:30 p.m. CDT

### **ASSESSMENT OF GENOTOXIC DNA DAMAGE IN DOGS FROM A HIGH POLLUTED AREA IN ITALY**

Davide De Biase, Valeria Baldassarre, Giuseppe Piegari, Ilaria d'Aquino, Francesco Prisco, Serenella Papparella, Orlando Paciello  
Department of Veterinary Medicine and animal production, University of Naples "Federico II", Naples, Italy

In the last decades, many concerns have raised about the adverse effects of environmental contaminants on human population. Dogs represent a warning sentinel for human health because they share the human environment and respond to many toxic insults in ways analogous to humans. The aim of this work was to standardize and validate methods to monitor environmental damage through the evaluation of biological markers such as genotoxic DNA damage by Comet assay and Micronuclei test and the immunocytochemical expression of iNOS as a marker for oxidative stress. We conducted a cross-sectional study employing exposed dogs living in a shelter in a high polluted area in Campania region (Italy). The study was conducted with the approval of the Ethics Committee of the University of Naples (PG/2021/0030881). The study population included 15 clinically healthy dogs, aged 4 to 10 years old, randomly sampled, with a minimum two-year presence in the shelter. The control group consisted of 5 healthy dogs living in a less polluted nearby area. Blood samples were collected for Comet assay and immunocytochemistry for iNOS evaluation. Epithelial buccal cells

were collected for micronuclei count. The results were compared with environmental pollution data. The expression of iNOS and genotoxic DNA damage were significantly higher in dogs from polluted area compared to controls. Our data show that dogs living in highly polluted areas have a higher risk to develop genotoxic DNA damage. Moreover, we suggest that iNOS expression, comet assay and micronuclei test may be reliable tool to assess environmental-related pathology.

Tuesday, November 2, 2021

12:30 p.m. – 1:30 p.m. CDT

### **CANCER TREATMENT INDUCED COGNITIVE IMPAIRMENT AND NEUROINFLAMMATION IN MICE**

Kimberly Demos-Davies, Allison Rogich, Jessica Lawrence, Davis Seelig  
University of Minnesota, Saint Paul, MN, USA

**Background:** Cancer survivors are increasingly identified with a syndrome of neurocognitive dysfunction termed cancer-related cognitive impairment (CRCI). Side effects of cancer therapy (chemotherapy and radiation therapy) have been implicated in causing CRCI. CRCI has been best documented in breast cancer survivors. The exact mechanism for the distant brain injury following cancer treatment is unknown.

**Objective:** To investigate the underlying mechanisms by which doxorubicin, an anthracycline that is standard of care in breast carcinoma treatment, and/or extracranial radiation trigger clinical and neuroinflammatory effects of CRCI.

**Methods:** Eight to ten week old SKH1 mice were administered a battery of behavior tests before treatment and 7 days post-treatment. Mice were treated with a single dose of radiation to their right hindlimb, an intraperitoneal injection of doxorubicin or both. After euthanasia, plasma, spleen and brains were collected for cytokine and immune cell analysis by flow cytometry and immunohistochemistry for GFAP and Iba1.

**Results/Conclusion:** Mice treated with doxorubicin, radiation or both showed equivalent hippocampal dependent memory deficits compared to control mice in the novel location recognition behavior test. Neuroinflammation was evident in the brains in all three mice treatment groups compared to control mice. Mice treated with doxorubicin had a significant increase in IL-6 plasma levels and changes in splenic lymphocyte percentages compared to controls. In conclusion, both standard of care therapies used in breast cancer patients cause neuroinflammation and cognitive changes in mice. This is the first study to report that mice treated with extracranial radiation, chemotherapy or both have comparable cognitive impairment.

Tuesday, November 2, 2021

12:30 p.m. – 1:30 p.m. CDT

### **MODELING HUMAN PROSTATE CANCER METASTASIS IN MICE VIA RESECTION OF SUBCUTANEOUS ALLOGRAFTS**

Lauren Peiffer, Angelo De Marzo, Karen Sfanos, Janielle Maynard  
Johns Hopkins University School of Medicine, Baltimore, MD, USA

**Background:** More than 80% of patients with advanced prostate cancer develop bone metastases, however, bone metastases rarely occur spontaneously in murine prostate cancer models. Most prostate cancer metastatic models involve intracardiac or intraosseous implantation of cancer cells, which bypass early stages of tumor invasion and metastasis.

**Objective:** Perform a pilot study to determine whether resection of subcutaneous allografts in immunocompetent mice results in spontaneous metastasis.

**Methods:** Three-month-old intact male FVB/NCrl mice (n=9) were inoculated subcutaneously with MyC-CaP cells. Tumors were surgically resected once they reached 1.5cm in any direction. After recovery, mice were monitored three times weekly for tumor recurrence. Animals were euthanized or died, and a full set of tissues were collected and fixed in 10% neutral buffered formalin for histopathologic examination, immunohistochemistry, and RNA in-situ hybridization.

**Results:** Tumors took an average of 44 days (range 23-61) to reach 1.5cm in any direction. All tumors were resectable. One mouse was euthanized due to multifocal dermatitis of unknown cause. The eight remaining mice were euthanized or died an average of 113 days (range 72-156) after tumor inoculation and 70 days (range 30-121) after tumor resection. Six mice developed tumor recurrence at the site of resection by 121 days post-resection and one mouse developed bone metastases positive for androgen receptor and human c-Myc. One mouse developed suspected metastases to the abdominal cavity (confirmatory stains pending).

**Conclusions:** Resection of subcutaneous allografts in mice extends time of tumor growth and results in tumor recurrence with metastases to bone and potentially the abdominal cavity.

### **Highlighted Posters: Clinical Pathology**

Saturday, October 30, 2021 | 4:30 p.m. – 4:33 p.m. CDT

Saturday, October 30, 2021

4:30 p.m. – 4:33 p.m. CDT

#### **CALCIUM AND PHOSPHORUS HOMEOSTASIS IN ADVANCED STAGES OF CANINE LEISHMANIASIS**

MARIO ALBERTO GONZÁLEZ, RAFAEL BARRERA, MARÍA GIL-MOLINO, BEATRIZ MACÍAS, JOSÉ IGNACIO CRISTÓBAL, PALOMA NICOLÁS-BARCELÓ, ÁNGELA DURÁN, ANA BELÉN GARCÍA, FRANCISCO JAVIER DUQUE

Departamento Medicina Animal, Universidad de Extremadura, Cáceres, Spain

**Background:** Chronic kidney disease (CKD) secondary to canine leishmaniasis (CL) is frequently associated with mineral and bone disorders (MBD). In CL, information about that is very scarce. Correct knowledge of calcium and phosphorus homeostasis is essential to improve the management of advanced CKD. Our objectives were: 1) To assess CKD-MBD in patients with advanced stages of LC, and 2) To evaluate the diagnostic utility of biomarkers analyzed.

**Methods:** Sixteen dogs classified in Leishvet (n(III)=7; n(IV)=9) were compared with control group (n(CG)=6). Hematology, biochemistry with plasmatic total calcium (PtCa) and phosphorus (PPho), and urinalysis were performed. CL was diagnosed by ELISA. Parathyroid hormone (PTH) (Human, Abcam®, UK) was determined using a sandwich ELISA kit; fibroblast growth factor-23 (FGF23) and osteocalcin (OC) were analyzed using the same method (Canine, Mybiosource®, U.S.A.). 25-hydroxyvitamin-D (VD) was measured using electrochemiluminescence immunoassay (Human, 800 Cobas® Roche Diagnostics, Switzerland). One-way ANOVA was used to compare CL groups (III and IV) with CG. A p-value of <0.05 was considered statistically significant.

**Results:** PPho (mg/dL) showed differences between CG and IV, (GC=4.2±0.7;IV=10.1±5.5); FGF23 (pg/mL) had differences between CG and IV (10478,6±9937,7 and 148,6±134,1, respectively); finally, for VD (ng/mL), III and IV presented differences compare with CG (CG=39,5±9,8;III=15,5±9,6;IV=9,6±3,4).

**Conclusions:** III and IV groups presented marked hypovitaminosis D. Marked phosphorus disorders were presented in IV group, exactly hyperphosphatemia and increased levels of FGF23. These biomarkers present a great potential for diagnosis and management of MBD in advanced stages of CL.

Saturday, October 30, 2021

4:33 p.m. – 4:36 p.m. CDT

### **SYSMEX XT-2000iV AND PROCYTE DX SCATTERGRAM FINDINGS IN A DOG WITH MASTOCYTEMIA**

Jelena Palic, Annabelle Heier, Els Acke

Vet Med Labor GmbH Division of IDEXX Laboratories, Kornwestheim, Germany

**Background:** Complete blood count (CBC) with morphologic evaluation was performed in the assessment of a 10-year-old female Golden Retriever with history of completely removed low-grade complex mammary carcinoma two months prior. In-house ProCyte Dx automated counts revealed moderate non-regenerative anemia, moderate eosinophilia and mild monocytosis.

**Objective:** To describe scattergrams from ProCyte Dx and Sysmex XT-2000iV with blood smear findings in a dog with mastocytomia.

**Methods:** CBC analysis performed on ProCyte Dx and Sysmex XT-2000iV, followed by blood smear evaluation.

**Results:** ProCyte Dx WBC scattergram showed the second cloud parallel and to the right from the monocyte dot plot location. Cells in that cloud were classified as either monocytes or neutrophils with no clear separation. On Sysmex XT-2000iV DIFF scattergram, neutrophil and eosinophil dot plots were present at the respective locations, appeared separated but could not be differentiated. Lymphocyte and monocyte dot plots were abnormal. In addition to the normal lymphocyte dot plot location, the second cloud of cells classified as lymphocytes was displayed to the right of the monocyte dot plot area. Cluster was also present in basophil dot plot area. Blood smear assessment detected mastocytemia with 16% degranulated mast cells. They had round nuclei with finely stippled chromatin, moderate amount of cytoplasm with numerous small uniform vacuoles and rare small magenta granules.

**Conclusion:** This is the first description of abnormal scattergrams from ProCyte Dx and Sysmex XT-2000iV correlated with blood smear examination from a dog with mastocytemia. In cases with abnormalities detected on scattergrams, blood smear examination is essential.

Saturday, October 30, 2021

4:36 p.m. – 4:39 p.m. CDT

## **HEPATOCTE DERIVED CFA-MICRORNAS AS SERUM BIOMARKERS OF HEPATIC FIBROSIS OR CIRRHOSIS IN DOGS**

Ahmed El-Sebaey<sup>1,2</sup>, Pavel Abramov<sup>1</sup>, Natalia Slesarenko<sup>1</sup>, Seidfatima Borunova<sup>1</sup>, Sergey Pozyabin<sup>1</sup>

<sup>1</sup>Moscow State Academy of Veterinary Medicine and Biotechnology – MVA by K. I., Skryabin, house 23, Academician Scriabin str., Moscow, Russian Federation, <sup>2</sup>Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt

**Background:** Liver fibrosis or cirrhosis are major complications of prolonged chronic hepatitis in which most dogs remain asymptomatic until progressive fatal liver failure or hepatocellular carcinoma (HCC) is developed. **Objective:** The goal of this prospective study was to evaluate the possible use of hepatocyte-derived *Canine familiaris* (cfa)-miRNAs-122, -21, 34a, 126, and -200c as accurate serum biomarkers to diagnose the early onset of fibrotic and cirrhotic complications in dogs diagnosed with chronic liver damage. **Methods:** Based on ultrasonographic, computed tomographic and histopathological findings, the relative expression of selected cfa-miRNAs was estimated in 60 serum samples [(20 healthy controls, 20 with liver fibrosis, 20 with cirrhosis] using quantitative RT-PCR and Qiagen® PCR kit. **Results:** Cfa-miRNA-122 and -21 were upregulated in dogs with hepatic fibrosis at significance levels of  $P < 0.01$  and  $P < 0.001$ , respectively compared to cirrhosis and control groups and at an area under the curve (AUC) of 0.96 and 0.99, respectively exhibited potential roles in distinguishing dogs with fibrosis from the control. Cfa-miRNA-200c was significantly ( $P < 0.01$ ) expressed only in dogs with cirrhosis compared to the fibrosis and control groups and at AUC of 0.87 showed high diagnostic performance in differentiating dogs with cirrhosis from the healthy group. **Conclusions:** Cfa-miRNA-122 and -21 are reliable

biomarkers for diagnosis of liver fibrosis while cfa-miRNA-200c is a diagnostic biomarker of cirrhosis, particularly at early stages which could help the clinician for early prediction or prevention of hepatic fibrosis leaden to end-stage cirrhosis-associated HCC in dogs.

Keywords: dogs, fibrosis, cirrhosis, RT-PCR, AUC, cfa- miRNAs.

Saturday, October 30, 2021

4:39 p.m. – 4:42 p.m. CDT

### **COMPARISON BETWEEN RBC VARIABLES IN CATS ACCORING TO FIV/FELV INFECTION STATUS**

Laura Victoria Contreras, Fernanda Andreola, Vanessa Eder, Ana Paula Borenstein, Kauana Kaefer, Taynara Fogaça, Lina Bilhalva, Bruno Almeida, Stella Valle  
Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

**Background:** Automated analyzers are widely used as the first source of information regarding CBC in clinics and hospitals. Leukocytes variables on FIV or FeLV infection have been described; however, there is less information about RBC variables.

**Objective:** To explore the difference between RBC variables of cats tested for FIV and FeLV using a rapid test (Snap FIV/FeLV combo test, IDEXX) in a veterinary teaching hospital.

**Methods:** Results of Snap tests and automated CBC (ProCyte Dx, IDEXX) from cats tested for FIV and FeLV from September of 2018 to June of 2021 were retrospectively evaluated. Data included records from 584 cats and RBC, HCT, HGB, MCV, MCH, MCHC, RDW, %RETIC, Reticulocyte count (RETIC) and reticulocyte hemoglobin (RETIC-HB) were considered. CBCs performed more than five days before or after the retroviral test were excluded. Cats were divided into four groups: Negative (G1), positive for FIV (G2), positive for FeLV (G3) and positive for both (G4). Kruskal-Wallis test was used to compare the distribution of variables between groups.

**Results:** Groups were composed as: G1: 448 cats (76.8%), G2: 30 (5.1%), G3: 94 (16.1%) and G4: 12 (2%). A difference was observed between G1-G2 and G3-G4 in RBC, PCV and HCT. MCHC was different between G1 vs G3-G4. MCV, MCH and RETIC-HB were different between G3 vs G1-G2.

**Conclusion:** This study provides information on RBC parameters on a wide population, which contribute to understand the link between retroviral infections and certain RBC alterations, and the potential use of these parameters as indicators of disease.

Saturday, October 30, 2021

4:45 p.m. – 4:48 p.m. CDT

### **NASAL COLONIZATION BY ENTAMOEBIA GINGIVALIS IN A 13-YEAR-OLD ITALIAN GREYHOUND**

Nina Kristen Randolph<sup>1</sup>, Camille McAloney<sup>1</sup>, Robert Ossiboff<sup>2</sup>, Matthew Cook<sup>1</sup>, Betsy Hernandez<sup>1</sup>, Jessica Hokamp<sup>1</sup>



<sup>1</sup>The Ohio State University, Columbus, OH, USA, <sup>2</sup>University of Florida, Gainesville, FL, USA

A 13-year-old 4.6 kg castrated male Italian Greyhound was presented for evaluation of bilateral epistaxis and mucoid nasal discharge of approximately 3 months duration. At presentation, the patient's CBC was unremarkable. His biochemical profile revealed mild azotemia as well as mild electrolyte abnormalities. Severe periodontal disease was noted on physical exam. Rhinoscopy revealed a tan plaque present in the left dorsal meatus of the nasal cavity. Vigorous flushing yielded granular white particulates suspended in the saline, which were cytologically examined. Marked neutrophilic inflammation, a diverse bacterial population, and organisms consistent with amoeba were identified. The remaining fluid was analyzed by PCR identifying *Entamoeba gingivalis*. The dog's clinical signs improved dramatically after initiating treatment with clindamycin. 6 weeks after diagnosis a dental cleaning with multiple extractions was performed. Four weeks after the dental procedure the dog was clinically normal. This report describes the clinical, hematologic, cytologic, and gross findings in a case of *Entamoeba gingivalis* infection in a dog.

Saturday, October 30, 2021

4:51 p.m. – 4:54 p.m. CDT

### **MELAMINE-CYANURIC-LIKE CRYSTALLURIA IN A DOG**

David Rotstein<sup>1,2</sup>, Sarah Peloquin<sup>2</sup>, Renate Reimschuessel<sup>2</sup>, Jake Guag<sup>2</sup>, Kathleen Proia<sup>2</sup>, Betsy Yakes<sup>3</sup>

<sup>1</sup>FDA Center for Veterinary Medicine, Rockville, MD, USA, <sup>2</sup>FDA Center for Veterinary Medicine Veterinary Laboratory Investigation and Response Network, Laurel, MD, USA,

<sup>3</sup>FDA Center for Food Safety and Nutrition, College Park, MD, USA

**Background:** FDA's Center for Veterinary Medicine (CVM), Veterinary Laboratory Investigation and Response Network (Vet-LIRN) initiated an investigation reported by a Vet-LIRN member lab about suspected melamine crystalluria in an 8.5-year-old FS Pit Bull Mix. The dog had intermittent urinary incontinence. Crystals had a golden brown "wagon wheel" spherulite appearance and were observed on two subsequent urine sediments. Vet-LIRN was contacted to evaluate crystal composition for melamine-cyanuric acid.

**Objective:** To investigate the crystal composition with traditional methods and Raman microspectroscopy, perform genetic testing, and determine possible dietary relationships.

**Methods:** Vet-LIRN investigation involved medical record review, exposure interview, serum chemistry analysis including uric acid and bile acids, urine uric acid: creatinine ratio, urine sediment formalin exposure microscopy and Raman spectroscopy, and genetic testing for hyperuricosuria (SLC2A9 mutation).

**Results:** Two types of crystals were detected with Raman spectroscopy – uric acid and sulfate. Urine sediment crystals dissolved within three minutes upon formalin exposure. Creatinine, BUN, uric acid, and bile acids were within reference ranges. The urine uric acid: creatinine ratio was 0.8 (ref. <0.5). The dog had the HU/HU genotype for hyperuricosuria.

**Conclusions:** The dog had a SLC2A9 mutation leading to hyperuricosuria; the dog food was unrelated. This mutation has been reported in Dalmatians, Pitbull Terrier, and American Staffordshire. Hyperuricosuria can result in formation of uric acid crystals which appear similar to melamine-cyanuric acid crystals. While spectroscopy was diagnostic, formalin treatment of a slide of urine sediment provides a simple and accessible method for ruling out melamine-cyanuric crystalluria.

Saturday, October 30, 2021

4:54 p.m. – 4:57 p.m. CDT

**PRECURSOR-DIRECTED IMMUNE-MEDIATED ANEMIA IN A CAT WITH PHAGOCYTOSED NUCLEAR MATERIAL IN CIRCULATING NEUTROPHILS**

Nina Randolph, Camille McAloney, Sam Evans

The Ohio State University College of Veterinary Medicine Department of Veterinary Biosciences, Columbus, OH, USA

Precursor-directed immune-mediated anemia (PIMA) is an autoimmune disorder in which erythroid hematopoietic cells are targeted and destroyed, resulting in a non-regenerative anemia. It can pose a diagnostic challenge, as the CBC findings are quite different from the classic presentation of immune-mediated hemolytic anemia (IMHA), which targets mature red blood cells. Although it is uncommon, PIMA is most frequently seen in dogs and rarely observed in cats. To the authors' knowledge, this is the first report of PIMA in a cat with phagocytosed nuclear material identified within neutrophils in peripheral blood. A 6 year old neutered male domestic short hair cat was presented for decreased appetite and lethargy; the referring veterinarian had noted icterus and elevated liver enzymes and referred to The Ohio State University's Veterinary Medical Center. The cat's CBC showed marked non-regenerative, normocytic, normochromic, anemia, marked thrombocytopenia, and mild leukocytosis characterized by mild mature neutrophilia (rare cytoplasmic basophilia consistent with toxic change) and mild monocytosis. Blood smear evaluation showed occasional round, basophilic inclusions in the neutrophils ("ragocyte"-like morphology) that were positive for DAPI staining, consistent with nuclear material. Neutrophils that displayed erythrophagia were also observed in peripheral blood. Bone marrow aspirates revealed marked erythroid hyperplasia with left shift and evidence of phagocytosis of erythroid precursors by macrophages. Histopathology of bone marrow core biopsy was supportive of a diagnosis of PIMA, and anemia and thrombocytopenia resolved with immunosuppressive therapy. These findings are consistent with a unique presentation of PIMA in a cat.

## Highlighted Posters: Diagnostic Pathology and Education

Saturday, October 30, 2021 | 4:30 p.m. – 4:33 p.m. CDT

Saturday, October 30, 2021

4:30 p.m. – 4:33 p.m. CDT

### **CERVICAL COMPRESSIVE MYELOPATHY SECONDARY TO GUDAIR VACCINE ASSOCIATED GRANULOMAS IN SHEEP**

Leah Manning<sup>1</sup>, Katie Eager<sup>1</sup>, Brendon O'Rourke<sup>1</sup>, Eve Hall<sup>2</sup>, Keith Walker<sup>1</sup>, Erika Bunker<sup>1</sup>, Angel Ngo<sup>1</sup>, Zoe Spiers<sup>1</sup>, Mukesh Srivastava<sup>1</sup>, Pedro Pinczowski<sup>1</sup>

<sup>1</sup>Elizabeth Macarthur Agricultural Institute, Department of Primary Industries, Menangle, NSW, Australia, <sup>2</sup>Murray Local Land Services, Holbrook, NSW, Australia

A commercial and stud sheep property in the Riverina region of New South Wales, Australia had a three-year history of gradually progressive neurological signs affecting approximately 1% of six to 12-month-old sheep. Animals had a wide-based stance, hindlimb weakness and ataxia, occasional forelimb knuckling and recumbency. Histopathology revealed Wallerian degeneration of the spinal cord of variable severity. Based on these findings, 'Cooma ataxia', a likely inherited degenerative thoracic myelopathy reported in sheep in NSW, was initially suspected. Subsequent necropsy on six affected sheep found all animals to have multifocal to coalescing, soft to firm, pale brown, variably demarcated masses within the deep cervical musculature, extending within the spinal canal causing marked compression of the cranial cervical spinal cord. Histologically these masses were granulomas with variable mineralisation and rare lipid vacuoles containing small numbers of acid-fast bacteria. There was marked Wallerian degeneration, most pronounced in the cranial cervical spinal cord. Aerobic cultures of the granulomas yielded no significant findings. A High Throughput Johnes (HTJ) PCR of granulomas from 2 animals was positive for *Mycobacterium avium* subspecies *paratuberculosis*. The HTJ PCR was also positive for cervical spinal cord from 5 of 6 affected animals, and negative for spinal cord from both vaccinated and unvaccinated control animals. It was concluded that the granulomas were due to improper vaccination technique of the Gudair vaccine, used for management of ovine Johne's disease. No clinically affected animals have been reported since changes to the vaccine procedure were implemented.

Saturday, October 30, 2021

4:33 p.m. – 4:36 p.m. CDT

### **ABERRANT (ECTOPIC) CD3 EXPRESSION IN A FELINE EXTRAMEDULLARY CUTANEOUS PLASMACYTOMA**

Michael Childress<sup>1</sup>, Caitlin Brown<sup>1</sup>, Tsang Lin<sup>1</sup>, Emily Hartman<sup>1</sup>, Peter Moore<sup>2</sup>, José Ramos-Vara<sup>1</sup>

<sup>1</sup>Purdue University, West Lafayette, IN, USA, <sup>2</sup>University of California Davis, Davis, CA, USA

Aberrant CD3 lineage expression has been reported in humans and rarely in dogs in a variety of B-cell lymphomas and plasmacytomas. We report a case of feline extramedullary plasmacytoma (FEP) with aberrant CD3 expression. A 14-year-old, neutered, Domestic Shorthair cat developed a subcutaneous mass on the abdomen.

The original punch biopsy specimens had a neoplastic proliferation of discrete round cells with variable amount of eosinophilic to amphophilic cytoplasm and slightly eccentric round nucleus with nucleolus. Rare binucleated cells were present. Mitotic count was 2-14 per 400x field (0.237 mm<sup>2</sup>). The differential diagnosis included extramedullary plasmacytoma (EP) with lymphoma or histiocytic sarcoma considered less likely. Immunohistochemistry for MUM 1 was positive in the majority of neoplastic cells (nuclear), whereas Iba 1 and Pax 5 were negative. A month later, and based on cytologic features and CD3 positivity, an excisional biopsy of this mass was classified as a T-cell lymphoma. The neoplastic cells also expressed MUM 1. Molecular clonality analysis of IgH2 and IgH3 (B cell) yielded clonal rearrangements; analysis of TRG (T cell) revealed a somewhat reduced repertoire, but ultimately polyclonal rearrangements in both biopsy specimens. Based on the histopathological, immunohistochemical, and molecular findings, this tumor was diagnosed as a plasmacytoma with aberrant expression of CD3. To our knowledge, this is the first report of FEP with aberrant (ectopic) CD3 expression.

Saturday, October 30, 2021

4:36 p.m. – 4:39 p.m. CDT

### **PERFORMANCE OF A BLOOD-BASED 'LIQUID BIOPSY' MULTI-CANCER EARLY DETECTION (MCED) TEST IN SEVEN COMMON CANINE CANCERS**

Andi Flory, Jason Chibuk, Lauren Holtvoigt, Katherine Lytle, Dana Tsui, Ilya Chorny, Jill Rafalko, Daniel Grosu, Kristina Kruglyak

PetDx, La Jolla, CA, USA

**Background/Objective:** Certain types of cancer are more common in dogs and account for the majority of cancer mortality in the species, notably: lymphoma, hemangiosarcoma, soft-tissue sarcoma, mast cell tumor, osteosarcoma, mammary gland carcinoma, and malignant melanoma. Recently, a blood-based multi-cancer early detection (MCED) test using next-generation sequencing was developed for use in dogs, and its performance for the detection of these seven common cancers was evaluated.

**Methods:** Blood samples from an all-comers cohort of 191 cancer-diagnosed dogs and 188 presumably cancer-free dogs were subjected to DNA extraction, proprietary library preparation, and next-generation sequencing. Sequencing data were analyzed using an internally developed bioinformatics pipeline to detect genomic alterations associated with the presence of cancer. The testing laboratory was blinded to the cancer status and type of cancer in these patients until after test results were issued.

**Results:** In 191 cancer-diagnosed subjects, the seven 'common' cancer types noted above accounted for 60% of cases (n=114). The test's overall detection rate across these cancers was 63% (72 of 114). Of 188 presumably cancer-free dogs, 2 were excluded after a cancer diagnosis and 6 were putative false positives, corresponding to a minimum specificity of 97%.

**Conclusions:** Genomic testing solutions being developed for canine cancer screening should enable detection of frequently encountered and clinically relevant malignancies.

A novel blood-based MCED test has demonstrated the ability to detect some of the most common canine cancers with high sensitivity and specificity. This test has several potential applications, including use as a routine screening tool for common malignancies.

Saturday, October 30, 2021

4:39 p.m. – 4:42 p.m. CDT

### **RETROSPECTIVE ANALYSIS OF REPRODUCTIVE LESIONS IN REPTILES**

Kelsie Dougherty, Brigid Troan, Greg Lewbart

North Carolina State University College of Veterinary Medicine, Raleigh, NC, USA

Reptiles including crested geckos (*Correlophus ciliates*), leopard geckos (*Eublepharis macularius*), and several species of turtles and snakes are commonly utilized in the pet trade. Increasing demands for exotic pets encourage captive breeding, which is fairly successful in some species such as the crested gecko. Captive breeding encourages better trade practices, including improved biosecurity and reduced capture demands from the wild. Understanding the reproductive health of captive reptiles is essential for captive breeding programs and to encourage best practices in caring for them. A retrospective analysis of reproductive lesions over the past 10 years in all reptiles seen through the North Carolina State University Diagnostic Laboratory Service was conducted. 48 cases were examined (37 snakes, 1 tortoise, 1 frilled lizard, and 11 geckos) with several lesion types represented including bacterial salpingitis and oophoritis (7/48), egg yolk coelomitis (4/48), neoplasia (10/48), and oviductal torsion (1/48). Granulomatous lesions were the most uncommon lesion found (2/48). The lesions represented were not necessarily the cause of death in animals examined, but were often deemed co-morbidities. During this investigation, it was found characterizing neoplasia in reptiles, especially in reproductive tissues, can be difficult and should be further explored to better understand their behavior. In conclusion, the most common reproductive lesion found in reptiles was neoplasia (primary or metastatic), followed closely by bacterial salpingitis and oophoritis, and reproductive cysts (ovarian and testicular).

Saturday, October 30, 2021

4:42 p.m. – 4:45 p.m. CDT

### **PAUCIBACILLARY MYCOBACTERIOSIS IN RELATED CAPTIVE RED SISKINS (*CARDUELIS CUCULLATA*)**

Brittany Beavis<sup>1</sup>, Andrew Cartoceti<sup>2</sup>, Steven Kubiski<sup>3</sup>, Jason Thornton<sup>1</sup>, Elise LaDouceur<sup>1</sup>

<sup>1</sup>Joint Pathology Center, Silver Spring, MD, USA, <sup>2</sup>Smithsonian National Zoo, Washington, DC, USA, <sup>3</sup>San Diego Zoo, San Diego, CA, USA

**Background:** Red siskins (*Carduelis cucullata*) are a species of finch in the Fringillidae family of Passeriformes. From 2016 to 2020, four related individuals within a captive breeding flock died or were euthanized and had histologic evidence of multi-systemic histiocytic disease.

**Methods:** Tissue samples were fixed in 10% neutral buffered formalin, processed routinely for histology, and stained with hematoxylin and eosin, Giemsa, Ziehl-Neelsen, and Fite-Faraco stains. Polymerase chain reaction (PCR) was performed on paraffin embedded tissue scrolls from all cases using degenerate *Mycobacterium* spp. primers.

**Results:** Histiocytic inflammation was present in coelomic membranes (4/4 cases), liver (4/4 cases), lungs (4/4 cases), heart (2/4 cases), dermis (2/4 cases), large vessels (1/4 cases), kidneys (1/4 cases), proventriculus (1/4 cases), skeletal muscle (1/4 cases), sinuses/nasal mucosa (1/4 cases), bone marrow (1/4 cases), adrenal gland (1/3 cases), and spleen (1/3 cases). Histiocytic inflammation was mild to moderate, except in the coelomic membranes of case Nos. 1 and 2, and in the heart and spleen of case 3, in which inflammation was severe. Acid-fast staining identified rare, intrahistiocytic acid-fast bacilli in all cases. PCR amplified mycobacterial DNA in only one case (19-0213) and had 85.4% similarity to *M. florentium*.

**Conclusion:** Paucibacillary mycobacteriosis has not been reported in passerines, which typically present with multibacillary infections. Coelomic membranes were frequently the most severely inflamed anatomic location. PCR could not definitively identify the causative mycobacterial species, likely due to low numbers of organisms present in these samples.

Saturday, October 30, 2021

4:45 p.m. – 4:48 p.m. CDT

## **ACUTE CEREBRAL TOXOPLASMOSIS IN A 2-YEAR-OLD QUARTER HORSE**

Andrew Oates, Terry Spraker

Colorado State University Veterinary Diagnostic Laboratory, Fort Collins, CO, USA

### **Background:**

A 2-year-old quarter horse mare was submitted for necropsy to the Colorado State University Veterinary Diagnostic Laboratory with a 5-day history of progressive neurologic signs including head pressing, right-sided head tilt, circling to the right, and lack of menace response. On gross examination of the brain, both the right and left lateral ventricles were filled and expanded by well-demarcated, pale green to tan masses. These were ~3 cm in diameter and gelatinous to firm with gritty mineralized foci.

### **Methods:**

The brain was immersion fixed in 10% neutral buffered formalin for >72 hours. Sections of the masses were trimmed and routinely processed to produce slides stained with hematoxylin and eosin (H&E), Brown-Hopps modified gram, Grocott's Methenamine Silver (GMS), acid fast, Luna, and Periodic acid Schiff (PAS). Immunohistochemistry for *Toxoplasma gondii* and *Neospora* were performed by the Animal Health Diagnostic Center at Cornell University, College of Veterinary Medicine.

### **Results:**

Histopathology of the lesion demonstrated severe, regionally extensive, necrotizing and granulomatous encephalitis with many intralesional tachyzoites. These organisms stained negative with gram, GMS, and acid fast; and stained positive with PAS, Luna, and with immunohistochemistry for *Toxoplasma gondii* antigen.

### **Conclusion:**

This case represents a rare instance of fulminant equine cerebral toxoplasmosis with intralesional tachyzoites.

Saturday, October 30, 2021

4:48 p.m. – 4:51 p.m. CDT

### **RESPIRATORY CRYPTOCOCCOSIS IN AN UMBRELLA COCKATOO (*CACATUA ALBA*)**

Camille Cordero-Aponte, Denae LoBato

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

A 45-year-old female intact Umbrella Cockatoo (*Cacatua alba*) died after presenting for respiratory distress and was submitted for necropsy to The University of Tennessee College of Veterinary Medicine. Gross post-mortem findings revealed bilateral translucent mucoid material within the oral cavity, and multiple soft, pale yellow, translucent, myxomatous nodules expanding the air sacs and lungs. Microscopically, these nodules consisted of extracellular round to oval yeasts with a large clear capsule morphologically consistent with *Cryptococcus* spp., with variable numbers of mixed inflammatory cells. *Cryptococcus neoformans* was confirmed as the infective species through polymerase-chain reaction (PCR). Previous reports of cryptococcosis in psittacine in other countries have described proliferative lesions affecting the beak with disseminated infection to coelomic organs; lesions were restricted to the lungs and air sacs in this case. The source of inoculation in this cockatoo is unknown. To our knowledge, we provide the first report of *Cryptococcus neoformans* infection in a psittacine in the U.S. In wild and domestic avian species with respiratory disease, cryptococcosis should be considered, even in the absence of proliferative beak lesions. Furthermore, this report can raise awareness for prevention of outbreaks and potential zoonotic transmission.

Saturday, October 30, 2021

4:51 p.m. – 4:54 p.m. CDT

### **OSTEOFLUOROSIS IN A FREE RANGING CALIFORNIA SEA LION**

Margaret Martinez<sup>1</sup>, Michelle Rivard<sup>1</sup>, Jaclyn Isbell<sup>1</sup>, Chelsea Sykes<sup>2</sup>, Robert Poppenga<sup>2</sup>, Pdraig Duignan<sup>1</sup>

<sup>1</sup>The Marine Mammal Center, Sausalito, CA, USA, <sup>2</sup>California Animal Health and Food Safety Laboratory, School of Veterinary Medicine, University of California, Davis, CA, USA

A free ranging subadult male California sea lion (CSL) was admitted to The Marine Mammal Center with a primary clinical differential of a musculoskeletal disease. Radiographs demonstrated periosteal new bone proliferation on the mandible and both

humeri. Despite care, the animal continued to decline, and euthanasia was elected. Necropsy revealed segmental circumferential periosteal new bone proliferation on the diaphysis of both femurs and humeri. New bone proliferation was also on both mandibles and the cranium. Histopathology established that the periosteal woven bone was formed from endochondral and a lesser extent intramembranous ossification radiating perpendicular to the pre-existing cortical bone, entrapping atrophied muscles bundles at the periphery, and with remodeling of the inner two thirds of the new bone trabeculae. Differentials for hyperostosis included a toxicity, congenital disease, and hypertrophic osteopathy. Total fluoride levels within various bones of the case ranged from 4,000 – 9,700 ppm dw, 2-3x higher than levels found to cause hyperostosis in adult cattle. Ribs from 4 CSLs of various ages and both sexes had an average total fluoride of 1,575 ppm dw, which is twice as high as the bone fluoride levels of normal adult cattle, but 6x lower than the rib fluoride levels in the present case. Therefore, this is the first case of osteofluorosis in a free ranging marine mammal. As the growth plates and teeth were not affected, this animal was likely exposed later in life; however, the exact source is not known as male CSLs have a large range and varied diet.

Saturday, October 30, 2021

4:54 p.m. – 4:57 p.m. CDT

## **WOMEN REPRESENTATION AND GENDER EQUALITY IN DIFFERENT ACADEMIC LEVELS IN VETERINARY SCIENCE**

Xinyue Liu, Rebecca Dunlop, Rachel Allavena, Chiara Palmieri

School of Veterinary Science, The University of Queensland, Gatton, Australia

### Background

Women's participation and completion at veterinary schools has increased globally for the past few decades. However, increased female graduates have not translated into similar patterns of academic staffing.

### Objectives

This study aimed to evaluate, and compare, the proportion of female academic staff in veterinary science faculties in Australia and New Zealand, Europe, and North America. The study assessed workplace gender distribution within each academic level and compared distributions between academic level in different veterinary science faculties and countries.

### Methods

The gender distribution within each academic level at eight accredited veterinary faculties in Australia and New Zealand, 38 accredited faculties in USA and Canada, and 98 accredited faculties in Europe were analyzed. For all analyses, general linear models were used, assuming a binomial distribution and with gender as the binomial response variable.

### Results



Women occupied 47.9%, 45.5% and 47.5% of the academic positions in Australia/New Zealand, USA/Canada and Europe, respectively. Compared to their male counterparts, female academics were more likely to hold the lower ranked positions. The gender distribution skewed toward men in the senior positions at or above Associate Professor level in all analyzed regions.

## Conclusions

The findings of this study confirm gender inequality in academic progression meaning there is a continued need to develop strategies to eliminate inequity in veterinary science faculties worldwide.

## Highlighted Posters: Natural Disease

Sunday, October 31, 2021 | 11:00 a.m. – 11:03 a.m. CDT

Sunday, October 31, 2021

11:00 a.m. – 11:03 a.m. CDT

### INVOLVEMENT OF FELIS CATUS PAPILLOMAVIRUS TYPE 2 IN THE TUMORIGENESIS OF FELINE MERKEL CELL CARCINOMA

Soma Ito<sup>1</sup>, James Chambers<sup>1</sup>, Ayumi Sumi<sup>1</sup>, Nanako Yamashita-Kawanishi<sup>1</sup>, Tetsuo Omachi<sup>2</sup>, Takeshi Haga<sup>1</sup>, Hiroyuki Nakayama<sup>1</sup>, Kazuyuki Uchida<sup>1</sup>

<sup>1</sup>University of Tokyo, Tokyo, Japan, <sup>2</sup>Diagnostic Laboratory Patho Labo, Shizuoka, Japan

**Background:** Merkel cell carcinoma (MCC) is a cutaneous neuroendocrine tumor and caused by polyomavirus in human. We recently demonstrated that cats with MCC often have other proliferative cutaneous lesions, such as Bowenoid *in situ* carcinoma (BISC), squamous cell carcinoma (SCC) and basal cell carcinoma (BCC). **Objective:** Based on this finding, we hypothesized that *Felis catus* papillomavirus (FcaPV) is involved in the pathogenesis of MCC, similar to SCC and BCC. In this study, we aimed to elucidate the relationship between feline MCC and FcaPV. **Methods:** Twenty-one feline MCC cases were examined. FcaPV2-specific PCR was carried out using DNA extracted from formalin-fixed paraffin-embedded tissues. To localize FcaPV2 oncogenes, *in situ* hybridization (ISH) was performed using a DNA probe for *E7*. Also, *E6* and *E7* mRNA expression was assessed by RNAscope ISH. Furthermore, immunoreactivity for tumor suppressor proteins was evaluated by immunohistochemistry. **Results:** PCR detected FcaPV2 DNA in 20/21 MCC tissues. ISH for *E7* revealed punctate nuclear signals within tumor cells in 19/21 MCC, and RNAscope revealed multiple dot-like nuclear and cytoplasmic signals in 15/21 MCC. Increased immunoreactivity for p16<sup>CDKN2A</sup> and decreased immunoreactivity for retinoblastoma protein (pRb) and p53 were immunohistochemically confirmed in 20/21 MCC. These findings were also observed in BISC, SCC, and BCC of MCC-affected cats. **Conclusions:** The present study suggests that FcaPV2 infection may be a major etiological factor of MCC in cats. Similar to other FcaPV-induced tumors, subsequent inhibition of pRb and p53 induced by integration

and expression of the viral oncogenes may be associated with feline MCC tumorigenesis.

Sunday, October 31, 2021

11:03 a.m. – 11:06 a.m. CDT

### **HISTOLOGIC FINDINGS IN EQUINE TEETH SUBMITTED FROM AN EQUINE REFERRAL HOSPITAL**

Sarah Cudd<sup>1</sup>, Brian Murphy<sup>2</sup>, James Brown<sup>3</sup>, Charles Schwarten<sup>1</sup>, Elise LaDouceur<sup>1</sup>  
<sup>1</sup>Joint Pathology Center, Silver Spring, MD, USA, <sup>2</sup>UC Davis School of Veterinary Medicine, Davis, CA, USA, <sup>3</sup>Virginia Tech, Marion DuPont Scott Equine Medical Center, Leesburg, VA, USA

**Background:** The histologic analysis of equine teeth by pathologists is increasingly common due to the rapidly growing field of equine dentistry. However, there are few references to aid in evaluating these teeth.

**Objective:** Review histologic findings in equine tooth biopsies to provide reference material for pathologists.

**Methods:** For control specimens, incisors and cheek teeth were removed from horses that were euthanized with no history of dental disease. Control and biopsy equine teeth were fixed whole in 10% neutral buffered formalin and decalcified in a rapid acid decalcification solution (RDO). Incisors, canine, and wolf teeth (vestigial MaxPM1) were decalcified 7-10 days. Cheek teeth were decalcified up to 21 days. Teeth were sectioned in coronal sections at occlusal, mid, and apical levels, processed routinely, sectioned at 5µm thickness, and stained with hematoxylin and eosin.

**Results:** One hundred and forty teeth, including 83 incisors, 6 canines, 1 wolf (PM1), 17 premolars, and 33 molars, were examined from 78 horses ranging from 3-31 years old (mean 14 years old). The most common diagnoses per horse were periodontitis (38.5% [30/78]), equine odontoclastic tooth resorption and hypercementosis (EOTRH; 37.2% [29/78]), pulpitis/pulp necrosis (29.5% [23/78]), reactive hypercementosis (19.2% [15/78]), fracture (17.9% [14/78]), caries (16.7% [13/78]), pulp stones (6.4% [5/78]), bacterial infection (5.1% [4/78]), within normal limits (5.1% [4/78]), ameloblastoma (1.3% [1/78]), squamous cell carcinoma (1.3% [1/78]), and odontodysplasia (1.3% [1/78]).

**Conclusions:** Inflammation and EOTRH were common diagnoses, while neoplasia and odontodysplasia were rare. Reactive hypercementosis was considered secondary to inflammation and/or fracture. Pulp stones were presumed incidental.

Sunday, October 31, 2021

11:06 a.m. – 11:09 a.m. CDT

### **HISTOLOGIC LESIONS OF CESTODIASIS IN OCTOPUSES**

Daniel Finnegan<sup>1</sup>, Michael Murray<sup>2</sup>, Salvatore Frasca, Jr.<sup>3</sup>, Michael Garner<sup>4</sup>, Elise LaDouceur<sup>1,4</sup>

<sup>1</sup>Joint Pathology Center, Silver Spring, MD, USA, <sup>2</sup>Monterey Bay Aquarium, Monterey, CA, USA, <sup>3</sup>Connecticut Veterinary Medical Diagnostic Laboratory, University of Connecticut, Storrs, CT, USA, <sup>4</sup>Northwest ZooPath, Monroe, WA, USA

**Background:** Previous reports of cestodiasis in cephalopods describe the anatomic location of infection and geographic location of the infected specimen. Although cestodiasis is well documented in cephalopods, particularly European species, there are no reports of detailed histologic findings of this infection.

Hypothesis: Cestodiasis causes histologic lesions in octopuses.

**Methods:** Northwest ZooPath archives were searched for cases of octopuses with cestode infections; HE slides were reviewed.

**Results:** Eight octopuses with cestodiasis were identified, including three common octopuses (*Octopus vulgaris*), two Caribbean reef octopuses (*Octopus briareus*), two two-spot octopuses (*Octopus bimaculoides*), and one giant Pacific octopus (*Enteroctopus dofleini*). Cestodes were present in the cecum (4/4 cases; 100%), intestines (4/6 cases; 67%), digestive gland (3/7 cases; 43%), renal appendage (1/5 cases; 20%), and chitinous alimentary tract (1/6 cases; 17%). In four cases (4/8; 50%), cestodes were invading tissue and associated with hemocytic inflammation and necrotic tracts in the digestive gland (3/3 cases), renal appendage (1/1 case), and/or intestines (2/4 cases). Inflammation was commonly mild. Cestodes were confined to the lumen without tissue invasion when present in the cecum and chitinous alimentary tract. The most common concurrent diseases included enteric *Aggregata* sp. infection, branchial rickettsial-like infection, enteric larval nematode infection, and digestive gland atrophy.

**Conclusions:** Cestodes can be pathogens in octopuses, particularly in the digestive gland, intestines, and renal appendage, where infection is often associated with mild inflammation and necrosis. Cestode infection in other portions of the alimentary tract was not associated with inflammation or tissue invasion.

Sunday, October 31, 2021

11:12 a.m. – 11:15 a.m. CDT

### **A NOVEL IDIOPATHIC HEPATITIS SYNDROME IN TEN HORSES FROM INDIANA**

Patrick Huang<sup>1,2</sup>, Margaret Miller<sup>1</sup>, Janice Kritchevsky<sup>1</sup>, Carla Olave<sup>1</sup>, Sandra Taylor<sup>1</sup>

<sup>1</sup>Purdue University, West Lafayette, IN, USA, <sup>2</sup>NIH Comparative Biomedical Scientist Training Program, Bethesda, MD, USA

**Background:** Hepatitis of unknown cause was diagnosed in 10 horses between January and March of 2021, increased from 1-5 cases/year in the preceding 5 years.

**Objective:** Correlation of histologic with clinical features in horses with idiopathic hepatitis.

**Methods:** Inclusion criteria were fever and increased serum gamma-glutamyl transferase (GGT) activity. Liver biopsies from 8/10 horses were histologically reviewed and graded for hepatocellular death, biliary epithelial injury, inflammation, and fibrosis.

**Results:** Five mares and 5 geldings (median age 14 years) representing 6 breeds presented to the Purdue University from 10 Indiana premises. Fever was at least biphasic in 9/10 horses. Besides increased GGT activity, common biochemical abnormalities included hyperbilirubinemia (8/10), hyperfibrinogenemia (7/9), increased sorbitol dehydrogenase activity (3/4), and increased serum amyloid A concentration (3/3). Histologic impression of the liver biopsies was chronic hepatitis. Lesions included piecemeal (7/8) and/or random hepatic necrosis (5/8) with mainly portal lymphohistiocytic inflammation and fibrosis (8/8), increased biliary profiles (6/8), and ductular reaction (1/8). Laboratory tests were negative for known causes of equine hepatitis. Hospitalized horses (6/10) received nonsteroidal anti-inflammatory drugs, acetaminophen, antibiotics, and/or omeprazole, with clinical resolution (10/10) over 4-12 weeks. However, histologic lesions persisted in 3 horses evaluated on follow-up examination up to 3 months after clinical resolution.

**Conclusions:** The diagnosis of chronic hepatitis in febrile horses suggests viral or other infection, but a cause was not identified despite culture and molecular tests for microbial pathogens. Further investigation is required to determine the pathogenesis and prognosis of this idiopathic equine hepatitis syndrome.

Sunday, October 31, 2021

11:15 a.m. – 11:18 a.m. CDT

### **IMPROVING METHODS OF CONTROL OF AMERICAN FOULBROOD IN HONEY BEES IN SASKATCHEWAN**

Michael Zabrodski<sup>1</sup>, Geoff Wilson<sup>2</sup>, Igor Moshynskyy<sup>1</sup>, Mohsen Sharafi<sup>1</sup>, Lara Reitsma<sup>1</sup>, Mateo Castano Ospina<sup>1</sup>, Jessica DeBruyne<sup>1</sup>, Alexandra Wentzell<sup>1</sup>, Sarah Wood<sup>1</sup>, Ivanna Kozii<sup>1</sup>, Colby Klein<sup>1</sup>, Jenna Thebeau<sup>1</sup>, Fatima Masood<sup>1</sup>, Igor Medici de Mattos<sup>1</sup>, Allyssa Cloet<sup>1</sup>, Brandele Brown<sup>1</sup>, Melanie Roulin<sup>1</sup>, Dana Liebe<sup>1</sup>, LaRhonda Sobchishin<sup>1</sup>, Tasha Epp<sup>1</sup>, Antonio Ruzzini<sup>1</sup>, Elemir Simko<sup>1</sup>

<sup>1</sup>Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada, <sup>2</sup>Ministry of Agriculture, Government of Saskatchewan, Prince Albert, SK, Canada

**Background:** American foulbrood (AFB) is a devastating disease of honey bee larvae caused by the spore-forming bacterium, *Paenibacillus larvae*. Disease often results in colony death by either natural disease progression or destruction by the beekeeper to

limit the spread of spores to other colonies. North American beekeepers rely heavily on routine antimicrobial metaphylaxis to prevent disease, but treatment fails to eliminate infectious spores. With the emergence of antibiotic-resistant strains of *P. larvae*, there is a need for alternative, evidence-based management tools to reduce reliance on antibiotics while maintaining sustainable beekeeping operations.

**Methods:** Province-wide surveillance of *P. larvae* spores in honey may be a proxy for evaluating yard-level AFB risk. Accordingly, we analyzed the spore content of pooled, extracted honey from 52 large-scale and 72 small-scale Saskatchewan (Canada) beekeepers, representing over 70,000 of the province's 110,000 honey bee colonies.

**Objective:** These results, in conjunction with data from an accompanying questionnaire, will establish prognostic reference ranges for honey to identify the immediate risk of AFB outbreaks in antibiotic-dependent management systems.

**Results:** To date, spores have been detected in 52.2% of large-scale honey samples at low (66.5%), medium (26.8%), or high (6.7%) concentrations. For small-scale samples, 28.2% have detectable spores at low (84.1%), medium (13.6%), or high (2.3%) concentrations.

**Conclusions:** Subsequent incidence of AFB was observed in 1/1 small-scale and 3/6 large-scale beekeepers with high spore concentrations, and 3/14 large-scale beekeepers with medium spore concentrations. Consistent with our current risk criteria, AFB has not been reported in beekeepers with low spore concentrations.

Sunday, October 31, 2021

11:18 a.m. – 11:21 a.m. CDT

## **REVIEW OF NEOPLASIA IN THE ALIMENTARY TRACT OF CAPTIVE BEARDED DRAGONS (POGONA SPP.)**

Elise LaDouceur<sup>1,2</sup>, Alexandria Argue<sup>1</sup>, Michael Garner<sup>2</sup>

<sup>1</sup>Joint Pathology Center, Silver Spring, MD, USA, <sup>2</sup>Northwest ZooPath, Monroe, WA, USA

**Background:** There are multiple reports of gastric neuroendocrine carcinoma in bearded dragons. Other types of alimentary neoplasia are rarely reported in this species. **Objective:** Describe the anatomic distribution and types of alimentary neoplasia in bearded dragons. **Methods:** Biopsy and autopsy specimens of alimentary neoplasia in captive bearded dragons were collated from the Northwest ZooPath and Joint Pathology Center archives. **Results:** Fifty one cases were identified in the stomach (n=26), oral cavity (n=18), intestines (n=13), esophagus (n=3), and cloaca (n=2). Round cell neoplasia was diagnosed in the alimentary tract in 14 cases, all of which had extra-alimentary involvement, including from most to least commonly in the liver, lung, spleen, kidney, heart, pancreas, gonad, skeletal muscle, trachea, adipose tissue, brain, eye, blood vessels, bone marrow, adrenal gland, thyroid gland, bone, nasal cavity, and mesentery. Oral neoplasms included sarcoma, adenomatous polyp, round cell neoplasia, fibromatous epulis of periodontal ligament origin, and myxoma. Esophageal neoplasms included round cell neoplasia. Gastric neoplasms included

neuroendocrine carcinoma, round cell neoplasia, adenocarcinoma, and sarcoma. Enteric neoplasms included round cell neoplasia, sarcoma, adenocarcinoma, and metastatic sarcoma. Cloacal neoplasms included round cell neoplasia and squamous cell carcinoma. **Conclusions:** Round cell neoplasia and gastric neuroendocrine carcinoma were the most common diagnoses. Round cell neoplasia was consistently present in extra-alimentary sites; all other neoplasms were primary alimentary neoplasms except for one metastatic sarcoma. Sarcomas and polyps were common in the oral cavity and rare to absent elsewhere. Adenocarcinoma was rare and only identified in the stomach and intestines.

Sunday, October 31, 2021

11:21 a.m. – 11:24 a.m. CDT

### **SYSTEMIC AVIAN POXVIRUS INFECTIONS ASSOCIATED WITH THE B1 SUBCLADE OF CANARYPOX VIRUS**

Devinn Sinnott<sup>1,2</sup>, Jennifer Burchell<sup>2</sup>, Carmel Witte<sup>2</sup>, Rachel Burns<sup>2</sup>, Steven Kubiski<sup>2</sup>

<sup>1</sup>University of California, Davis, Davis, CA, USA, <sup>2</sup>San Diego Zoo Wildlife Alliance, San Diego, CA, USA

**Background:** Avian poxviruses classically manifest as two forms: cutaneous (“dry”) pox affecting the skin, and diphtheritic (“wet”) pox affecting the oropharyngeal and upper respiratory and gastrointestinal mucosa. Systemic viral spread beyond the skin and mucous membranes is rarely reported.

**Objective:** We evaluated the histopathologic lesions of systemic avian poxvirus over a 20-year period at a zoological institution and assessed strain and host factor variations between systemic and dry/wet poxvirus infections.

**Methods:** Hematoxylin and eosin-stained slides were reviewed for all suspected systemic avian poxvirus cases identified in a medical record search. In-situ hybridization was used to assess the intralesional presence of poxvirus DNA. Polymerase chain reaction targeting two loci (REV LTR flanking region and core P4b protein gene) was performed using frozen tissues to identify viral strains.

**Results:** Twenty-two cases of systemic avian poxvirus were identified. Two histopathologic patterns emerged: 1) histiocytic inflammation in multiple organs with intrahistiocytic viral inclusions, and 2) severe, localized dry or wet pox lesions with viral inclusions within dermal and subepithelial macrophages. In-situ hybridization confirmed the presence of poxvirus DNA within macrophages in both patterns. Sequences of the REV LTR flanking region from all systemic avian poxvirus cases were identical to a previously described condorpox virus. Sequences of the core P4b protein gene from all systemic avian poxvirus cases grouped into cluster 2 of the B1 subclade of canarypox viruses.

**Conclusion:** The definitive factors leading to systemic avian poxvirus infections are uncertain but may involve particular strain variations in combination with various possible host factors.

Sunday, October 31, 2021

11:24 a.m. – 11:27 a.m. CDT

## **AN OUTBREAK OF CHLAMYDIOSIS IN CRITICALLY ENDANGERED CAPTIVE BREEDING PUERTO RICAN PARROTS (*AMAZONA VITTATA*)**

HL Shivaprasad<sup>1</sup>, Karine Laroucau<sup>2</sup>, Rachid Aaziz<sup>2</sup>, Fabien Vorimore<sup>2</sup>, Chiara Palmieri<sup>3</sup>

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### Background

Puerto Rican parrots (*Amazona vittata*) is the only remaining native parrot in Puerto Rico, listed as critically endangered species since 1994. Conservation efforts are thus critical to save this bird from extinction, including prompt identification and treatment of infectious threats.

### Objective

This study summarizes the main pathologic findings, diagnostic plan and treatment of chlamydiosis in Puerto Rican Parrots.

### Methods

An outbreak of chlamydiosis was diagnosed by gross examination, histopathology, IHC, and combined-PCR/high-resolution melting assay in an aviary containing 250 adults and 100 juvenile critically endangered Puerto Rican Parrots housed in captive breeding program in the territory of Puerto Rico.

### Results

The disease was characterized by depression, ruffled feathers, dehydration and death of 20 parrots over a period of two weeks. Postmortem examination revealed fibrinous thickening of the air sacs, hepatomegaly, splenomegaly and occasionally thickening of the pericardial sac and renomegaly. Histopathology revealed fibrinous airsacculitis, pericarditis, conjunctivitis, hepatitis, splenitis, nephritis, synovitis, osteomyelitis, sinusitis, associated with elementary bodies of *Chlamydia* sp. in the cytoplasm of macrophages and epithelial cells, further confirmed by IHC. *Chlamydia psittaci* genotype A was isolated in cell culture and identified as group III pigeon by PCR/high resolution melting assay. Treatment of birds with Doxycycline in the drinking water for 45 days eradicated the disease.

### Conclusions

Chlamydiosis should be considered as one of the differential diagnosis in Puerto Rican parrots showing non-specific clinical signs and unexplained mortality. Pigeons in the

vicinity that had access to the aviary were probably the most likely source of chlamydia to the parrots.

## Highlighted Posters: Experimental Disease, Industrial and Toxicologic Pathology

Tuesday, November 2, 2021 | 12:00 p.m. – 12:03 p.m. CDT

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12:00 p.m. – 12:03 p.m. CDT

### DOES BLUEBERRY POLLEN PREDISPOSE HONEY BEES TO EUROPEAN FOULBROOD DISEASE?

Ivanna Kozii<sup>1</sup>, Igor de Mattos<sup>1</sup>, Melanie Roulin<sup>1</sup>, Sarah Wood<sup>1</sup>, Colby Klein<sup>1</sup>, Michael Zabrodski<sup>1</sup>, Mohsen Sharafi<sup>1</sup>, Jenna Thebeau<sup>1</sup>, Dana Liebe<sup>1</sup>, Jessica Debruyne<sup>1</sup>, Brandele Brown<sup>1</sup>, Fatima Masood<sup>1</sup>, Larhonda Sobchishin<sup>1</sup>, Igor Moshynskyy<sup>1</sup>, Meghan Millbrath<sup>2</sup>, Robyn McCallum<sup>3</sup>, Marta Guarna<sup>4</sup>, Patricia Wolf Veiga<sup>5</sup>, Eric Gerbrandt<sup>6</sup>, Elemir Simko<sup>1</sup>

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European foulbrood (EFB) is an important disease of honey bees caused by *Melissococcus plutonius*, a bacterium that infects larval midgut and outcompetes the larva for nutrients. The recent increased incidence of EFB in honey bee colonies involved in blueberry pollination has been associated with lower acidity and protein contents of blueberry pollen compared to polyfloral pollen. However, this association is putative and has not been tested.

Our objective was to *i)* establish an in-vivo model of EFB infection and *ii)* compare the effects of blueberry and polyfloral pollen on survival and weight of bees inoculated with incremental doses of *M. plutonius*.

We inoculated 5-day-old honey bee larvae with 0, 5000 and 45,000 colony forming units (CFU) of *M. plutonius*. These larvae were raised within restricted mesh-lined compartments inside a hive containing either blueberry or polyfloral pollen. Capping rate (survival) and weight at emergence were recorded.

We have successfully established an *in-vivo* model for EFB research. We found that larval capping rate inversely correlated with *M. plutonius* infection doses. Additionally, bees inoculated with the highest dose of *M. plutonius* and raised on blueberry pollen had a significant 6.6% decreased survival rate compared to those raised on polyfloral pollen.

Our *in vivo* model of EFB is well suited to study *M. plutonius* infection in honey bees. The results of this study suggest that blueberry pollen diet may be associated with increased mortality of bee larvae exposed to high infectious dose of *M. plutonius*.



Tuesday, November 2, 2021  
12:03 p.m. – 12:06 p.m. CDT

### **INFECTING AMBLYOMMA AMERICANUM TICKS WITH CYTAUXZON FELIS VIA DIRECT INJECTION**

Tzushan Yang<sup>1</sup>, Mason Reichard<sup>2</sup>, Henry Marr<sup>1</sup>, Leah Cohn<sup>3</sup>, Laura Nafe<sup>2,3</sup>, Nathan Whitehurst<sup>1</sup>, Adam Birkenheuer<sup>1</sup>

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**Background:** *Cytauxzoon felis* is a tick-borne hemoprotozoa that causes life-threatening disease in cats in the United States. Currently, platforms for *C. felis* research are limited to natural or experimental infection of domestic cats.

**Objective:** Develop an alternative model by infecting *Amblyomma americanum* ticks with *C. felis* via direct injection.

**Methods:** *A. americanum* adults were injected with *C. felis*-infected feline erythrocytes through the anal pore or percutaneously into the hemocoel. RNAscope<sup>®</sup> *in situ* hybridization (ISH) was used to visualize the parasites within the ticks at different time points after injection. Eight weeks after injection, ticks were tested for *C. felis* RNA via RT-qPCR and fed on 3 naïve cats to assess their ability to transmit *C. felis*.

**Results:** ISH signals were observed in ticks up to 3 weeks after injection, but the number of hybridization signals notably decreased over time. Prior to the transmission challenge, 50% of the sampled ticks were positive for *C. felis* RNA. Despite successful tick attachment and feeding activity during the transmission challenge, none of the cats became infected with *C. felis*.

**Conclusion:** In this study, the injected *C. felis* remained alive in ticks but failed to progress to infective sporozoites after injection. This outcome may be associated with uncharacterized differences in *C. felis* life cycle or the lack of the feeding and molting process in our model. Nonetheless, our study demonstrated the potential of using ticks to study *C. felis*, and the utility and concept of such model should continue to be refined and explored.

Tuesday, November 2, 2021  
12:06 p.m. – 12:09 p.m. CDT

### **SYSTEMIC AND RESPIRATORY INFLAMMATORY RESPONSES FROM PREDISPOSING FACTORS OF BOVINE RESPIRATORY DISEASE IN BEEF CALVES**

Seyed Saeid Tabatabaei<sup>1</sup>, DeLenn Burrows<sup>1</sup>, Monica Baquero<sup>1</sup>, Ksenia Vulikh<sup>1</sup>, Derek Haley<sup>2</sup>, Jeff Caswell<sup>1</sup>

<sup>1</sup>Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, <sup>2</sup>Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

**Background:** The literature suggests that predisposing factors for bovine respiratory disease (BRD) such as weaning, transport, and viral infection cause immunosuppression, which predisposes to subsequent bacterial infection. However, our recent work suggests that a dysregulated pro-inflammatory response plays a role in BRD development.

**Objective:** Characterize systemic and local respiratory inflammatory responses after transport or abrupt weaning in calves, to determine if these risk factors cause a heightened response to inflammatory stimuli.

**Methods:** Three groups of calves were considered: two-stage (low-stress) weaning with nose flaps (n=5), abrupt weaning (n=6), and abrupt weaning and transportation (n=6). Blood and bronchoalveolar lavage fluid (BALF) samples were collected on arrival and 24h after an aerosolized inflammatory stimulus on day 1. Differential cell counts were performed on blood. Cell counts, cytokine concentrations (IL-1b, IL-6, IL-8), T cell subpopulations, and total MHC II expression were evaluated in BALF.

**Results:** In BALF, there were higher IL-8 concentrations, percentages of neutrophils and gamma delta T cells, and MHC II expression on leukocytes in abruptly weaned and transported calves compared to those with two-stage weaning.

**Conclusions:** Our results suggest that abrupt weaning and transportation promote an increased response to an aerosolized inflammatory stimulus. These findings suggest a novel mechanism by which well-known risk factors predispose to BRD.

Tuesday, November 2, 2021  
12:09 p.m. – 12:12 p.m. CDT

## **EQUINE ALVEOLAR MACROPHAGES AND BLOOD MONOCYTE-DERIVED MACROPHAGES RESPOND DIFFERENTLY TO BARN DUST-ANALOGUE CHALLENGE**

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### Background

Severe equine asthma (SEA) is a non-infectious inflammatory disease of the lower respiratory tract caused by exposure to respirable barn dusts. Inhalation of a mixture of *Aspergillus fumigatus* spores, lipopolysaccharide, and silica microspheres (FLS) as barn dust analogue causes SEA exacerbation in susceptible horses. Alveolar macrophages (AMs) are lung-resident macrophages and the predominant immune cell in the healthy equine lower respiratory tract. Additionally, blood monocytes are recruited to inflamed tissues, including the lung, where they differentiate into monocyte-derived macrophages (MDMs). However, responses of AMs and MDMs to barn dust mimics are unclear.

### Objectives

To assess how AMs and MDMs react to FLS exposure.

## Methods

AMs and MDMs from six healthy horses were incubated with FLS in serum-free cell culture medium for six hours, using medium without FLS as control. Cell surface markers and cytokine production after FLS exposure were analyzed by flow cytometry and direct cytokine measurement, respectively.

## Results

Flow cytometry results showed that MDMs but not AMs had reduced surface expression of CD206 after FLS exposure. Cell culture supernatant from non-FLS exposed AMs, but not MDMs contained IL-8, indicating constitutive production by AMs. FLS-exposure induced production of TNF- $\alpha$ , IL-8, IL-1 $\beta$  and INF- $\gamma$  in AMs, and production of TNF- $\alpha$ , IL-8 and IL-10 in MDMs.

## Conclusions

These results indicate unique immunophenotypes and cytokine profiles in two different types of equine macrophages. The results emphasize that AMs and MDMs are not interchangeable for *in vitro* investigations of the pathogenesis of SEA, and provide a rationale for further functional characterization.

Tuesday, November 2, 2021

12:12 p.m. – 12:15 p.m. CDT

### **UPREGULATED INFLAMMATORY PATHWAYS IN THE SPINAL CORD OF SIV-INFECTED MACAQUES**

Kathleen Mulka<sup>1</sup>, Suzanne Queen<sup>1</sup>, Audrey Knight<sup>1,2</sup>, Lisa Mangus<sup>1</sup>, Sarah Beck<sup>1</sup>, Megan McCarron<sup>1</sup>, Clarisse Solis<sup>1</sup>, Carlo Colantuoni<sup>1</sup>, Joseph Mankowski<sup>1</sup>

<sup>1</sup>Johns Hopkins University, Baltimore, MD, USA, <sup>2</sup>University of North Carolina, Chapel Hill, NC, USA

**Background:** HIV-associated peripheral neuropathy is one of the most prevalent clinical manifestations of neurological disease associated with HIV infection even with anti-retroviral treatment. The spinal cord is a key relay point for sensory pathways, and studies have shown increased glial activation and pro-inflammatory signaling in HIV-infected patients although evaluation of this tissue in humans has been limited. In contrast, the SIV/monkey model allows for evaluation of the CNS at strategic time points throughout infection. Our previous work has shown myelitis and increased CD68 expression by microglia in the spinal cord of infected animals.

**Objective:** Our objective was to compare gene expression patterns in the spinal cord of SIV-infected and uninfected macaques by global RNA sequencing to characterize immunoregulatory pathways.

**Methods:** We isolated RNA from the spinal cord of SIV-infected and uninfected animals, prepared cDNA libraries followed by sequencing, and analyzed the relative mRNA expression in these groups.

**Results:** Differential expression analysis using DESeq2 revealed 355 genes upregulated in SIV infection and 219 downregulated genes. Pathway analysis using Enrichr indicated that upregulated genes in SIV-infected spinal cord were aligned with interferon and viral response pathways. Additionally, this upregulated gene set significantly overlapped with those expressed in myeloid cells and microglia. Downregulated genes were involved in cholesterol and collagen biosynthesis, and TGF-beta regulation of extracellular matrix.

**Conclusions:** These findings identify activated immune pathways in the spinal cord immunologic compartment that may contribute to neuropathy, and implicate microglia and myeloid cells as key players involved in the spinal cord response to SIV.

Tuesday, November 2, 2021  
12:15 p.m. – 12:18 p.m. CDT

### **PATHOLOGY OF LYMPHOPROLIFERATIVE DISEASE VIRUS INFECTION IN NATURALLY AND EXPERIMENTALLY INFECTED TURKEYS**

Chloe Goodwin<sup>1,2</sup>, Kayla Adcock<sup>2</sup>, Mark Ruder<sup>2</sup>, Rebecca Poulson<sup>2</sup>, Kevin Niedringhaus<sup>3</sup>, Daniel Mead<sup>2</sup>, Heather Fenton<sup>4</sup>, Kevin Keel<sup>3</sup>, Nicole Nemeth<sup>1,2</sup>

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<sup>4</sup>School of Veterinary Medicine, Ross University, Basseterre, Saint Kitts and Nevis

**Background:** Lymphoproliferative disease virus (LPDV) is an oncogenic retrovirus associated with lymphoid proliferation and round cell neoplasia in wild and domestic turkeys. Since its initial detection in wild turkeys in 2009, high LPDV prevalence among apparently healthy wild turkeys across eastern North America has raised questions about transmission, pathogenesis, and risk to population health.

**Objective:** To experimentally reproduce LPDV infection and disease in domestic turkeys and compare lesions with naturally infected, wild turkeys.

**Methods:** Twenty-five, 4-week-old, domestic turkeys were LPDV-inoculated via various routes: oral, nasal, crop, or subcutaneous or coelomic injection. Blood was collected weekly until euthanasia and necropsy at 12 weeks post-inoculation. Pathology from diagnostic cases of 195 wild turkeys that were LPDV PCR-positive from 2009 to 2021 were reviewed.

**Results:** 11/25 (48%) inoculated birds had DNAemia at varying times from 1-12 weeks post-infection, representing all inoculation routes. All birds (sham and inoculated) displayed lymphoid proliferation including perivascular lymphocytic cuffs to discrete lymphoid follicles, most frequently liver, adrenal gland, and pancreas. Three inoculated birds had round cell neoplasia in spleen, intestine, adrenal gland, and/or bone marrow (27%). Sixty-three (32%) wild turkeys exhibited similar lesions but with skin most commonly affected (34/63; 54%). Round cell neoplasia was in 30/195 (15%) naturally infected wild turkeys.

**Conclusions:** Experimental LPDV infection was achieved via multiple routes in domestic turkeys and caused similar pathology to naturally infected wild turkeys. As with spontaneous LPDV infection in wild turkeys, neoplastic lesions were uncommon but raise questions about LPDV pathogenesis, diagnostic strategies, and epidemiology in wild turkeys.

Tuesday, November 2, 2021  
12:18 p.m. – 12:21 p.m. CDT

### **CLINICAL AND MORPHOLOGIC CHARACTERIZATION OF THE CENTRAL NERVOUS SYSTEM OF MICE INFECTED WITH VARIOUS STRAINS OF CHIKUNGUNYA VIRUS**

Jonathan Nagel<sup>1,2</sup>, Audrey Knight<sup>3</sup>, Elizabeth Anderson<sup>1</sup>, Hannah Atkins<sup>1,3</sup>, Stephanie Montgomery<sup>1,3</sup>, Victoria Baxter<sup>1,3</sup>

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**Background:** Chikungunya virus (CHIKV) infection in people often manifests as rash and arthritis and occasionally encephalomyelitis. CHIKV neurovirulence is poorly understood and lacks a well-established preclinical model.

**Objective:** We aim to establish a model of CHIKV neurovirulence by examining the clinical and histomorphologic manifestations of intracranial inoculation with different CHIKV strains in mice.

**Methods:** 4-6-week-old C57BL/6J mice were intracranially inoculated with PBS or one of three clinical CHIKV strains: SL15649, AF15561, SM2013, or neuroadapted SL15649 (MANV). Mice were monitored daily for clinical disease, and brains and spinal cords were harvested 3-29 days post-infection. Tissues were assessed for viral titers and histomorphology, including H&E and immunohistochemistry [cleaved caspase 3 (cC3), IBA1, GFAP, B220, CD4/CD8].

**Results:** Neurological signs (hunched posture, abnormal gait, abnormal tail posture) and weight loss were more prominent in mice infected with SL15649 than AF15561 and SM2013. Microscopic examination revealed brain inflammation was primarily lymphohistiocytic with variable amounts of CD4 and CD8 positive cells. Inflammation was focused in the corpus callosum and more severe in mice infected with SL15649 and AF15561. Other brain morphologic findings included cavitation, astrocyte and microglia proliferation, apoptosis (cC3 positive), and neuronal necrosis (cC3 negative). Spinal cord inflammation was rare, mild, and predominantly lymphohistiocytic. Mice infected with MANV developed more severe weight loss, neurological signs, and

encephalitis with higher peak titers than clinical CHIKV strains.

**Conclusions:** Clinical and morphologic findings in CHIKV-infected mice vary by CHIKV strain and are more severe following neuroadaptation, aiding in the development of a pre-clinical CHIKV model.